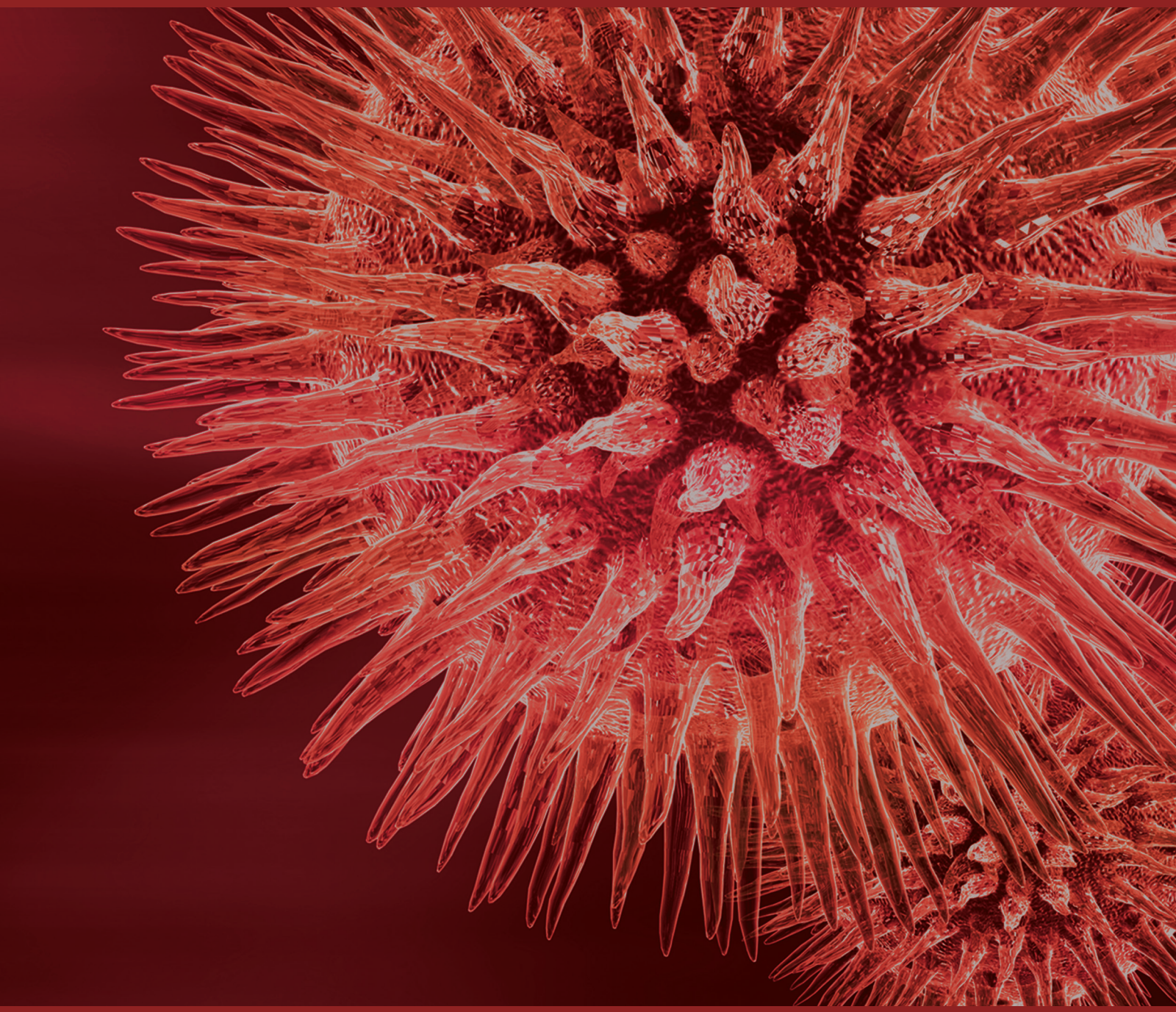
BioMed Research International

Genetics of Exercise and Sedentary Behaviors

Guest Editors: Eco de Geus, J. Timothy Lightfoot, Martine Thomis, Jaakko Kaprio, and Meike Bartels



Genetics of Exercise and Sedentary Behaviors

BioMed Research International

Genetics of Exercise and Sedentary Behaviors

Guest Editors: Eco de Geus, J. Timothy Lightfoot, Martine Thomis, Jaakko Kaprio, and Meike Bartels

Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 805476, 12 pages
http://dx.doi.org/10.1155/2014/805476

Research Article

Genetic and Environmental Influences on the Allocation of Adolescent Leisure Time Activities

Brett C. Haberstick, Joanna S. Zeiger, and Robin P. Corley

Institute for Behavioral Genetics, University of Colorado Boulder, Campus Box 447, Boulder, CO 80309-0447, USA

Correspondence should be addressed to Brett C. Haberstick; brett.haberstick@colorado.edu

Received 19 November 2013; Revised 6 February 2014; Accepted 14 April 2014; Published 20 May 2014

Academic Editor: Meike Bartels

There is a growing recognition of the importance of the out-of-school activities in which adolescents choose to participate. Youth activities vary widely in terms of specific activities and in time devoted to them but can generally be grouped by the type and total duration spent per type. We collected leisure time information using a 17-item leisure time questionnaire in a large sample of same- and opposite-sex adolescent twin pairs ($N = 2847$). Using both univariate and multivariate genetic models, we sought to determine the type and magnitude of genetic and environmental influences on the allocation of time toward different leisure times. Results indicated that both genetic and shared and nonshared environmental influences were important contributors to individual differences in physical, social, intellectual, family, and passive activities such as watching television. The magnitude of these influences differed between males and females. Environmental influences were the primary factors contributing to the covariation of different leisure time activities. Our results suggest the importance of heritable influences on the allocation of leisure time activity by adolescents and highlight the importance of environmental experiences in these choices.

1. Introduction

Adolescents are confronted with a large number of choices regarding their use of free or leisure time. Of the many options, they could choose to participate in extracurricular activities such as competitive sports teams or relatively unstructured activities such as socializing with their peers or solitary, passive, or sedentary activities such as watching television. Some adolescents may allocate a large amount of time to family, caring for younger members or doing housework, while others work in paid employment situations outside the home. How adolescents allocate their time is of importance as participation in social, physical, and passive types of activities has been associated with the quality of academic performance, physical and psychological health, and behavioral problems both concurrently and at subsequent ages [1–4].

Much of the research into how adolescents spend their time outside of school and work has focused on leisure time activities. So, appropriately, recent estimates from the American Time Use Survey (ATUS) suggest that both male and female adolescents (ages 15–19 years) in the United States spend more than five hours a day on leisure time activities, making leisure time second only to personal care activities (e.g., sleeping, bathing, and dressing) as a proportion of daily life, exceeding time spent at both school and work [5]. However, the definition of what constitutes leisure time varies between studies with regard to the classification of these activities. Although there is an absence of a standard definition in the literature, leisure time is operationalized as being either the amount of time spent engaged in activities or type of activity, but it could also be classified by its psychological and emotional impact [6]. Further, all aspects of leisure time will differ as a function of gender, race, age, and cultural influences [4, 7, 8].

Leisure time activities have been assessed in a number of ways, but self-report questionnaires remain the most widely used method for assessing a wide variety of leisure time activities [9]. In contrast to technology-based tools (e.g., accelerometry), self-report questionnaires provide a low cost and easy to use method for encompassing the diversity of adolescent out-of-school activities. Response bias is one important limitation, however, and may be due to pressures

to respond in a socially acceptable manner. Both physical activity and sedentary behaviors have been shown to be sensitive to this type of bias [10, 11], though not always [12].

Family, economic circumstances, and peers have all been implicated as meaningful influences on the allocation of leisure time [1, 13–15]. However, heritable influences may also be an important influence. Twin studies are well suited for investigating the extent to which genetic and environmental influences contribute to individual differences in how siblings of the same family allocate leisure time. Heritable influences are implicated when the monozygotic (MZ) twin correlation is twice the dizygotic (DZ) twin correlation, as MZ twins share all of their genes in common while DZ twins share only half or 50%. Shared environmental influences are those experiences that make siblings of the same family similar and are suggested when the DZ twin correlation is greater than half the MZ twin correlation. In situations where the DZ twin correlation is less than half the MZ twin correlation, nonadditive genetic or dominance contributions to individual differences are suggested.

Twin and family studies have primarily focused on leisure time physical activity and passive activities such as watching television. In general, the magnitude of genetic influences on individual differences ranges between zero and 85% with differences as a function of age, sex, the duration of physical activity, and means of assessment [16–23]. Shared environmental factors appear to have important influences on physical activity during childhood and early adolescence, with their effects diminishing into late adolescence and young adulthood [16]. To our knowledge, only a few twin studies of passive leisure time activities [24–26] have been reported and generally implicate increasing heritable influences as children age and decreasing contributions from environments that make siblings of the same family more alike.

Although physical and passive activities are both widely engaged in by adolescents, they are only two of a variety of activities in which adolescents participate. Other important activities include social, family, and intellectual pursuits. Along these lines, our analyses were designed to examine two questions. First, to what extent do genetic and environmental influences contribute to individual differences in adolescent leisure time activities? Second, to what extent do the genetic and environmental influences on one leisure time activity also influence other activities?

2. Methods

2.1. Subjects. A total of 2847 adolescent twins from 1429 families (11 families with only 1 twin) were drawn from the Longitudinal Twin Study [27] and the Community Twin Study [28, 29]. Zygosity status was determined by a combination of tester ratings and DNA polymorphisms, with discrepancies between testers and DNA results resolved by a second round of genotyping. Nine hundred twenty-four DZ same-sex twins (50.2% female), 1374 MZ twins (54.9% female), 547 DZ opposite-sex twins (50.3% female), and one pair of males whose zygosity was ambiguous due to DNA refusal completed questionnaires at an average age of 15.1 years (S.D. 2.2 years). The percentages of 12- to 18-year-olds in the total sample were roughly comparable (ranging from 10.0% to 22.9%), with higher proportions of 12- and 14-year-olds. The sample was self-identified as 86.5% White, with 7.8% endorsing multiple racial origins. Approximately 10% of the sample was identified ethnically as Hispanic.

2.2. Measures. Leisure time activity was measured with 17 items drawn from a questionnaire expanded from [30]. Leisure time activities were rated on a six-point Likert scale ranging from no hours spent (0), one hour or less (1), two to three hours (2), four to five hours (4), six to seven hours (5), or eight or more hours (6) spent on an activity after school or work and on weekends. Two items about the amount of time (hours) per week spent watching television on weekdays and weekends were summed into a single variable. A total leisure time composite representing the sum of all 17 items was created for comparison purposes after transformation of the Likert items into hour equivalents.

2.3. Analyses. Age trends within sex for each item were examined by regression serially on linear, quadratic, and cubic age terms. To determine which items formed coherent scales, unstandardized residuals after taking out significant age effects were then subjected to an exploratory factor analysis (principal components, varimax rotation) using SPSS, version 22. Based on these results, five leisure time scales were created and included: physical, social, intellectual, family, and passive activities. These five scales represent sums of age-corrected residual items.

Twin correlations and genetic models were estimated with sex as a covariate in the statistical software Mx [31]. Two genetic models were utilized for the current analyses: sex limitation and Cholesky decomposition [31, 32]. When based on data from same-sex sibling pairs, the sex limitation model examines whether the magnitude of heritable and environmental contributions to leisure time allocation differs between males and females (quantitative sex differences). When data are also available from opposite-sex sibling pairs, additional sex-specific parameters can be included in order to examine whether different factors contribute to leisure time activities in one sex but not the other (qualitative sex differences).

Although useful, univariate twin models may not provide enough statistical power to choose between genetic and environmental influences on a particular leisure time activity domain and may provide an overestimation of the heritability. When additional variables have been measured from the same individual, multivariate models can be more statistically powerful as they make use of all the covariance with other leisure time activity domains. Therefore, we also fit a Cholesky decomposition model to our data. This model examines the extent that genetic and environmental influences contribute to the covariation of different leisure time activities and is a simple restatement of that latent factor structure designated in our univariate models. Latent genetic (A) and environmental (C and E) influences are stratified into influences that are common to leisure time activities and those that are specific or residual to one activity (Figure 1).

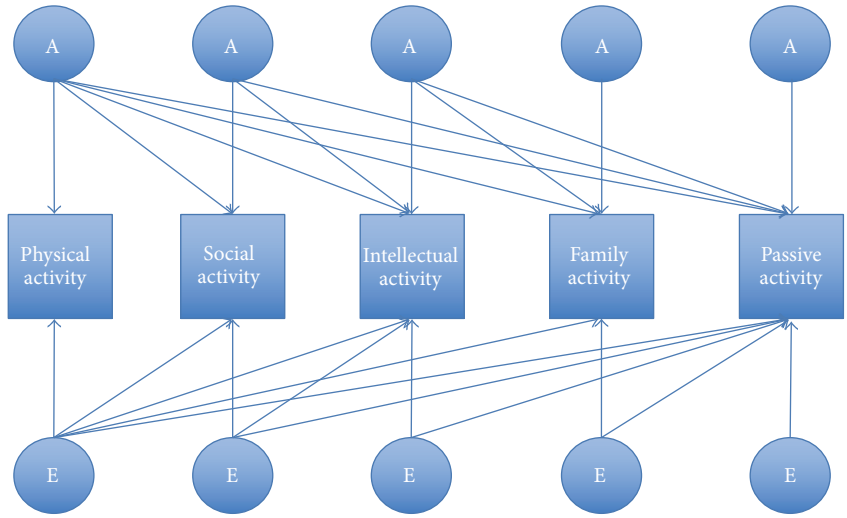


Figure 1: Cholesky decomposition model. This model decomposes the covariance between different leisure time activity domains into that due to genetic (G), including additive (A) genetic, and environmental, including shared (C) and nonshared (E), influences.

From this model, it is possible to obtain the genetic (environmental) correlation, which indexes the extent that genetic (environmental) influences are common to different leisure time activity domains.

The fit of our genetic models was evaluated using maximum-likelihood estimation. Our baseline model included the additive genetic (A) and nonshared environmental (E) latent factors and either a nonadditive genetic (D) or shared environment (C) factor, as both are confounded in sibling-based models. The significance of model parameters was evaluated by a comparison of twice log-likelihood (−2LL) for models with or without the parameters, with the difference distributed as a chi-square distribution and the degrees of freedom being equal to the difference between the number of parameters estimated. A nonsignificant difference in chi-square ($\Delta\chi^2$) between two models indicates that the parameters dropped from the more parsimonious model were not significantly different from zero. Models were accepted on the basis of the Akaike information criterion {AIC) [33] as calculated by subtracting twice the difference in the degrees of freedom from the difference chi-square between any particular model and the fullest, that is, least parsimonious, model considered. The AIC indexes the extent that a given model offers the most parsimonious, but adequate, explanation to the data, though limitations to using the AIC as a primary criterion in evaluating model fit do exist [34].

3. Results

For each of the 17 leisure time activities, the percentage of adolescents who spent no time per week doing an activity, eight or more hours per week doing an activity, and the mean number of hours per week is reported in Table 1. Of the 17 activities, three were not engaged in by over half of the sample, with the least frequently reported activity being taking care of younger family members (76%). Only three percent of the sample reported not watching television during an ordinary week. Conversely, one-third of the sample reported viewing television for more than eight hours per week. Spending time with friends, doing schoolwork, and taking part in organized sport were the activities that the highest proportions of adolescents in this sample spent eight or more hours doing per week, and it was to an extent similar for males and females. For most items, residuals after correcting for age trends within sex are highly correlated with the uncorrected hours per week (Table 1). The exceptions are for the two friends items and talking on the telephone where hours spent per week increased significantly with age in both sexes.

Summing across items yielded a total leisure score with means of 44.1 (standard deviation, S.D. = 16.7) hours for males and 43.5 (S.D. = 17.0) hours for females, or approximately six hours per day. Scores on a total leisure time scale ranged between 2 and 120 hours, with a slight upward skewness. The 1.2% of the sample who reported spending total leisure times of 90 hours or more per week was elevated on every item but had ranges comparable to the sample as a whole.

Principal component analyses yielded five leisure time scales with eigenvalues above one and factor loading ≥0.40. These included physical, social, intellectual, family, and passive activities. Factor loadings for each of the 17 leisure time activities are provided in Table 2. All but two items, doing things with family and sitting and listening to music, could be clearly allocated to a particular scale. These two items were subsequently allocated to the family and passive scales, respectively, in order to preserve a simple structure in the scales and to create scales that reflected clear domains of leisure activities. Cronbach alphas ranged from 0.36 for the passive scale to 0.69 for the physical activity scale.

Phenotypic correlations for males and females for the five leisure time scales are shown in Table 3. Generally, the phenotypic correlations between the five leisure time scales were positive and small (0.08) to moderate (0.35) in magnitude for both sexes. Negative small phenotypic correlations (from −0.03 to −0.01) were observed between the physical

Table 1: Descriptive statistics for 17 leisure time activity items.

Leisure time items	0 hours (%)	8+ hours (%)	Males Mean (S.D.)	Females Mean (S.D.)	R^2
Taking part in an organized sport or recreation program?	29	17	3.69 (3.12)	3.23 (3.05)	1.00
Working out as part of a personal exercise program	39	5	2.25 (2.50)	1.87 (2.22)	0.99
Playing pickup games like basketball, touch football, and so forth?	44	4	2.38 (2.45)	1.44 (2.15)	0.97
Practicing different physical activities?	39	6	2.33 (2.56)	1.96 (2.42)	0.99
Going out with friends or dating?	25	13	3.23 (2.87)	3.37 (2.85)	0.89
Sitting around with friends?	12	19	4.36 (2.83)	4.03 (2.81)	0.95
Talking on the telephone?	33	6	1.69 (2.09)	2.58 (2.56)	0.96
Doing your schoolwork?	12	16	3.85 (2.71)	4.43 (2.77)	0.98
Reading for fun?	48	3	1.18 (1.82)	1.79 (2.26)	0.98
Doing things with a club?	70	3	0.89 (1.85)	1.01 (1.94)	0.99
Spending time on a hobby?	38	5	2.25 (2.52)	1.90 (2.27)	1.00
Doing things with your family?	15	7	3.21 (2.31)	3.25 (2.39)	0.99
Taking care of younger family members?	76	2	0.62 (1.48)	0.92 (1.95)	1.00
Doing household chores?	27	2	1.92 (1.80)	2.01 (1.86)	1.00
Total hours watching television weekday plus weekend	3	30	6.74 (4.65)	6.17 (4.53)	0.99
Just sitting around doing nothing?	50	2	1.44 (1.93)	1.23 (1.88)	0.99
Just sitting and listening to music?	32	5	2.07 (2.26)	2.27 (2.40)	0.98

Note: S.D.: standard deviation; R^2: correlation between the raw data and residual data.

Table 2: Factor loadings of residualized leisure time activity hours on principal components.

Leisure time items	PC1	PC2	PC3	PC4	PC5
Taking part in an organized sport or recreation program?	**0.72**	−0.05	0.23	−0.13	−0.10
Working out as part of a personal exercise program	**0.54**	0.26	0.08	0.10	−0.14
Playing pickup games like basketball, touch football, and so forth?	**0.73**	0.13	−0.04	0.16	0.07
Practicing different physical activities?	**0.76**	0.09	0.20	0.08	0.05
Going out with friends or dating?	0.16	**0.80**	0.04	−0.02	−0.06
Sitting around with friends?	0.08	**0.76**	0.12	−0.10	−0.02
Talking on the telephone?	0.12	**0.64**	−0.08	0.12	0.14
Doing your schoolwork?	0.15	0.01	**0.48**	−0.11	−0.22
Reading for fun?	−0.18	−0.01	**0.58**	0.30	−0.08
Doing things with a club?	0.17	−0.04	**0.59**	−0.08	−0.01
Spending time on a hobby?	0.15	0.19	**0.58**	0.13	0.17
Doing things with your family?	0.25	0.02	0.40	**0.38**	0.11
Taking care of younger family members?	0.02	−0.04	−0.07	**0.77**	−0.06
Doing household chores?	0.12	0.11	0.14	**0.72**	0.08
Total hours watching television weekday plus weekend	0.07	−0.01	−0.09	−0.02	**0.74**
Just sitting around doing nothing?	−0.11	0.08	0.02	0.01	**0.73**
Just sitting and listening to music?	−0.07	0.48	0.06	0.20	**0.42**

Note: PC: principal component.

activity, passive, and intellectual scales for both males and females. This suggested the possibility of different etiological influences on the hours allocated to these three types of leisure time activities.

Twin correlations for each leisure time scale are shown in Table 4. Overall, MZ male and female twins were more similar than same-sex and opposite-sex DZ twins. This pattern of correlations suggests genetic influences on the amount of time spent engaging in different types of leisure time activities. The greater than half MZ twin correlation for physical activity and passive leisure time activities for male DZ twins suggests that environmental influences shared by siblings of the same family are important sources of individual differences. The lower opposite-sex than same-sex DZ twin correlations suggest the possibility of different latent influences for males and females.

Table 3: Phenotypic correlations between five leisure time activities for males (below the diagonal) and females (above the diagonal).

Leisure time scale	Physical activity	Social activity	Intellectual activity	Family activity	Passive activity
Physical activity	—	0.23	0.35	0.20	**−0.03**
Social	0.29	—	0.08	0.11	0.22
Intellectual	0.26	0.17	—	0.26	−0.07
Family	0.28	0.14	0.29	—	0.10
Passive	**−0.01**	0.17	**−0.02**	0.11	—

Note: all phenotypic correlations are significant at $P < 0.01$, except bolded cells, which indicate a nonsignificant correlation.

Table 4: Twin correlations (95% confidence intervals) for MZ and DZ same- and opposite-sex twin pairs.

Zygosity	Leisure time activity scales				
	Physical activity	Social activity	Intellectual activity	Family activity	Passive activity
MZM	0.51 (.42, .59)	0.50 (.41, .57)	0.41 (.31, .49)	0.50 (.41, .57)	0.21 (.41, .32)
MZF	0.52 (.42, .59)	0.54 (.46, .61)	0.39 (.30, .47)	0.54 (.46, .61)	0.54 (.47, .61)
DZM	0.42 (.31, .52)	0.21 (.09, .32)	0.19 (.06, .32)	0.21 (.09, .32)	0.24 (.09, .32)
DZF	0.28 (.15, .39)	0.30 (.18, .41)	0.23 (.11, .34)	0.30 (.18, .41)	0.30 (.18, .41)
OSDZ-MF	0.07 (−.09, .23)	0.27 (.12, .41)	0.09 (−.05, .23)	0.19 (.03, .33)	0.13 (−.02, .28)
OSDZ-FM	0.12 (−.07, .29)	0.42 (.26, .54)	0.03 (−.15, .21)	0.06 (−.13, .23)	0.23 (.05, .39)

Note: MZ: monozygotic; DZ: dizygotic; OS: opposite-sex; M: male; F: female.

3.1. Univariate Genetic Modeling. Table 5 summarizes the results from our baseline (full) and best-fitting univariate models. The baseline model allowed A, C, and E latent influences to be estimated separately between males and females (quantitative sex differences). Sex-limited genetic influences were also included in our baseline model to test whether the same genes contribute to leisure time activities between males and females (qualitative sex differences). The model fit for each of the baseline models of five leisure time activity scales is provided in Table 5.

Against the baseline model, we next compared the fit of models that equated the latent A, C, and E parameters for males and females. For each of the scales, genetic and environmental influences could not be equated between the sexes without a significant deterioration in model fit ($P \geq 0.01$). Results from models that equated the DZ and OSDZ twin genetic correlations to be equal indicated that the same genes were influencing leisure time activities in both sexes ($P \leq 0.24$). Similar results were obtained from models that equated shared environment correlations between DZ and OSDZ twins ($P \leq 0.20$). These findings indicated that although the allocation of leisure time activity was influenced by the same genes in males and females, the magnitude of their impact was different between the sexes.

To refine our baseline model further, we next compared the fit of a series of nested submodels that dropped either A, C, or both latent factors in males and females separately. For males, C influences were found to be important contributors to the hours spent engaged in physical ($\Delta\chi^2 = 15.95$, $\Delta df = 1$, $P > 0.001$, and AIC = 13.95) and passive leisure time activities ($\Delta\chi^2 = 4.885$, $\Delta df = 1$, $P > 0.03$, and AIC = 2.88). The best-fitting models for the social, intellectual, and family leisure time scales included only A and E influences. For females, C influences were important influences on intellectual ($\Delta\chi^2 = 5.53$, $\Delta df = 1$, $P = 0.02$, and AIC = 3.53) and passive ($\Delta\chi^2 = 4.88$, $\Delta df = 1$, $P = 0.027$, and AIC = 2.88) leisure time activities but not for physical ($\Delta\chi^2 = 0.74$, $\Delta df = 1$, $P = 0.39$, and AIC = −1.27), family ($\Delta\chi^2 = 2.74$, $\Delta df = 1$, $P = 0.10$, and AIC = 0.74), and social ($\Delta\chi^2 = 2.43$, $\Delta df = 1$, $P = 0.12$, and AIC = 0.44) leisure time activities, where C influences could be dropped from the model for females without a significant deterioration in model fit. The best-fitting model and variance component estimates for each of the five activity scales are shown in Table 5.

3.2. Multivariate Genetic Modeling. We next investigated the extent of familial specificity in the genetic and environmental influences on physical, social, intellectual, family, and passive leisure time activities. Because our univariate models indicated that shared environmental influences were important sources of individual differences, we included A, C, and E latent factors in a five-variable Cholesky decomposition model. Latent factors were allowed to differ between the sexes.

The overall fit of our Cholesky model was −2LL = 87184.10, df = 14075. Parameter estimates for the genetic

Table 5: Model fit statistics and variance component estimates (95% confidence intervals) for baseline (full) and best-fitting (best) model of five leisure time activity scales for males and females.

Model	Model fit statistics							Variance component estimates		
	−2LL	df	$\Delta\chi^2$	Δdf	P value	AIC		a^2	c^2	e^2
Physical activity										
Full model	19074.67	2825	—	—	—	—	Males	0.15 (.00, .42)	0.36 (.13, .53)	0.48 (.41, .53)
							Females	0.49 (.23, .60)	0.05 (.00, .28)	0.46 (.40, .54)
Best model	19074.77	2827	0.74	2	0.69	−3.26	Males	0.07 (.00, .25)	0.43 (.27, .53)	0.50 (.43, .57)
							Females	0.54 (.47, .60)	—	0.46 (.40, .53)
Social										
Full model	17737.58	2826	—	—	—	—	Males	0.50 (.41, .56)	0.00 (.00, .00)	0.51 (.44, .59)
							Females	0.49 (.23, .61)	0.06 (.00, .28)	0.45 (.39, .53)
Best model	17739.71	2829	2.14	3	0.55	−3.86	Males	0.50 (.42, .57)	—	0.50 (.43, .58)
							Females	0.55 (.49, .61)	—	0.45 (.39, .51)
Intellectual										
Full model	17389.10	2826	—	—	—	—	Males	0.41 (.15, .50)	0.00 (.00, .00)	0.59 (.51, .68)
							Females	0.30 (.02, .47)	0.10 (.00, .32)	0.61 (.53, .71)
Best model	17390.91	2828	1.81	2	0.41	−2.20	Males	0.40 (.32, .49)	—	0.59 (.51, .68)
							Females	0.16 (.01, .40)	0.20 (.00, .35)	0.64 (.56, .73)
Family										
Full model	15966.60	2826	—	—	—	—	Males	0.31 (.00, .40)	0.00 (.00, .30)	0.69 (.60, .79)
							Females	0.23 (.00, .49)	0.20 (.00–.42)	0.57 (.50, .66)
Best model	15969.82	2829	3.22	3	0.36	−2.78	Males	0.31 (.21, .39)	—	0.69 (.61, .79)
							Females	0.44 (.36, .50)	—	0.56 (.50, .64)
Passive										
Full model	18186.62	2826	—	—	—	—	Males	0.03 (.00, .33)	0.21 (.00, .31)	0.76 (.66, .85)
							Females	0.30 (.07, .55)	0.23 (.01, .43)	0.46 (.40, .54)
Best model	18186.95	2828	0.33	2	0.85	−3.66	Males	—	0.23 (.15, .30)	0.77 (.70, .85)
							Females	0.35 (.15, .51)	0.19 (.05, .77)	0.46 (.40, .54)

Note: −2LL: −2 log-likelihood; df: degrees of freedom; AIC: Akaike information criterion; *a*: additive genetic; *c*: shared environment; *e*: nonshared environment.

Table 6: Parameter estimates (95% confidence intervals) for additive genetic (a), shared environment (c), and nonshared environmental (e) influences on five leisure time activities for males.

	Physical activity	Social activity	Intellectual activity	Family activity	Passive activity
$a1$	0.11 (.00, .33)				
$a2$	−0.10 (−.36, .24)	**0.39 (.24, .49)**			
$a3$	0.06 (−.21, .40)	−0.39 (−1.31, .19)	**0.35 (.18, .46)**		
$a4$	0.02 (−.24, .37)	−0.30 (−1.21, .37)	**0.39 (.03, .77)**	**0.21 (.02, .35)**	
$a5$	−1.69 (−1.69, 1.69)	0.54 (−.12, 1.04)	6.48 (.78, 6.48)	−0.27 (−1.89, .90)	**0.09 (.02, .30)**
$c1$	**0.40 (.20, .54)**				
$c2$	**0.52 (.24, .75)**	**0.09 (.03, .21)**			
$c3$	0.10 (−.22, .36)	**0.32 (.01, .92)**	**0.06 (.05, .20)**		
$c4$	0.33 (.02, .57)	0.05 (−.48, .52)	−0.16 (−.48, .11)	**0.09 (.01, .26)**	
$c5$	5.01 (5.01, 5.01)	−0.06 (−.41, .42)	−0.35 (−.35, −.24)	0.00 (−1.15, 1.02)	**0.16 (.01, .26)**
$e1$	**0.49 (.42, .57)**				
$e2$	**0.58 (.39, .78)**	**0.52 (.44, .60)**			
$e3$	0.84 (.61, 1.12)	1.06 (.68, 1.68)	**0.59 (.51, .68)**		
$e4$	**0.64 (.45, .87)**	1.23 (.79, 1.01)	**0.79 (.56, 1.04)**	1.28 (.68, 2.42)	
$e5$	−2.31 (−2.31, 2.31)	**0.52 (.19, .91)**	−5.13 (−5.13, −.89)	1.28 (.67, 2.42)	**0.75 (.73, .83)**

Note: bold indicates significant parameter estimates.

and environmental contributions to individual differences in five leisure time activities and the covariance between the different activities are shown in Table 6 for males and Table 7 for females. Bolded estimates indicate that a parameter is statistically significant as judged by its 95% confidence interval (95% CI). As shown, the magnitude of heritable and environmental effects varied for each of the five leisure time activities (along the diagonal), with similar magnitudes to those obtained from our baseline or full univariate models. For males, the covariation of different leisure time activities appears to be largely due to environmental influences, as only family leisure time activities evidenced the influence of genetic factors that also contributed to intellectual activities. For females, genetic contributions to physical activity were also found to influence intellectual leisure time activities. Nonshared environmental influences were the largest contributors to the covariation of different leisure time activities.

In order to understand the proportion of variance different leisure time activities shared, we estimated the genetic and environmental correlations that are presented in Tables 8 and 9 for males and females, respectively. Bolded estimates indicate that a parameter is statistically significant as judged by its 95% confidence interval (95% CI). As shown, few leisure time activities shared common genetic influences for either sex. For males, similar genetic influences appear to be contributing to both intellectual and family leisure time activities (r_g = 0.41, 95% CI: −0.04, 0.1.0) whereas for females common genes contributed to both social and intellectual activities (r_g = 0.51, 95% CI: −0.49, 0.1.0). None of the five leisure time activities were influenced by common shared environmental contributions. For both males and females, however, nonshared environmental experiences on different leisure time activities were often common to each other. The exception to this is the nonsignificant nonshared environmental correlation between physical leisure time activity and passive activities.

4. Discussion

Leisure time activities are an important part of many adolescents' days and are often the predominate choice the majority of children make. Because leisure time activities

Table 7: Parameter estimates (95% confidence intervals) for additive genetic (a), shared environment (c), and nonshared environmental (e) influences on five leisure time activities for females.

	Physical activity	Social activity	Intellectual activity	Family activity	Passive activity
$a1$	**0.43 (.25, .60)**				
$a2$	0.16 (−.29, .80)	**0.35 (.31, .49)**			
$a3$	**0.45 (.21, .64)**	0.71 (−1.33, 2.81)	**0.23 (.06, .41)**		
$a4$	0.26 (−.15, .59)	1.01 (−.15, 2.51)	0.39 (−.12, .86)	**0.20 (.02, .41)**	
$a5$	2.48 (2.48, 2.48)	0.45 (−.10, .97)	0.73 (−1.95, .73)	0.01 (−2.04, 1.64)	**0.30 (.07, .52)**
$c1$	0.10 (.00, .25)				
$c2$	0.33 (−.19, .69)	**0.19 (.07, .33)**			
$c3$	0.01 (−.11, .21)	−1.24 (−4.44, −.09)	**0.14 (.01, .31)**		
$c4$	0.02 (−.23, .31)	−0.74 (−2.29, .21)	−0.03 (−.44, .40)	**0.22 (.04, .42)**	
$c5$	−0.06 (−.06, −.06)	0.29 (−.17, .80)	1.55 (−.85, 1.55)	−0.23 (−.66, 1.79)	0.33 (−.19, .69)
$e1$	**0.47 (.40, .54)**				
$e2$	**0.50 (.30, .75)**	**0.46 (.40, .53)**			
$e3$	**0.54 (.39, .70)**	1.53 (.80, 4.40)	**0.63 (.62, .71)**		
$e4$	0.73 (.47, 1.06)	0.72 (.30, 1.44)	**0.63 (.43, .87)**	0.76 (.30, 1.69)	
$e5$	−1.42 (−1.42, 1.42)	0.26 (.06, .48)	−1.28 (−1.28, −.38)	0.76 (.28, 1.69)	**0.47 (.41, .54)**

Note: bold indicates significant parameter estimates.

have been linked with healthy and unhealthy lifestyles as well as educational attainment [1–4], we sought to determine etiology of individual differences in the allocation of leisure time activities. To do so, we examined self-reported hours spent engaged in five leisure time activity domains among adolescent same- and opposite-sex twin pairs. Using a twin design to understand the etiology of individual differences in the allocation of leisure time allowed us to investigate three questions. First, to what extent do genes and environments contribute to individual differences in the allocation of leisure time? Second, are there sex differences in the heritable and environmental influences on the time spent engaged in leisure time activities? Lastly, to what extent are genetic and environmental influences specific to a particular activity domain or shared across different domains?

Our first two questions sought to determine the type and magnitude of genetic and environmental influences on the allocation of leisure time and whether they differed between sexes. Existing twin studies have implicated genetic influences on exercise and sport participation and general physical activity. The estimates of the size of genetic effects vary between studies, ranging from zero to 85% [16–23, 35]. Sex differences in the magnitude of genetic contributions have also been implicated, with greater effects among males than females, especially during adolescence [35–37]. Shared environmental contributions to physical activity levels have also been suggested in adolescent samples [16, 23, 24, 38] with estimates ranging between 25% and 75%. Against this literature, results from our study are broadly consistent with findings that implicate genetic and shared environmental influences on adolescent physical activity, though they differ in respect to the magnitude of genetic effects. One possible reason for this could be that twin siblings played the same or different sports. Though they had different amounts of practice or playing time, they live in a household where there is an emphasis placed on sports participation. Though it was not possible to determine if this was the case in our data, a scenario such as this would be expected to result in DZ twin correlations greater than half the MZ twin correlation. A further possibility could be changes in the role genetic and environmental influences have during adolescence [35].

Declining physical activity during adolescence [39–41] has sparked a growing interest in the etiological influences on sedentary or passive leisure time activities. The increase in the

Table 8: Genetic, shared, and nonshared environmental correlations (95% confidence intervals) for five leisure time activities for males.

	1	2	3	4
*a*2	−0.13 (−.80, .22)			
*a*3	−0.08 (−.37, .42)	−0.15 (−.60, .08)		
*a*4	0.04 (−.68, .59)	−0.14 (−.67, .24)	**0.41 (.04, 1.0)**	
*a*5	0.25 (−.50, .83)	−0.49 (−.10, .97)	−0.80 (−1.0, −.11)	−0.20 (−1.0, .51)
*c*2	0.77 (−.14, 1.0)			
*c*3	0.16 (−.52, .92)	0.66 (−.11, 1.0)		
*c*4	0.48 (.03, 1.0)	0.09 (−.55, .91)	−0.66 (−1.0, .50)	
*c*5	−0.30 (−.98, −.01)	−0.09 (−.74, .50)	0.08 (−.78, .96)	0.00 (−1.0, .72)
*e*2	**0.33 (.23, .41)**			
*e*3	**0.39 (.30, .47)**	**0.29 (.20, .38)**		
*e*4	**0.30 (.22, .39)**	**0.29 (.19, .37)**	**0.34 (.25, .43)**	
*e*5	0.06 (−.03, .15)	**0.15 (.05, .24)**	**0.17 (.07, .27)**	**0.19 (.09, .28)**

Note: bold indicates significant parameter estimates.

Table 9: Genetic, shared, and nonshared environmental correlations (95% confidence intervals) for five leisure time activities for females.

	1	2	3	4
*a*2	0.10 (−.20, .44)			
*a*3	**0.51 (.49, 1.0)**	0.20 (−.39, .69)		
*a*4	0.18 (−.13, .63)	0.44 (−.05, .1.0)	0.48 (−.11, .99)	
*a*5	−0.18 (−.50, .04)	0.30 (−.08, .71)	−0.18 (−.99, .33)	0.00 (−.92, .66)
*c*2	0.56 (−.77, .87)			
*c*3	0.04 (−.50, .48)	−0.61 (−.1.0, .09)		
*c*4	0.02 (−.41, .38)	−0.42 (−.90, .13)	−0.04 (−.75, .69)	
*c*5	0.01 (−.36, .37)	0.30 (−.18, .80)	−0.56 (−.62, −.22)	0.10 (−.72, .72)
*e*2	**0.24 (.15, .34)**			
*e*3	**0.36 (.27, .44)**	**0.23 (.13, .32)**		
*e*4	**0.29 (.20, .37)**	**0.16 (.07, .25)**	**0.28 (.19, .36)**	
*e*5	0.08 (−.02, .17)	**0.12 (.03, .21)**	**0.16 (.15, .24)**	**0.15 (.06, .24)**

Note: bold indicates significant parameter estimates.

hours spent engaged in passive leisure time activities has been linked to poor metabolic syndrome profiles [42–44] as well as poorer psychosocial functioning [45–47]. Further, previous work has suggested that physical and passive activities are not two ends of a spectrum of activity and that the two types of behaviors are relatively distinct [48–50]. To date, there have been few heritability studies of sedentary behaviors. In general, they implicate heritable and environmental influences with estimates that vary widely between males and females [24–26]. Consistent with this previous literature, we found both genetic and shared environmental contributions to the hours spent engaged in passive leisure time activities and that their magnitudes differed between the sexes. Differences between males and females could reflect family expectations.

Because adolescents have wider choices in how to spend their leisure time, we also examined the genetic and environmental influences on additional leisure time activities. These included social, intellectual, and family activities that are thought to provide important opportunities for adolescents to develop skills and interests and promote growth [2, 51–53]. To our knowledge, the etiology of individual differences in these three leisure time activities has not been examined previously. From both our univariate and multivariate analyses, we found that each activity domain evidenced small to moderate genetic influences and large nonshared environmental effects, which may reflect measurement error. Difference in the parameter estimates may be due to the lower statistical power from considering each measured activity domain separately as in the case of our univariate models. Shared environments, such as home and school experiences, were also important influences on intellectual activities for females but not males. That intellectual leisure time activities evidence the impact of environmental experiences shared by siblings of the same family suggests that educational programs and opportunities as well as the parental prioritization of learning that are directed towards females would be expected to offer an opportunity or target for intervention efforts to improve or further enhance these activities.

Our third question sought to investigate the extent that genetic and environmental influences on one activity domain also influenced other activity domains. A common observation from our Cholesky model was that environmental influences, both shared and nonshared, were the primary etiological influences in the covariation of different leisure time activities. In the case of physical activity and social activities among males, an example of a shared or common environmental experience could be a team's emphasis on social interactions amongst its members and the large amounts of time they spend together in an athletic environment. Similarly, the relationship between social and passive activities among males could reflect an adolescent choice of activity while spending time with peers. Further, results from our multivariate models indicated that genetic influences on leisure time activities were largely specific to that activity. Further, results from our Cholesky model indicated that genetic influences on leisure time activities were largely specific to that activity. Among males, our results suggest that the time allocation to intellectual and family leisure time activities has similar genetic influences. Among females, a similar relationship was identified for physical activity and social leisure time activities. This could reflect genetically influenced interaction styles or personality characteristics that are involved in these activities. Future studies, though, are needed to examine these possibilities.

Although our results are largely consistent with the extant literature, a number of limitations need to be considered when generalizing these results. First, leisure time activities were assessed via self-report which may be impacted by recall bias. Similarly, social desirability or a tendency to report more socially favorable responses may have resulted in an overreporting of the time allocated to the different activities [9]. Second, though the passive leisure time activity scale assessed watching television, it did not include other measures of computer and video game usage that are common activities of many adolescents. Third, though we controlled age and sex effects on leisure time activities, we did not control possible differences in sociodemographic influences. Lastly, our measure of leisure time activities may reflect those engaged in by American youth and thereby limit their generalizability.

In conclusion, we sought to determine the type and magnitude of heritable and environmental influences on adolescent leisure time allocations and the extent to which the genetic and environmental influences on one leisure time activity domain also influenced other activity domains. To this end, our findings suggest that both genes and environments, especially those environmental experiences shared by siblings of the same home, are important contributors to how leisure time is allocated. Importantly, though the magnitudes of genetic and environmental influences appear to differ between males and females, having an awareness of the extent that leisure time allocations are environmentally influenced may offer opportunities for effective public health messaging and interventions designed to change unhealthy behaviors as well as to promote certain types of activities. That environmental influences also contribute to the covariation of leisure time activities suggests the possibility of influencing more than a single choice of how children allocate their leisure time.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors wish to thank Drs. John K. Hewitt, Michael C. Stallings, Matthew B. McQueen, and Sally Ann Rhea for their support of this work. Funding support was provided by Grants P60-DA011015 and R01-HD010333.

References

[1] J. L. Mahoney and H. Stattin, "Leisure activities and adolescent antisocial behavior: the role of structure and social context," *Journal of Adolescence*, vol. 23, no. 2, pp. 113–127, 2000.

[2] J. S. Eccles and B. L. Barber, "Student council, volunteering, basketball, or marching band: what kind of extracurricular involvement matters?" *Journal of Adolescent Research*, vol. 14, no. 1, pp. 10–43, 1999.

[3] A. C. Fletcher, P. Nickerson, and K. L. Wright, "Structured leisure activities in middle childhood: links to well-being," *Journal of Community Psychology*, vol. 31, no. 6, pp. 641–659, 2003.

[4] K. Cuypers, K. de Ridder, K. Kvaloy et al., "Leisure time activities in adolescence in the presence of susceptibility genes for obesity: risk or resilience against overweight in adulthood? The HUNT study," *BMC Public Health*, vol. 12, article 820, 2012.

[5] *American Time Use Sruvey, 2012 Results*, USDL-13-1178, Bureau of Labor Statistics, U.S. Department of Labor, 2013.

[6] E. H. Sharp, L. L. Caldwell, J. W. Graham, and T. A. Ridenour, "Individual motivation and parental influence on adolescents' experiences of interest in free Time: a longitudinal examination," *Journal of Youth and Adolescence*, vol. 35, no. 3, pp. 340–353, 2006.

[7] K. F. Cuypers, S. Krokstad, L. T. Holmen, S. M. Knudtsen, L. O. Bygren, and J. Holmen, "Patterns of receptive and creative cultural activities and their association with perceived health, anxiety, depression, and satisfaction with life among adults," *Journal of Epidemiology and Community Health*, vol. 66, no. 8, pp. 698–703, 2012.

[8] J. F. Sallis, "Epidemiology of physical activity and fitness in children and adolescents," *Critical Reviews in Food Science and Nutrition*, vol. 33, no. 4-5, pp. 403–408, 1993.

[9] J. F. Sallis and B. E. Saelens, "Assessment of physical activity by self-report: status, limitations, and future directions," *Research Quarterly for Exercise and Sport*, vol. 71, no. 2, pp. 1–14, 2000.

[10] S. A. Adams, C. E. Matthews, C. B. Ebbeling et al., "The effect of social desirability and social approval on self-reports of physical activity," *The American Journal of Epidemiology*, vol. 161, no. 4, pp. 389–398, 2005.

[11] R. Jago, T. Baranowski, J. C. Baranowski, K. W. Cullen, and D. I. Thompson, "Social desirability is associated with some physical activity, psychosocial variables and sedentary behavior but not self-reported physical activity among adolescent males," *Health Education Research*, vol. 22, no. 3, pp. 438–449, 2007.

[12] R. W. Motl, E. McAuley, and C. DiStefano, "Is social desirability associated with self-reported physical activity?" *Preventive Medicine*, vol. 40, no. 6, pp. 735–739, 2005.

[13] S. L. Hofferth and J. F. Sandberg, "How American children spend their time," *Journal of Marriage and Family*, vol. 63, no. 2, pp. 295–308, 2001.

[14] R. W. Larson and S. Verma, "How children and adolescents spend time across the world: work, play, and developmental opportunities," *Psychological Bulletin*, vol. 125, no. 6, pp. 701–736, 1999.

[15] S. L. Hutchinson, C. K. Baldwin, and L. L. Caldwell, "Differentiating parent practices related to adolescent behavior in the free time context," *Journal of Leisure Research*, vol. 35, no. 4, pp. 396–422, 2003.

[16] J. H. Stubbe, D. I. Boomsma, and E. J. C. de Geus, "Sports participation during adolescence: a shift from environmental to genetic factors," *Medicine and Science in Sports and Exercise*, vol. 37, no. 4, pp. 563–570, 2005.

[17] S. Aaltonen, A. Ortega-Alonso, U. M. Kujala, and J. Kaprio, "A longitudinal study on genetic and environmental influences on leisure time physical activity in the finnish twin cohort," *Twin Research and Human Genetics*, vol. 13, no. 5, pp. 475–481, 2010.

[18] M. Eriksson, F. Rasmussen, and P. Tynelius, "Genetic factors in physical activity and the equal environment assumption—the Swedish young male twins study," *Behavior Genetics*, vol. 36, no. 2, pp. 238–247, 2006.

[19] G. E. Duncan, J. Goldberg, C. Noonan, A. V. Moudon, P. Hurvitz, and D. Buchwald, "Unique environmental effects on physical activity participation: a twin study," *PLoS ONE*, vol. 3, no. 4, Article ID e2019, 2008.

[20] L. Mustelin, J. Joutsi, A. Latvala, K. H. Pietilainen, A. Rissanen, and J. Kaprio, "Genetic influences on physical activity in young adults: a twin study," *Medicine & Science in Sports & Exercise*, vol. 37, no. 4, pp. 563–570, 2012.

[21] P. W. Franks, E. Ravussin, R. L. Hanson et al., "Habitual physical activity in children: the role of genes and the environment," *The American Journal of Clinical Nutrition*, vol. 82, no. 4, pp. 901–908, 2005.

[22] C. Huppertz, M. Bartels, I. E. Jansen et al., "A twin-sibling study on the relationship between exercise attitudes and exercise behavior," *Behavior Genetics*, vol. 44, no. 1, pp. 45–55, 2014.

[23] C. Huppertz, M. Bartels, C. E. M. van Beijsterveldt, D. I. Boomsma, J. J. Hudziak, and E. J. C. de Geus, "Effect of shared environmental factors on exercise behavior from age 7 to 12 year," *Medicine & Science in Sports & Exercise*, vol. 44, no. 10, pp. 2025–2032, 2012.

[24] A. Fisher, C. H. M. van Jaarsveld, C. H. Llewellyn, and J. Wardle, "Environmental influences on children's physical activity: quantitative estimates using a twin design," *PLoS ONE*, vol. 5, no. 4, Article ID e10110, 2010.

[25] M. den Hoed, Soren Brage, J. H. Zhao et al., "Heritability of objectively assessed daily physical activity and sedentary behavior," *The American Journal of Clinical Nutrition*, vol. 98, pp. 1317–1325, 2013.

[26] N. van der Aa, M. Bartels, S. J. te Vele, D. I. Boomsma, E. J. C. de Geus, and J. Brug, "Genetic and environmental influences on individual differences in sedentary behavior during adolescence," *Archives of Pediatrics and Adolescent Medicine*, vol. 160, no. 6, pp. 509–514, 2012.

[27] R. N. Emde and J. K. Hewitt, *Infancy to Early Childhood: Genetic and Environmental Influences on Developmental Change*, Oxford University Press, New York, NY, USA, 2001.

[28] S. A. Rhea, A. A. Gross, B. C. Haberstick, and R. P. Corley, "Colorado twin registy: an update," *Twin Research and Human Genetics*, vol. 16, no. 1, pp. 351–357, 2013.

[29] S. A. Rhea, A. A. Gross, B. C. Haberstick, and R. P. Corley, "Colorado twin registry," *Twin Research and Human Genetics*, vol. 9, no. 6, pp. 941–949, 2006.

[30] R. Jessor and S. L. Jessor, *Problem Behavior and Psychosocial Development: A Longitudinal Study of Youth*, Academic Press, 1977.

[31] M. C. Neale, S. M. Boker, G. Xie, and H. Maes, *Mx: Statistical Modeling*, Department of Psychiatry, Medical College of Virginia, Richmond, Va, USA, 1999.

[32] M. C. Neale and L. R. Cardon, *Methodology for Genetic Study of Twins and Families*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1992.

[33] H. Akaike, "Factor analysis and AIC," *Psychometrika*, vol. 52, no. 3, pp. 317–332, 1987.

[34] P. F. Sullivan and L. J. Eaves, "Evaluation of analyses of univariate discrete twin data," *Behavior Genetics*, vol. 32, no. 3, pp. 221–227, 2002.

[35] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *PLoS ONE*, vol. 1, no. 1, article e22, 2006.

[36] G. Beunen and M. Thomis, "Genetic determinants of sports participation and daily physical activity," *International Journal of Obesity*, vol. 23, supplement 3, pp. S55–S63, 1999.

[37] S. Carlsson, T. Andersson, P. Lichtenstein, K. Michaëlsson, and A. Ahlbom, "Genetic effects on physical activity: results from the Swedish twin registry," *Medicine and Science in Sports and Exercise*, vol. 38, no. 8, pp. 1396–1401, 2006.

[38] J. M. Vink, D. I. Boomsma, S. E. Medland et al., "Variance components models for physical activity with age as modifier: a comparative twin study in seven countries," *Twin Research and Human Genetics*, vol. 14, no. 1, pp. 25–34, 2011.

[39] D. J. Aaron, K. L. Storti, R. J. Robertson, A. M. Kriska, and R. E. LaPorte, "Longitudinal study of the number and choice of leisure time physical activities from mid to late adolescence: implications for school curricula and community recreation programs," *Archives of Pediatrics and Adolescent Medicine*, vol. 156, no. 11, pp. 1075–1080, 2002.

[40] R. Telama and X. Yang, "Decline of physical activity from youth to young adulthood in Finland," *Medicine and Science in Sports and Exercise*, vol. 32, no. 9, pp. 1617–1622, 2000.

[41] S. M. Dovey, "Continuity and change in sporting and leisure time physical activities during adolescence," *The British Journal of Sports Medicine*, vol. 32, no. 1, pp. 53–57, 1998.

[42] C. L. Li, J. D. Lin, S. J. Lee, and R. F. Tseng, "Associations between the metabolic syndrome and its components, watching television and physical activity," *Public Health*, vol. 121, no. 2, pp. 83–91, 2007.

[43] A. E. Mark and I. Janssen, "Relationship between screen time and metabolic syndrome in adolescents," *Journal of Public Health*, vol. 30, no. 2, pp. 153–160, 2008.

[44] D. Martinez-Gomez, J. Tucker, K. A. Heelan, G. J. Welk, and J. C. Eisenmann, "Associations between sedentary behavior and blood pressure in young children," *Archives of Pediatrics and Adolescent Medicine*, vol. 163, no. 8, pp. 724–730, 2009.

[45] G. J. Norman, B. A. Schmid, J. F. Sallis, K. J. Calfas, and K. Patrick, "Psychosocial and environmental correlates of adolescent sedentary behaviors," *Pediatrics*, vol. 116, no. 4, pp. 908–916, 2005.

[46] K. H. Schmitz, L. A. Lytle, G. A. Phillips, D. M. Murray, A. S. Birnbaum, and M. Y. Kubik, "Psychosocial correlates of physical activity and sedentary leisure habits in young adolescents: the teens eating for energy and nutrition at school study," *Preventive Medicine*, vol. 34, no. 2, pp. 266–278, 2002.

[47] P. Gordon-Larsen, R. G. McMurray, and B. M. Popkin, "Adolescent physical activity and inactivity vary by ethnicity: the National Longitudinal Study of Adolescent Health," *Journal of Pediatrics*, vol. 135, no. 3, pp. 301–306, 1999.

[48] L. Perusse, A. Tremblay, C. Leblanc, and C. Bouchard, "Genetic and environmental influences on level of habitual physical activity and exercise participation," *The American Journal of Epidemiology*, vol. 129, no. 5, pp. 1012–1022, 1989.

[49] N. Owen, E. Leslie, J. Salmon, and M. J. Fotheringham, "Environmental determinants of physical activity and sedentary behavior," *Exercise and Sport Sciences Reviews*, vol. 28, no. 4, pp. 153–158, 2000.

[50] M. S. Bray, J. M. Hagberg, L. Pérusse et al., "The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update," *Medicine and Science in Sports and Exercise*, vol. 41, no. 1, pp. 35–73, 2009.

[51] R. W. Larson, "Toward a psychology of positive youth development," *The American Psychologist*, vol. 55, no. 1, pp. 170–183, 2000.

[52] A. C. Fletcher, P. Nickerson, and K. L. Wright, "Structured leisure activities in middle childhood: links to well-being," *Journal of Community Psychology*, vol. 31, no. 6, pp. 641–659, 2003.

[53] D. M. Hansen, R. W. Larson, and J. B. Dworkin, "What adolescents learn in organized youth activities: a survey of self-reported developmental experiences," *Journal of Research on Adolescence*, vol. 13, no. 1, pp. 25–55, 2003.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 209140, 9 pages
http://dx.doi.org/10.1155/2014/209140

Research Article

A Study of Sedentary Behaviour in the Older Finnish Twin Cohort: A Cross Sectional Analysis

Maarit Piirtola,[1,2] Jaakko Kaprio,[1,3,4] and Annina Ropponen[5]

[1] *Department of Public Health, Hjelt Institute, University of Helsinki, P.O. Box 41 (Mannerheimintie 172), 00014 Helsinki, Finland*
[2] *UKK Institute, Centre for Health Promotion Research, Tampere, Finland*
[3] *Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, P.O. Box 30 (Mannerheimintie 166), 00300 Helsinki, Finland*
[4] *Institute for Molecular Medicine (FIMM), University of Helsinki, P.O. Box 20 (Tukholmankatu 8), 00014 Helsinki, Finland*
[5] *Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, 00250 Helsinki, Finland*

Correspondence should be addressed to Maarit Piirtola; maarit.piirtola@helsinki.fi

Received 12 November 2013; Revised 17 February 2014; Accepted 6 March 2014; Published 15 April 2014

Academic Editor: Meike Bartels

The aim of the study was to investigate the effects of age, sex, and body mass index (BMI) on total sitting time among the Finnish twin cohort. Also, heritability and environmental factors were analysed. The final sample included 6713 twin individuals 53–67 years of age (46% men). Among them there were 1940 complete twin pairs (732 monozygotic [MZ] and 1208 dizygotic [DZ] twin pairs). Sedentary behaviour was queried with a self-reported questionnaire with multiple-choice questions about sitting time at different domains. The mean total sitting time per day was 6 hours 41 minutes (standard deviation: 2 hours 41 minutes). The total sitting time was less in women than in men ($P = 0.002$). Older age was associated with less total sitting time ($P < 0.001$). Those with higher body mass index had higher total sitting time in age and sex adjusted analysis ($P < 0.001$). MZ pairs were more similar for sitting time than DZ pairs, with initial estimates of heritability for the total sitting time of 35%.The influence of shared environmental factors was negligible (1%), while most (64%) of the variation could be ascribed to unique environmental factors, the latter including measurement error.

1. Introduction

Sedentary behaviour, measured as sitting time, is one of the major global public health concerns [1, 2]. A high amount of sitting is independently associated with overweight [3, 4] and cardiometabolic risk [4]. In addition, a high amount of sitting time increases all-cause and cardiovascular disease-related mortality independent of whether a person is meeting physical activity guidelines [5–7]. Thus, actions to investigate the backgrounds and genetics of sedentary behaviour need to be studied further in order to implement effective preventive actions [8].

Sedentary behaviour has been defined in various ways [9]. It has been suggested that sedentary behaviour can be a paradigm of its own, distinctive to that of moderate- to vigorous-intensity physical activity, with independent effects on health [2]. Thus, sedentary behaviour is not simply the absence of moderate- to vigorous-intensity physical activity [2] or even the presence of light physical activity [9]. Recently one of the globally accepted suggestions for the use of the term sedentary behaviour had been given by the Sedentary Behaviour Research Network, including the definition of sedentary behaviour "as any waking behaviour characterized by an energy expenditure ≤1.5 metabolic equivalents (METs) while in sitting or reclining posture" [10]. The Network also suggests using the term inactive in describing "those who are performing insufficient amount of moderate- to vigorous-intensity physical activity (MVPA) (i.e., not meeting specific physical activity guidelines)" [10].

In addition to variation in definitions, sedentary behaviour can also be measured in various ways. Even though there is a recommendation that sedentary behaviour should be

monitored by incorporation of both self-reported and device-based measures [11], no consensus exists for a golden rule of method to measure sedentary behaviour [11].

In the observational research, sedentary behaviour has been investigated with self-report questions about sitting time during different sedentary activities such as watching TV or using the computer and sitting at work or in vehicles [12, 13]. Especially in large population-based studies, those involving thousands of participants, the self-reported sitting time is a useful method despite its inaccuracy of the total amount of physical activity and the potential recall bias [11].

Understanding why some people are physically active and others inactive or behaving in a sedentary way is important in planning public health interventions [14]. It has been shown that age, health status, self-efficacy, and motivation in addition to social and physical environmental factors are associated with physical activity levels [14]. In two recently published Finnish population-based studies using self-administered questionnaires, the mean sitting times per day in men and women 25 to 60 years of age varied between 5.5 to 6.9 hours and 5.2 to 6.4 hours, respectively [15, 16]. The total sedentary times in these Finnish cohorts [15, 16] were less than reported in a large multiethnic cohort study with over 130,000 older subjects where the average daily sitting time was 8.0 hours in men and 8.2 in women [7]. It has been reported that differences in sitting times are based on ethnicity, age, educational level, and body mass index (BMI) [17].

Another potential factor related to physical activity, inactivity, and sedentary behaviour is family background and genetic predisposition [18]. It has been shown that when exercise participation is determined as 60 minutes/week at a minimum intensity of 4 METs, interindividual genetic differences accounted for 48–71% of variance in both sexes [19]. A recently published review presented a variance for genetics that was widely spread, as heritability estimates ranged from 0 to 85% for physical activity and from 25 to 60% for physical inactivity depending on definitions of physical activity levels, population, age, and other differences between studies [18]. Among adolescents, genetics has explained 72% to 85% of variance in exercise participation determined as sedentary, moderate, or vigorous exercise by METs [20]. The contribution of genetic factors to variation in sedentary behaviour frequency per week among 12–20 year-old boys was 35% to 48% and 19% to 34% among same aged girls [21]. In addition, the influence of shared environmental factors decreased along age in adolescence [21]. In another study, the role of genetics in MVPA was 59% among adolescents and 12% in young adults [22]. In adolescents, genetics explained 34% of the variance of sedentary time (per week), whereas shared environmental (household) explained 10% and unique environmental factors 56% of the variation [22]. The influence of genetics in sedentary behaviour in young adults was 28% of variance suggesting a somewhat increasing impact of unique environmental factors along age. In older adult twins, genetics explained 31% of the time spent in sedentary behaviour (≤1.5 METs) whereas it was larger (47%) for MVPA [23]. The effect of unique environment (i.e., the exposures and experiences mainly related to adulthood) was 52–55% of variance [23]. It is possible that genetic influences are different in inactivity and sedentary behaviours compared to physical activity but there is an inconsistency between previously published results [18, 23].

In this paper, the focus was to describe the latest data collection of physical activity and sedentary behaviour in the Finnish twin cohort [24, 25]. The main aim was to investigate the relative contribution of genetics and environmental factors of sedentary behaviour in 53 to 67 year-old men and women from the Finnish twin cohort study. In addition, the effects of age, sex, and body mass index (BMI) on total sitting time were investigated.

2. Materials and Methods

2.1. Participants. The population-based data of the older Finnish twin cohort was available for 16,269 same-sex twin pairs [26]. The extensive longitudinal data includes four waves of surveys (baseline in 1975 and three follow-up surveys in 1981, 1990, and 2011/12) [24–26]. During the fourth wave (October 2011 to June 2012) all twins born 1945–1957 (n = 11 738) originally identified to the cohort in 1974 and known to be alive in 2011 with an address in Finland were sent the questionnaire survey, either in Finnish or in Swedish. All subjects, except 13 individuals, had answered at least one of the earlier surveys (1975 and/or 1981 and/or 1990) along the follow-up. The vital status of the original cohort members was updated in 2011 from the national Finnish population register. All surviving twins received the survey irrespective of their cotwin's status. The data collection of the fourth wave is mainly described in the review article in 2013 [25]. The protocol was designed and performed according to the principles of the Helsinki Declaration and was approved by the Ethical Committee of the Helsinki University Central Hospital.

2.2. Methods. To maintain continuity the original questions (such as in 1975 and 1981) were used wherever possible, despite the development of better measurements for some topics. The questionnaire of the fourth wave included comprehensive questions about self-reported health, functional capacity, and lifestyle factors, described in more detail in the review published in 2013 [25].

2.2.1. Physical Activity. All four surveys included questions about the quality and quantity of leisure-time physical activities: amount (per year), duration (per one session), frequency (per month), and intensity of leisure-time physical activity as well as daily time of commuting by physically active means (including walking, jogging, and cycling) to and from work (minutes per day) (see Appendix A). Also physical workload of the present or previous work was asked.

2.2.2. Sedentary Behaviour. Sedentary behaviour has been queried in the fourth questionnaire with multiple-choice questions about sitting time during different activities (see Appendix B). The participants have answered how many hours, on average, they are sitting per day: (1) in office or

similar places, (2) at home watching TV or videos, (3) at home at the computer, (4) in a vehicle, and (5) elsewhere. Each question had four alternatives: (a) less than an hour, (b) an hour–less than two hours, (c) two hours–less than four hours, and (d) four hours or more. We assumed the intensity of each of these sedentary activities to be no more than 1.5 METs [9]. Total daily sitting time was the sum of the midpoints of the specific sitting categories, using 30 minutes for "less than an hour," 90 minutes for "an hour–less than two hours," 180 minutes for "two hours–less than four hours," and 300 minutes for "four hours or more." For those twin individuals ($n = 4034$) who were not working at the moment of the survey, sitting time at work was denoted as zero minutes.

2.3. Statistical Methods and Data Analysis. The total sitting time parameter has been developed based on five sitting activities by developing a summary variable (sitting time in hours and/or in minutes). Only those twin individuals reporting sitting times in all five sitting domains, including those not at work with zero sitting time on that item, were chosen for the final analyses. The normality of the sitting summary variable was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The total sitting sum variable had the skewness value of 0.676 and kurtosis of 0.345 indicating that sitting time was not fully, normally distributed ($P < 0.001$) (Figure 1). However, tests of normality are extremely robust [27] and our relatively large sample size will result in a minor departure from normality being significant. Furthermore, the methods used in analyzing twin data are robust to minor deviation of normality. The original cohort had been a sample of twin pairs and that was taken into account, and robust standard errors were derived to obtain correct confidence intervals and P values [28]. The chi-squared test, Spearman correlation, and independent-sample t-test were used in the descriptive analyses. In the linear regression models, with 95% confidence intervals (95% CI), the effects of sex and age were analysed together. Age was used as a continuous parameter in the analyses.

Body mass index (BMI) was calculated by individual's weight and height ($\mathrm{kg}*\mathrm{m}^{-2}$). BMI values 20 or less as well as BMI 36 or more were combined to be the first and the last categories for descriptive purposes. The sex and age adjusted linear regression model with 95% confidence intervals was used in analysing the association between the total sitting time and BMI.

To investigate the heritability of physical activity, the phenotype was assumed to have an underlying, continuous liability. Heritability was analysed by calculating pairwise correlation coefficients by zygosity and sex and further comparing the results of monozygotic twins (MZ) to same-sex dizygotic twins (DZ). As MZ twins are genetically alike, that is, share the same genomic sequence, while DZ twins share on average 50% of the their segregating genes, increased similarity of MZ pairs versus DZ pairs is taken as evidence for the presence of genetic effects. In addition, Falconer's formula [29] was used to calculate the proportion of variance estimated by the ratio of MZ and DZ twin correlations explained by additive genetics ($h^2 = 2(r_{\mathrm{MZ}} - r_{\mathrm{DZ}})$), shared environmental factors ($c^2 = 2r_{\mathrm{DZ}} - r_{\mathrm{MZ}}$), and unique environmental factors ($e^2 = 1 - h^2 - c^2$) based on MZ correlations being twice that or less compared to DZ correlations.

All analyses were performed with the Stata version 12 or the IBM SPSS version 21. In all analyses, significance was considered to be $P < 0.05$.

3. Results

The fourth questionnaire was returned by 8406 twin individuals (3750 men, 4656 women) resulting in a response rate of 72%. Complete data of sedentary behaviour (those answered in all five sitting domains) was available for 6713 participants (3082 men, 3631 women). The data covered 80% of those who returned the questionnaire.

There were more women (61% versus 54%, $P = 0.009$) in those having missing information at least in one sitting domain compared with those with information in all five domains. Those with any missing information were older (mean age 62 years versus 60 years, $P = 0.000$), not working full time (81% versus 41%, $P = 0.000$), and they were more likely obese, BMI > 30 (19.1% versus 16%, $P = 0.009$). There were no significant differences in the amount of leisure-time physical activity between those with or without missing information about their sitting times ($P = 0.068$).

The final analysis sample ($n = 6713$) comprised 310 complete monozygotic male pairs (MZM), 422 monozygotic female pairs (MZW), 527 dizygotic male pairs (DZM), and 681 dizygotic female pairs (together 1940 pairs). The average age of the twins was 60 years (range 53 to 67 years) in both sexes and their mean BMI was 26 (range 15 to 48). At the time of the survey, 59% of the twin individuals reported working full time. The physical workload of the present work was mainly sedentary work in 38% of the twins; 12% of twins had work which involved standing and walking but no other physical activity; 41% had work which in addition to standing and walking required lifting and carrying; and 8% did heavy physical work, whereas 1% of the twins reported a mixed combination of all these kinds of work loading conditions.

The mean sitting time per day was 6 hours 41 minutes (SD: 2 h 41 minutes) (Figure 1). In men, the mean total sitting time was 6 hours 46 min (SD: 2 h 50 minutes) and in women 6 hours 34 minutes (SD: 2 hours 34 minutes). One quarter of the twins reported sitting 4.5 hours or less per day, a half 4.5–6.5 hours a day, and 10% at least 10.5 hours a day. The sitting times during different sitting activities by sex are described in Table 1.

There were 6% of the individuals who reported not exercising any kind of physical activity during their leisure time. Of the sample, 22% reported a small amount of exercise and the rest 72% were exercising at least a moderate amount of exercise per year. The amount of leisure-time exercise by sex is described in Table 1. In the preliminary analyses, there was no evidence for differences in physical activity levels regarding the total daily sitting time (data not shown).

In the linear regression analyses, the total sitting time of women was less than sitting time of men (regression

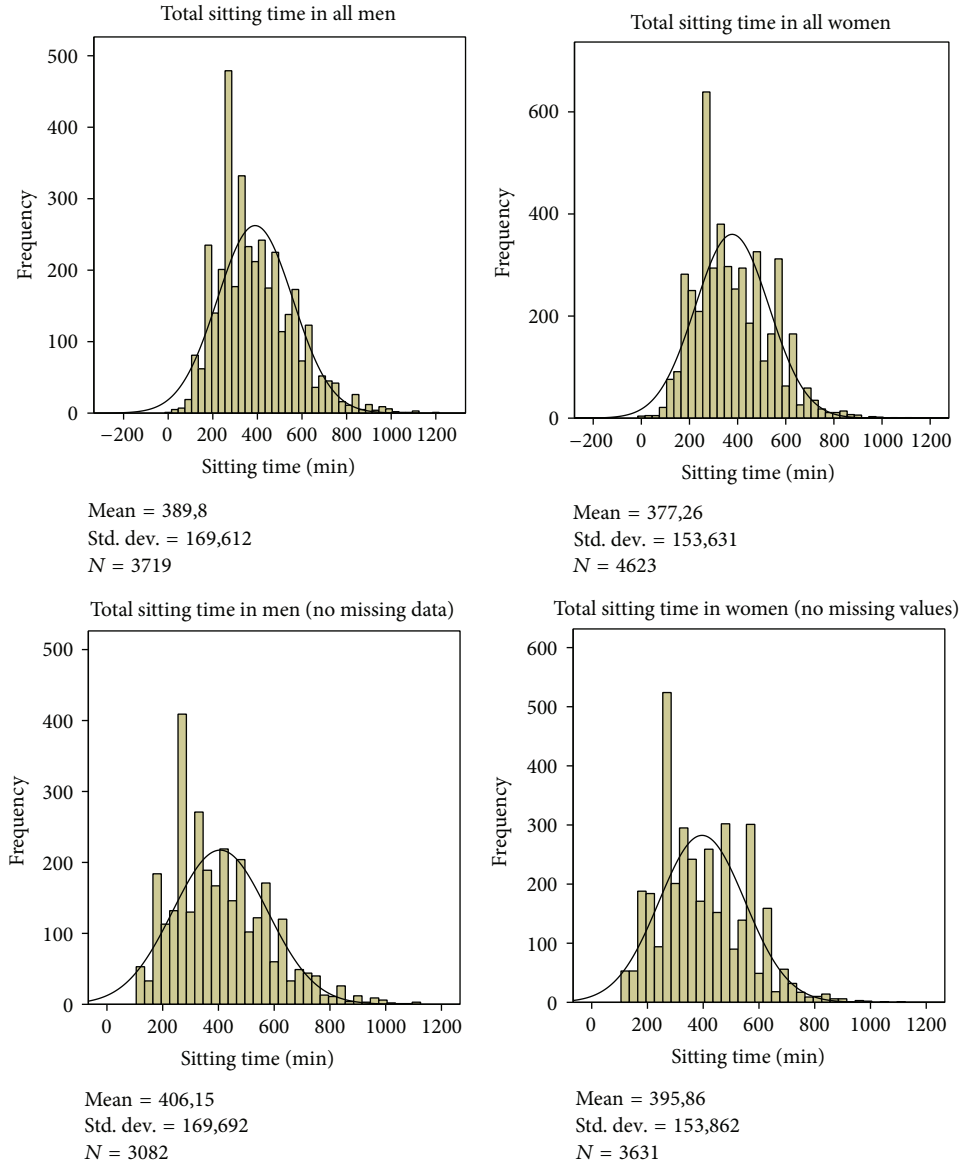


Figure 1: Distribution of total sitting time (minutes) by sex and data included. Total sum score includes all five domains: work, commuting, watching TV, computer use, and others. No missing data includes those with no missing data on any single sitting domain. For those not working, sitting time at work was denoted as zero minutes.

coefficients: −13.01 minutes [95% CI: −21.30, −4.83]) and increase of age decreased the sitting time (regression coefficients: −9.67 minutes per year [95% CI: −10.72, −8.63]). In the analyses of sitting time in different activities (Table 2), sex had no effect on the sitting time at work but sitting at work decreased with age. Both sexes had an equally long sitting time at home watching TV or videos, and age increased this activity. However, men had significantly longer sitting time at the computer at home as they did for sitting time in vehicles compared to women. Increase of age increased computer related sitting time but decreased the amount of vehicle-related sitting time. In other activities, both sexes were sitting an equal amount of time but age increased the sitting time.

As an example of the sedentary behaviour risk factors, the association of body mass index with the total sitting time was analysed. The association of BMI and total time of sitting seemed to be linear in direction that those with higher BMI had also higher total sitting time (regression coefficients 2.78 minutes per BMI unit [95% CI: 1.77, 3.79], and correlation coefficient 0.064) (Figure 2).

In general, the pairwise correlations of MZ twins were double compared to correlation of DZ twins suggesting genetic influences on sedentary behaviour (Table 3). The correlation coefficients were similar for men and women. MZ pairs were more similar for sitting time than DZ pairs, with initial estimates of heritability for the total sitting time being 35%. The influence of shared environmental factors was

Table 1: Domain-based sitting times and the amount of leisure-time physical exercise by sex (n = 6713*).

Activities	Men (n = 3082) Mean time (SD)	Women (n = 3631) Mean time (SD)
Sitting time		
At work**	2 h 52 min (1 h 53 min)	2 h 58 min (2 h 56 min)
At home watching TV or videos	2 h 25 min (1 h 12 min)	2 h 25 min (1 h 11 min)
At home at the computer	57 min (52 min)	50 min (43 min)
In a vehicle	59 min (59 min)	43 min (33 min)
Elsewhere	48 min (45 min)	48 min (45 min)
Sum of sitting time***	6 h 46 min (2 h 50 min)	6 h 36 min (2 h 34 min)
	n (%)	n (%)
Amount of leisure-time physical exercise****		
Practically none	252 (8)	150 (4)
A little	754 (25)	716 (20)
A moderate amount	1369 (45)	1722 (48)
Quite a lot or a great deal	695 (23)	1038 (29)

* Data from those twin individuals who had no missing values in any of the sedentary domains.
** Data from those twin individuals (n = 3970) who reported working full time at the moment of the survey.
*** In calculating the total sum of sitting, sitting at office (work) was denoted as zero minutes for those twins (n = 2728) who reported not working at the moment of the survey (missing information in 15 individuals).
**** Missing information in 17 individuals.

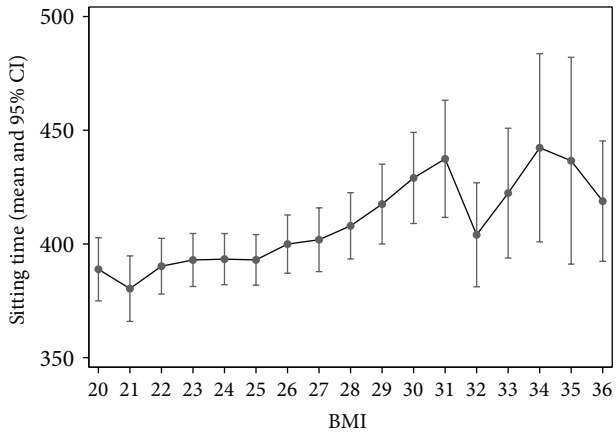


Figure 2: Association of BMI and regression model predicted mean of the total sitting time (minutes per day), with 95% confidence intervals. BMI values 20 or less as well as values 36 or more were combined to be the first and the last categories.

Table 2: Influence of sex and age on sitting time in five different sitting domains in the linear regression model (regression coefficients with 95% confidence intervals). Men were used as the reference sex.

Sitting domain	Coefficient	95% CI	P value
At work*			
Female sex	6.40	0.50, 12.30	0.034
Age per year	−11.92	−12.64, −11.20	0.000
At home watching TV or videos			
Female sex	1.10	−2.57, 4.76	0.558
Age	2.28	1.77, 2.79	0.000
At home at the computer			
Female sex	−6.70	−9.13, −4.26	0.000
Age	0.52	0.18, 0.87	0.003
In a vehicle			
Female sex	−16.86	−19.31, −14.41	0.000
Age	− 1.16	−1.46, − 0.86	0.000
Elsewhere			
Female sex	0.93	−1.29, 3.14	0.413
Age	0.74	0.44, 1.05	0.000

* For those twins who were not working at the moment of survey, sitting time at work was denoted as zero minutes (n = 2728).

negligible (1%), while most (64%) of the variation could be ascribed to unique environmental factors, the latter including measurement error.

4. Discussion

In this sample of 6713 twin individuals, 53–67 years of age, the total amount of sitting was on average 6 hours 41 minutes per day. In a Finnish population-based study of 30–45 years of age, the mean sedentary time (time spent viewing TV, using the computer, reading, listening to music/radio, and in other types of relaxation) was slightly less, a little over 5 hours, [16] but the time spent in vehicles was not inquired into. Another study, involving those of 25–64 years of age, has also shown similar estimates based on the mean self-reported sitting times (including sitting times at work and during leisure time, at home, while visiting friends, studying,

Table 3: Pairwise correlations for sitting time by zygosity.

	Number of pairs	Correlation coefficient
MZ total	732	0.364
DZ total	1208	0.188
Male MZ	310	0.355
Female MZ	422	0.372
Male DZ	527	0.211
Female DZ	681	0.166

MZ: monozygotic; DZ: dizygotic.

and travelling) during a day: 6.9 hours (SD: 3.5) in men and 6.4 (SD: 3.3) hours in women [15].

In this study, the association between sitting time and age indicated that those of a younger age had higher total sitting time. The association of aging in sedentary behaviour and physical activity patterns is still poorly known [30]. Some evidence exists that younger adults are more active in moderate to vigorous physical activity than older adults [31] but knowledge is lacking on changes in the daily proportion of age-specific sedentary time. Among those with an average 79 years of age, sedentary behaviour explained 24% of the daily functions [30]. The changes in leisure-time activities after working age can only be speculated. In this study, 59% of the twin individuals reported working full time during the survey. It is possible that after working age the mean activity level may increase because of more active hobbies and a decreasing amount of sitting in vehicles to and from work, at least for a few years. This hypothesis needs to be studied further with long-term follow-up studies.

Our results also indicate that the increase in total sitting time is associated with increase of BMI. This is in line with other studies where a high amount of TV viewing time has been related to higher BMI and waist circumference [3, 16]. On the other hand, lower BMI has been related to a higher physical activity level in the aged [30].

There is evidence that genetics has at least a moderate influence on physical activity levels, and age seems to be a regulator of the activity heritability [21, 32, 33]. In adolescents (13–19 years of age), genetic factors explained 72–85% of the variance in exercise behaviour [20]. In another study, genetics of sedentary behaviour in 12-year-old boys was 35% and 19% in girls, whereas the proportion of variance explained by genetics increased to 48% in 20-year-old boys and to 34% in 20-year-old girls, respectively [21]. To the best of our knowledge, our study is among the first ones to study the relative contribution of heritability and environmental factors to sedentary behaviour, measured as total sitting time among older adults. In the present study, the influence of heritability for the total sitting time was 35%, and most (64%) of the variation could be ascribed to unique environmental factors. The role of heritability was of equal importance for women and men in this study. The proportion of heritability has been shown to be alike also in a study measuring daily activities with an accelerometer device [23]. However, the genetic component of physical inactivity has reported to be stronger in a comprehensive review [18] compared to our results. It has been reported that the heritability of physical activity decreases with age [33]. However, more sophisticated analyses need to be performed to confirm these results, also using relevant adjusting or stratified variables.

It seems that environmental factors play an important role in sitting time that may be related to adulthood choices or other factors unique to each individual such as occupation and leisure-time activities. The strong influence (50% to 72%) of unique environmental factors on physical activity has also been reported in other twin studies [22, 34]. These findings suggest that factors influencing our sedentary behaviour should be further elucidated. If the adulthood choices or habits, but also built environments are really of importance for the sitting time, as our preliminary results suggest, there might be possibilities to target public health campaigns to increase the public awareness of sedentary behaviour and/or to target both individual and community-based interventions in order to minimize sedentary behaviour and increase more activity and health enhancing behaviour. Societal policies in urban planning, the work environment, accessing leisure-time facilities, and many others are probably of great importance in their impact on total sitting time. However, we can also explore the role of earlier life circumstances, personality factors, life events, and health status using the cohort data available to us.

One of the main strengths of this study is the large population-based twin data and a high participation rate. The twin study design enables analysing the genetic component of sitting time. Also the generalizability of this twin data should be good since earlier reports have shown that the twins do not differ from the general population in terms of several traits including behaviour [35] or morbidity and mortality [25]. Thus, our study gives new information and aspects in analysing both the prevalence of sedentary behaviour by sitting activity and the relative role of heritability in sitting time.

Recommendations exist that monitoring self-reported sedentary behaviour should include overall sitting time in various activities [11]. In this study, the questionnaire included questions of sitting time in several activities; at work, at home, during commuting, and in all other activities from which we calculated the total time of sitting. Hence, we would like to assume that we have captured well the sitting time during a day. Even though there are both validity and reliability problems in self-rating methods reporting sedentary behaviour [36], there is also evidence that those who are reporting more sedentary behaviour in the self-rating questionnaires are also behaving more sedentary in the objectively measured studies [13]. Previous studies have used predominantly only TV viewing time or TV viewing alongside related "screen time" activities such as computer and video-based time as an indicator of sedentary behaviour [7, 11]. For example, long TV viewing time has been associated with overweight [3], mortality related to all-causes and cardiovascular diseases [7] as well as mental health [37] independent of many other risk factors or health behaviours. In addition, sitting most of the day has been shown to cause negative effects on insulin sensitivity and plasma lipids [38]. It is, however, noticeable that not all kinds of sitting are harmful

to our health [37]. Further studies of the age-related sedentary behaviour heritability are needed.

5. Conclusion

The amount of sitting time decreases with increasing age but seems to increase along with BMI among older adults. Heritability seems to have a modest role in sitting time with no difference by sex.

Appendices

A. Questions about Physical Exercise

What is your daily time of commuting by physically active means (including walking, jogging, cycling, and/or cross-country skiing) to and from work in total?

(a) less than 15 minutes
(b) 15 minutes–less than half an hour
(c) half an hour–less than an hour
(d) an hour or more
(e) I am not presently at work

Leisure-time physical exercise (exercise that does not occur at work or while commuting to and from work). Here are five alternatives that describe the amount of your leisure-time exercise. Which one applies best to you when considering the amount of exercise you get during the year as a whole?

(a) practically none
(b) a little
(c) a moderate amount
(d) quite a lot
(e) a great deal

How long does one session of your leisure-time physical exercise last an average?

(a) less than 15 minutes
(b) 15 minutes–less than half an hour
(c) half an hour–less than an hour
(d) an hour to less than two hours
(e) over two hours

Presently how many times per month do you engage in physical exercise during your leisure time?

(a) less than once a month
(b) 1-2 times a month
(c) 3–5 times a month
(d) 6–10 times a month
(e) 11–19 times a month
(f) more than 20 times a month

Is your leisure-time physical exercise about as intensive on average as

(a) walking
(b) alternatively walking and jogging
(c) jogging (light run)
(d) running

B. Question about Sitting Time (Sedentary Behaviour)

How many hours per day do you sit on average?

(1) in office or similar places (e.g., during a working day):

(a) less than an hour
(b) an hour–less than two hours
(c) two hours–less than four hours
(d) four hours or more

(2) at home watching TV or videos:

(a) less than an hour
(b) an hour–less than two hours
(c) two hours–less than four hours
(d) four hours or more

(3) at home at the computer:

(a) less than an hour
(b) an hour–less than two hours
(c) two hours–less than four hours
(d) four hours or more

(4) in a vehicle:

(a) less than an hour
(b) an hour–less than two hours
(c) two hours–less than four hours
(d) four hours or more

(5) elsewhere:

(a) less than an hour
(b) an hour–less than two hours
(c) two hours–less than four hours
(d) four hours or more

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Acknowledgments

Data collection was supported by the Academy of Finland Center of Excellence in Complex Disease Genetics (Grant nos. 213506 and 129680 to Jaakko Kaprio) and the Academy of Finland (Grant nos. 265240 and 263278 to Jaakko Kaprio). Analysis was supported by a grant from the Ministry for Education and Culture of Finland to Annina Ropponen. The authors would like to warmly thank the dedicated staff and researchers in our group who took part in the planning and data collection of wave four. A great thanks is due to all the twins who have participated in these studies over the years.

References

[1] I. M. Lee, E. J. Shiroma, F. Lobelo, P. Puska, S. N. Blair, and P. T. Katzmarzyk, "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy," *The Lancet*, vol. 380, no. 9838, pp. 219–229, 2012.

[2] M. T. Hamilton, D. G. Hamilton, and T. W. Zderic, "Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease," *Diabetes*, vol. 56, no. 11, pp. 2655–2667, 2007.

[3] S. Inoue, T. Sugiyama, T. Takamiya, K. Oka, N. Owen, and T. Shimomitsu, "Television viewing time is associated with overweight/obesity among older adults, independent of meeting physical activity and health guidelines," *Journal of Epidemiology*, vol. 22, no. 1, pp. 50–56, 2012.

[4] A. E. Staiano, D. M. Harrington, T. V. Barreira, and P. T. Katzmarzyk, "Sitting time and cardiometabolic risk in US adults: associations by sex, race, socioeconomic status and activity level," *British Journal of Sports Medicine*, vol. 48, no. 3, pp. 213–219, 2014.

[5] N. Owen, G. N. Healy, C. E. Matthews, and D. W. Dunstan, "Too much sitting: the population health science of sedentary behavior," *Exercise and Sport Sciences Reviews*, vol. 38, no. 3, pp. 105–113, 2010.

[6] K. I. Proper, A. S. Singh, W. Van Mechelen, and M. J. M. Chinapaw, "Sedentary behaviors and health outcomes among adults: a systematic review of prospective studies," *American Journal of Preventive Medicine*, vol. 40, no. 2, pp. 174–182, 2011.

[7] Y. Kim, L. R. Wilkens, S. Y. Park, M. T. Goodman, K. R. Monroe, and L. N. Kolonel, "Association between various sedentary behaviours and all-cause, cardiovascular disease and cancer mortality: the Multiethnic Cohort Study," *International Journal of Epidemiology*, vol. 42, no. 4, pp. 1040–1056, 2013.

[8] H. W. Kohl, C. L. Craig, E. V. Lambert et al., "The pandemic of physical inactivity: global action for public health," *The Lancet*, vol. 380, no. 9838, pp. 294–305, 2012.

[9] R. R. Pate, J. R. O'Neill, and F. Lobelo, "The evolving definition of 'sedentary'," *Exercise and Sport Sciences Reviews*, vol. 36, no. 4, pp. 173–178, 2008.

[10] M. Tremblay, "Letter to the editor: standardized use of the terms 'sedentary' and 'sedentary behaviours'," *Applied Physiology, Nutrition and Metabolism*, vol. 37, no. 3, pp. 540–542, 2012.

[11] G. N. Healy, B. K. Clark, E. A. H. Winkler, P. A. Gardiner, W. J. Brown, and C. E. Matthews, "Measurement of adults' sedentary time in population-based studies," *American Journal of Preventive Medicine*, vol. 41, no. 2, pp. 216–227, 2011.

[12] H. J. Helmerhorst, S. Brage, J. Warren, H. Besson, and U. Ekelund, "A systematic review of reliability and objective criterion-related validity of physical activity questionnaires," *International Journal of Behavioral Nutrition and Physical Activity*, vol. 9, p. 103, 2012.

[13] J. M. Schuna Jr., W. D. Johnson, and C. Tudor-Locke, "Adult self-reported and objectively monitored physical activity and sedentary behavior: NHANES 2005–2006," *International Journal of Behavioral Nutrition and Physical Activity*, vol. 10, no. 1, p. 126, 2013.

[14] A. E. Bauman, R. S. Reis, J. F. Sallis, J. C. Wells, R. J. Loos, and B. W. Martin, "Correlates of physical activity: why are some people physically active and others not?" *The Lancet*, vol. 380, no. 9838, pp. 258–271, 2012.

[15] K. Borodulin, C. Zimmer, R. Sippola, T. E. Mäkinen, T. Laatikainen, and R. Prättälä, "Health behaviours as mediating pathways between socioeconomic position and body mass index," *International Journal of Behavioral Medicine*, vol. 19, no. 1, pp. 14–22, 2012.

[16] I. Heinonen, H. Helajärvi, K. Pahkala et al. et al., "Sedentary behaviours and obesity in adults: the Cardiovascular Risk in Young Finns Study," *BMJ Open*, vol. 3, no. 6, 2013.

[17] D. M. Harrington, T. V. Barreira, A. E. Staiano, and P. T. Katzmarzyk, "The descriptive epidemiology of sitting among US adults, NHANES 2009/2010," *Journal of Science and Medicine in Sport*, 2013.

[18] D. M. de Vilhena e Santos, P. T. Katzmarzyk, A. F. Seabra, and J. A. Maia, "Genetics of physical activity and physical inactivity in humans," *Behavior Genetics*, vol. 42, no. 4, pp. 559–578, 2012.

[19] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *PLoS ONE*, vol. 1, no. 1, article e22, 2006.

[20] N. van der Aa, E. J. De Geus, T. C. van Beijsterveldt, D. I. Boomsma, and M. Bartels, "Genetic influences on individual differences in exercise behavior during adolescence," *International Journal of Pediatrics*, vol. 2010, Article ID 138345, 8 pages, 2010.

[21] N. van der Aa, M. Bartels, S. J. te Velde, D. I. Boomsma, E. J. de Geus, and J. Brug, "Genetic and environmental influences on individual differences in sedentary behavior during adolescence: a twin-family study," *Archives of Pediatrics and Adolescent Medicine*, vol. 166, no. 6, pp. 509–514, 2012.

[22] M. C. Nelson, P. Gordon-Larsen, K. E. North, and L. S. Adair, "Body mass index gain, fast food, and physical activity: effects of shared environments over time," *Obesity*, vol. 14, no. 4, pp. 701–709, 2006.

[23] M. den Hoed, S. Brage, J. H. Zhao et al., "Heritability of objectively assessed daily physical activity and sedentary behavior," *The American Journal of Clinical Nutrition*, 2013.

[24] J. Kaprio and M. Koskenvuo, "Genetic and environmental factors in complex diseases: the older Finnish Twin Cohort," *Twin Research*, vol. 5, no. 5, pp. 358–365, 2002.

[25] J. Kaprio, "The Finnish Twin Cohort Study: an update," *Twin Research and Human Genetics*, vol. 16, no. 1, pp. 157–162, 2013.

[26] J. Kaprio, M. Koskenvuo, and S. Sarna, "Cigarette smoking, use of alcohol, and leisure-time physical activity among same-sexed adult male twins," *Progress in clinical and biological research*, vol. 69, pp. 37–46, 1981.

[27] T. Lumley, P. Diehr, S. Emerson, and L. Chen, "The importance of the normality assumption in large public health data sets," *Annual Review of Public Health*, vol. 23, pp. 151–169, 2002.

[28] R. L. Williams, "A note on robust variance estimation for cluster-correlated data," *Biometrics*, vol. 56, no. 2, pp. 645–646, 2000.

[29] D. S. Falconer and T. F. C. Mackay, *Introduction To Quantitative Genetics*, Longmans Green, Essex, UK, 4th edition, 1996.

[30] S. Lord, S. F. M. Chastin, L. McInnes, L. Little, P. Briggs, and L. Rochester, "Exploring patterns of daily physical and sedentary behaviour in community-dwelling older adults," *Age and Ageing*, vol. 40, no. 2, Article ID afq166, pp. 205–210, 2011.

[31] M. G. Davis and K. R. Fox, "Physical activity patterns assessed by accelerometry in older people," *European Journal of Applied Physiology*, vol. 100, no. 5, pp. 581–589, 2007.

[32] T. Moore-Harrison and J. T. Lightfoot, "Driven to be inactive? The genetics of physical activity," *Progress in molecular biology and translational science*, vol. 94, pp. 271–290, 2010.

[33] J. M. Vink, D. I. Boomsma, S. E. Medland et al., "Variance components models for physical activity with age as modifier: a comparative twin study in seven countries," *Twin Research and Human Genetics*, vol. 14, no. 1, pp. 25–34, 2011.

[34] G. E. Duncan, J. Goldberg, C. Noonan, A. V. Moudon, P. Hurvitz, and D. Buchwald, "Unique environmental effects on physical activity participation: a twin study," *PLoS ONE*, vol. 3, no. 4, Article ID e2019, 2008.

[35] L. Pulkkinen, I. Vaalamo, R. Hietala, J. Kaprio, and R. J. Rose, "Peer reports of adaptive behavior in twins and singletons: is twinship a risk or an advantage?" *Twin Research*, vol. 6, no. 2, pp. 106–118, 2003.

[36] B. K. Clark, T. Sugiyama, G. N. Healy, J. Salmon, D. W. Dunstan, and N. Owen, "Validity and reliability of measures of television viewing time and other non-occupational sedentary behaviour of adults: a review," *Obesity Reviews*, vol. 10, no. 1, pp. 7–16, 2009.

[37] M. Hamer and E. Stamatakis, "Prospective study of sedentary behavior, risk of depression, and cognitive impairment," *Medicine and Science in Sports and Exercise*, vol. 46, no. 4, pp. 718–723, 2014.

[38] B. M. Duvivier, N. C. Schaper, M. A. Bremers et al., "Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable," *PLoS ONE*, vol. 8, no. 2, Article ID e55542, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 931820, 8 pages
http://dx.doi.org/10.1155/2014/931820

Review Article

Factors behind Leisure-Time Physical Activity Behavior Based on Finnish Twin Studies: The Role of Genetic and Environmental Influences and the Role of Motives

Sari Aaltonen,[1,2] Urho M. Kujala,[2] and Jaakko Kaprio[1,3,4]

[1] *Department of Public Health, The Hjelt Institute, University of Helsinki, P.O. Box 41, Mannerheimintie 172, 00014 Helsinki, Finland*
[2] *Department of Health Sciences, University of Jyväskylä P.O. Box 35, 40014 Jyväskylä, Finland*
[3] *Institute for Molecular Medicine, University of Helsinki, P.O. Box 21, Tukholmankatu 8, 00014 Helsinki, Finland*
[4] *Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland*

Correspondence should be addressed to Sari Aaltonen; sari.s.aaltonen@helsinki.fi

Received 29 October 2013; Revised 18 February 2014; Accepted 6 March 2014; Published 8 April 2014

Academic Editor: Meike Bartels

Different approaches are being taken to clarify the role of various factors in the development of physical activity behaviors. Genetic studies are a new area of physical activity research and also the motives for physical activity have been widely studied. The purpose of this paper is to review the findings emerging from the longitudinal genetic studies on leisure-time physical activity and to evaluate the associations between motivational factors and leisure-time physical activity. The focus is to review recent findings of longitudinal Finnish twin studies. The results of the latest longitudinal Finnish twin studies point to the existence of age-specific genetic and environmental influences on leisure-time physical activity. Variations in environmental factors seem to explain the observed deterioration in leisure-time physical activity levels. A decline in genetic influences is seen first from adolescence to young adulthood and again from the age of thirty to the mid-thirties. In the Finnish twin participants, mastery, physical fitness, and psychological state were the major motivation factors associated with consistent leisure-time physical activity behavior. The results also indicate that intrinsic motivation factors may be important for engagement in leisure-time physical activity.

1. Introduction

Epidemiological studies have revealed that physical activity can reduce risks for obesity as well as preventing several chronic diseases and even reducing mortality [1–6]. However, a substantial proportion of individuals, especially those living in the most developed countries, do not participate in sufficient physical activities and thus fail to gain the subsequent health benefits [7, 8]. If we are to understand why some subjects fail to engage in regular physical activity in leisure time, then we need to clarify which factors underlie individual differences in physical activity behavior.

It is known that many different factors play a role in leisure-time physical activity behavior. Leisure-time physical activity level may partly be determined on the basis of personal traits, needs, and interests and partly on external factors such as environment and availability factors [9–11]. Some of these factors may make it easier or harder for certain individuals to achieve high levels of physical activity. However, it is important to remember that environmental and genetic factors always work in conjunction. In the last decades, serious attempts have been made to clarify the role of different factors in physical activity behavior. Studies have concentrated on the correlates (i.e., factors associated with physical activity) and the determinants of physical activity (i.e., factors associated with a causal relationship). No clear consensus has been achieved, although several factors such as age, sex, previous physical activity, self-efficacy, and health status do seem to be associated with current physical activity level [11].

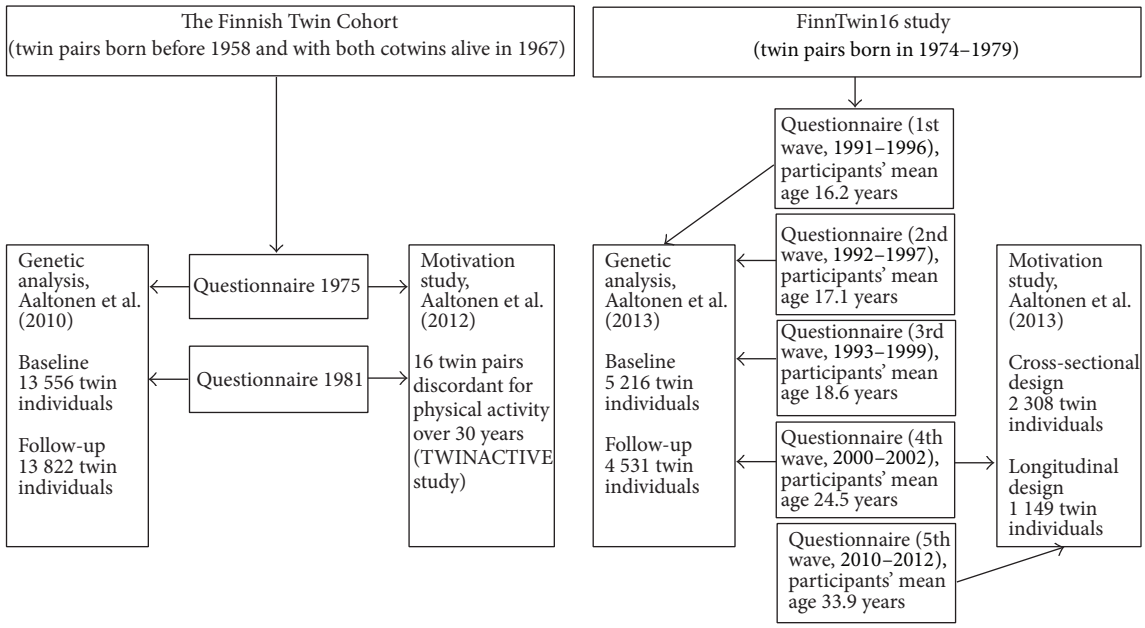


Figure 1: Participants in the Finnish twin studies originally described by Aaltonen et al. [27, 28, 65, 66].

Genetic studies are one of the new areas of physical activity research. This is logical because individual's genetic characteristics seem to be a possible determinant of physical activity [11] and advances in genetic technologies permit identification of individual genes or gene systems associated with a trait such as physical activity. These studies have attempted to determine the genetic architecture of factors contributing to an individual's propensity to be physically active. This includes estimating the overall role of genetic factors (in contrast to all nongenetic factors). If genetic factors are shown to be relevant, work is done to identify the genes and the mode of action of the genes in physical activity. The overall contribution of genetic factors to variation in physical activity is often examined by conducting twin studies. Twin study designs are popular in behavioral genetics, as they provide an opportunity to disentangle the effects of genes from those of the environment [12, 13]. In addition to genetics, motivation is a personal characteristic that also may be one of the key factors to help understand why some people spend their leisure time undertaking physical activity. This may be the reason why motives have been widely studied.

Although there are cross-sectional studies examining the associations between the genetic and environmental influences, motives, and leisure-time physical activity, longitudinal studies have been less frequently conducted. However, the advantages of longitudinal study designs are that causal associations can be better revealed and that the true effects of aging may be demonstrated. To date, little is also known about whether the motives for physical activity change over the life course. Another poorly characterized area is the difference in motivational factors between active and inactive individuals. The Finnish twin cohorts offered a great opportunity to utilize longitudinal study design and conduct comparison between physically active and inactive twins. The main aim of the present paper is to review the recent findings on genetic and environmental influences on the longitudinal changes of leisure-time physical activity behavior as revealed in the Finnish twin studies: first, from adolescence to young adulthood and, second, over a 6-year follow-up period in adulthood. Furthermore, the motives for leisure-time physical activity among consistently physically active and inactive people from the Finnish twin studies are presented. The present paper is based on the Ph.D. thesis of the first author, Aaltonen [14].

Physical activity has been defined to be body movements produced by the skeletal muscles, which cause a substantial increase in energy demands over resting energy expenditure [15]. However, the term physical activity is often used interchangeably with the terms exercise or sports although that is not correct or recommended [15]. The choice of term (physical activity, exercise, or sports) may impact the results of the genetic analyses and motivational studies. In this review, we have therefore used the same terms used in the original papers.

2. Genetic and Environmental Influences on Leisure-Time Physical Activity

In quantitative genetic modeling, physical activity is assumed to be made up of genetic and environmental contributions. Environmental influences can be divided into shared environmental influences, representing the effects of environmental factors shared, for example, by the cotwins in a pair. Specific environmental influences represent unique environmental influences and specific environmental influences result in differences between the cotwins of a pair [16]. A number of twin studies using the quantitative genetic

modeling have shown that genetic influences play an important role in explaining individual differences in leisure-time physical activity [17–26]. However, the different studies have found very different patterns. The largest of these studies has pooled data on leisure-time exercise behavior from seven different countries (GenomEUtwin project) and found that the heritability of exercise participation ranged from 48% to 71%, with the exception of Norwegian men where it was only 27% [22]. As this investigation indicates, it is clear that there is heterogeneity in the results of studies related to genetic influences on leisure-time physical activity. It can be assumed that a significant proportion of the heterogeneity may derive not only from changes in the genetic contribution to this trait in different aged individuals but also from culture-specific, sex-specific, and period-specific effects. Physical activity assessment methods may also have an influence on the heterogeneity of results. Heritability is always assessed at a particular time and age, and above all, heritability is an estimate of the genetic influences to individual differences on a population level.

Longitudinal study designs are needed to reveal the age-specific genetic influences on leisure-time physical activity. However, only a few studies have investigated the genetic and environmental influences on longitudinal leisure-time physical activity before the Finnish twin studies were published [27, 28]. Simonen et al. [29] reported change across the lifespan in heritability estimates for leisure-time physical activity in adult male twin pairs. A recent comparative study in twins aged 19 to 50 from seven countries that collaborated in the GenomEUtwin project was not a pure longitudinal study, but it revealed also age-related changes in heritability [30].

Earlier studies have also reported a shift between genetic and environmental influences in the time periods between childhood and adolescence and between adolescence and young adulthood, although at different times in different studies and in different directions. In Dutch boys, genetic influences on leisure-time exercise behavior were fluctuating from age of 7 years to age of 12 years, while in girls genetic influences were more stable [31]. In this study, shared environmental influences mainly explained the largest part of the variance in leisure-time exercise behavior between childhood and early adolescence. The decline in the heritability estimate was noted in longitudinal studies by both van der Aa et al. [32] and Eriksson et al. [33]. Genetic influences on leisure-time physical activity declined from early adolescence to late adolescence in both sexes in Dutch twins [32] and decline was also seen during a 4-year followup among young Swedish men in their twenties [33]. In contrast to these studies, Stubbe et al. [34] found in their longitudinal study that between the age of 13 and the age of 16 years genetic influences were not important, whereas between the age of 19 and the age of 20 years genetic influences largely explained the individual differences in leisure-time sports participation.

2.1. Genetic and Environmental Influences on Longitudinal Leisure-Time Physical Activity in Finnish Twin Studies. The participants of the Finnish twin studies examined for genetic and environmental influences of leisure-time physical activity are drawn from two cohorts: the FinnTwin16 study (twins born between 1975 and 1979) and the older Finnish Twin Cohort (twins born before 1958 and both cotwins alive in 1967) (Figure 1). Both cohorts were identified from the Central Population Registry of Finland with the purpose of forming a national resource for genetic epidemiological studies [35–39]. The longitudinal quantitative genetic analyses of these cohorts published by Aaltonen et al. [27, 28] produced results, which corroborate the findings of much of the previous work in this field; that is, the heritability of leisure-time physical activity behavior ranged between 27% and 71% as summarized above. In the studies by Aaltonen et al. [27, 28], the heritability of leisure-time physical activity ranged between ~30% and ~52%.

In addition, these results of the Finnish twin studies confirmed the existence of age-specific changes in the genetic and environmental influences on leisure-time physical activity. The results revealed a change in the pattern of genetic and environmental influences in the progress of leisure-time physical activity: first, from adolescence to adulthood [27] and, second, from the age of thirty to the mid-thirties [28]. The summary of the final models for leisure-time physical activity has been presented in Figure 2.

In the study of the younger Finnish twins, the relative role of additive genetic influences remained rather stable during adolescence only changing from 43% to 52% [27]. However, the heritability estimate declined in the period from adolescence to young adulthood to around 30%. This decrease in genetic influences is parallel to the indications that leisure-time physical activity level declines with age [8, 40–42]. Shared environmental influences, in turn, also showed relative stability during adolescence, but in contrast to genetic influences they increased markedly in young adulthood, especially in women. Additive genetic, shared environmental, and specific environmental correlations between the baseline results in adolescence and follow-up results in young adulthood are shown in Figure 2.

In adulthood, around the age of thirty, additive genetic influences were also moderate, at 44%, while a slight decline was also seen in the mid-thirties, when additive genetic influences were estimated to be 34% [28]. In this study, the additive genetic correlation for leisure-time physical activity was greater for men, 0.79, than for women, 0.64, but the environmental correlation between the two time points did not differ substantially between the sexes (Figure 2). The longitudinal phenotypic correlation in men was 0.45, of which 74% was due to longitudinal additive genetic influences, while in women the longitudinal phenotypic correlation was 0.38, of which 60% was due to longitudinal additive genetic influences.

Based on these longitudinal quantitative studies among Finnish twins, both shared and specific environmental influences affected leisure-time physical activity up to adulthood, but only specific environmental influences were further present in adulthood in the thirties and mid-thirties. In contrast to the consistent expression of an important group of genes observed in adulthood, new additive genetic,

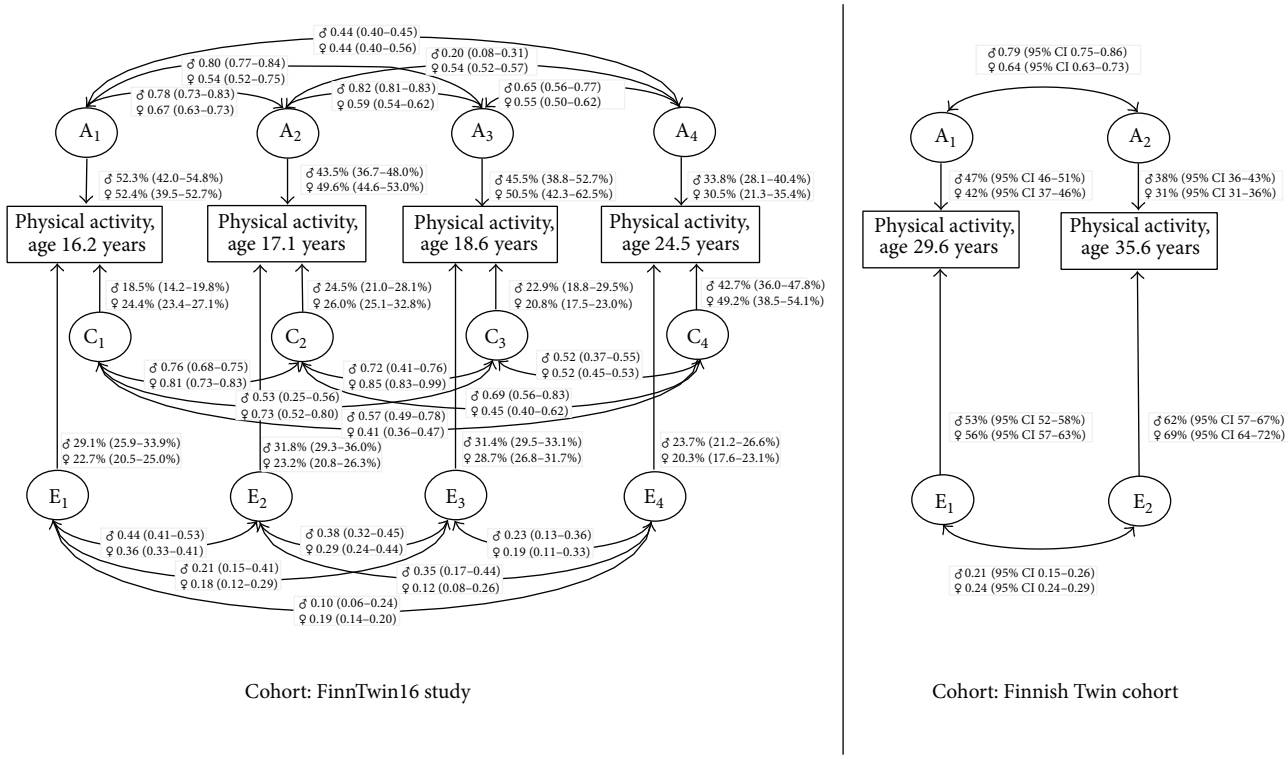


Figure 2: The summary of the final genetic models for leisure-time physical activity between both ages of 16.2 and 24.5 years and ages of 29.6 and 35.6 years in Finnish twin studies. It is important to note that the cohorts used in the models between ages of 16.2 and 24.5 years and between ages of 29.6 and 35.6 years are not identical. Genetic and environmental influences are shown as percentages; upper value is for men and lower value is for women. Confidence intervals (95% CI) are shown in the parentheses. Additive genetic, shared environmental, and specific environmental correlations between the baseline and follow-up results are shown as curved arrows. The more detailed summaries for models are presented in the publications of Aaltonen et al. [27, 28].

shared, and specific environmental influences emerged at each follow-up point in adolescence and in young adulthood.

3. Motives for Leisure-Time Physical Activity

In addition to genetics, motivation is a personal characteristic that also may be one of the key factors for understanding why some people are physically active in their leisure time. Many studies have been published on what motivates individuals to undertake physical activity. Several of these studies have reported that, regardless of age, gender, or level of physical activity, health is an important factor motivating participation in leisure-time physical activity among adults [43–49]. For instance, among the citizens of the European Union member states, almost half of those aged over 15 years reported good health as the most important reason for participation in physical activity [44]. Despite the general importance of health as a factor motivating leisure-time physical activity, it seems to be a factor which varies by region [50]. In addition to health benefits, appearance [51], fitness [48], enjoyment [48], and body image [52] are features which are highly linked to physical activity among young adults. However, it is important to remember that motives may change during the stages of adoption of some form of physical exercise [53]. Differences may also exist according to exercise type [54, 55], gender, and age [46, 53, 56, 57].

So far, only some of the published studies have examined differences in motivational factors between physically active and inactive people, but none of these studies has been longitudinal. Studies have been based on the hypothesis that the level of leisure-time physical activity is explained by differences in motivational factors. One study did indicate that physical activity was mostly associated with environmental factors, whereas inactivity was linked with sociodemographic factors [58]. Overall, when physically active people were compared to physically inactive people, health, fitness, and enjoyment were identified as the major motivational factors for leisure-time physical activity in the active people [46, 48, 59]. Social reasons were highlighted by physically active and inactive people in the recent study of Costello et al. [60]. In this study, physically inactive people wanted leisure-time physical activity to be purposeful and fun, while the active participants enjoyed exercise regardless of its purpose. The randomized controlled study of Silva et al. [61] found that women whose intervention focused on promoting autonomous forms of exercise regulation and intrinsic motivation showed higher physical activity levels than controls.

The role of family and genetic factors in motivation for physical activity is poorly studied; further, links between physical activity, genetic influences, and motivational factors remain unraveled. A recent animal study suggested that voluntary running motivation may be inherent [62]. In a study by Huppertz et al. [63], exercise attitude components explained 28% of the variance in leisure-time exercise behavior. In bivariate modeling, all the genetic and all but two unique environmental correlations between attitude components and exercise behavior suggested a causal relationship between exercise attitude and leisure-time exercise behavior. The authors concluded that both exercise attitudes and exercise behavior are heritable and are partly correlated through pleiotropic genetic effects. It thus seems plausible that family and genetic factors influence motives for physical activity.

3.1. Motives for Leisure-Time Physical Activity Based on Finnish Twin Studies. The motives for undertaking leisure-time physical activity were also studied using data from the FinnTwin16 study (younger twins born between 1975 and 1979) and the Finnish Twin Cohort (older twins born before 1958 and both cotwins alive in 1967) (Figure 1). Participants from the FinnTwin16 study were analyzed as individuals in their mid-thirties. The cotwin control study design was used when twin pairs (mean age 60.4 years) discordant for leisure-time physical activity over 30 years from the Finnish Twin Cohort were analysed. In these studies, physical fitness, psychological state, and enjoyment were the highest scored reasons for engaging in leisure-time physical activity when motivational factors were assessed by the Recreational Exercise Motivation Measure (REMM) [64]. Thus, the same factors seem to be important for engagement in leisure-time physical activity among both younger and older adults in Finland. These were also the factors that the physically active participants rated higher than the physically inactive participants. The findings of the importance of physical and psychological health as motivational factors are also in agreement with earlier findings by other researchers presented above.

However, a major result of the Finnish twin studies related to motives confirmed the importance of motivational factors in separating leisure-time physical activity behavior. When motives for leisure-time physical activity were measured among older Finnish twin pairs who have been discordant for leisure-time physical activity over 30 years, the motivational factors of mastery, physical fitness, and psychological state were subdimensions that differed significantly between the consistently physically active twins and their consistently physically inactive cotwins [65] (Figure 3). The same results were obtained when the consistently active twin individuals were compared to the consistently inactive twin individuals in the FinnTwin16 study [66] (Figure 4). These younger twin individuals had been either consistently physically active or consistently physically inactive for at least the last ten years. Moreover, motivational factors related to appearance, enjoyment, and willingness to be fitter or look better than others and the social aspect of physical activity differed also significantly between the younger twin individuals in the FinnTwin16 study [66] (Figure 4). The results did not substantially differ according to gender.

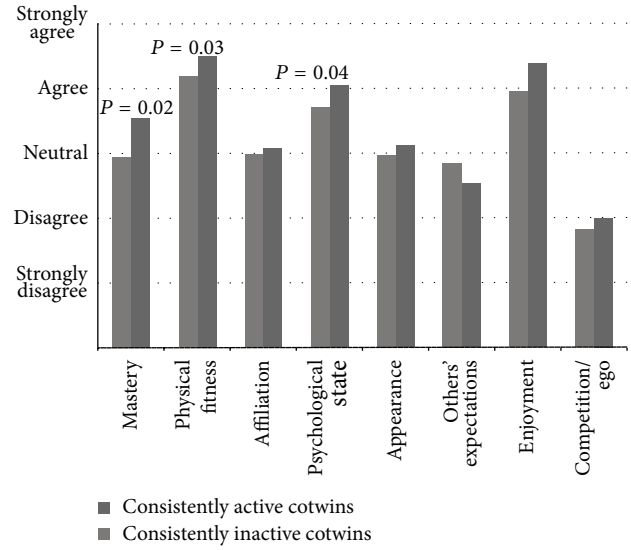


Figure 3: Differences in the subdimensions of the REMM measurement scale among twin pairs who have been discordant for leisure-time physical activity over 30 years (twins from the Finnish Twin Cohort) [65]. The dark grey columns of the histogram represent twins who have been physically active over 30 years and the light grey columns represent their inactive cotwins. The names of the subdimensions are shown below the columns and the answer options are shown on the left hand side of the histogram. The *P* values above the columns indicate that there is a statistical difference between the active and inactive cotwins. The *P* value is shown only if a significant difference between the groups was detected.

In the Finnish twin studies, both younger twin individuals and older twin pairs rated conforming to others' expectations as the least meaningful motivating factors for undertaking leisure-time physical activity. Conforming to others' expectations is one of the subdimensions of the REMM. The older inactive twins in the Finnish Twin Cohort emphasized compliance with other peoples expectations slightly more than their active cotwins within the pair. The same result was found among younger twin individuals in the FinnTwin16 study. However, the difference was statistically significant only between the consistently active and consistently inactive twin individuals in the FinnTwin16 study and between the consistently active and consistently inactive women in their mid-thirties in the FinnTwin16 study [66]. No statistically significant difference was seen between the consistently active and consistently inactive men in their mid-thirties in the FinnTwin16 study [66] or between the twin pairs who have been discordant for leisure-time physical activity over 30 years in the Finnish Twin Cohort [65]. The measure of effect size also revealed that the difference between the groups was of low magnitude. The subdimension of conforming to others' expectations clearly reflects the extrinsic type of motivation. This suggests that genetic factors may contribute to the relationship of physical activity and motivations, but this has not been formally modelled.

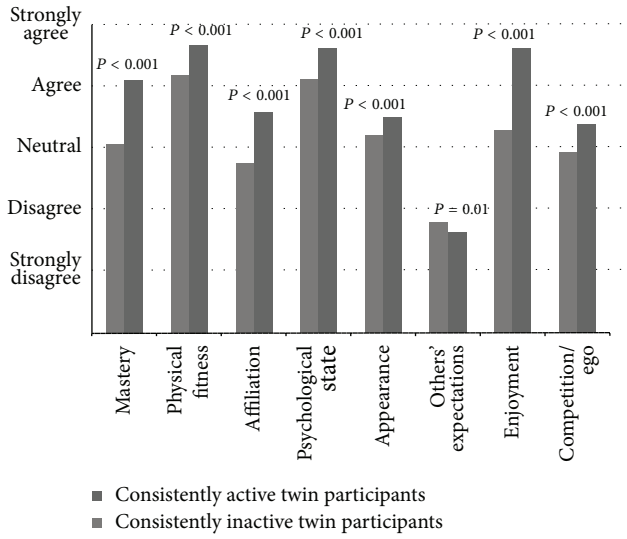


Figure 4: Differences in the subdimensions of the REMM measurement scale among consistently physically active and inactive twin individuals in their mid-thirties (twins from the FinnTwin16 study) [66]. The dark grey columns of the histogram represent twin individuals who have been physically active for at least the last ten years and the light grey columns represent twin individuals who have been inactive for the same period of time. The names of the subdimensions are shown below the columns and the answer options are shown on the left hand side of the histogram. The *P* values above columns indicate that there is a statistical difference between the active and inactive cotwins.

4. Conclusions

In conclusion, several studies have provided evidence that both genetic and environmental influences and motives are associated with physical activity behavior. Furthermore, the latest longitudinal studies among Finnish twins deepened the understanding of regular, consistent leisure-time physical activity behavior. Specifically, the results of the longitudinal Finnish twin studies found evidence for the existence of age-specific genetic and environmental influences on leisure-time physical activity. Such age-specific genetic effects need to be carefully considered when designing and analyzing molecular genetic studies to identify specific genes and factors affecting the expression of genes, such as through epigenetic mechanisms. In addition, the results of the Finnish twin studies revealed differences in motivational factors influencing leisure-time physical activity between consistently physically active and inactive people. The results also indicated that intrinsic motivation factors are important for engagement in leisure-time physical activity.

The results of the present review suggested that variations in environmental factors seemed to explain the observed deterioration in leisure-time physical activity levels. Measures promoting leisure-time physical activity may be even more important for women than for men, because of the greater role of environmental influences in women shown by these Finnish twin studies. Furthermore, the transitional period from adolescence to young adulthood should be seen as a strategic point to stimulate leisure-time physical activity that would also lead to an active lifestyle in later adulthood.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The research work has been supported by the Finnish Ministry of Education (Sari Aaltonen and Urho M. Kujala), Tekes—the Finnish Funding Agency for Technology and Innovation (Grant no. 1104/10 to Urho M. Kujala), the Academy of Finland Center of Excellence in Complex Disease Genetics (Grants nos. 213506 and 129680 to Jaakko Kaprio), the Juho Vainio Foundation (Sari Aaltonen and Karri Silventoinen), and the Yrjö Jahnsson Foundation (Sari Aaltonen). The data collection of the FinnTwin16 study has been supported by the U.S. National Institute of Alcohol Abuse and Alcoholism (Grants nos. AA-12502, AA-00145, and AA-09203 to Richard J. Rose) and the Academy of Finland (Grants nos. 100499, 205585, and 141054 to Jaakko Kaprio).

References

[1] J. N. Morris, R. Pollard, and M. G. Everitt, "Vigorous exercise in leisure-time: protection against coronary heart disease," *The Lancet*, vol. 2, no. 8206, pp. 1207–1210, 1980.

[2] R. S. Paffenbarger Jr., R. T. Hyde, A. L. Wing, and C.-C. Hsieh, "Physical activity, all-cause mortality, and longevity of college alumni," *The New England Journal of Medicine*, vol. 314, no. 10, pp. 605–613, 1986.

[3] U. M. Kujala, J. Kaprio, S. Sarna, and M. Koskenvuo, "Relationship of leisure-time physical activity and mortality: the Finnish twin cohort," *Journal of the American Medical Association*, vol. 279, no. 6, pp. 440–444, 1998.

[4] D. E. Laaksonen, L. Niskanen, H.-M. Lakka, T. A. Lakka, and M. Uusitupa, "Epidemiology and treatment of the metabolic syndrome," *Annals of Medicine*, vol. 36, no. 5, pp. 332–346, 2004.

[5] C. E. Garber, B. Blissmer, M. R. Deschenes et al., "Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise," *Medicine and Science in Sports and Exercise*, vol. 43, no. 7, pp. 1334–1359, 2011.

[6] F. W. Booth, C. K. Roberts, and M. J. Laye, "Lack of exercise is a major cause of chronic diseases," *Comprehensive Physiology*, vol. 2, no. 2, pp. 1143–1211, 2012.

[7] M. Pratt, C. A. Macera, and C. Blanton, "Levels of physical activity and inactivity in children and adults in the United States: current evidence and research issues," *Medicine and Science in Sports and Exercise*, vol. 31, no. 11, pp. S526–S533, 1999.

[8] M. A. Martinez-Gonzalez, J. J. Varo, J. L. Santos et al., "Prevalence of physical activity during leisure time in the European Union," *Medicine and Science in Sports and Exercise*, vol. 33, no. 7, pp. 1142–1146, 2001.

[9] C. Bouchard and R. J. Shephard, "Physical activity, fitness and health: the model and key concepts," in *Physical Activity, Fitness and Health. International proceedIngs and Consensus Statement*,

C. Bouchard, R. J. Shephard, and T. Stephens, Eds., pp. 77–88, Human Kinetics, Champaign, Ill, USA, 1994.

[10] C. Bouchard, S. N. Blair, and W. L. Haskell, "Why study physical activity and health?" in *Physical Activity and Health*, C. Bouchard, S. N. Blair, and W. L. Haskell, Eds., pp. 3–19, Human Kinetics, Champaign, Ill, USA, 2007.

[11] A. E. Bauman, R. S. Reis, J. F. Sallis et al., "Correlates of physical activity: why are some people physically active and others not?" *The Lancet*, vol. 380, no. 9838, pp. 258–271, 2012.

[12] D. Boomsma, A. Busjahn, and L. Peltonen, "Classical twin studies and beyond," *Nature Reviews Genetics*, vol. 3, no. 11, pp. 872–882, 2002.

[13] J. van Dongen, P. E. Slagboom, H. H. Draisma, N. G. Martin, and D. I. Boomsma, "The continuing value of twin studies in the omics era," *Nature Reviews Genetics*, vol. 13, no. 9, pp. 640–653, 2012.

[14] S. Aaltonen, *Leisure-Time Physical Activity in a Finnish Twin Study: Genetic and Environmental Influences as Determinants and Motives as Correlates*, Studies in Sport, Physical Education and Health 195, University of Jyväskylä, 2013, https://jyx.jyu.fi/dspace/bitstream/handle/123456789/42018/978-951-39-5326-3.pdf?sequence=2.

[15] C. J. Caspersen, K. E. Powell, and G. Christenson, "Physical activity, exercise and physical fitness: definitions and distinctions for health-related research," *Public Health Reports*, vol. 100, no. 2, pp. 126–131, 1985.

[16] F. V. Rijsdijk and P. C. Sham, "Analytic approaches to twin data using structural equation models," *Briefings in bioinformatics*, vol. 3, no. 2, pp. 119–133, 2002.

[17] J. Kaprio, M. Koskenvuo, and S. Sarna, "Cigarette smoking, use of alcohol, and leisure-time physical activity among same-sexed adult male twins," *Progress in Clinical and Biological Research*, vol. 69, pp. 37–46, 1981.

[18] M. Aarnio, T. Winter, U. M. Kujala, and J. Kaprio, "Familial aggregation of leisure-time physical activity—a three generation study," *International Journal of Sports Medicine*, vol. 18, no. 7, pp. 549–556, 1997.

[19] G. Beunen and M. Thomis, "Genetic determinants of sports participation and daily physical activity," *International Journal of Obesity*, vol. 23, no. 3, pp. S55–S63, 1999.

[20] J. A. R. Maia, M. Thomis, and G. Beunen, "Genetic factors in physical activity levels: a twin study," *American Journal of Preventive Medicine*, vol. 23, no. 2, pp. 87–91, 2002.

[21] S. Carlsson, T. Andersson, P. Lichtenstein, K. Michaëlsson, and A. Ahlbom, "Genetic effects on physical activity: results from the Swedish Twin Registry," *Medicine and Science in Sports and Exercise*, vol. 38, no. 8, pp. 1396–1401, 2006.

[22] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *PLoS ONE*, vol. 1, no. 1, article e22, 2006.

[23] J. H. Stubbe and E. J. C. de Geus, "Genetics of exercise behavior," in *Handbook of Behavior Genetics*, K. Yong-Kyu, Ed., pp. 343–358, Springer, New York, NY, USA, 2009.

[24] D. M. de Vilhena e Santos, P. T. Katzmarzyk, A. F. Seabra, and J. A. Maia, "Genetics of physical activity and physical inactivity in humans," *Behavior Genetics*, vol. 42, no. 4, pp. 559–578, 2012.

[25] L. Mustelin, J. Joutsi, A. Latvala, K. H. Pietilainen, A. Rissanen, and J. Kaprio, "Genetic influences on physical activity in young adults. A twin study," *Medicine & Science in Sports & Exercise*, vol. 44, no. 7, pp. 1293–1301, 2012.

[26] S. Carlsson, A. Ahlbom, P. Lichtenstein, and T. Andersson, "Shared genetic influence of BMI, physical activity and type 2 diabetes: a twin study," *Diabetologia*, vol. 56, no. 5, pp. 1031–1035, 2013.

[27] S. Aaltonen, A. Ortega-Alonso, U. M. Kujala, and J. Kaprio, "Genetic and environmental influences on longitudinal changes in leisure-time physical activity from adolescence to young adulthood," *Twin Research and Human Genetics*, vol. 16, no. 2, pp. 535–543, 2013.

[28] S. Aaltonen, A. Ortega-Alonso, U. M. Kujala, and J. Kaprio, "A longitudinal study on genetic and environmental influences on leisure time physical activity in the finnish twin cohort," *Twin Research and Human Genetics*, vol. 13, no. 5, pp. 475–481, 2010.

[29] R. Simonen, E. Levälahti, J. Kaprio, T. Videman, and M. C. Battié, "Multivariate genetic analysis of lifetime exercise and environmental factors," *Medicine and Science in Sports and Exercise*, vol. 36, no. 9, pp. 1559–1566, 2004.

[30] J. M. Vink, D. I. Boomsma, S. E. Medland et al., "Variance components models for physical activity with age as modifier: a comparative twin study in seven countries," *Twin Research and Human Genetics*, vol. 14, no. 1, pp. 25–34, 2011.

[31] C. Huppertz, M. Bartels, C. E. Van Beijsterveldt, D. I. Boomsma, J. J. Hudziak, and E. J. De Geus, "Effect of shared environmental factors on exercise behavior from age 7 to 12 years," *Medicine and Science in Sports and Exercise*, vol. 44, no. 10, pp. 2025–2032, 2012.

[32] N. van der Aa, E. J. De Geus, T. C. van Beijsterveldt, D. I. Boomsma, and M. Bartels, "Genetic influences on individual differences in exercise behaviour during adolescence," *International Journal of Pediatrics*, vol. 2010, Article ID 138345, 8 pages, 2010.

[33] M. Eriksson, F. Rasmussen, and P. Tynelius, "Genetic factors in physical activity and the equal environment assumption—the Swedish young male twins study," *Behavior Genetics*, vol. 36, no. 2, pp. 238–247, 2006.

[34] J. H. Stubbe, D. I. Boomsma, and E. J. C. De Geus, "Sports participation during adolescence: a shift from environmental to genetic factors," *Medicine and Science in Sports and Exercise*, vol. 37, no. 4, pp. 563–570, 2005.

[35] J. Kaprio, S. Sarna, M. Koskenvuo, and I. Rantasalo, "The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program," *Progress in Clinical and Biological Research*, vol. 24, pp. 179–184, 1978.

[36] J. Kaprio, M. Koskenvuo, and R. J. Rose, "Population-based twin registries: illustrative applications in genetic epidemiology and behavioral genetics from the Finish twin cohort study," *Acta Geneticae Medicae et Gemellologiae*, vol. 39, no. 4, pp. 427–439, 1990.

[37] J. Kaprio and M. Koskenvuo, "Genetic and environmental factors in complex diseases: the older Finnish twin cohort," *Twin Research*, vol. 5, no. 5, pp. 358–365, 2002.

[38] J. Kaprio, "Twin studies in Finland 2006," *Twin Research and Human Genetics*, vol. 9, no. 6, pp. 772–777, 2006.

[39] J. Kaprio, "The Finnish twin cohort study: an update," *Twin Research and Human Genetics*, vol. 16, no. 1, pp. 157–162, 2013.

[40] C. J. Crespo, S. J. Keteyian, G. W. Heath, and C. T. Sempos, "Leisure-time physical activity among US adults: results from the Third National Health and Nutrition Examination Survey," *Archives of Internal Medicine*, vol. 156, no. 1, pp. 93–98, 1996.

[41] J. F. Sallis, "Age-related decline in physical activity: a synthesis of human and animal studies," *Medicine and Science in Sports and Exercise*, vol. 32, no. 9, pp. 1598–1600, 2000.

[42] P. C. Hallal, L. B. Andersen, F. C. Bull et al., "Global physical activity levels: surveillance progress, pitfalls, and prospects," *The Lancet*, vol. 380, no. 9838, pp. 247–257, 2012.

[43] B. Ashford, S. Biddle, and M. Goudas, "Participation in community sports centres: motives and predictors of enjoyment," *Journal of Sports Sciences*, vol. 11, no. 3, pp. 249–256, 1993.

[44] H.-J. F. Zunft, D. Friebe, B. Seppelt et al., "Perceived benefits and barriers to physical activity in a nationally representative sample in the European Union," *Public Health Nutrition*, vol. 2, no. 1A, pp. 153–160, 1999.

[45] G. S. Kolt, R. P. Driver, and L. C. Giles, "Why older Australians participate in exercise and sport," *Journal of Aging and Physical Activity*, vol. 12, no. 2, pp. 185–198, 2004.

[46] M. Dacey, A. Baltzell, and L. Zaichkowsky, "Older adults' intrinsic and extrinsic motivation toward physical activity," *American Journal of Health Behavior*, vol. 32, no. 6, pp. 570–582, 2008.

[47] J. A. M. Murcia, C. M. Galindo, and P. M. Pardo, "Motivations and reasons for exercising in water gender and age differences in a sample of Spanish exercisers," *International Journal of Aquatic Research and Education*, vol. 2, pp. 237–246, 2008.

[48] C. H. P. Sit, J. H. Kerr, and I. T. F. Wong, "Motives for and barriers to physical activity participation in middle-aged Chinese women," *Psychology of Sport and Exercise*, vol. 9, no. 3, pp. 266–283, 2008.

[49] E. Caglar, Y. Canlan, and M. Demir, "Recreational exercise motives of adolescents and young adults," *Journal of Human Kinetics*, vol. 22, no. 1, pp. 83–89, 2009.

[50] R. J. Iannotti, R. Chen, H. Kololo, G. Petronyte, E. Haug, and C. Roberts, "Motivations for adolescent participation in leisure-time physical activity: international differences," *Journal of Physical Activity and Health*, vol. 10, no. 1, pp. 106–112, 2013.

[51] M. Kilpatrick, E. Hebert, and J. Bartholomew, "College students' motivation for physical activity: differentiating men's and women's motives for sport participation and exercise," *Journal of American College Health*, vol. 54, no. 2, pp. 87–94, 2005.

[52] L. Brudzynski and W. P. Ebben, "Body image as a motivator and barrier to exercise participation," *International Journal of Exercise Science*, vol. 3, no. 1, pp. 14–24, 2010.

[53] C. M. Frederick and R. M. Ryan, "Differences in Motivation for sport and exercise and their relations with participation and mental health," *Journal of Sport Behavior*, vol. 16, no. 3, pp. 124–146, 1993.

[54] R. M. Ryan, C. M. Frederick, D. Lepes, N. Rubio, and K. M. Sheldon, "Intrinsic motivation and exercise adherence," *International Journal of Sport Psychology*, vol. 28, no. 4, pp. 335–354, 1997.

[55] A. de Andrade Bastos, A. Salguero, R. González-Boto, and S. Marquez, "Motives for participation in physical activity by Brazilian adults," *Perceptual and Motor Skills*, vol. 102, no. 2, pp. 358–367, 2006.

[56] M. E. Finkenberg, J. M. DiNucci, S. L. McCune, and E. D. McCune, "Analysis of course type, gender, and personal incentives to exercise," *Perceptual and Motor Skills*, vol. 78, no. 1, pp. 155–159, 1994.

[57] D. L. Gill, L. Williams, D. A. Dowd, C. M. Beaudoin, and J. J. Martin, "Competitive orientations and motives of adult sport and exercise participants," *Journal of Sport Behavior*, vol. 19, pp. 307–318, 1996.

[58] P. Gordon-Larsen, R. G. McMurray, and B. M. Popkin, "Determinants of adolescent physical activity and inactivity patterns," *Pediatrics*, vol. 105, no. 6, p. E83, 2000.

[59] R. M. Reid and I. McGowan, "A longitudinal psycho-physiological study of active and inactive men," *British Journal of Sports Medicine*, vol. 20, no. 4, pp. 174–177, 1986.

[60] E. Costello, M. Kafchinski, J. Vrazel, and P. Sullivan, "Motivators, barriers, and beliefs regarding physical activity in an older adult population," *Journal of Geriatric Physical Therapy*, vol. 34, no. 3, pp. 138–147, 2011.

[61] M. N. Silva, P. N. Vieira, S. R. Coutinho et al., "Using self-determination theory to promote physical activity and weight control: a randomized controlled trial in women," *Journal of Behavioral Medicine*, vol. 33, no. 2, pp. 110–122, 2010.

[62] M. D. Roberts, J. D. Brown, J. M. Company et al., "Phenotypic and molecular differences between rats selectively bred to voluntarily run high vs. low nightly distances," *American Journal of Physiology*, vol. 304, no. 11, pp. 1024–1035, 2013.

[63] C. Huppertz, M. Bartels, I. E. Jansen et al., "A twin-sibling study on the relationship between exercise attitudes and exercise behavior," *Behavior Genetics*, vol. 44, no. 1, pp. 45–55, 2014.

[64] H. Rogers and T. Morris, "An overview of the development and validation of the Recreational Exercise Motivation Measure (REMM)," in *Proceedings of the 10th the European Congress of Sport Psychology*, 2003.

[65] S. Aaltonen, T. Leskinen, T. Morris et al., "Motives for and barriers to physical activity in twin pairs discordant for leisure time physical activity for 30 years," *International Journal of Sports Medicine*, vol. 33, no. 2, pp. 157–163, 2012.

[66] S. Aaltonen, M. Rottensteiner, J. Kaprio, and U. M. Kujala, "Motives for physical activity among active and inactive persons in their mid-30s," *Scandinavian Journal of Medicine & Science in Sports*, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 845207, 9 pages
http://dx.doi.org/10.1155/2014/845207

Clinical Study

Genotype by Energy Expenditure Interaction and Body Composition Traits: The Portuguese Healthy Family Study

D. M. Santos,[1] P. T. Katzmarzyk,[2] V. P. Diego,[3] T. N. Gomes,[1] F. K. Santos,[1] J. Blangero,[3] and J. A. Maia[1]

[1] *CIFI2D, Faculty of Sports, University of Porto, Rua Dr. Plácido Costa 91, 4200-450 Porto, Portugal*
[2] *Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808, USA*
[3] *Texas Biomedical Research Institute, San Antonio, Texas 78227-5301, USA*

Correspondence should be addressed to D. M. Santos; d.monteiro.santos13@gmail.com

Received 21 November 2013; Revised 12 February 2014; Accepted 25 February 2014; Published 25 March 2014

Academic Editor: Martine Thomis

Background and Aims. Energy expenditure has been negatively correlated with fat accumulation. However, this association is highly variable. In the present study we applied a genotype by environment interaction method to examine the presence of Genotype x by Total Daily Energy Expenditure and Genotype x by Daily Energy Expenditure interactions in the expression of different body composition traits. *Methods and Results*. A total of 958 subjects from 294 families of *The Portuguese Healthy Family Study* were included in the analysis. TDEE and DEE were assessed using a physical activity recall. Body fat percentages were measured with a bioelectrical impedance scale. GxTDEE and GxDEE examinations were performed using SOLAR 4.0 software. All BC traits were significantly heritable, with heritabilities ranging from 21% to 34%. The GxTDEE and GxDEE interaction models fitted the data better than the polygenic model for all traits. For all traits, a significant GxTDEE and GxDEE interaction was due to variance heterogeneity among distinct levels of TDEE and DEE. For WC, GxTDEE was also significant due to the genetic correlation function. *Conclusions*. TDEE and DEE are environmental constraints associated with the expression of individuals' BC genotypes, leading to variability in the phenotypic expression of BC traits.

1. Introduction

The hypothesis that the development of many complex traits are the result of the interplay between genetic background and environmental influences has long been postulated [1] and has been referred to as genotype-by-environment interaction (GxE) [2]. Under such a hypothesis it is expected that genetic effects are dynamically modulated by environmental exposures.

This concept has been used to study obesity for several decades [3], and there is a wealth of data confirming that environmental factors, whether related to nutritional habits and/or physical activity/exercise patterns, play key roles in the accumulation of body fat [4, 5]. However, within a population sharing the same physical activity (PA) habits (in terms of levels and patterns), interindividual variability in body composition is widely observed [6].

Genetic epidemiology research suggests that genetic factors account for 50% to 90% [7] of the total interindividual variability in body fat accumulation. It remains, however, to be explained how environmental and behavioral factors, such as PA, affect the genetic influence on body composition. Twin-based studies have shown that genetic factors influence weight changes following different exercise patterns [8–10]. For example, results from the Swedish Young Male Twins study [5] indicated that for those twins with genetic susceptibility for obesity, engaging in an active lifestyle, had a preventive effect on accumulating fat. Accordingly, Mustelin et al. [11] found an inverse additive genetic correlation between PA and BMI in both genders with correlations of −0.22 and −0.08 for females and males, respectively. More recently, an association study identified significant interactions between individual genes and self-reported PA, suggesting, for example, that the effect of the *FTO rs9939609*

polymorphism on body fat accumulation is exacerbated by low levels of PA [12]. Also, it has been shown that PA decreases the impact of *FTO* gene variants on obesity [13]. In a study with Danish and Finnish twin samples [14], the results follow the same trend with an inverse association between PA and WC and BMI and % body fat as well as evidence that PA decreases both genetic and environmental variances of BMI and waist circumference. Moreover, using a GxE model, McCaffery et al. [15] found that BMI is, on average, lower among those individuals that engage in vigorous activities and that vigorous exercise significantly modified the additive genetic component of BMI, confirming the presence of a GxE interaction. Using an animal model, Noland et al. [16] found that even when exposed to a high fat diet, rats with inherited low oxidative capacity were heavier and hypertriglyceridemic when compared to high oxidative capacity rats. As such, it is highly likely that differences in PA patterns and levels may have different impacts on body composition changes within the same population. Accordingly, to better explain why some people become obese while others do not, it is important to understand how PA interacts with genotype and influences its association with body fat.

In the present study, using a nuclear family design, we bring together information on body composition and energy expenditure aiming (1) to estimate the magnitude of the genetic effects on body composition (BC) traits and (2) to examine the Genotype x Total Daily Energy Expenditure (GxTDEE) and Genotype x Daily Energy Expenditure (GxDEE) interactions that may affect the impact of PA on BC traits. Our main hypothesis is that the genetic regulation of BC is affected by distinct levels of PA/EE.

2. Materials and Methods

2.1. Study Population

The Portuguese Healthy Family Study, from the Portuguese *Estudo de Famílias Saudáveis Portuguesas* (FAMS), investigates the relationship among metabolic syndrome indicators, physical activity, physical fitness, and body composition in nuclear Caucasian families. Children and adolescents aged 10 to 18 years were recruited in schools from the north and central regions of mainland Portugal and were approached to freely participate in the study with their siblings and parents. Children with chronic diseases (such as asthma and diabetes), physical handicaps, or psychological disorders that might impair their daily routines and physical activities within schools and/or sports clubs were excluded. Given that families with 3 or more children are scarce in the Portuguese population [17], the sample comprises 294 families with only one or two siblings (see Table 1). The ethics committee of the Faculty of Sport, University of Porto, approved the study and written informed consent, and assent was obtained from all subjects.

2.2. Data Collection

2.2.1. Physical Activity. Using a 3-day physical activity recall [18], a trained technician interviewed each subject, recording the dominant activity for each 15-min period during 24 h by using a list of categorized activities. Categories from 1 to 9 refer to increasing levels of energy expenditure (METs) of each activity in which category 1 indicates very low energy expenditure such as sleeping or resting in bed and category 9 refers to highly demanding physical work such as high-intensity sports. Approximate median energy cost for each of the nine categories in kcal/kg/15 min was used to compute the daily energy expenditure (DEE) for each individual. The number of 15-min periods for each category was first summed over the 3-day period and weighted by its own median energy cost. DEE was then calculated by summing over the median energy cost of all nine categories and dividing by 3 days. TDEE was then computed by multiplying DEE by subjects' body weights. This method has been previously validated for children and adults [18].

2.2.2. Anthropometric Measurements. The standardized procedures of Lohman et al. [19] were used to measure height with a Siber Hegner anthropometer (GMP instruments), and body composition was measured with a bioelectric impedance scale (TANITA BC-418 MA; Segmental Body Composition Analyser Tanita, Corporation, Tokyo, Japan). Two body composition traits were estimated—%body fat (%FAT) and %trunk fat (%TFAT). This impedance scale has been validated previously with Dual-Energy X-ray Absorptiometry—DXA [20], a gold standard method for body composition measurement. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Waist circumference was measured at the end of a normal expiration just above the iliac crest, using a nonelastic Holtain tape.

2.2.3. Statistical Analysis. Univariate quantitative genetic procedures as implemented in SOLAR [21] under a special class of the multivariate linear model, namely, the variance components (VC) approach, were used to estimate additive genetic and environmental VCs for each of the BC traits. Prior to all modeling, TDEE, age, age^2, sex, age-by-sex, and age^2-by-sex were used as covariates in a preliminary VC model. Residuals were thus derived for each trait and were normalized using an inverse normal transformation, as previously advocated [22, 23]. Heritability estimates (h^2) were computed using a maximum likelihood approach to estimate variance components under the standard polygenic model as implemented in SOLAR v.4.3.1 software [21].

Hypothesis Testing. In order to assess the influence of distinct levels of energy expenditure in body composition genetic regulation we established two main hypotheses.

(1) The genetic background of body composition traits is dependent on changes in total daily energy expenditure [TDEE (kcal/day)];

(2) The genetic background of body composition traits is dependent on changes in daily energy expenditure [DEE (kg/kcal/day)].

To test for GxTDEE and GxDEE interactions, basic initial hypotheses were formulated regarding the variance/covariance relationship of a BC indicator between

Table 1: Sample descriptive characteristics (means ± standard deviations).

	Fathers (n = 180)	Mothers (n = 253)	Sons (n = 265)	Daughters (n = 260)
Age (yrs)	45.4 ± 5.2	43.5 ± 4.5	14.7 ± 2.8	14.4 ± 2.8
Height (cm)	170.0 ± 6.7	158.6 ± 5.7	162.2 ± 12.9	156.3 ± 9.7
Weight (kg)	80.1 ± 13.2	66.9 ± 10.2	58.0 ± 16.2	53.6 ± 12.7
TDEE (kcal/day)	3561.8 ± 962.7	2788.4 ± 527.6	2280.6 ± 774.4	2024.9 ± 568.4
DEE (kg/kcal/day)	44.25 ± 8.8	41.92 ± 6.3	39.15 ± 7.4	39.03 ± 10.6
BMI (kg/m^2)	27.7 ± 4.1	26.6 ± 3.9	21.5 ± 4.1	21.7 ± 3.9
%FAT	23.0 ± 5.7	33.7 ± 5.9	20.0 ± 6.5	27.8 ± 6.2
%TFAT	24.6 ± 6.5	29.9 ± 7.0	16.9 ± 6.8	22.5 ± 7.6
WC (cm)	92.3 ± 10.6	81.0 ± 9.0	72.8 ± 10.4	68.4 ± 8.6

TDEE: total daily energy expenditure; DEE: daily energy expenditure; BMI: body mass index; %FAT: fat percentage; %TFAT: trunk fat percentage; WC: waist circumference.

family members with different levels of energy expenditure. With regard to GxTDEE and GxDEE interactions, the fundamental null hypothesis is that the expression of a polygenotype (i.e., aggregate of all genotypes related to the expression of a phenotype) is independent of TDEE and/or DEE levels. It can be shown from first principles that if there are no GxTDEE and/or GxDEE interactions, the same BC indicator measured in subjects with different levels of TDEE and/or DEE will have a genetic correlation of 1.0 and the genetic variance will be homogeneous across all levels of TDEE and/or DEE [24, 25]. On the contrary, if GxTDEE and/or GxDEE interactions are present, the genetic correlation will be significantly less than 1.0 and/or the genetic variance will not be the same among all levels of TDEE and/or DEE.

The foregoing requires that we model the variance and correlation as functions of TDEE and/or DEE levels. For the genetic variance function (and similarly for the environmental variance function), we modeled the variance using an exponential function to ensure positivity, which is required since any variance is a squared term [24, 25]: $\sigma_g^2 = \exp[\alpha_g + \gamma_g(\mathrm{EE})]$, where α_g and γ_g are parameters to be estimated. An additional justification for the exponential function is suggested by the alternative name of this approach, namely, the log-linear model of the variance: $\ln \sigma_g^2 = \alpha_g + \gamma_g(\mathrm{EE})$. That is, on taking the natural logarithm of the variance modeled as an exponential function, we have the equation of a straight line. In this form, the variance homogeneity null hypothesis obviously holds for a slope-term equal to 0: $\gamma_g = 0$. For the genetic correlation function, we modeled the genetic correlation as an exponential decay function of the pairwise differences in TDEE and/or DEE levels: $\rho_g = \exp(-\lambda|\mathrm{EE}_i - \mathrm{EE}_j|)$, where λ is a parameter to be estimated as a function of the difference in TDEE and/or DEE levels between any two individuals i and j. Here, we also have an elegant reexpression of the interaction null hypothesis, in this case regarding the genetic correlation, in that a genetic correlation equal to 1 is equivalent to $\lambda = 0$. This is because for $\lambda = 0$, we have $\rho_g = \exp(-\lambda|\mathrm{EE}_i - \mathrm{EE}_j|) = e^0 = 1$. At the same time we employed a similar variance function for the residual environment variance as a function of the TDEE and/or DEE environments because it guards against bias in the detection of additive genetic variance heterogeneity. Allowing for variance heterogeneity (i.e., model the variance as a function) in only the additive genetic variance would lead to a bias in the relevant parameter estimate because it is possible in theory for there to be heterogeneity in the residual environmental variance as well. Thus, allowing for heterogeneity in both the additive genetic variance and residual environmental variance can be said to guard against this bias. Also, since our statistical genetic model assumes from the outset that the genetic and residual environmental effects are uncorrelated we did not posit a corresponding environmental correlation function. Allowing for a residual environmental correlation function on the same environmental variable as that for the genetic correlation function would violate the said assumption.

For reasons detailed in Diego et al. [24], the likelihood ratio test statistics (LRTs) to test $\gamma_g = 0$ and $\lambda = 0$ are, respectively, distributed as χ_1^2 and are as follows: a chi-square random variable with 1 degree of freedom (d.f.) and $((1/2)\chi_0^2 + (1/2)\chi_1^2)$, a 50 : 50 mixture of chi-square random variable with a point-mass at 0, denoted by χ_0^2, and a chi-square with 1 d.f. Prior to examination of these hypotheses, however, we first confirmed if the overall GxTDEE and/or GxDEE interaction models provided a better fit to the data than the standard so-called polygenic model. The LRT for these comparisons can be shown to be distributed as $((1/2)\chi_2^2 + (1/2)\chi_3^2)$ [26]. Under the null hypothesis, the GxTDEE and/or GxDEE models can be thought of as reparameterized models, where the additive genetic variance is equal to exp(alphaG) and the residual environmental variance is equal to exp(alphaE). Taking this into account, on comparison with the polygenic model, the full GxTDEE and/or GxDEE models have three additional parameters, namely, the gamma parameters for the additive genetic and residual environmental variance functions and the lambda parameter for the genetic correlation function. The two gamma parameters give rise to LRTs that are each distributed as χ_1^2, and the lambda parameter gives rise to an LRT that is distributed as the mixture $((1/2)\chi_0^2 + (1/2)\chi_1^2)$. The sum of these chi-squares gives $((1/2)\chi_2^2 + (1/2)\chi_3^2)$.

3. Results

The basic descriptive data for TDEE, DEE, and BC traits in fathers, mothers, sons, and daughters are presented in Table 1.

Table 2: Family structures.

	FM4	FM3	FM2	FM1	FM	M3	M2	M1	M	F3	F2	F	2	1	Total
n	3	21	105	41	4	7	44	24	2	2	7	1	16	17	294
%	1.02	7.14	35.71	13.95	1.36	2.38	14.97	8.16	0.68	0.68	2.38	0.34	5.44	5.78	100

FM4: father + mother + 4 offspring; FM3: father + mother + 3 offspring; FM2: father + mother + 2 offspring; FM1: father + mother + 1 offspring; FM: father + mother; M3: mother + 3 offspring; M2: mother + 2 offspring; M1: mother + 1 offspring; M: mother; F3: father + 3 offspring; F2: father + 2 offspring; F: father; 2: two siblings; 1: one sibling.

Table 3: Heritability estimates (h^2), standard-errors, and corresponding 95% confidence intervals (95% CI) of the different phenotypes in The Portuguese Healthy Family Study.

Trait	h^2 (95% CI)	Std. error	*P* value
BMI	0.25 (0.14, 0.37)	0.07	<0.001
%FM	0.25 (0.14, 0.37)	0.07	<0.001
%TFM	0.21 (0.10, 0.32)	0.07	<0.001
WC	0.34 (0.22, 0.45)	0.07	<0.001

BMI: body mass index; %FM: fat percentage; %TFM: trunk fat percentage; WC: waist circumference.

Information from 294 families comprising 180 fathers, 253 mothers, 265 sons, and 260 daughters was included. The average family size was 3.3 subjects (Table 2). Total daily energy expenditure follows the expected trend with fathers presenting the highest values, followed by mothers, sons, and daughters, which can be explained by the greater weight of fathers and mothers. These differences are heavily diminished for daily energy expenditure, which does not account for the individuals' weight. However, significant differences were still observed between all classes of relatives for DEE [$F(3,795) = 16.126$, $P < 0.001$]. As expected, %FAT was higher in females than in males. Sons and daughters' average BMIs were very similar.

Heritability estimates (h^2) presented in Table 3 were all highly significant ($P < 0.001$), ranging from 0.21 (95% CI: 0.14, 0.37) for %TFAT to 0.34 (95% CI: 0.22, 0.45) for WC meaning that the phenotypic expression of BC traits is in part due to moderate-to-strong additive genetic factors, which is a compelling argument to pursue further specific analysis of their genetic architecture.

The polygenic model was compared to the GxTDEE and/or GxDEE interaction models by means of a log-likelihood ratio test (see Table 4). The GxTDEE and GxDEE interaction models were significantly better than the polygenic model for all the BC traits implying that the GxTDEE and/or GxDEE models fit the data better than the polygenic model for each of these four traits. This means that interindividual variability in the phenotypic expression of these body composition traits is to some degree explained by an interaction between genotype and energy expenditure. As such, different genotype architectures lead to distinct expressions of body composition under the same energy expenditure levels. In Table 5 we present the parameter estimates relevant to interpreting GxE interaction, namely, the gamma and lambda parameters.

Table 4: Results of log-likelihood ratio tests (LRT) and respective *P* values contrasting a polygenic model versus a GxTDEE and GxDEE model for each of the body composition traits.

Trait	Polygenic LnL	LnL	LRT	*P* value
		GxTDEE		
BMI	−387.781	−338.660	98.243	<0.0001
%FM	−386.643	−370.572	32.144	<0.0001
%TFM	−387.543	−380.396	14.294	0.002
WC	−380.061	−319.731	120.660	<0.0001
		GxDEE		
BMI	−387.781	−356.720	62.124	<0.0001
%FM	−386.630	−378.016	17.227	<0.0001
%TFM	−387.432	−380.609	13.646	0.002
WC	−379.868	−344.650	70.436	<0.0001

BMI: body mass index; %FM: fat mass percentage; %TFM: trunk fat mass percentage; WC: waist circumference; LnL: log-likelihoods; LRT: likelihood ratio test.

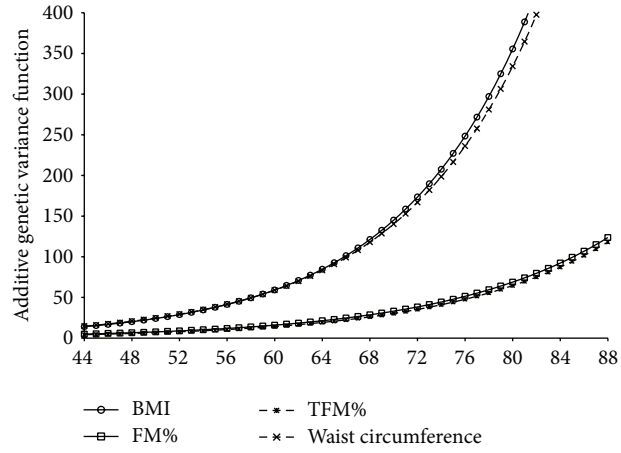


Figure 1: Genotype x Daily Energy Expenditure genetic variance. BMI: body mass index; FM%: fat mass percentage; TFM%: trunk fat mass percentage; WC: waist circumference.

Verification of GxTDEE and/or GxDEE interactions was made by comparing both full models to their constrained alternatives for BMI, % FAT, % TFAT, and WC.

The significant results for variance heterogeneity and genetic correlation are shown in Figures 1 and 2. All traits were significantly influenced for both GxTDEE and GxDEE models. The significance of the GxDEE model was due to the rejection of the genetic variance (σ_g^2) (Figure 1) homogeneity hypothesis, whereas the significance of the GxTDEE model

Table 5: Lambda and Gamma parameter estimates for each of the body composition traits under the GxTDEE and the GxDEE models.

Trait	Lambda*	Gamma*	Lambda LRT	Gamma LRT
		GxTDEE		
BMI	0.0008 (0.0004, 0.0017)	—	6.243	—
%FM	0.0014 (0.0008, 0.0026)	—	11.597	—
%TFM	0.0012 (0.0006, 0.0026)	—	7.031	—
WC	0.00009 (0.0005, 0.0014)	0.0006 (0.0004, 0.0007)	11.909	12.711
		GxDEE		
BMI	—	0.0896 (0.0733, 0.1076)	—	13.261
%FM	—	0.0731 (0.0525, 0.0949)	—	12.116
%TFM	—	0.0755 (0.0527, 0.0989)	—	9.950
WC	—	0.0868 (0.0710, 0.1043)	—	27.063

*Maximum likelihood parameter estimate followed by the lower and upper bounds for a 95% confidence interval computed following standard methods. BMI: body mass index; %FM: fat mass percentage; %TFM: trunk fat mass percentage; WC: waist circumference; LRT: likelihood ratio test.

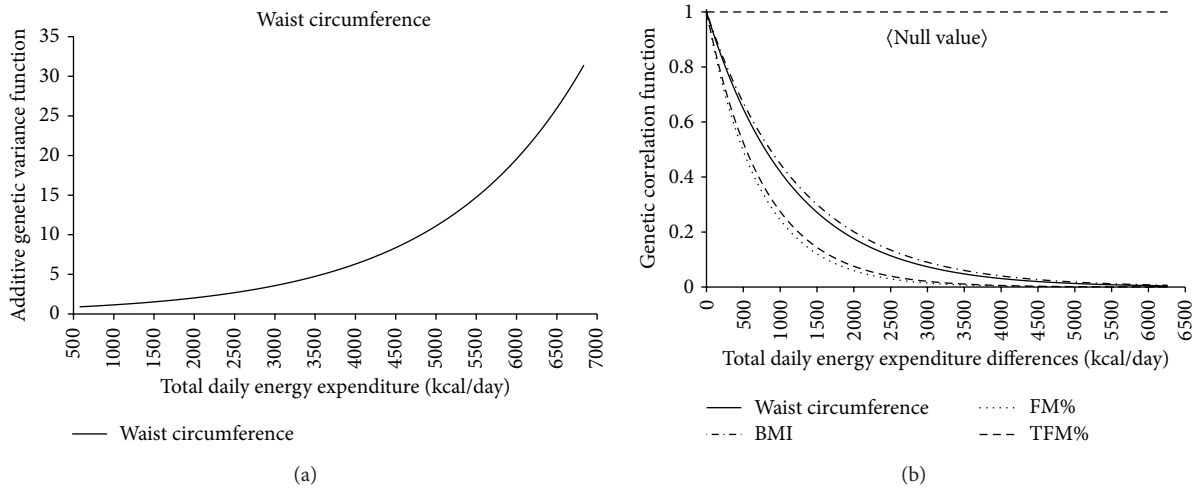


Figure 2: Genotype x Total Daily Energy Expenditure genetic variance (a) and genetic correlation (b). BMI: body mass index; FM%: fat mass percentage; TFM%: trunk fat mass percentage. Genetic correlation function refers to the genetic correlation for the same trait under different TDEE environments.

was due to the rejection of the null hypotheses of a genetic correlation (ρ_g) equal to 1 (Figure 2). The only exception was WC in which the genetic variance (σ_g^2) homogeneity hypothesis was also rejected under the GxTDEE model (Figure 2(b)). This means, for instance, despite variance homogeneity for BMI, %FAT, and %TFAT under the GxTDEE model, that a significant interaction with TDEE was still present because the genetic correlation of these traits under distinct TDEE levels was not equal to 1. For example, if the genetic correlation between BMI under TDEE of 2500 kcal/day and a TDEE of 1500 kcal/day is 0.6, then we may speculate that if the TDEE environments differ then different genes are being activated and are being responsible for body composition expression. The null hypothesis of homogeneity in the genetic variance implies a straight line graph (i.e., slope equal to 0) at the level of the natural logarithm of the heritability given that the variances are modeled as exponential functions. Thus, Figure 2(a) shows that the genetic variance does vary as a function of the energy expenditure environment. Specifically, the genetic variance increases with increasing levels of energy expenditure, which means that the higher the TDEE values, the greater the differences in the set of genes activated that are responsible for WC expression. As for Figures 1 and 2(b), the null hypothesis of a genetic correlation equal to 1 is graphically depicted by the horizontal line where the genetic correlation function equals 1. This means that under the null hypothesis the genetic correlation is not to be regarded as a function of differences in the environmental measure. Exponential curves that decay away from the null value simply indicate that the genetic correlation is in these cases a function of differences in the environmental measure.

Figure 3 shows the simultaneous representation of the variance and correlation functions for WC, demonstrating that GxTDEE interaction for WC is a joint function of genetic

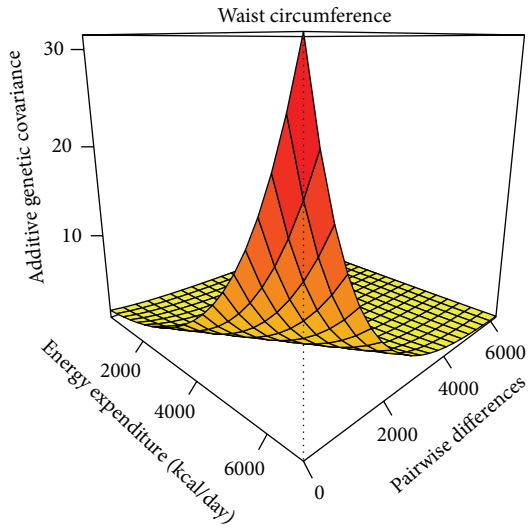


Figure 3: Genetic covariance function for waist circumference under the GxTDEE model.

variance heterogeneity and a genetic correlation different than one. In the figure, pairwise differences refer to the differences between subjects in their TDEE levels.

4. Discussion

This study, based on a Portuguese sample of families, aimed to quantify the genetic variance of different BC traits as well as to examine the GxTDEE and GxDEE interactions in modulating the manifestation of these traits in family members. Our results not only confirm the importance of genetic factors in governing the expression of these BC traits, with all h^2 being significant, but also most importantly showed the importance of both GxTDEE and GxDEE interactions in fat accumulation. To the extent of our knowledge this is the first effort to apply a GxE interaction analysis, using a nuclear family-design study, to test the hypothesis that individual differences in phenotypic expression of BC traits are conditioned by their EE levels; that is, the interindividual variability in different body composition traits is genetically driven and mediated by physical activity exposure.

Body composition heritability estimates reported here were all statistically significant which is in agreement with previous results [27–32]. Waist circumference was the most heritable of the four traits ($h^2 = 0.34$), and its value is comparable to the estimates of 0.38 found in the Linosa study [32] and 0.39 found in a study with 533 nuclear families from Spain [28]. The heritability of BMI ($h^2 = 0.25$) is lower than those from Spain ($h^2 = 0.44$ [28]). The same tendency was observed for body fat with our moderate heritability estimate of 0.25 contrasting with 0.69 in a Swedish sample [33], 0.48 in Nigeria, 0.54 in Jamaica, 0.57 in USA [31], and 0.64 for males and 0.56 for females in a Chinese sample [34]. These discrepancies are usually attributable to different sampling strategies and sample sizes, distinct statistical approaches used to estimate h^2, and use of distinct adjustments (different covariates). For instance, in our study, all of the h^2 estimations were controlled for the effect of TDEE which might explain this discrepancy of results. In summary, this wealth of data merely affirms the well-known dictum that heritability estimates are sample specific. Although our h^2 estimates are somewhat lower than the ones previously reported, we still have from 1/5 to 1/3 of residual variance of BC traits explained by genetic factors, which is a compelling argument to further examine the underlying genetic architecture.

Over the years, researchers have been keen on studying the associations of different environmental exposures with BC [35]. This has mostly been done using a regression-based approach for the detection of phenotypic-level associations between traits among family members [34, 36]. Despite its usefulness in quantifying the degree and sign of the association between distinct BC phenotypes, correlations provide little information regarding the putative mechanisms that underlie such associations. GxE interaction analysis holds the promise of verifying if the association between an environmental factor (e.g., EE) and body fat accumulation is genetically driven, which may be of importance in understanding why people respond differently to physical exercise intervention programs [37].

In the present report, we chose to analyze the potential effects of EE on genotype determination of body composition traits in two different ways: assess the effects of (i) total daily energy expenditure (kcal/min) and (ii) daily energy expenditure (kg/kcal/min). The rationale behind the two different approaches is that TDEE is an absolute measure that is known to be significantly influenced by the effects of age on BMI [38], mainly due to the greater weight of older subjects that is here well observed since there are substantial differences in TDEE between generations, meaning that the differences track with age. Thus, the further analysis of GxDEE allows avoiding the bias related with the influence of greater weight on energy expenditure and a possible age effect on the results of GxTDEE.

The results showed that all BC traits were significantly influenced by both GxDEE and GxTDEE interactions through the rejection of the hypothesis of the genetic correlation being equal to 1 or/and the hypothesis of variance homogeneity. This means that the genotype effects are not exactly the same under different energy expenditure conditions, as they are not fully correlated between distinct DEE and/or TDEE environments. Generally, distinct trends were observed for the two models as the GxDEE interaction was significant due to the rejection of the genetic variance (σ_g^2) homogeneity hypothesis and the GxTDEE interaction due to the rejection of the null hypotheses of a genetic correlation (ρ_g) equal to 1. Waist circumference was the only trait to be significantly influenced by the two hypotheses and only under the GxTDEE model.

As regards the GxDEE model and the expression of WC under the GxTDEE model, the results presented here show that the genetic variance increases with increasing levels of DEE (and TDEE for WC), which may lead us to speculate that there are genes involved in the expression of body composition traits that are only "triggered" at higher levels

of DEE. This particular set of results is not in line with the majority of the previous studies on the interaction between energy expenditure and obesity, in which increasing EE levels have been found to diminish the genetic effects on obesity-related traits. However, the research by Lappalainen et al. [39] also failed to find an association between exercise and the effect of FTO gene on weight changes, in a 4-year followup of 522 overweight or obese subjects randomized to control and lifestyle intervention groups. This evidence poses an argument for the necessity of continuing efforts to unravel the effects of EE at a genetic level that might influence different BC traits. The results under the GxTDEE model indicate that the greater the differences in TDEE levels, the lower the genetic correlations, meaning that the genes influencing body composition traits differ under different TDEE levels. So, in contrast with the GxDEE model, the significance of this model is due to the influence of different genes under distinct levels of TDEE and not to an increase in the additive effects of genes under higher levels of DEE. Previously, physical inactivity was found to upregulate the expression of a number of genes in skeletal muscle tissue in a mice model, which leads to a speculation that the same may be true for obesity markers [40, 41]. In humans, physical inactivity before and after bed rest has been associated with higher levels of tumor necrosis factor α (TNF-α) [42], which is a potent mediator of gene expression related to inflammation by activating nuclear factor kappaB (NFκB) signalling [43, 44]. On the basis of these data, individuals at different ends of the spectrum of physical activity would be expected to express different sets of genes, one set more associated with subclinical inflammation and the other set less so. In turn, these different sets of genes being expressed across the physical activity spectrum would result in a decay of the genetic correlation away from complete correlation.

GxEE influence on body composition traits has also been studied using DNA analysis [45, 46]. For example, Li et al. [45] genotyped 12 SNPs in obesity-susceptibility loci of 20,430 individuals from the EPIC-Norfolk cohort and reported that each additional BMI-increasing allele significantly increased the risk of obesity in the whole population, but significantly ($p_{\text{interaction}} = 0.015$) more in inactive individuals [OR = 1.158 ($CI_{95\%}$ = 1.118–1.199)] than in active individuals [OR = 1.095 ($CI_{95\%}$ = 1.068–1.123)]. However, in the active group this increase was only 379 g, leading to the conclusion that being active may reduce the genetic predisposition to obesity by 40%. Also, the *FTO* gene was found, when comparing active to nonactive individuals, to have a diminished influence on BMI (0.25 BMI increase per risk allele in active individuals versus 0.44 BMI increase per risk allele in nonactive individuals) and WC (0.64 cm increase per risk allele in active individuals versus 1.04 cm increase per risk allele in nonactive individuals) [46]. More recently, in a robust meta-analysis of 218,166 adults and 19,268 children the results showed that the association between FTO and obesity is diminished by 27% from the effect of PA [13].

This issue is highly challenging and important considering that in many countries researchers and policy makers are trying to deal with the obesity epidemic and associated morbidities not only from a health standpoint but also from a financial view given the public burden in costs of obesity related morbidities [47, 48]. This epidemic has been mostly connected to a fast changing environment (referred to as "obesogenic") characterized by inducing low levels of energy expenditure and persuasive ways of increasing caloric intake that together constitute a difficult challenge to our genome [45, 46], but our results highlight that genetic adaptability to energy expenditure environments is probably more important than the environment itself. This has been proven previously in a highly cited experimental study with MZ twins [8] in which the variance in response to an overfeeding program of 100 days was three times greater between-pairs than within-pairs for BC traits. The same trend was observed when MZ twins were subjected to an exercise protocol over a 93-day period. Once again, and under controlled nutrient intake, the differences in weight loss were more pronounced between-pairs than within-pairs [10]. Both of these studies substantiate that the more genetically similar individuals are, the more similar they react to the same environment.

We think that our results add to the efforts in trying to disentangle these matters and help to substantiate the latter arguments by suggesting that the phenotypic expression of BC traits is the result of joint effects of genes, EE levels (environment), and their interactions.

Despite the relevance of the present results, some limitations should be acknowledged. Firstly, the sample used may not be representative of the general Portuguese population. Secondly, the method chosen to estimate BC traits, in our case bioelectrical impedance analysis, even though having been previously validated with DXA [20], is not free from bias in its results although the precision of the equipment is ±1%. Nevertheless, this method has been widely used as a BC analyzer in many studies [49–51]. Also, our sample is made of 294 families, which compares with 319 families from the Viva la Familia Study [30] but is somewhat smaller than 533 families from Spain [28]. However, we feel that the joint effects of the size of our sample, the use of state of the art statistical procedures, and the novelty of the analysis in PA genetic epidemiology research are strengths of the present study that warrant consideration.

5. Conclusions

In conclusion, the present results showed that the genetic expression of BC traits is significantly influenced by energy expenditure levels. Accordingly, physical activity may be considered an environmental variable that promotes interindividual differences in BC traits through genetic mediation. This is valuable information for health practitioners. More efforts should be devoted to not only identify specific loci that control different BC traits but also test if these loci are regulated or not by different PA levels.

Acronyms

BMI: Body mass index
%TFM: Trunk fat mass percentage
%FM: Fat mass percentage

TDEE: Total daily energy expenditure
DEE: Daily energy expenditure
EE: Energy expenditure
GxE: Genotype by environment interaction
PA: Physical activity
BC: Body composition
WC: Waist circumference.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the FCT—Fundação para a Ciência e a Tecnologia. The authors thank them for granting this research (PTDC/DES/67569/2006, FCOMP-01-0124-FEDEB-09608, and SFRH/BD/65290/2009). The authors thank the participants in the healthy family study.

References

[1] J. B. Haldane, "The interaction of nature and nurture," *Annals of Eugenics*, vol. 13, no. 3, pp. 197–205, 1946.

[2] T. F. C. Mackay, "The genetic architecture of quantitative traits," *Annual Review of Genetics*, vol. 35, pp. 303–339, 2001.

[3] C. Bouchard, "Gene-environment interactions in the etiology of obesity: defining the fundamentals," *Obesity*, vol. 16, no. 3, pp. S5–S10, 2008.

[4] M. C. Nelson, P. Gordon-Larsen, K. E. North, and L. S. Adair, "Body mass index gain, fast food, and physical activity: effects of shared environments over time," *Obesity*, vol. 14, no. 4, pp. 701–709, 2006.

[5] N. Karnehed, P. Tynelius, B. L. Heitmann, and F. Rasmussen, "Physical activity, diet and gene-environment interactions in relation to body mass index and waist circumference: the Swedish young male twins study," *Public Health Nutrition*, vol. 9, no. 7, pp. 851–858, 2006.

[6] N. A. King, M. Hopkins, P. Caudwell, R. J. Stubbs, and J. E. Blundell, "Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss," *International Journal of Obesity*, vol. 32, no. 1, pp. 177–184, 2008.

[7] H. H. M. Maes, M. C. Neale, and L. J. Eaves, "Genetic and environmental factors in relative body weight and human adiposity," *Behavior Genetics*, vol. 27, no. 4, pp. 325–351, 1997.

[8] C. Bouchard, A. Tremblay, J.-P. Despres et al., "The response to long-term overfeeding in identical twins," *The New England Journal of Medicine*, vol. 322, no. 21, pp. 1477–1482, 1990.

[9] A. Macdonald and A. Stunkard, "Body-mass indexes of British separated twins," *The New England Journal of Medicine*, vol. 322, no. 21, p. 1530, 1990.

[10] C. Bouchard, A. Tremblay, J. P. Després et al., "The response to exercise with constant energy intake in identical twins," *Obesity research*, vol. 2, no. 5, pp. 400–410, 1994.

[11] L. Mustelin, K. Silventoinen, K. Pietiläinen, A. Rissanen, and J. Kaprio, "Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins," *International Journal of Obesity*, vol. 33, no. 1, pp. 29–36, 2009.

[12] C. H. Andreasen, K. L. Stender-Petersen, M. S. Mogensen et al., "Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation," *Diabetes*, vol. 57, no. 1, pp. 95–101, 2008.

[13] T. O. Kilpeläinen, L. Qi, S. Brage et al., "Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children," *PLoS Medicine*, vol. 8, no. 11, Article ID e1001116, 2011.

[14] K. Silventoinen, A. L. Hasselbalch, T. Lallukka et al., "Modification effects of physical activity and protein intake on heritability of body size and composition," *American Journal of Clinical Nutrition*, vol. 90, no. 4, pp. 1096–1103, 2009.

[15] J. M. McCaffery, G. D. Papandonatos, D. S. Bond, M. J. Lyons, and R. R. Wing, "Gene × environment interaction of vigorous exercise and body mass index among male Vietnam-era twins," *American Journal of Clinical Nutrition*, vol. 89, no. 4, pp. 1011–1018, 2009.

[16] R. C. Noland, J. P. Thyfault, S. T. Henes et al., "Artificial selection for high-capacity endurance running is protective against high-fat diet-induced insulin resistance," *American Journal of Physiology*, vol. 293, no. 1, pp. E31–E41, 2007.

[17] M. J. V. Rosa and P. Chitas, Portugal: os números. Lisboa 2010.

[18] C. Bouchard, A. Tremblay, and C. Leblanc, "A method to assess energy expenditure in children and adults," *American Journal of Clinical Nutrition*, vol. 37, no. 3, pp. 461–467, 1983.

[19] T. G. Lohman, A. F. Roche, and R. Martorel, *Anthropometric Standardization Reference Manual*, Human Kinetics Books, Champaign, Ill, USA, 1988.

[20] A. Pietrobelli, F. Rubiano, M.-P. St-Onge, and S. B. Heymsfield, "New bioimpedance analysis system: improved phenotyping with whole-body analysis," *European Journal of Clinical Nutrition*, vol. 58, no. 11, pp. 1479–1484, 2004.

[21] L. Almasy and J. Blangero, "Multipoint quantitative-trait linkage analysis in general pedigrees," *American Journal of Human Genetics*, vol. 62, no. 5, pp. 1198–1211, 1998.

[22] J. Blangero, V. P. Diego, T. D. Dyer et al., "kernel of truth: statistical advances in polygenic variance component models for complex human pedigrees," *Advances in Genetics*, vol. 81, pp. 1–31, 2013.

[23] V. P. Diego, D. L. Rainwater, X.-L. Wang et al., "Genotype × adiposity interaction linkage analyses reveal a locus on chromosome 1 for lipoprotein-associated phospholipase A2, a marker of inflammation and oxidative stress," *American Journal of Human Genetics*, vol. 80, no. 1, pp. 168–177, 2007.

[24] V. P. Diego, L. Almasy, T. D. Dyer, J. M. Soler, and J. Blangero, "Strategy and model building in the fourth dimension: a null model for genotype x age interaction as a Gaussian stationary stochastic process," *BMC Genetics*, vol. 4, supplement 1, p. S34, 2003.

[25] J. Blangero, "Statistical genetic approaches to human adaptability," *Human Biology*, vol. 81, no. 5-6, pp. 523–546, 2009.

[26] V. P. Diego, *Genotype x Age Interaction, and the Insulin-Like Growth Factor I Axis in the San Antonio Family Heart Study: A Study in Human Senescence*, Graduate School of Binghamton University, New York, NY, USA, 2005.

[27] A. Poveda, A. Jelenkovic, I. Salces, M. E. Ibañez, and E. Rebato, "Heritability variations of body linearity and obesity indicators during growth," *Homo*, vol. 63, no. 4, pp. 301–310, 2012.

[28] A. Jelenkovic, A. Poveda, and E. Rebato, "Quantitative genetics of human morphology and obesity-related phenotypes in nuclear families from the Greater Bilbao (Spain): comparison with other populations," *Annals of Human Biology*, vol. 38, no. 4, pp. 471–478, 2011.

[29] R. A. Mathias, M. Deepa, R. Deepa, A. F. Wilson, and V. Mohan, "Heritability of quantitative traits associated with type 2 diabetes mellitus in large multiplex families from South India," *Metabolism*, vol. 58, no. 10, pp. 1439–1445, 2009.

[30] N. F. Butte, G. Cai, S. A. Cole, and A. G. Comuzzie, "Viva la Familia Study: genetic and environmental contributions to childhood obesity and its comorbidities in the Hispanic population," *American Journal of Clinical Nutrition*, vol. 84, no. 3, pp. 646–654, 2006.

[31] A. Luke, X. Guo, A. A. Adeyemo et al., "Heritability of obesity-related traits among Nigerians, Jamaicans and US black people," *International Journal of Obesity*, vol. 25, no. 7, pp. 1034–1041, 2001.

[32] A. Bellia, E. Giardina, D. Lauro et al., ""The Linosa Study": epidemiological and heritability data of the metabolic syndrome in a Caucasian genetic isolate," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 19, no. 7, pp. 455–461, 2009.

[33] H. Wagner, H. Melhus, N. L. Pedersen, and K. Michaelsson, "Genetic influence on bone phenotypes and body composition: a Swedish twin study," *Journal of Bone and Mineral Metabolism*, vol. 31, no. 6, pp. 681–689, 2013.

[34] R. Liu, X. Liu, L. M. Arguelles et al., "A population-based twin study on sleep duration and body composition," *Obesity*, vol. 20, no. 1, pp. 192–199, 2012.

[35] L. Pérusse and C. Bouchard, "Genotype-environment interaction in human obesity," *Nutrition Reviews*, vol. 57, no. 5, pp. S31–S38, 1999.

[36] K. M. Rose, B. Newman, E. J. Mayer-Davis, and J. V. Selby, "Genetic and behavioral determinants of waist-hip ratio and waist circumference in women twins," *Obesity Research*, vol. 6, no. 6, pp. 383–392, 1998.

[37] C. Bouchard and T. Rankinen, "Individual differences in response to regular physical activity," *Medicine and Science in Sports and Exercise*, vol. 33, no. 6, pp. S446–S451, 2001.

[38] B. L. Wajchenberg, "Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome," *Endocrine Reviews*, vol. 21, no. 6, pp. 697–738, 2000.

[39] T. J. Lappalainen, A.-M. Tolppanen, M. Kolehmainen et al., "The common variant in the FTO gene did not modify the effect of lifestyle changes on body weight: the finnish diabetes prevention study," *Obesity*, vol. 17, no. 4, pp. 832–836, 2009.

[40] M. T. Hamilton, D. G. Hamilton, and T. W. Zderic, "Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease," *Diabetes*, vol. 56, no. 11, pp. 2655–2667, 2007.

[41] L. Bey, N. Akunuri, P. Zhao, E. P. Hoffman, D. G. Hamilton, and M. T. Hamilton, "Patterns of global gene expression in rat skeletal muscle during unloading and low-intensity ambulatory activity," *Physiological Genomics*, vol. 13, pp. 157–167, 2003.

[42] L. Hojbjerre, M. P. Sonne, A. C. Alibegovic et al., "Impact of physical inactivity on adipose tissue low-grade inflammation in first-degree relatives of type 2 diabetic patients," *Diabetes Care*, vol. 34, no. 10, pp. 2265–2272, 2011.

[43] J. Van de Voorde, B. Pauwels, C. Boydens, and K. Decaluwe, "Adipocytokines in relation to cardiovascular disease," *Metabolism*, vol. 62, no. 11, pp. 1513–1521, 2013.

[44] A. Taube, R. Schlich, H. Sell, K. Eckardt, and J. Eckel, "Inflammation and metabolic dysfunction: links to cardiovascular diseases," *American Journal of Physiology*, vol. 302, no. 11, pp. H2148–H2165, 2012.

[45] S. Li, J. H. Zhao, J. Luan et al., "Physical activity attenuates the genetic predisposition to obesity in 20,000 men and women from EPIC-Norfolk prospective population study," *PLoS Medicine*, vol. 7, no. 8, Article ID e1000332, 2010.

[46] K. S. Vimaleswaran, S. Li, J. H. Zhao et al., "Physical activity attenuates the body mass index-increasing influence of genetic variation in the FTO gene," *American Journal of Clinical Nutrition*, vol. 90, no. 2, pp. 425–428, 2009.

[47] L. Bahia, E. S. Coutinho, L. A. Barufaldi et al., "The costs of overweight and obesity-related diseases in the Brazilian public health system: cross-sectional study," *BMC Public Health*, vol. 12, p. 440, 2012.

[48] J. Cawley and C. Meyerhoefer, "The medical care costs of obesity: an instrumental variables approach," *Journal of Health Economics*, vol. 31, no. 1, pp. 219–230, 2012.

[49] H. Du, D. Bennett, L. Li et al., "Physical activity and sedentary leisure time and their associations with BMI, waist circumference, and percentage body fat in 0.5 million adults: the China Kadoorie Biobank study," *The American Journal of Clinical Nutrition*, vol. 97, no. 3, pp. 487–496, 2013.

[50] N. Cummings, K. A. Shields, J. E. Curran et al., "Genetic variation in SH3-domain GRB2-like (endophilin)-interacting protein 1 has a major impact on fat mass," *International Journal of Obesity*, vol. 36, no. 2, pp. 201–206, 2012.

[51] Z. Pausova, C. Syme, M. Abrahamowicz et al., "A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in french canadians," *Circulation: Cardiovascular Genetics*, vol. 2, no. 3, pp. 260–269, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 591717, 9 pages
http://dx.doi.org/10.1155/2014/591717

Research Article

The Dopaminergic Reward System and Leisure Time Exercise Behavior: A Candidate Allele Study

Charlotte Huppertz,[1,2] Meike Bartels,[1,2,3] Maria M. Groen-Blokhuis,[1] Conor V. Dolan,[1] Marleen H. M. de Moor,[1] Abdel Abdellaoui,[1,3] Catharina E. M. van Beijsterveldt,[1] Erik A. Ehli,[4] Jouke-Jan Hottenga,[1] Gonneke Willemsen,[1,2] Xiangjun Xiao,[5] Paul Scheet,[6] Gareth E. Davies,[4] Dorret I. Boomsma,[1,2,3] James J. Hudziak,[7] and Eco J. C. de Geus[1,2,3]

[1] *Department of Biological Psychology, VU University Amsterdam, Van der Boechorststraat 1, 1081 BT Amsterdam, The Netherlands*
[2] *EMGO+ Institute for Health and Care Research, VU University Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands*
[3] *Neuroscience Campus Amsterdam, VU University Medical Center, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*
[4] *Avera Institute for Human Genetics, Avera McKennan Hospital and University Health Center, 3720 W. 69th Street Suite 200, Sioux Falls, SD 57108, USA*
[5] *Department of Community and Family Medicine, Geisel School of Medicine, Dartmouth College, 1 Rope Ferry Road, Hanover, NH 03755-1404, USA*
[6] *Department of Epidemiology, University of Texas M.D., Anderson Cancer Center, Unit 1340, P.O. Box 301439, Houston, TX 77230-1439, USA*
[7] *Department of Psychiatry, Medicine, and Pediatrics, Vermont Center for Children, Youth and Families, College of Medicine, University of Vermont, UHC Campus, Arnold 3, 1 South Prospect, Burlington, VT 05401, USA*

Correspondence should be addressed to Charlotte Huppertz; c.huppertz@vu.nl

Received 6 December 2013; Revised 20 January 2014; Accepted 20 January 2014; Published 9 March 2014

Academic Editor: J. Timothy Lightfoot

Purpose. Twin studies provide evidence that genetic influences contribute strongly to individual differences in exercise behavior. We hypothesize that part of this heritability is explained by genetic variation in the dopaminergic reward system. Eight single nucleotide polymorphisms (SNPs in DRD1: rs265981, DRD2: rs6275, rs1800497, DRD3: rs6280, DRD4: rs1800955, DBH: rs1611115, rs2519152, and in COMT: rs4680) and three variable number of tandem repeats (VNTRs in *DRD4*, upstream of *DRD5*, and in *DAT1*) were investigated for an association with regular leisure time exercise behavior. *Materials and Methods*. Data on exercise activities and at least one SNP/VNTR were available for 8,768 individuals aged 7 to 50 years old that were part of the Netherlands Twin Register. Exercise behavior was quantified as weekly metabolic equivalents of task (MET) spent on exercise activities. Mixed models were fitted in SPSS with genetic relatedness as a random effect. *Results*. None of the genetic variants were associated with exercise behavior ($P > .02$), despite sufficient power to detect small effects. *Discussion and Conclusions*. We did not confirm that allelic variants involved in dopaminergic function play a role in creating individual differences in exercise behavior. A plea is made for large genome-wide association studies to unravel the genetic pathways that affect this health-enhancing behavior.

1. Introduction

Despite its well-known health benefits both in youth [1] and in adults [2, 3], regular leisure time exercise behavior drops from childhood to adolescence and reaches unacceptable low proportions in adulthood, with the majority of people in the United States and Europe not engaging in regular exercise activities at the recommended level [4–6]. Twin studies have shown that a substantial part of the variation in exercise behavior between individuals can be explained with genetic factors [7]. However, there is no definite evidence on *which* genes are implicated in the take-up and maintenance of

exercise behavior [8, 9]. A few significant associations have been found, but replication studies are scarce and the functional meaning of those genes is often not straightforward [10].

It is likely that a large part of the heritability of leisure time exercise behavior is due to genes that influence the affective reaction to exercise [11]. Feelings of reward and punishment have been hypothesized to be crucial agents in the take-up and maintenance of exercise behavior [11, 12]. The net rewarding effects of exercise may have to outweigh the net aversive effects to a substantial degree for the behavior to be repeated [11]. As part of an intervention study, Williams et al. [13] investigated the relationship between acute affective responses during a moderate-intensity exercise test on a treadmill and subsequent exercise behavior 6 months and 12 months after the baseline assessment. They found large individual differences in the affective reactions to the exercise test, with some of the participants reporting a more positive affect during (versus *before*) the test, some of them reporting a more negative affect, and some showing no change. Importantly, individuals characterized by positive affect during the exercise test were more likely to be engaged in exercise behavior at 6 and 12 months of follow-up.

Reward is governed by the mesolimbic reward system that involves dopaminergic pathways [14]. Associations between those pathways and physical activity behavior have been found both in animal models and in humans. It is well established that physical activity affects the dopaminergic system in some way. For instance, Greenwood et al. [15] showed that in rodents, acute rewarding effects of exercise were linked to changes in dopaminergic functioning. The *reversed* case, where dopaminergic functioning affects physical activity behavior and thus acts as a potential *determinant* of exercise behavior, has been less studied and deserves closer attention. Knab et al. [16] examined voluntary wheel running in mice. Both a high-active strain of mice (C57L/J) and a low-active strain of mice (C3H/HeJ) were divided into two groups: one group had free access to running wheels for 21 days and the other did not. After 21 days, the high-active strain and the low-active strain differed in the expression of two dopaminergic genes (*drd1* and *th*), *irrespective* of access to the running wheels. Assuming that expression was controlled in part by *cis*-acting variants, this suggests that innate differences in dopaminergic functioning can affect physical activity behavior. A review on the role of the dopamine system as a determinant of physical activity can be found in Knab and Lightfoot [17].

There are not many studies in humans that have investigated the effect of genetic variants in dopaminergic genes on physical activity. Jozkow et al. [18] found no significant association between two polymorphisms and the level of physical activity in a group of adult men. Two variants were investigated: rs6275 in the *DRD2* gene ($N = 371$) and a 48-base pair variable number of tandem repeat (VNTR) in the *DRD4* gene ($N = 397$). Simonen et al. [19] examined the association between rs6275 in DRD2 and physical activity in participants of the Quebec Family Study (QFS, $N = 721$) and replicated it in participants of the HERITAGE Family Study ($N = 275$ African American and 497 Caucasian participants). They found that Caucasian women that were homozygous for the T allele had been significantly less active during the past year than CT heterozygotes and CC homozygotes. Thomson et al. [20] examined the association between rs1800955 in the *DRD4* gene and risk-taking behavior in sports by measuring general and ski/snowboarding-specific sensation seeking behavior in 503 male and female skiers and snowboarders. They found a significant association between the studied polymorphism and sport-specific sensation seeking, with higher sensation seeking scores in the CC homozygotes. Thus, part of the genetic variation that causes differences in exercise behavior may indeed reside in the dopaminergic midbrain reward systems, although the evidence is not compelling.

There are currently several strategies to detect genetic variants involved in the heritability of behavioral traits—the two most frequently used techniques are (i) genome-wide association studies (GWAS) where markers are placed across the length of the entire genome, ranging in density from a few hundreds of thousands to millions [21, 22], and (ii) candidate gene studies [23], where polymorphisms are typed in genes of putative biological relevance. Both techniques have strengths and weaknesses–for instance, a GWAS allows for unexpected gene discovery by taking an agnostic approach to the selection of single nucleotide polymorphisms (SNP); it is limited, however, by requiring very large samples to overcome the multiple testing penalty and by the difficulty of explaining association results when identified SNPs are intergenic. Candidate gene studies, on the other hand, rely on polymorphisms in (close proximity to) genes of interest, ideally with known effects on gene function. While this limits the ability to discover novel polymorphisms, it provides interpretability within an a priori theoretical framework and greatly reduces the multiple testing burden.

For the present study, we selected the latter approach. Eight SNPs (rs265981, rs6275, rs1800497, rs6280, rs1800955, rs1611115, rs2519152, and rs4680) and three VNTRs (a 48-bp VNTR in exon III of *DRD4*, a dinucleotide repeat 18.5 kb upstream of *DRD5*, and a 40-bp VNTR in the 3′ UTR of *DAT1*) were chosen based on their known function in the dopaminergic reward system.

Dopamine receptors relay signals from one nerve cell to a neighboring nerve cell. At least five subtypes have been identified (dopamine receptors D1 to D5) that are encoded by dopamine receptor genes (*DRD1* to *DRD5*, resp.). The receptors D1 and D5 are grouped in the D1-like family which increase the cellular response [increased cyclic adenosine monophosphate (cAMP) production], whereas D2, D3, and D4 are grouped in the D2-like family and decrease the cellular response (decreased cAMP production). We selected four SNPs and two VNTRs that affect the dopamine receptors for this study: *rs265981* is located within the *DRD1* gene and has two possible alleles, A (minor) and G (major). The A allele has been associated with a decrease of *DRD1* expression levels and thus worse dopamine transmission compared to the G allele [24]. *rs6275* (minor allele A and major allele G) is a synonymous SNP located within the *DRD2* gene. The G allele has been associated with increased *DRD2* expression levels [25]. The rs1800497 polymorphism (minor allele A and major

allele G) lies within the ankyrin repeat and kinase domain containing 1 gene (ANKK1), downstream of and in linkage disequilibrium with the *DRD2* gene [26, 27]. The A allele has been associated with a reduced number of dopamine D2 receptors and thus increased dopamine transmission [28, 29] and higher reward responsiveness [30]. *rs6280* lies within the *DRD3* gene and is translated to one of two amino acids in the D3 receptor protein: glycine (minor allele C) or serine (major allele T), with glycine having a higher affinity for dopamine compared to serine [31] and thus decreasing dopamine transmission. *rs1800955* (minor allele C and major allele T) is located in close proximity to the *DRD4* gene and has been shown to influence promoter activity, with the C allele potentially enhancing activity compared to the T allele [32, 33]. A VNTR in exon III of the *DRD4* gene was investigated consisting of 48 base pairs with varying repeats ranging from 2 to 11. The 7-repeat allele has been shown to have a lower affinity for dopamine compared to the other repeats [34], thus increasing dopamine transmission [35]. A VNTR 18.5 kb upstream of the *DRD5* transcription start site consists of a dinucleotide polymorphism with alleles ranging from 130 to 166 base pairs and has been hypothesized to be in strong linkage disequilibrium with one or more functional variants in the DRD5 gene. The 148 allele has been associated with decreased *DRD5* expression levels [36].

Dopamine β-hydroxylase (DBH) converts dopamine to norepinephrine and is encoded by the *DBH* gene. *rs1611115* (minor allele T and major allele C) is located in the promoter region of the *DBH* gene. This polymorphism has been shown to account for 30–50% of the variance in DBH activity. More specifically, the C allele has been associated with higher plasma levels of DBH and thus lower dopamine levels [37, 38]. The *rs2519152* polymorphism (minor allele C and major allele T) is situated within the *DBH* gene and the T allele has been associated with lower DBH activity and thus higher dopamine levels compared to the C allele [39].

Finally, two genes were selected based on their association with dopamine reuptake and dopamine degradation: the *DAT1 (=SLC6A3)* gene and the *COMT* gene, respectively. The *dopamine active transporter* is encoded by the *DAT1* gene and clears dopamine from the synapse by depositing it back into the cells. A VNTR in the 3′ untranslated region of the *DAT1* gene was investigated that consists of a 40-base pair repeat with three alleles: 440, 480, and 520. We investigated the effect of the 480 allele in the present study as it has been associated with higher expression of the transporter, resulting in higher dopamine reuptake and thus lower levels of dopamine [40, 41]. *Catechol-O-methyltransferase* is encoded by the *COMT* gene and degrades dopamine. The SNP rs4680 (minor allele A and major allele G) lies within the *COMT* gene and is either translated to methionine (Met) or valine (Val), depending on the allelic variant that an individual has (G versus A, resp.). The COMT-Met enzyme degrades dopamine slower than the COMT-Val enzyme does and therefore results in higher dopamine levels [42], thereby increasing reward responsiveness and reward seeking [43].

The aim of the present study was to specifically test candidate alleles with a known function in the dopaminergic reward system for their association with regular leisure time exercise behavior, assuming that higher dopamine levels and stronger dopamine transmission are associated with higher reward sensitivity and thus more exercise behavior. The specific hypotheses are summarized in Table 1.

2. Materials and Methods

2.1. Participants. Data originated from twins and their family members that agreed to participate in longitudinal research of the Netherlands Twin Register (NTR) which has been set up to investigate individual differences in human behavior. The data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Centre. The final sample consisted of 8,768 individuals (3,900 families), of which 38% were males and 62% were females, with a mean age of 32.5 years (SD = 12.3, age range = 7–50 years).

Twins and their families are involved in research projects: for 7-, 10-, and 12-year-olds, both mothers and fathers are invited to fill in surveys on their twins' health, lifestyle, and behavior. From 13 years onwards, the twins and their siblings are invited to complete self-report surveys. When reaching adulthood (18 years), the twins are asked to fill in surveys every 2-3 years and additional family members are invited to take part in research projects (siblings, parents, adult offspring, and spouses). Characteristics and recruitment of participants are described in van Beijsterveldt et al. [44] and Willemsen et al. [45]. Individuals with diseases or disabilities that may prevent them from being physically active were excluded from the present study. Only individuals with a Dutch/Western European background were included that had genotype data available and at least one measure of exercise behavior (see below).

2.2. Phenotyping. For this study, we focused on regular *leisure time* exercise behavior since we were interested in *voluntary* (leisure time) physical activity that might be affected by individual differences in reward sensitivity. Participants (or their parents, for <13-year-olds) were asked to indicate (1) which exercise activities they participated in and (if any) (2) for how many years, (3) how many months a year, (4) how many times a week, and (5) how many minutes each time they participated in the respective activity. Test-retest reliability of this questionnaire was high (>0.82) in previous studies [46, 47] and it has been associated with other exercise phenotypes [48]. Our focus was *regular leisure time* exercise behavior, explicitly excluding irregular activities such as sailing camps or ski holidays (by requiring activities to be conducted for at least 3 months a year and for at least half a year), non-leisure activities such as transportation (e.g., cycling or walking to get somewhere), gardening, house cleaning, and–for younger participants–compulsory physical education classes. Each activity was recoded into its metabolic equivalent of task (MET), reflecting energy expenditure during a specific activity as a multiple of energy expended at rest (approximately one kcal/kg/h). For individuals younger than 18 years old, Ridley et al's. [49] compendium of energy expenditures for youth was applied; for individuals of 18 years or older,

Table 1: Allele-specific hypotheses.

Gene	Variant	Allele	Expected effect	Reference number	Effect on dopamine level*	Effect on exercise behavior*
DRD1	rs265981	A	Decreased DRD1 expression levels	[24]	↓	↓
DRD2	rs6275	G	Increased DRD2 expression levels	[25]	↓	↓
	rs1800497	A	Reduced number of (inhibitory) D2 receptors	[28, 29]	↑	↑
DRD3	rs6280	C	Higher affinity for dopamine → decreased transmission	[31]	↓	↓
DRD4	rs1800955	C	Increased DRD4 expression levels	[32, 33]	↓	↓
	VNTR: 7 allele		Lower affinity for dopamine → increased transmission	[34, 35]	↑	↑
DRD5	VNTR: 148 allele		Decreased DRD5 expression levels	[36]	↓	↓
DBH	rs1611115	C	Higher DBH activity	[37, 38]	↓	↓
	rs2519152	T	Lower DBH activity	[39]	↑	↑
DAT1	VNTR: 480 allele		Higher DAT activity → higher reuptake	[40, 41]	↓	↓
COMT	rs4680	G	Methionine → slower degradation of dopamine	[42]	↑	↑

* ↑: increase; ↓: decrease.

Ainsworth's compendium of physical activities was used [50]. The product of the MET score, weekly frequency, and duration was summed over all exercise activities that an individual was engaged in, resulting in one summary score, namely, "weekly MET hours spent on exercise activities." If an individual participated in more than 120 MET hours a week ($N = 31$ of the final sample), the score was truncated at 120 MET hours.

Exercise data of several longitudinal assessments were combined into one score. First, exercise data of individuals that were >50 years old were changed to missing and exercise data (of the respective assessment only) were removed when participants were injured at the time of survey completion. Subsequently, the data were combined by creating a new "weekly MET hours"-variable based on the most recent questionnaire of adults. Missing values were then replaced with older data of those individuals–preferentially, with data at an adult age and, if unavailable, with data of adolescents and children (step by step, one batch of questionnaires at a time). The association analysis was thus run on the joint exercise-variable that was composed of adults' data ($N = 7,349$), adolescents' data ($N = 997$), and children's data ($N = 422$).

2.3. Genotyping and Imputation. Genotype data were available from several projects within the NTR. Eight SNPs (rs265981, rs6275, rs1800497, rs6280, rs1800955, rs1611115, rs2519152, and rs4680) and three VNTRs (a 48-bp VNTR in exon III of *DRD4*, a dinucleotide repeat 18.5 kb upstream of *DRD5*, and a 40-bp VNTR in the 3′ UTR of *DAT1*) were selected for this candidate gene study based on their known function in the dopaminergic reward system. For some individuals, genotype data were available from fingerprint sets that included 30–38 SNPs and 5–7 VNTRs in candidate genes (see [44]). For other individuals (partly overlapping with the fingerprint-sample), SNP data were available based on imputed genome-wide SNP arrays.

In the fingerprint set, samples were excluded based on low sample call rate, sex errors, inconsistencies between duplicate samples, Mendelian errors, and erroneous IBS/IBD relationships. In the imputed dataset, samples were filtered on the same criteria, as well as on excessive heterozygosity. If samples were present in both the fingerprint and the imputed dataset, they were included only if they survived quality control (QC) in both sets.

In the fingerprint set, SNPs and VNTRs were tested for Hardy-Weinberg Equilibrium (HWE), Mendelian error rate, SNP/VNTR call rate, concordance rate for duplicate samples, and allele frequency difference with a reference set (HapMap CEU). In the genome-wide SNP dataset, SNPs were filtered on the following criteria before imputation: HWE P value >.00001, minor allele frequencies (MAF) >.01, Mendelian error rate <.02, SNP call rate >.95, SNP concordance rate >.99, and allele frequency difference with the reference set <.20. Haplotype phasing and imputation of missing genotyped SNPs was done in MACH 1.0 and subsequent imputation of the missing SNPs was done with Minimach using 1000G as a reference set (March 2012 phase 3 release, all ethnicity panels). After imputation, SNPs were tested for HWE, Mendelian error rate, allele frequency difference with the reference set, and imputation quality (R^2). For two SNPs (rs1611115 and rs1800955), we decided to use the fingerprint data only, since they showed a low R^2 and/or a high rate of Mendelian errors in the imputed set as well as a low concordance between the fingerprint set and the imputed set (<95%). MAF and HWE for the final data set are depicted in Table 2. Allele frequencies were similar to those in public data bases (e.g., HapMap CEU).

In individuals with genome-wide SNP data, information on ancestry was based on Principal Component Analysis [51]. For the remaining individuals, ancestry information was derived from questionnaire information on birth country of the parents. Individuals who were from non-Western European origin were excluded. The final sample consisted of 8,768 individuals with both phenotype data and genotype data on at least one variant. For the VNTRs and two SNPs (rs1611115 and rs1800955), data were derived from the fingerprint chip only.

Table 2: Number of individuals with complete genotype and phenotype data (N), their mean age (SD), mean weekly MET hours for the three combinations of alleles (SD; the number of individuals across the three allele codings), minor allele frequencies (MAF), the P value of the test for Hardy-Weinberg Equilibrium (HWE), and the P value of the main effect of the variant on exercise behavior, for each SNP/VNTR separately.

Gene	Variant	N	Age μ (sd)	0 μ (sd; N)	1 μ (sd; N)	2 μ (sd; N)	MAF	HWE	P value*
DRD1	rs265981	7873	33.28 (12.13)	*GG*: 12.51 (17.54; 3069)	*GA*: 12.39 (18.18; 3771)	*AA*: 12.57 (18.20; 1033)	0.37	0.02	0.942
DRD2	rs6275	7734	33.23 (12.14)	*GG*: 12.41 (18.07; 3812)	*GA*: 12.44 (17.48; 3262)	*AA*: 13.40 (19.38; 660)	0.30	0.31	0.672
	rs1800497	8756	32.46 (12.27)	*GG*: 12.92 (18.33; 5714)	*GA*: 13.18 (18.63; 2684)	*AA*: 14.52 (20.24; 358)	0.19	0.06	0.357
DRD3	rs6280	7734	33.23 (12.14)	*CC*: 12.27 (18.30; 734)	*CT*: 12.72 (18.65; 3272)	*TT*: 12.37 (17.23; 3728)	0.31	0.68	0.878
DRD4	rs1800955	2152	23.94 (11.25)	*TT*: 17.34 (22.54; 680)	*TC*: 18.04 (22.30; 1103)	*CC*: 18.13 (21.29; 369)	0.43	0.03	0.365
	7 allele	2476	23.34 (10.98)	18.29 (22.72; 1624)	19.69 (23.24; 756)	15.75 (17.88; 96)	0.19	0.49	0.854
DRD5	148 allele	2480	23.34 (10.98)	17.58 (23.02; 607)	19.17 (22.29; 1302)	18.33 (23.02; 571)	0.49	0.01	0.477
DBH	rs1611115	3140	24.38 (11.21)	*TT*: 15.90 (19.34; 137)	*TC*: 18.23 (23.14; 1035)	*CC*: 17.96 (21.85; 1968)	0.21	0.95	0.737
	rs2519152	8139	32.77 (12.28)	*CC*: 12.45 (16.91; 1752)	*CT*: 12.61 (17.76; 3948)	*TT*: 13.52 (20.08; 2439)	0.46	0.04	0.028
DAT1	480 allele	2464	23.33 (10.98)	19.22 (21.11; 162)	18.20 (20.77; 925)	18.87 (24.19; 1377)	0.25	0.69	0.882
COMT	rs4680	8755	32.46 (12.27)	*GG*: 13.79 (18.62; 1779)	*GA*: 13.16 (18.73; 4339)	*AA*: 12.40 (18.04; 2637)	0.45	0.94	0.085

*Fixed effects: sex, age, sex × age interaction, SNP/VNTR; random effect: latent genetic factor.

For two other SNPs (rs6275 and rs6280), data were derived from the imputed set only. For the remaining SNPs (rs265981, rs1800497, rs2519152, and rs4680), data were derived from the fingerprint chip for about 37% of the individuals and were complemented with data from the imputed set for 63% of the individuals. Concordance between genotyped and imputed SNP data in the individuals with both fingerprint chip and genome-wide data was higher than 95%.

2.4. Statistical Analyses. The SNPs were coded based on the presence of one or two of each of the two alleles in the called genotype (0 = allele 1 homozygote, 1 = heterozygote, and 2 = allele 2 homozygote). For the SNPs, the exact combination of alleles corresponding to 0, 1, and 2 can be found in Table 2. VNTRs, particularly the ones in the DRD4 and DRD5 genes, are highly polymorphic. Based on the literature, we decided to focus on specific repeats and the coding was based on the presence or absence of those repeats. For the VNTR in the DRD4 gene, this resulted in the following coding: 0 = no 7 allele, 1 = one 7 allele, and 2 = two 7 alleles. For the DRD5 gene, it was 0 = no 148 allele, 1 = one 148 allele, and 2 = two 148 alleles. For the DAT1 gene, it was 0 = no 480 allele, 1 = one 480 allele, and 2 = two 480 alleles.

As a first step, the analyses were performed for each genetic variant separately. Mixed models were run in SPSS for Windows (version 20.0, SPSS Inc.) and were based on maximum likelihood estimation. The dependent variable was weekly MET hours. The following variables were included as fixed effects: sex (0 = males, 1 = females), age (z-score), sex x age interaction, and the respective SNP/VNTR. We tested whether correction for a number of possible confounders had a significant effect on the results, namely, ancestry differences within the Dutch population (3 principal components), ancestry differences based on the 1000 Genomes project (6 principal components), differences due to batch effects (1 principal component), and a dummy variable to correct for differences between genotyping platforms.

As the next steps, (1) multiple variants were included into a single mixed model to test their effects simultaneously and (2) mixed models were run with a polygenic risk score computed as the sum of the alleles that are hypothesized to increase dopamine level ("effect alleles") across multiple variants. As data were derived from family members (twins, siblings, parents, and spouses of twins), we added genetic relatedness as a random effect to the models. The chosen alpha level was .05/11 (Bonferroni correction for 11 tests; alpha = .0045).

To get an indication of the power to detect genetic effects, simulated data was used, as this allows us to accommodate the large variation in family composition and the truncation of

the phenotype distribution (*R* code available upon request). Due to differences in sample sizes and family structures between the "fingerprint data only" and the "(fingerprint data with additional) imputed data", the power was calculated for four genetic variants: (1) the SNP with the smallest sample size (rs1800955, N = 2,152), (2) the SNP with the largest sample size within the five variants that we had fingerprint data for only (rs1611115, N = 3,140), (3) the SNP with the smallest sample size within the six variants that included imputed data (rs6275, N = 7,734), and (4) the SNP with the largest sample size (rs1800497, N = 8,756). Thus, we approximated the *upper* and *lower bounds* of power within (a) five variants that were derived from the fingerprint set and (b) six variants that were derived from the imputed/combined set. The power calculations were based on 1000 replications and the chosen alpha level was .05/11. For the smaller data set, the power ranged from .36 (95% confidence interval: .33–.39) to .58 (.55–.61) to detect an effect explaining 0.5% of the phenotypic variance. The power to detect an effect explaining 1% of the variance ranged from .78 (95% confidence interval: .75–.80) to .91 (.89–.92). For the larger data set, the power ranged from .69 (95% confidence interval: .66–.72) to .75 (.72–.77) to detect an effect explaining 0.25% of the variance. The power to detect an effect explaining 0.5% of the variance ranged from .96 (.94–.97) to .97 (.96–.98). These estimates are conservative as age and sex were not taken into account.

3. Results

Table 2 depicts—for each genetic variant—the number of individuals with complete genotype and phenotype data, their mean age (SD), the mean weekly MET hours across the three allele codings (SD; the number of individuals across the three allele codings), and the P value for the main effect of the respective SNP or VNTR. The table also includes the specific combinations of alleles for each SNP (not for the VNTRs). The sample size is lower for those variants that were collected with the fingerprint chip only (all VNTRs, rs1800955, and rs1611115) compared to the remaining variants that were derived from the fingerprint chip and complemented with imputed data, or derived from the imputed data only. Also, the fingerprint data were derived from relatively young participants. The P values in the table are based on the model that included sex, age, sex × age interaction, and the respective variant as fixed effects and familial relatedness as a random effect. Main effects of sex and age were significant ($P < .001$) with males and younger participants showing higher levels of exercise behavior and so was the sex x age interaction ($P < .004$). Importantly, none of the SNPs or VNTRs had a significant effect on exercise behavior ($P > .02$).

In additional analyses, we (1) added possible confounders (differences in ancestry, batch effect, and genotyping platforms) to the model and (2) reran the analyses on dosage scores (in which the uncertainty of imputation is taken into account). The effect of each SNP and VNTR remained non-significant. Next, multiple variants were included into a single mixed model to investigate their joint effect. As the VNTRs and two SNPs (rs1800955, rs1611115) were derived from the fingerprint chip only, the number of individuals dropped to less than 2,000 individuals when including only individuals that had been genotyped on all variants. Therefore, a potential overall effect was tested in two steps. *First*, all variants were included, reducing the sample size to 1,954 individuals with full genotypic and phenotypic data. *Second*, only SNPs were included that we had imputed data for (mostly *in addition to* the fingerprint data; rs265981, rs6275, rs1800497, rs6280, rs2519152, and rs4680), resulting in 7,734 individuals with full genotypic and phenotypic data. In both cases, the joint effect of the variants was non-significant ($\chi^2 = 15.65$, df = 11, and $\chi^2 = 3.99$, df = 6, resp.).

Finally, the analyses on the polygenic risk scores also failed to show a significant association ($P > .15$). Mixed models on the sum of the effect alleles across multiple variants were again run in two steps. First, the complete set of variants was included and, second, only the variants we had the larger sample size for were included.

4. Discussion

This study aimed to investigate the genetic basis of regular leisure time exercise behavior. Eight SNPs (rs265981, rs6275, rs1800497, rs6280, rs1800955, rs1611115, rs2519152, and rs4680) and three VNTRs (a 48-bp VNTR in exon III of *DRD4*, a dinucleotide repeat 18.5 kb upstream of *DRD5*, and a 40-bp VNTR in the 3′ UTR of *DAT1*) with a known function in the dopaminergic reward system were investigated. None of them was significantly associated with exercise behavior.

It is well established from twin studies that exercise behavior is a heritable trait [11]. Twin studies allow the decomposition of variance of any phenotype into variance due to *genetic effects* and variance due to *environmental effects* (genetic effects + environmental effects = 100% of the variance). In children, genetic effects have been shown to explain slightly more than 20% of the variance in exercise behavior [52]. This heritability rises dramatically to 70–80% in adolescence [53] and stabilizes at about 50–60% in adulthood [54]. However, it is not clear yet *which* genes contribute to individual differences in exercise behavior.

A priori, genetic variation in the dopaminergic signaling pathway provided a promising source for the biological basis of this phenotype. Dopaminergic neurotransmission is implicated in the experience of reward which in turn is likely to be a crucial agent in the take-up and maintenance of exercise behavior [17]. Engaging in exercise itself has been related to changes in dopaminergic transmission [15] and individual differences in the dopaminergic reward system, more specifically in genetic variants that affect the system, have previously been linked to differences in physical activity both in rodents [16] and in humans [19]. Admittedly, some of this previous evidence implicating dopaminergic genes looked at more general forms of physical activity (e.g., parts of [19]) instead of the trait of self-initiated exercise behavior used here [55]. We focused on voluntary exercise behavior for two reasons. First, we hypothesized that the pleasure someone experiences when performing an exercise activity is a crucial determinant of the voluntary take-up and maintenance of regular exercise habits [10]. Secondly, excellent test-retest reliability has been

established for assessing leisure time exercise behavior by survey [46, 47], probably because recall is relatively easy as those activities are not only self-initiated but often clearly defined in time. In contrast, general physical activity is harder to assess reliably by questionnaires or recall interviews. It has been shown that self-reported physical activity corresponds only poorly with actual physical activity [56]. Reliability of self-reported physical activity may improve when focusing on activities that require moderate to vigorous effort, as these are more salient to the person. Nonetheless, even then recall will not be perfect. It may be hard, for instance, to recall the exact duration of nonvoluntary physical activity at work (lifting and effortful manual labor) or activities like bicycling to work or effortful household activities (vacuum cleaning). Instead, more objective measurement instruments should be applied, such as accelerometers or doubly labeled water.

Our study was founded on the solid expectation that we would find an association between known functional allelic variations in the dopaminergic signaling pathway and the narrow, but well-defined, trait of regular leisure time exercise behavior. This expectation was clearly not borne out by the results. Do our findings rule out a role for the dopaminergic system in individual differences in regular leisure time exercise behavior? There are a number of reasons why this conclusion would be premature.

First, the selected SNPs and VNTRs might not have covered all genetic variation within the dopaminergic genes examined, specifically in the case of low linkage disequilibrium between variants within a gene. We opted to choose alleles with known functional effects and/or previously reported effects on relevant phenotypes instead of examining the larger set of SNPs tagging the major haplotypes within dopaminergic genes [57]. Also, by focusing on eight genes, we covered only a small portion of the total dopamine signaling pathway. Already there are many other proteins known to be involved in this signaling pathway [14] and probably an even larger amount still eludes us. By definition, a candidate gene approach will miss these uncharted parts of the signaling cascade.

Second, one might argue that the effect sizes of the genetic variants measured here may have been too low to detect even with the substantial sample sizes available to us. Exercise behavior is a very complex phenotype and is likely to be affected by a lot of genes, each of which has only a small effect. These small effects might not be detectable in a sample of less than ten thousands of individuals. For six of the eleven variants, data of around 8,000 individuals were available and for the remaining five variants, data of around 2,500 individuals were available. A power analysis revealed that—for the larger samples—the power to detect an effect explaining 0.5% of the phenotypic variance was very good, and the power to detect an effect explaining 0.25% of the variance was acceptable, taking into account multiple testing, family structures, and the phenotypic distribution. For the smaller samples, power was more modest, but still the power to detect an effect explaining 1% of the phenotypic variance ranged between .78 and .91. Apart from increasing sample size, power could be increased by using intermediate phenotypes [12]. For instance, genetic association with reward sensitivity in the context of exercise activities or exercise motivation could be investigated as intermediate biological precursors instead of the exercise behavior per se. These are potentially more directly related to the genetic mechanisms, thereby decreasing residual variance that might cover an effect. Replication of our study in large, independent cohorts would increase the confidence in our results.

Third, we should bear in mind that dopaminergic neurotransmission may mediate the effect of entirely different genetic variants on exercise behavior, in the absence of a direct effect of dopaminergic genes. For instance, there might be genetic variants that increase exercise ability, thereby triggering increased dopaminergic neurotransmission during exercise activities as it is rewarding to perform an activity that one is good at. In this case, genetic variants within the dopaminergic pathway may not be directly involved, but dopaminergic neurotransmission may still indirectly convey genetic effects on exercise behavior.

5. Conclusions

We did not confirm our hypothesis that allelic variants involved in dopaminergic function create individual differences in exercise behavior. This leads us to plea for a large scale GWAS on leisure time exercise behavior involving more research groups as the success of GWAS efforts clearly scales with the number of participants. Currently, leisure time exercise behavior is less frequently assessed than general physical activity, in spite of the potentially less favorable psychometric properties of the latter. We believe that a GWAS effort on leisure time exercise behavior is worth pursuing. In order to pick up effects, assessing intermediate phenotypes such as exercise motivation should be considered. An inactive lifestyle is one of the major public health burdens nowadays and interventions that aim to tackle the problem are mostly unsuccessful. Given the substantial heritability of leisure time exercise behavior, it is of outmost importance to better understand its biological basis in order to improve intervention on this health-enhancing lifestyle.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank the members of the twin families registered with the Netherlands Twin Register for their continued support of scientific research. Funding for this study was obtained from the National Institutes of Health (NIH, Grand Opportunity Grant "DETECT" 1RC2 MH089995, NIDDK; NIH 1R01 DK092127-01); the Netherlands Organization for Scientific Research (NWO) and MagW/ZonMW Grants 912-10-020, 463-06-001, 451-04-034,400-05-717, Spi-56-464-14192, Center for Medical Systems Biology (CSMB, NWO Genomics); the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI–NL, 184.021.007); the VU University Institute for Health and Care Research (EMGO+);

the European Science Council (ERC 230374); and the Avera Institute, Sioux Falls, South Dakota (USA).

References

[1] I. Janssen and A. G. LeBlanc, "Systematic review of the health benefits of physical activity and fitness in school-aged children and youth," *International Journal of Behavioral Nutrition and Physical Activity*, vol. 7, article 40, 2010.

[2] C. E. Garber, B. Blissmer, M. R. Deschenes et al., "Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise," *Medicine and Science in Sports and Exercise*, vol. 43, no. 7, pp. 1334–1359, 2011.

[3] D. E. R. Warburton, S. Charlesworth, A. Ivey, L. Nettlefold, and S. S. D. Bredin, "A systematic review of the evidence for Canada's physical activity guidelines for adults," *International Journal of Behavioral Nutrition and Physical Activity*, vol. 7, article 39, 2010.

[4] N. Armstrong and W. van Mechelen, "Are young people fit and active?" in *Young and Active? Young People and Health-Enhancing Physical Activity—Evidence and Implications*, S. J. H. Biddle, J. F. Sallis, and N. Cavill, Eds., pp. 69–97, Health Education Authority, London, UK, 1998.

[5] M. A. Martinez-Gonzalez, J. J. Varo, J. L. Santos et al., "Prevalence of physical activity during leisure time in the European Union," *Medicine and Science in Sports and Exercise*, vol. 33, no. 7, pp. 1142–1146, 2001.

[6] R. P. Troiano, D. Berrigan, K. W. Dodd, L. C. Mâsse, T. Tilert, and M. Mcdowell, "Physical activity in the United States measured by accelerometer," *Medicine and Science in Sports and Exercise*, vol. 40, no. 1, pp. 181–188, 2008.

[7] J. H. Stubbe and E. J. C. de Geus, "Genetics of exercise behavior," in *Handbook of Behavior Genetics*, Y. K. Kim, Ed., pp. 343–358, Springer, New York, NY, USA, 2009.

[8] C. Bouchard and E. P. Hoffman, Eds., *Genetic and Molecular Aspects of Sport Performance*, Blackwell Publishing, Chichester, UK, 2011.

[9] T. Rankinen, S. M. Roth, M. S. Bray et al., "Advances in exercise, fitness, and performance genomics," *Medicine and Science in Sports and Exercise*, vol. 42, no. 5, pp. 835–846, 2010.

[10] E. J. C. de Geus and M. H. M. de Moor, "A genetic perspective on the association between exercise and mental health," *Mental Health and Physical Activity*, vol. 1, no. 2, pp. 53–61, 2008.

[11] E. J. C. de Geus and M. H. M. de Moor, "Genes, exercise, and psychological factors," in *Genetic and Molecular Aspects of Sport Performance*, C. Bouchard and E. P. Hoffman, Eds., pp. 294–305, Blackwell Publishing, Chichester, UK, 2011.

[12] A. Bryan, K. E. Hutchison, D. R. Seals, and D. L. Allen, "A transdisciplinary model integrating genetic, physiological, and psychological correlates of voluntary exercise," *Health Psychology*, vol. 26, no. 1, pp. 30–39, 2007.

[13] D. M. Williams, S. Dunsiger, J. T. Ciccolo, B. A. Lewis, A. E. Albrecht, and B. H. Marcus, "Acute affective response to a moderate-intensity exercise stimulus predicts physical activity participation 6 and 12 months later," *Psychology of Sport and Exercise*, vol. 9, no. 3, pp. 231–245, 2008.

[14] J.-M. Beaulieu and R. R. Gainetdinov, "The physiology, signaling, and pharmacology of dopamine receptors," *Pharmacological Reviews*, vol. 63, no. 1, pp. 182–217, 2011.

[15] B. N. Greenwood, T. E. Foley, T. V. Le et al., "Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway," *Behavioural Brain Research*, vol. 217, no. 2, pp. 354–362, 2011.

[16] A. M. Knab, R. S. Bowen, A. T. Hamilton, A. A. Gulledge, and J. T. Lightfoot, "Altered dopaminergic profiles: implications for the regulation of voluntary physical activity," *Behavioural Brain Research*, vol. 204, no. 1, pp. 147–152, 2009.

[17] A. M. Knab and J. T. Lightfoot, "Does the difference between physically active and couch potato lie in the dopamine system?" *International Journal of Biological Sciences*, vol. 6, no. 2, pp. 133–150, 2010.

[18] P. Jozkow, M. Slowinska-Lisowska, L. Laczmanski, and M. Medras, "DRD2 C313T and DRD4 48-bp VNTR polymorphisms and physical activity of healthy men in Lower Silesia, Poland (HALS study)," *Annals of Human Biology*, vol. 40, no. 2, pp. 186–190, 2012.

[19] R. L. Simonen, T. Rankinen, L. Pérusse et al., "A dopamine D2 receptor gene polymorphism and physical activity in two family studies," *Physiology and Behavior*, vol. 78, no. 4-5, pp. 751–757, 2003.

[20] C. J. Thomson, C. W. Hanna, S. R. Carlson, and J. L. Rupert, "The -521 C/T variant in the dopamine-4-receptor gene (DRD4) is associated with skiing and snowboarding behavior," *Scandinavian Journal of Medicine & Science in Sports*, vol. 23, no. 2, pp. e108–e113, 2013.

[21] J. Flint, "Gwas," *Current Biology*, vol. 23, no. 7, pp. 265–266, 2013.

[22] P. M. Visscher, M. A. Brown, M. I. McCarthy, and J. Yang, "Five years of GWAS discovery," *The American Journal of Human Genetics*, vol. 90, no. 1, pp. 7–24, 2012.

[23] H. K. Tabor, N. J. Risch, and R. M. Myers, "Candidate-gene approaches for studying complex genetic traits: practical considerations," *Nature Reviews Genetics*, vol. 3, no. 5, pp. 391–397, 2002.

[24] F. Zhu, C.-X. Yan, Q. Wang et al., "An association study between dopamine D1 receptor gene polymorphisms and the risk of schizophrenia," *Brain Research*, vol. 1420, pp. 106–113, 2011.

[25] A. Doehring, N. V. Hentig, J. Graff et al., "Genetic variants altering dopamine D2 receptor expression or function modulate the risk of opiate addiction and the dosage requirements of methadone substitution," *Pharmacogenetics and Genomics*, vol. 19, no. 6, pp. 407–414, 2009.

[26] M. J. Neville, E. C. Johnstone, and R. T. Walton, "Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1," *Human Mutation*, vol. 23, no. 6, pp. 540–545, 2004.

[27] N. R. Mota, E. V. Araujo Jr., V. R. Paixão-Côrtes, M. C. Bortolini, and C. H. Bau, "Linking dopamine neurotransmission and neurogenesis: the evolutionary history of the NTAD (NCAM1-TTC12-ANKK1-DRD2) gene cluster," *Genetics and Molecular Biology*, vol. 35, supplement 4, pp. 912–918, 2012.

[28] A. Laakso, T. Pohjalainen, J. Bergman et al., "The A1 allele of the human D2 dopamine receptor gene is associated with increased activity of striatal L-amino acid decarboxylase in healthy subjects," *Pharmacogenetics and Genomics*, vol. 15, no. 6, pp. 387–391, 2005.

[29] Y. Zhang, A. Bertolino, L. Fazio et al., "Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 51, pp. 20552–20557, 2007.

[30] S. H. Lee, B.-J. Ham, Y.-H. Cho, S.-M. Lee, and S. H. Shim, "Association study of dopamine receptor D2TaqI A polymorphism and reward-related personality traits in healthy Korean

young females," *Neuropsychobiology*, vol. 56, no. 2-3, pp. 146–151, 2008.

[31] K. Lundstrom and M. P. Turpin, "Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D3 receptor with the Semliki Forest virus system," *Biochemical and Biophysical Research Communications*, vol. 225, no. 3, pp. 1068–1072, 1996.

[32] J. Shi, E. S. Gershon, and C. Liu, "Genetic associations with schizophrenia: meta-analyses of 12 candidate genes," *Schizophrenia Research*, vol. 104, no. 1–3, pp. 96–107, 2008.

[33] Y. Okuyama, H. Ishiguro, M. Toru, and T. Arinami, "A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia," *Biochemical and Biophysical Research Communications*, vol. 258, no. 2, pp. 292–295, 1999.

[34] V. Asghari, S. Sanyal, S. Buchwaldt, A. Paterson, V. Jovanovic, and H. H. M. van Tol, "Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants," *Journal of Neurochemistry*, vol. 65, no. 3, pp. 1157–1165, 1995.

[35] G. Guo, K. E. North, P. Gorden-Larsen, C. M. Bulik, and S. Choi, "Body mass, DRD4, physical activity, sedentary behavior, and family socioeconomic status: the add health study," *Obesity*, vol. 15, no. 5, pp. 1199–1206, 2007.

[36] N. Lowe, A. Kirley, Z. Hawi et al., "Joint analysis of the DRD5 marker concludes association with attention-deficit/hyperactivity disorder confined to the predominantly inattentive and combined subtypes," *The American Journal of Human Genetics*, vol. 74, no. 2, pp. 348–356, 2004.

[37] C. P. Zabetian, G. M. Anderson, S. G. Buxbaum et al., "A quantitative-trait analysis of human plasma-dopamine β-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus," *The American Journal of Human Genetics*, vol. 68, no. 2, pp. 515–522, 2001.

[38] J. F. Cubells and C. P. Zabetian, "Human genetics of plasma dopamine β-hydroxylase activity: applications to research in psychiatry and neurology," *Psychopharmacology*, vol. 174, no. 4, pp. 463–476, 2004.

[39] Y. Tang, S. G. Buxbaum, I. Waldman et al., "A single nucleotide polymorphism at DBH, possibly associated with attention-deficit/hyperactivity disorder, associates with lower plasma dopamine β-hydroxylase activity and is in linkage disequilibrium with two putative functional single nucleotide polymorphisms," *Biological Psychiatry*, vol. 60, no. 10, pp. 1034–1038, 2006.

[40] S. V. Faraone, T. J. Spencer, B. K. Madras, Y. Zhang-James, and J. Biederman, "Functional effects of dopamine transporter gene genotypes on in vivo dopamine transporter functioning: a meta-analysis," *Molecular Psychiatry*, 2013.

[41] J. Yacubian, T. Sommer, K. Schroeder et al., "Gene-gene interaction associated with neural reward sensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 19, pp. 8125–8130, 2007.

[42] J. Chen, B. K. Lipska, N. Halim et al., "Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mrna, protein, and enzyme activity in postmortem human brain," *The American Journal of Human Genetics*, vol. 75, no. 5, pp. 807–821, 2004.

[43] T. M. Lancaster, D. E. Linden, and E. A. Heerey, "COMT val158met predicts reward responsiveness in humans," *Genes, Brain, and Behavior*, vol. 11, no. 8, pp. 986–992, 2012.

[44] C. E. M. van Beijsterveldt, M. Groen-Blokhuis, J. J. Hottenga et al., "The Young Netherlands Twin Register (YNTR): longitudinal twin and family studies in over 70,000 children," *Twin Research and Human Genetics*, vol. 16, no. 1, pp. 252–267, 2013.

[45] G. Willemsen, J. M. Vink, A. Abdellaoui et al., "The Adult Netherlands Twin Register: twenty-five years of survey and biological data collection," *Twin Research and Human Genetics*, vol. 16, no. 1, pp. 271–281, 2013.

[46] M. H. M. de Moor, D. I. Boomsma, J. H. Stubbe, G. Willemsen, and E. J. C. de Geus, "Testing causality in the association between regular exercise and symptoms of anxiety and depression," *Archives of General Psychiatry*, vol. 65, no. 8, pp. 897–905, 2008.

[47] J. H. Stubbe, M. H. M. de Moor, D. I. Boomsma, and E. J. C. de Geus, "The association between exercise participation and well-being: a co-twin study," *Preventive Medicine*, vol. 44, no. 2, pp. 148–152, 2007.

[48] M. H. M. de Moor and E. J. C. de Geus, "Genetic influences on regular exercise behavior," in *Lifstyle Medicine*, J. M. Rippe, Ed., pp. 1367–1378, Taylor & Francis Group, LLC, Boca Raton, FL, USA, 2013.

[49] K. Ridley, B. E. Ainsworth, and T. S. Olds, "Development of a compendium of energy expenditures for youth," *International Journal of Behavioral Nutrition and Physical Activity*, vol. 5, article 45, 2008.

[50] B. E. Ainsworth, W. L. Haskell, M. C. Whitt et al., "Compendium of physical activities: an update of activity codes and MET intensities," *Medicine and Science in Sports and Exercise*, vol. 32, supplement 9, pp. S498–S504, 2000.

[51] A. Abdellaoui, J. J. Hottenga, P. de Knijff et al., "Population structure, migration, and diversifying selection in the Netherlands," *European Journal of Human Genetics*, vol. 21, no. 11, pp. 1277–1285, 2013.

[52] C. Huppertz, M. Bartels, C. E. M. van Beijsterveldt, D. I. Boomsma, J. J. Hudziak, and E. J. C. de Geus, "Effect of shared environmental factors on exercise behavior from age 7 to 12 years," *Medicine and Science in Sports and Exercise*, vol. 44, no. 10, pp. 2025–2032, 2012.

[53] N. van der Aa, E. J. C. de Geus, C. E. M. van Beijsterveldt, D. I. Boomsma, and M. Bartels, "Genetic influences on individual differences in exercise behavior during adolescence," *International Journal of Pediatrics*, vol. 2010, Article ID 138345, 8 pages, 2010.

[54] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *PLoS ONE*, vol. 1, no. 1, article e22, 2006.

[55] E. Kostrzewa and M. J. Kas, "The use of mouse models to unravel genetic architecture of physical activity: a review," *Genes, Brain, and Behavior*, vol. 13, no. 1, pp. 87–103, 2014.

[56] S. A. Prince, K. B. Adamo, M. E. Hamel, J. Hardt, S. Connor Gorber, and M. Tremblay, "A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review," *International Journal of Behavioral Nutrition and Physical Activity*, vol. 5, article 56, 2008.

[57] X. Xu, C. Rakovski, X. Xu, and N. Laird, "An efficient family-based association test using multiple markers," *Genetic Epidemiology*, vol. 30, no. 7, pp. 620–626, 2006.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 950516, 9 pages
http://dx.doi.org/10.1155/2014/950516

Research Article

Comparison of Gene and Protein Expressions in Rats Residing in Standard Cages with Those Having Access to an Exercise Wheel

Helaine M. Alessio,[1] Hayden Ansinelli,[1] Caitlyn Threadgill,[1] and Ann E. Hagerman[2]

[1] *Department of Kinesiology and Health, Miami University, Oxford, OH 45056, USA*
[2] *Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA*

Correspondence should be addressed to Helaine M. Alessio; alessih@miamioh.edu

Received 30 October 2013; Revised 23 December 2013; Accepted 14 January 2014; Published 25 February 2014

Academic Editor: J. Timothy Lightfoot

Lifelong physical inactivity is associated with morbidity in adulthood, possibly influenced by changes in gene and protein expressions occurring earlier in life. mRNA (Affymetrix gene array) and proteomic (2D-DIGE MALDI-TOF/MS) analyses were determined in cardiac tissue of young (3 months) and old (16 months) Sprague-Dawley rats housed with no access to physical activity (SED) versus an exercise wheel (EX). Unfavorable phenotypes for body weight, dyslipidemia, and tumorogenesis appeared more often in adult SED versus EX. No differentially expressed genes (DEGs) occurred *between* groups at 3 or 16 months. *Within* groups, SED and EX shared 215 age-associated DEGs. In SED, ten unique DEGs occurred with age; three had cell adhesion functions (fn1, lgals3, ncam2). In EX, five unique DEGs occurred with age; two involved hypothalamic, pituitary, and gonadal hormone axis (nrob2, xpnpep2). Protein expression involved in binding, sugar metabolic processes, and vascular regulation declined with age in SED (KNT1, ALBU, GPX1, PYGB, LDHB, G3P, PYGM, PGM1, ENOB). Protein expression increased with age in EX for ATP metabolic processes (MYH6, MYH7, ATP5J, ATPA) and vascular function (KNT1, ALBU, GPX1). Differences in select gene and protein expressions within sedentary and active animals occurred with age and contributed to distinct health-related phenotypes in adulthood.

1. Introduction

Major advances in biomedical research and human health and disease have occurred in large part through animal experiments. The shorter life expectancy of rats (~2 years) provides an advantage when studying age-related changes that occur over the lifespan [1, 2]. The human and rat genome are very similar, both containing 25,000–30,000 protein-coding genes [3, 4], although most gene functions (~95%) are unknown [5]. Thus far, approximately 80% of rat genes have been reported to have ortholog (genes that evolved from a common ancestral gene) counterparts in humans [6] and, to date, less than 5% of genes in the rat genome have been determined to lack an analogous gene in the human genome. Additionally, almost every human gene discovered to be associated with disease has an ortholog in the rat genome. Despite the biological similarities, outcomes of animal research usually do not account for molecular or physiological alterations that may occur over the life span due to extremely low physical activity levels in laboratory animals used in research [7, 8]. Laboratory rats housed in standard 0.02 m^3 cages move on average 160 $m \cdot day^{-1}$, compared with over 10,000 $m \cdot day^{-1}$ in animals that have access to a running wheel [9]. This very low activity coupled with ad libitum food access, likely contributed to findings from previous studies of unhealthy phenotypes including excessive weight gain, tumor development, abnormal blood lipids, hypertension, oxidative stress, and poor spatial maze performance in animals having no access to physical activity outside of a standard cage compared to those with access to an exercise wheel [9–14]. In these studies documenting phenotype differences after lifelong physical inactivity or activity, neither microarray nor proteomics was examined.

In this study, mRNA and protein expression in young and old animals confined to standard cages were compared with age-matched animals that voluntarily exercised on an exercise

wheel placed inside the cages. Health-related phenotypes in these same animals were previously reported [9, 10] with most health risk traits developing so gradually, that they did not appear clinically relevant until adulthood. It is possible that ephemeral shifts in gene or protein expressions that occur throughout the life span may provide a blueprint for age-associated disease phenotypes evident in adulthood. We know of no studies that have investigated both global gene and protein expression in cardiac tissue in laboratory animals with and without access to regular physical activity. It would be useful to understand if any genes and proteins express differently in animals that are housed solely in a cage compared with animals with access to an exercise wheel, given the phenotype data in adult rats already reported.

Microarray gene analyses provided identification at the transcriptome level of genes that were over- or underexpressed comparing cardiac tissue of young and old animals with very different daily physical activity levels. Proteomic analysis provided protein abundance in cardiac tissue. Over the course of a typical lifespan, the accumulation of exercise-induced effects on genes has been shown to offset many age-related gene expression increases reported in cardiac tissue of sedentary mice [15]. Bronikowski's study reported an overall downregulation of expression for genes involved with inflammation and stress responses in a regular exercise compared with a sedentary group. It is possible that limiting regular physical activity in laboratory animals by the confines inherent in standard cages affects the expression of a number of genes and proteins in critical networks, such as inflammation and stress, which are associated with age- and sedentary-related phenotypes. In this study, gene expressions, functional networks, protein levels, and clusters were compared in 3-month- and 16-month-old male Sprague-Dawley rats that were either chronically inactive due to being housed solely in a cage or were regularly active by voluntarily running on an exercise wheel located inside the cage.

2. Materials and Methods

Male Sprague-Dawley rats ($N = 24$; age = 3 wk; Charles River, Wilmington, MA) were divided into two groups consisting of animals with similar mean starting weights: (1) SED ($n = 12$): resided in pairs in standard cages with no access to a running wheel throughout the study; mean starting weight = 63.6 ± 1 g, and (2) EX ($n = 12$): resided in pairs in standard cages for 24 hours and then were placed individually in a different standard sized cage equipped with a running wheel for 24 hours, with cage switching repeated every day throughout the study; mean starting weight = 59.4 ± 1 g. This schedule provided EX animals regular access to physical activity on a running wheel while controlling for the effect of paired versus individual housing. Animals were housed in standard polypropylene cages (0.454 m × 0.238 m × 0.200 m) equipped with corn cob bedding (Bed-O-Cobs), in climate-controlled rooms (24 ± 2°C) with a 12-h light/dark cycle in a normal day cycle, and free access to water and food (Purina Lab Diet 5001 Rodent Chow). The study was conducted in accordance with ethical procedures and policies and was approved by the Institutional Animal Care and Use Committee.

2.1. Activity and Exercise Protocols and Measurements. Handling procedures were the same for all animals and described previously [9, 10]. SED animals resided in pairs in standard cages with no items added for enrichment. EX animals resided in pairs in the same standard cages and then were placed in standard cages equipped with running wheels every other day. This provided both groups with socialization as well as regular access to a running wheel for the EX group. Animal movement in the cages was monitored by a 24-hour surveillance VDI-2000BI B/W CCD IR camera, using infrared light. Two animals from each group were randomly selected each month and then marked with a permanent marking pen for tracking purposes. Recorded videos were evaluated by two individuals trained to trace movement inside the cage following the marked animals. Daily distance in SED was determined by averaging both total distance measures covered in 24 hours and recorded in meters. Daily distance in EX was determined by wheel revolutions converted to meters·day^{-1} in each EX animal.

SED and EX rats were sacrificed at 3 mo and 16 mo in a rested condition by decapitation and exsanguination. Serum was collected and separated following centrifugation (2000 ×g for 15 min). Organs and tissues were harvested immediately and were deep-frozen at −80°C for RNA and protein isolation described below.

2.2. Gene Expression Analyses. Global gene expression was measured using RNA from the frozen heart tissue. Briefly, RNA was extracted from 20–30 mg samples cut from the left ventricles using Qiagen RNeasy (Valencia, CA) RNA extraction kits. The absorbance ratios (A260/A280) were determined using a ND-1000 UV/VIS spectrophotometer (Nanodrop Technologies, Inc., Montchanin, DE) and were in a range from 2.04 to 2.17. The integrity of each sample was further assessed with an Agilent BioAnalyzer system by the Biomedical Genomics Core at the Research Institute at Nationwide Children's Hospital (Columbus, OH) and determined to be of high quality before processing. All samples passed quality control and were pooled within each treatment group to yield 4 gene chips per treatment group, with RNA from 3 animals on each chip, to ensure correct quantity of total RNA. The pooling of RNA samples allowed for representation in a cost-effective analysis. Samples were labeled with the Affymetrix Whole Transcript Labeling system and then hybridized to the Affymetrix GeneChip Rat Gene 1.0 ST Array (Santa Clara, CA). The design of the Rat Gene 1.0 ST Array is based primarily on a subset of GeneChip Rat Exon 1.0 ST Array probes that map to well-supported exons of known genes. The array comprised more than 700,000 unique 25-mer oligonucleotide features constituting more than 27,000 gene-level probe sets. Data was preprocessed using the RMA approach for background correction, normalization, and probe set summarization using the Bioconductor affy package in R.

Significance Analysis of Microarrays (SAM) used the Bioconductor Siggenes package to identify differentially expressed genes (DEGs) between 3 mo and 16 mo old animals for each treatment group. A two-class unpaired analysis with a

false discovery rate (FDR) of 10% was used to maximize sensitivity and minimize the effect on accuracy. Comparisons were made between 3 mo EX versus 3 mo SED and between 16 mo EX versus 16 mo SED. Microarray results are available in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) under the GEO accession no. GPL6247. Samples can be located with GEO accession nos. GSM1244311, GSM1244312, GSM1244313, GSM1244314, GSM1244319, GSM1244320, GSM1244321, GSM1244322, GSM1244335, GSM1244336, GSM1244337, GSM1244338, GSM1244343, GSM1244344, GSM1244345, and GSM1244346.

2-dimensional difference gel electrophoresis (2D-DIGE), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and protein cluster analyses identification were performed by Applied Biomics, Inc. (Hayward, CA). A small portion (10 mg) of cardiac tissue from the left ventricle of each animal was washed with 10 mM Tris-HCl, 5 mM magnesium acetate, pH 8.0 three times to remove any contaminating blood. Then 200 μL of 2D cell lysis buffer (30 mM Tris-HCl, pH 8.8, containing 7 M urea, 2 M thiourea, and 4% CHAPS) was added to the tissue. The mixture was sonicated at 4°C followed by shaking for 30 min at room temperature and centrifugation for 30 min at 21,000 g. Protein concentration in the supernatants was measured using Bio-Rad protein assay method.

For each cardiac sample, 30 μg of protein was mixed with 1.0 μL of diluted CyDye and kept in the dark on ice for 30 min. A protocol described by Berkelman and Stenstedt [16] was followed. Samples from each pair were labeled with Cy3 and Cy5 dyes, respectively. An internal standard was made up of equal amounts of protein from each sample and labeled with Cy2 and run on every gel. So, in each gel, there were 3 samples that were individually labeled: an internal standard labeled with Cy2, the first sample labeled with Cy3, and the second sample labeled with Cy5. The labeling reaction was stopped by adding 1.0 μL of 10 mM lysine to each sample and incubating in dark on ice for additional 15 min. The labeled samples were then mixed together. The 2X 2D sample buffer (8 M urea, 4% CHAPS, 20 mg/mL dithiothreitol (DTT), 2% pharmalytes, and trace amount of bromophenol blue), 100 μL destreak solution, and rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 20 mg/mL DTT, 1% pharmalytes, and trace amount of bromophenol blue) were added to the labeling mix to bring the total volume to 250 μL before loading the labeled samples into the strip holder.

After loading the labeled samples, isoelectric focusing (IEF) was run following the protocol provided by Amersham BioSciences. Upon finishing the IEF, the immobilized pH gradient (IPG) strips were incubated in the freshly made equilibration buffer-1 (50 mM tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% sodium dodecyl sulfate (SDS), trace amount of bromophenol blue, and 10 mg/mL DTT) for 15 minutes with gentle shaking. Then the strips were rinsed in the freshly made equilibration buffer-2 (50 mM Tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% SDS, trace amount of bromophenol blue, and 45 mg/mL DTT) for 10 min with gentle shaking. Next the IPG strips were rinsed in the SDS-gel running buffer before transferring into 12% SDS-gels. The SDS-gels were run at 15°C until the dye front reached the end of the gels.

Gel images were scanned immediately following the SDS polyacrylamide gel electrophoresis (SDS-PAGE) using Typhoon TRIO (GE Healthcare). Scanned images were then analyzed by Image Quant software (version 6.0, GE Healthcare), followed by cross-gel analysis using DeCyder software (version 6.5, GE Healthcare). Fold changes of the protein expression levels were obtained from in-gel DeCyder analysis. Specific proteins were identified by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS) and meeting criteria of 1.3-fold and $P < 0.05$ comparing old and young in SED and EX groups and correlated with protein spot numbers with an altered density. A follow-up completed the cluster pathway analysis, using a public bioinformatics tool available from the National Institutes of Health (NIH) called The Database for Annotation, Visualization and Integrated Discovery (DAVID).

DAVID, a public bioinformatics tool available from the NIH, was used to explore functionality and meaning of the list of proteins entered into the analyses [17]. All proteins entered into the analysis were first annotated into >40 annotation categories or clusters, including GO terms, protein-protein interactions, protein functional domains, disease associations, biopathways, sequence general features, homologies, gene functional summaries, and gene tissue expressions. DAVID software uses a novel algorithm to measure the relationships among the annotation terms based on the degrees of their coassociation genes to group the similar, redundant, and heterogeneous annotation contents from the same or different resources into annotation groups.

3. Results

3.1. Daily Physical Activity of SED and EX Groups. Distance covered in 24 hours for both SED and EX animals peaked at 2 mo of age and then, as demonstrated previously [9, 10], declined with age. However, the difference in daily physical activity between the two groups was stark. Mean daily distance of 161 ± 26 m·day^{-1} in the SED group was consistent over the life span. In contrast, animals in the EX group had a mean peak distance of 5548 ± 273 m·day^{-1} at 2 mo old on the running wheel. When housed in the standard cage with no exercise wheel every other day EX animals covered only 111 ± 10 m·day^{-1}. When in the cage equipped with a running wheel, daily activity other than wheel running was negligible and averaged 45 m·day^{-1}.

With increasing age, animals in the EX group became less active when housed in the cage with the running wheel, as shown by mean distances that decreased after 2 months with a peak of 5,548 m·day^{-1} to approximately 2000 m·day^{-1} at 6 mo and 500–1000 m·day^{-1} between 6 and 16 mo (Figure 1). Exercise intensity was not measured in this study; however, previous reports indicated that regular wheel running reduced body mass, visceral fat, and positively impacted lipid and amino acid metabolism [18–20], while not affecting oxidative capacity in rat muscle. We previously reported heart : body weight ratios as 0.370 ± 0.007 in EX and 0.340 ± .0007 in SED,

Table 1: Number of differentially expressed genes (DEGs) comparing 16 mo and 3 mo animals residing solely in a cage (SED) or with access to an exercise wheel (EX), measured by gene array analysis on mRNA isolated from heart tissue.

Comparison group	Reference group	Total number of DEGs	Number of upregulated genes	Number of downregulated genes	Number of DEGs with >2-fold increase	>Number of DEGs with >2-fold decrease
SED 16 mo	SED 3 mo	228	123	105	23	15
EX 16 mo	EX 3 mo	230	133	97	19	20

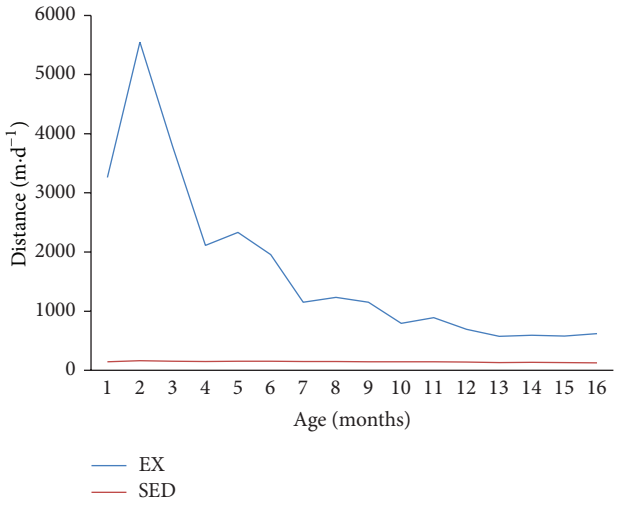


Figure 1: Mean daily distance covered (m·d^{-1}) in rats over time that were housed in standard cages (SED) and rats when housed individually in standard cages equipped with running wheels (EX).

which were not found to reach statistical significance. The main indicators that regular physical activity of the EX group resulted in physiological differences compared with the SED group have been previously reported [9, 10], and included EX having a significantly lower body weight throughout the life span with final mean body weights of 683 ± 14 versus 737 ± 18 g, lower prolactin levels (4.2 ± 0.6 versus 9.9 ± 2.4 ng·mL^{-1}), a more favorable oxidative stress balance represented by GSH : GSSG (189 versus 117), and fewer tumors (54% fewer) which were all benign cysts in EX compared with more tumors, including thyroid carcinoma and malignancy in SED.

3.2. Microarray Gene Expression. Microarray results comparing SED toEX at 16 mo and 3 mo showed no effect of exercise treatment, controlling for age, with no differentially expressed genes (DEGs). This surprising finding indicated that gene expression for animals that exercised on a running wheel was indistinguishable from gene expression for their sedentary counterparts of the same age. Phenotype changes that were obvious at 16 months in the absence of gene expression differences *between* groups may be the result of a relatively small number of modified gene expressions that occurred briefly, earlier in life. These gene expressions may have initiated the translation of key health-related proteins and then returned to a baseline transcription level. For animals in either group, there were unique changes in gene expression with age, supporting the hypothesis that exercise exerts an effect on gene expression early in life, even though physiological consequences may not be apparent until late in life. There was a significant age effect *within* SED and EX groups (Table 1) and a comparison of gene expressions in 16 mo and 3 mo old rats within SED and EX resulted in 228 DEGs in SED and 230 DEGs in EX, with 215 of those gene expressions shared by both groups. Genes with the largest disparate expressions between young and old in SED and EX were involved in vascular function, homeostasis, oxidative stress, and cholesterol (e.g., Col3a1, Emb, Atp2b2, Fmo1, Cyp2e1, Nox4, InhA, Chrna1, and Cyp1a1).

Table 2 displays the names of common genes expressed at least 2-fold differently between 16 mo and 3 mo old animals in SED or EX groups. The four largest DEGs occurred for the following genes: Atp2b2 (functions in intracellular calcium homeostasis), Cyp1a1 (roles in drug metabolism, synthesis of cholesterol, steroids, lipids, and NADPH-dependent electron transport pathway), Chrna1 (functions in acetylcholine binding/channel gating), and Col3a1 (roles in connective tissue and vascular function). None of these genes have previously been associated with physical activity, although Col3a1 was suspected to be associated with muscle cramping [21]. Table 3 displays fifteen unique DEGs with fold changes of at least 1.5-fold between 16 mo and 3 mo old animals in both groups. Ten unique DEGs were identified for SED, with three having cell adhesion functions (e.g. Fn1, Lgals3, and Ncam1). Only five unique genes were associated with EX, with two involved in the hypothalamic, pituitary, and gonadal hormone axis and obesity (e.g., Nrob2 and Xpnpep2). Candidate genes that were previously associated with voluntary exercise, including glucose transporter 4 (Glut4) [22], nescient helix loop helix 2 (Nhlh2) [23], and dopamine receptor 1 (Drd1) [24], were not identified as DEGs in the current study.

In a review of gene candidates that regulate physical activity, Lightfoot [25] describes a strong case for two candidate genes, Drd1, which regulates dopamine levels, and Nhlh2, which affects B-endorphin levels. Both gene candidates meet four standards of evidence from research including (1) having functional relevance to a known trait, (2) localizing within an identified quantitative trait loci, (3) having a possible genomic structural variation in the gene that may give rise to a functional difference in a protein, and (4) demonstrating a difference in gene expression as well as a difference in trait. Lightfoot states that other genes are likely to emerge as strong candidates, recognizing that epigenetic

Table 2: Common differentially expressed genes in SED and EX groups comparing 16 mo and 3 mo old animals.

Gene Symbol	Gene Name	Gene Function	Fold Change SED	Fold Change EX
col3a1	Collagen type III alpha 1	Connective tissue and vascular function	−1.5*	3.6#
emb	Embigin	Cell growth and homeostasis	−1.7*	2.0#
atp2b2	ATPase Calcium transporting plasma membrane 2	Intracellular calcium homeostasis	1.7#	−8.9*
fmo1	Flavin containing monoxidase 1	Oxidative metabolism	1.8#	−2.2*
cyp2e1	Cytochrome P450 family, 2, subfamily E polypeptide	Metabolism, cholesterol, and other lipid regulation	2.0#	−1.6*
npr3	Natriuretic peptide receptor C/guanylate cyclase C	Regulates blood volume and pressure cardiac function, metabolism	2.0#	2.3#
nax4	NADPH oxidase 4	Generates superoxide, functions as an oxygen sensor, apoptosis	−2.1*	1.5#
nadph oxidase 4	NADPH oxidase 4	Generates superoxide, functions as an oxygen sensor, apoptosis	−2.1*	−1.7*
inha	Inhibin alpha	Hypothalamic, pituitary, gonadal hormone secretion, germ cell development and maturation, erythoid differentiation, insulin secretion, nerve cell survival, embryonic axial development or bone growth	−2.6*	1.7#
chrna1	Cholinergic receptor, nicotinic, alpha 1	Plays a role in acetylcholine binding/channel gating	4.0#	2.3#
cyp1a1	Cytochrome P450, family 1, subfamily A polypeptide 1	Drug metabolism and synthesis of cholesterol, steroids and other lipids, involved in an NADPH-dependent electron transport pathway	−4.1*	−1.6*

#: Up-regulation; *: down-regulation.

forces, including acetylation and methylation, may also be strong regulators of traits associated with physical activity. Furthermore, combinations of genes that are up- and down-regulated can communicate in networks that ultimately translate for proteins that regulate cell function. In the current study, the largest DEGs expressed with age in both SED and EX were Atp2b2 (SED: 1.7 versus EX: −8.9) and Col3a1 (SED = −1.5 versus EX: +3.6). Other DEGs, whether shared or unique in SED and EX, were approximately 2-fold different or less. These data suggest resilience in gene expression over time, with only a small number of genes modified as a result of either chronic inactivity or activity.

3.3. 2D-DIGE, SDS PAGE, and MALDI-TOF-MS. 2D-DIGE and SDS PAGE distinguished 103 proteins that met statistical significance when comparing old and young animals in SED and EX groups. Of the 103 spots, 58 of these proteins were identified by MALDI-TOF-MS (Figures 2 and 3). Significant spots on the gel were identified independently in each comparison, and the number of significant spots varies by comparisons. Some spots could be significant in multiple comparisons. Supplementary Table 1 (see Supplementary Material available online at http://dx.doi.org/10.1155/2014/950516) lists the names of the DIGE-identified proteins in cardiac tissue that changed in abundance, identified by MALDI-TOF/MS and meeting criteria of 1.3-fold and $P < 0.05$, comparing old and young in SED and EX groups, and correlated with protein spot numbers with altered densities in Figures 2 and 3, respectively. Relevant information about the match quality, molecular weight, isoelectric point, protein scores, and confidence levels is provided in Supplementary Table 1. Supplementary Table 2 lists protein spot numbers that correspond to the names in Supplementary Table 1. Also listed are the comparative ratios in SED old versus young and EX old versus young, along with their P values. Fold changes in specific proteins, as well as functional categories in old and young animals in both SED and EX are shown in Figure 4.

Results of the DAVID bioinformatics cluster analyses showed that, compared with EX, SED had higher proteins levels in old versus young that were associated with mitochondrial membrane, motor activity, and muscle contraction categories and lower protein levels for binding, sugar metabolic processes, and vascular regulation (e.g., KNT1, ALBU, GPX1, PYGB, LDHB, G3P, PYGM, PGM1, and ENOB). Compared with SED, the EX group had higher protein levels in only two functional categories: ATP metabolic processes (e.g., MYH6, MYH7, ATP5J, and ATPA) and vascular function (e.g., KNT1, ALBU, and GPX1).

4. Discussion

Global gene expression and 2D-DIGE were combined in this study to compare gene and protein expressions in physically inactive animals that resided solely in a standard cage with physically active animals having regular access to an exercise wheel. Exercise training-induced changes in contractile,

Table 3: Genes that exhibit unique fold changes in animals in SED and EX groups, for SED and EX, 16 mo versus 3 mo.

Gene symbol	Gene name	Gene function	Fold change SED	Fold change EX
myoc	Myocilin, trabecular meshwork inducible glucocorticoid response	Cytoskeletal function and is expressed in many occular tissues	1.7[#]	
fn1	Fibronectin 1	Involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape	−1.5[*]	
s100a10	S100 calcium binding protein A10	Regulator of protein phosphorylation, involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation	−1.5[*]	
lgals3	Lectin, galactoside-binding, soluble, 3	Involved in acute inflammatory responses including neutrophil activation and adhesion, chemoattraction of monocytes macrophages, opsonization of apoptotic neutrophils, and activation of mast cells	−1.6[*]	
il33	Interleukin 33	Induces T-helper type 2-associated cytokines	1.6[#]	
vcan	Versican	May play a role in intercellular signaling and in connecting cells with the extracellular matrix. May take part in the regulation of cell motility, growth, and differentiation. Binds hyaluronic acid.	−1.6[*]	
emp1	Epithelial membrane protein 1		−1.5[*]	
nr1d1	Nuclear receptor subfamily 1, group D, member 1		−1.5[*]	
ncam1	Neural cellular adhesion molecule	Cell adhesion molecule involved in neuron-neuron adhesion, neurite fasciculation, and outgrowth of neurites	1.6[#]	
fhl3	Four and a half LIM domains 3	May be involved in tumor suppression, repression of MyoD expression, and repression of IgE receptor expression	−1.6[*]	
nr0b2	nuclear receptor subfamily 0, group B, member 1	Component of a cascade required for the development of the hypothalamic-pituitary-adrenal-gonadal axis		−1.5[*]
ptgfr	Prostaglandin F receptor	Receptor for prostaglandin F2-alpha (PGF2alpha). The activity of this receptor is mediated by G proteins which activate a phosphatidylinositol-calcium second messenger system. Initiates luteolysis in the corpus luteum		1.8[#]
xpnpep2	Inhibin alpha	Hypothalamic, pituitary, gonadal hormone secretion, germ cell development and maturation, erythroid differentiation, insulin secretion, nerve cell survival, embryonic axial development, or bone growth		1.5[#]
abca1	Cholinergic receptor, nicotinic, alpha 1	Plays a role in acetylcholine binding/channel gating		1.5[#]
pla2g2a	Cytochrome P450, family 1, subfamily A polypeptide 1	Drug metabolism and synthesis of cholesterol, steroids, and other lipids, involved in an NADPH-dependent electron transport pathway		−1.7[*]

[#]: upregulation; [*]: downregulation.

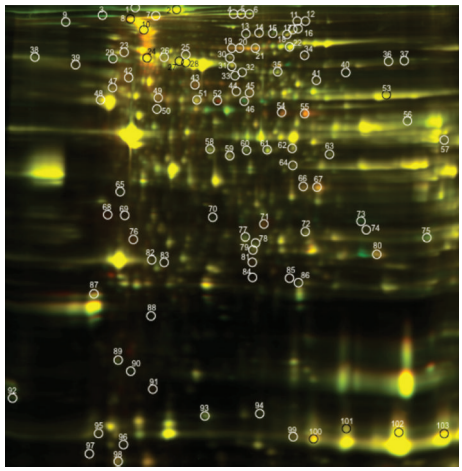


Figure 2: 2-dimensional gel electrophoresis image of overlay comparing 3-month-old SED and 3-month-old EX animals. Proteins ($n = 103$) showing different expressions (>1.5, $P < 0.05$) are circled.

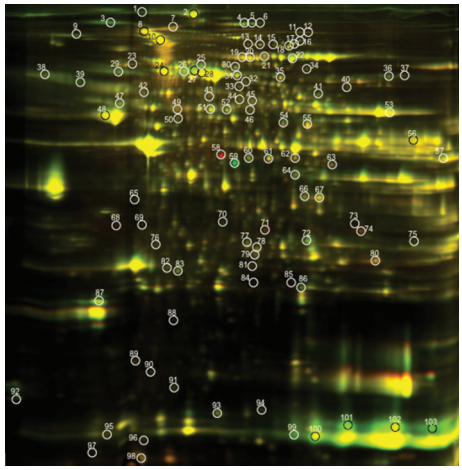


Figure 3: 2-dimensional gel electrophoresis image of overlay comparing 16 mo old SED and 16 mo old EX animals. Proteins ($n = 103$) showing different expressions (>1.5, $P < 0.05$) are circled.

mitochondrial, and transporter protein expression in rat skeletal muscle have been previously described [26, 27]. Yamaguchi et al. [28] identified 13 protein expression differences between high-intensity exercise trained and sedentary animals, several of which had previously unknown functions related to regular exercise. Several studies have reported that 65–73% of the variance in protein abundance may be explained by mRNA concentration [29, 30]; however, due to miRNA interactions, posttranslational modifications protein degradation, and sometimes unpredictable protein response to stimuli, mRNA alone is not always a good predictor of protein abundance [29, 30].

Although no DEGs were observed when comparing same-aged animals in different activity treatment groups, 2D-DIGE and SAGE analyses revealed 103 proteins at different measurable levels in 16 mo old SED versus 16 mo old EX rats and 3 mo SED versus 3 mo old SED rats. Comparing EX and SED, similar age-associated protein abundances were found for ETFD, HBB1, APOA1, MLRV, SPA3K, and FHL2 in both groups, indicating a more powerful age rather than physical activity influence on these protein expressions. Proteins that were expressed differently in old versus young SED compared with old versus young EX, indicating a more powerful influence of physical activity on protein expression, included NDUF3, ATP5J, G3P, GPX1, HSP7C, MCCA, PYGB, IDH3B, PYGM, ALDH2, ACADS, LDHB, MYL3, ALBU, and TTHY. These proteins represent a small fraction of the total number of the tens of thousands of proteins estimated in the entire organism. In our investigation, health-related differences between exercised and sedentary animals were not clinically obvious until the rats were 12 months old. After this age, animals housed in standard cages without access to a running wheel, exhibited undesirable phenotypes such as high blood cholesterol, high body weight, hypertension, greater number of tumors, and compromised spatial maze performance, compared with their more active counterparts [9, 10, 14]. The top proteins that were differentially expressed when comparing SED (old versus young) to EX (old versus young) groups (e.g., NDUF3, ATP5J, G3P, GPX1, and HSP7C) encompassed a wide variety of regulatory functions, including metabolism, oxidative stress, glycosaminoglycan binding, response to vitamin A, cell morphogenesis, activation of protein kinase activity, fatty acid biosynthesis, and contractile activity. Other proteins with large differences in expression between SED and EX had unknown functions related to exercise (e.g., four and a half LIM domains protein 2, ES1 protein homolog).

A surprisingly small number of genes and proteins have been found to be involved in cell regulation, with single proteins having multiple functions [5]. In previous studies only ~1.5% of genes from cardiac tissue in rats were differentially expressed when comparing animals at rest with those following an acute (one-time) bout of exercise [12, 27]. One study reported that sedentary female rat muscle exhibited 52 significant changes in gene transcription after one hour of vigorous exercise compared with skeletal muscle collected from resting animals [31]; however, any effect of these gene expressions on protein abundance was not investigated. In the present study, cardiac gene expressions were measured using tissue from animals sacrificed in a rested state, and so any change in expression, up or down, which may have occurred due to acute physical activity, were likely to have returned to baseline upon recovery. The small number of DEGs and proteins comparing old to young animals with different lifelong activity levels contrasted with large differences in phenotypes described previously in these animals (e.g., higher body weight, blood pressure, and tumor formation in less active) that did not emerge until early adulthood (12 months old) [9, 10].

Gene and protein expressions do not always clearly link to specific health- or disease-associated phenotypes. Yet the tissue-specific protein complement of the genome usually governs the function of the cell and in so doing regulates the phenotype. The emerging field of proteomics identifies and quantifies proteins that are translated, whether or not they can be predicted from DNA or mRNA analyses [28–30, 32].

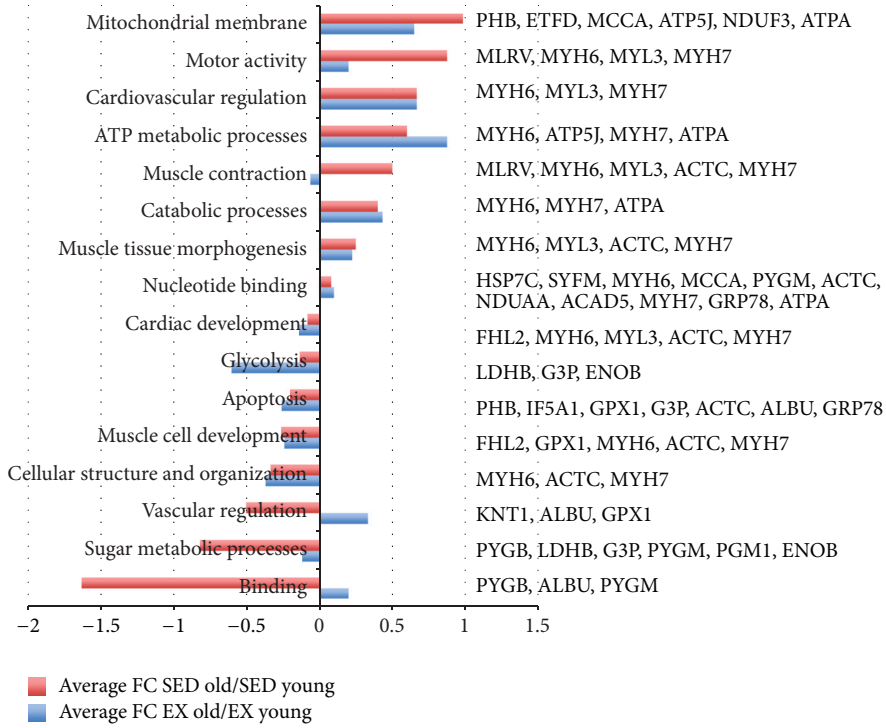


Figure 4: Functional category, protein names, and fold changes in SED and EX, old and young, FC = fold change.

The value of proteomic data ultimately depends upon its predictive ability of certain phenotypes. It is unclear if health-related phenotypes such as blood pressure, body weight, tumors, and oxidative stress can be traced to changes in a small number of genes or gene clusters, or entire networks that communicate with a small number or cluster of specific proteins.

5. Conclusion

The impact of environment, specifically, chronic inactivity imposed by space limitations of standard cages on gene expression and protein levels, is becoming recognized as an important consideration in animal research. Providing an exercise wheel is a simple, yet effective way to influence gene and protein expressions likely to influence health and disease traits in adulthood. Nevertheless, most animal studies neglect including information about physical activity and typically confine animals to standard cages, forcing them to be sedentary. Results from this study suggest that ephemeral changes in a small number of DEGs occur early in life and contribute to different protein abundances associated with healthy and disease traits that become evident in adulthood, differing substantively between physically inactive and inactive animals. It is currently unclear whether the predominant influence on protein synthesis stems from large changes in a small number of genes, or small changes in a large number of genes located in specific gene networks. Specific proteins expressed in lower and higher levels as a result of physical inactivity or activity are likely to affect health status, especially as one ages. Mechanisms by which lifetime physical inactivity or regular activity acts to affect the expression of specific genes and proteins remain a logical direction for future research, and the importance of housing and access to physical activity deserves further attention in future animal studies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Institute on Aging, 1R15AG029653-01A1, Miami University Committee on Faculty Research, and University Summer Scholars program.

References

[1] R. D. Emes, L. Goodstadt, E. E. Winter, and C. P. Ponting, "Comparison of the genomes of human and mouse lays the foundation of genome zoology," *Human Molecular Genetics*, vol. 12, no. 7, pp. 701–709, 2003.

[2] K. Paigen, "A miracle enough: the power of mice," *Nature Medicine*, vol. 1, no. 3, pp. 215–220, 1995.

[3] N. A. Datson, J. van der Perk, E. R. de Kloet, and E. Vreugdenhil, "Expression profile 30,000 genes in rat hippocampus using SAGE," *Hippocampus*, vol. 11, no. 4, pp. 430–444, 2001.

[4] J. C. Venter, M. D. Adams, E. W. Myers et al., "The sequence of the human genome," *Science*, vol. 291, no. 5507, pp. 1304–1351, 2001.

[5] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, "Membrane structure," in *Molecular Biology of the Cell*, pp. 10–25, Garland Science, New York, NY, USA, 5th edition, 2007.

[6] R. A. Gibbs, G. M. Weinstock, M. L. Metzker et al., "Genome sequence of the Brown Norway rat yields insights into mammalian evolution," *Nature*, vol. 428, no. 6982, pp. 493–521, 2004.

[7] A. Bye, M. A. Høydal, D. Catalucci et al., "Gene expression profiling of skeletal muscle in exercise-trained and sedentary rats with inborn high and low VO2max," *Physiological Genomics*, vol. 35, no. 3, pp. 213–221, 2008.

[8] B. Martin, S. Ji, S. Maudsley, and M. P. Mattson, "'Control' laboratory rodents are metabolically morbid: why it matters," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 14, pp. 6127–6133, 2010.

[9] H. M. Alessio, A. E. Hagerman, S. Nagy et al., "Exercise improves biomarkers of health and stress in animals fed ad libitum," *Physiology & Behavior*, vol. 84, no. 1, pp. 65–72, 2005.

[10] H. M. Alessio, N. B. Schweitzer, A. M. Snedden, P. Callahan, and A. E. Hagerman, "Revisiting influences on tumor development: focusing on laboratory housing," *Journal of the American Association for Laboratory Animal Science*, vol. 48, no. 3, pp. 258–262, 2009.

[11] M. P. Mattson and R. Wan, "Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems," *The Journal of Nutritional Biochemistry*, vol. 16, no. 3, pp. 129–137, 2005.

[12] M. L. Simonsen, H. M. Alessio, P. White, D. L. Newsom, and A. E. Hagerman, "Acute physical activity effects on cardiac gene expression," *Experimental Physiology*, vol. 95, no. 11, pp. 1071–1080, 2010.

[13] N. B. Schweitzer, H. M. Alessio, A. E. Hagerman et al., "Access to exercise and its relation to cardiovascular health and gene expression in laboratory animals," *Life Sciences*, vol. 77, no. 18, pp. 2246–2261, 2005.

[14] N. B. Schweitzer, H. M. Alessio, S. D. Berry, K. Roeske, and A. E. Hagerman, "Exercise-induced changes in cardiac gene expression and its relation to spatial maze performance," *Neurochemistry International*, vol. 48, no. 1, pp. 9–16, 2006.

[15] A. M. Bronikowski, P. A. Carter, T. J. Morgan et al., "Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart," *Physiological Genomics*, vol. 12, no. 2, pp. 129–138, 2003.

[16] T. Berkelman and T. Stenstedt, "2-D electrophoresis: principles and methods," Amersham Biosciences, 2002.

[17] D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources," *Nature Protocols*, vol. 4, no. 1, pp. 44–57, 2008.

[18] R. N. Cortright, M. P. Chandler, P. W. R. Lemon, and S. E. Dicarlo, "Daily exercise reduces fat, protein and body mass in male but not female rats," *Physiology & Behavior*, vol. 62, no. 1, pp. 105–111, 1997.

[19] J. L. Miles, K. Huber, N. M. Thompson, M. Davison, and B. H. Breier, "Moderate daily exercise activates metabolic flexibility to prevent prenatally induced obesity," *Endocrinology*, vol. 150, no. 1, pp. 179–186, 2009.

[20] E. A. Gulve, K. J. Rodnick, E. J. Henriksen, and J. O. Holloszy, "Effects of wheel running on glucose transporter (GLUT4) concentration in skeletal muscle of young adult and old rats," *Mechanisms of Ageing and Development*, vol. 67, no. 1-2, pp. 187–200, 1993.

[21] K. O'Connell, M. Posthumus, M. P. Schwellnus, and M. Collins, "Collagen genes and exercise-associated muscle cramping," *Clinical Journal of Sport Medicine*, vol. 23, no. 1, pp. 64–69, 2013.

[22] T.-S. Tsao, J. Li, K. S. Chang et al., "Metabolic adaptations in skeletal muscle overexpressing GLUT4: effects on muscle and physical activity," *The FASEB Journal*, vol. 15, no. 6, pp. 958–969, 2001.

[23] D. J. Good, C. A. Coyle, and D. L. Fox, "Nhlh2: a basic helix-loop-helix transcription factor controlling physical activity," *Exercise and Sport Sciences Reviews*, vol. 36, no. 4, pp. 187–192, 2008.

[24] A. M. Knab, R. S. Bowen, A. T. Hamilton, A. A. Gulledge, and J. T. Lightfoot, "Altered dopaminergic profiles: implications for the regulation of voluntary physical activity," *Behavioural Brain Research*, vol. 204, no. 1, pp. 147–152, 2009.

[25] J. T. Lightfoot, "Current understanding of the genetic basis for physical activity," *The Journal of Nutrition*, vol. 141, no. 3, pp. 526–530, 2011.

[26] F. W. Booth, M. V. Chakravarthy, and E. E. Spangenburg, "Exercise and gene expression: physiological regulation of the human genome through physical activity," *The Journal of Physiology*, vol. 543, no. 2, pp. 399–411, 2002.

[27] R. J. Tunstall, K. A. Mehan, G. D. Wadley et al., "Exercise training increases lipid metabolism gene expression in human skeletal muscle," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 283, no. 1, pp. E66–E72, 2002.

[28] W. Yamaguchi, E. Fujimoto, M. Higuchi, and I. Tabata, "A DIGE proteomic analysis for high-intensity exercise-trained rat skeletal muscle," *The Journal of Biochemistry*, vol. 148, no. 3, pp. 327–333, 2010.

[29] P. Lu, C. Vogel, R. Wang, X. Yao, and E. M. Marcotte, "Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation," *Nature Biotechnology*, vol. 25, no. 1, pp. 117–124, 2007.

[30] U. T. Shankavaram, W. C. Reinhold, S. Nishizuka et al., "Transcript and protein expression profiles of the NCI-60 cancer cell panel: an integromic microarray study," *Molecular Cancer Therapeutics*, vol. 6, no. 3, pp. 820–832, 2007.

[31] R. H. Lambertucci, A. C. Levada-Pires, L. V. Rossoni, R. Curi, and T. C. Pithon-Curi, "Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats," *Mechanisms of Ageing and Development*, vol. 128, no. 3, pp. 267–275, 2007.

[32] C. J. Jeffery, "Moonlighting proteins: old proteins learning new tricks," *Trends in Genetics*, vol. 19, no. 8, pp. 415–417, 2003.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 361048, 9 pages
http://dx.doi.org/10.1155/2014/361048

Research Article

Differential Gene Expression in High- and Low-Active Inbred Mice

Michelle Dawes,[1,2] Trudy Moore-Harrison,[3] Alicia T. Hamilton,[3] Tyrone Ceaser,[3] Kelli J. Kochan,[4] Penny K. Riggs,[4] and J. Timothy Lightfoot[1,2,3]

[1] *Department of Health and Kinesiology, Texas A&M University, College Station, TX 77843, USA*
[2] *Sydney and JL Huffines Institute for Sports Medicine and Human Performance, Texas A&M University, College Station, TX 77843, USA*
[3] *Department of Kinesiology, University of North Carolina Charlotte, Charlotte, NC 28223, USA*
[4] *Department of Animal Science, Texas A&M University, College Station, TX 77843, USA*

Correspondence should be addressed to Michelle Dawes; dawesmic@hlkn.tamu.edu

Received 15 October 2013; Accepted 15 December 2013; Published 16 January 2014

Academic Editor: Jaakko Kaprio

Numerous candidate genes have been suggested in the recent literature with proposed roles in regulation of voluntary physical activity, with little evidence of these genes' functional roles. This study compared the haplotype structure and expression profile in skeletal muscle and brain of inherently high- (C57L/J) and low- (C3H/HeJ) active mice. Expression of nine candidate genes [*Actn2*, *Actn3*, *Casq1*, *Drd2*, *Lepr*, *Mc4r*, *Mstn*, *Papss2*, and *Glut4* (a.k.a. *Slc2a4*)] was evaluated via RT-qPCR. SNPs were observed in regions of *Actn2*, *Casq1*, *Drd2*, *Lepr*, and *Papss2*; however, no SNPs were located in coding sequences or associated with any known regulatory sequences. In mice exposed to a running wheel, *Casq1* ($P = 0.0003$) and *Mstn* ($P = 0.002$) transcript levels in the soleus were higher in the low-active mice. However, when these genes were evaluated in naïve animals, differential expression was not observed, demonstrating a training effect. Among naïve mice, no genes in either tissue exhibited differential expression between strains. Considering that no obvious SNP mechanisms were determined or differential expression was observed, our results indicate that genomic structural variation or gene expression data alone is not adequate to establish any of these genes' candidacy or causality in relation to regulation of physical activity.

1. Introduction

The benefits of physical activity on health and disease have been demonstrated convincingly [1]. Despite this evidence, physical activity continues to decline in humans [2, 3], with data suggesting that less than 5% of adults complete moderate activity on a regular basis and 25% of adults are not active at all during their leisure time. Physical inactivity is a risk factor for many health outcomes such as cardiovascular disease, diabetes, some forms of cancer, and obesity [4].

Studies of both human and animal models strongly suggest that genetic factors play a role in physical activity with little common environmental effect [5–15]. Heritability of physical activity has been observed to widely range from 20% to 92% in humans and mice, depending on the heritability index used, the activity measurement employed, the sex and age of the subject, and species, among other factors. While copious evidence exists that genetics are associated with the determination of physical activity levels, little direct evidence supports involvement of specific genetic mechanisms in activity regulation.

Recently, several putative candidate genes have been proposed to play roles in physical activity; however, there has been no definite consensus about what constitutes "sufficient evidence" to define a candidate gene. Traditional experimental approaches most often have used the single criterion of functional relevance as the standard for candidate gene declaration [16]. DiPetrillo et al. [17] suggested that a candidate gene can be declared when a potential candidate gene exhibits at least three lines of evidence as to its involvement in the phenotype of study, which includes location within a known QTL, differences in gene expression, the aforementioned

"functional relevance," and/or alteration in the phenotype with manipulation of the gene. An example of this approach can be seen with two candidate genes for physical activity—dopamine receptor 1 (*Drd1*) and nescient helix loop helix 2 (*Nhlh2*)—which have shown functional relevance to activity [18, 19], interval-specific haplotype differences in animals exhibiting differential phenotypes [20], localization within identified activity single-effect and epistatic QTL [10, 12, 21–24], expression differences between high- and low-active animals [25], and/or a change in phenotype with gene manipulation [18, 26]. However, unlike *Drd1* and *Nhlh2*, the majority of potential candidate genes suggested to be associated with physical activity have little evidence to support their candidacy [27].

Therefore, the purpose of this study was to examine the interval-specific haplotype structure and gene expression of the nine previously suggested [10–12, 27], but weakly supported, candidate genes in high- (C57L/J) and low-active (C3H/HeJ) mice in both central brain (nucleus accumbens) and peripheral musculoskeletal (soleus) tissues, with the goal of adding additional lines of evidence to support these genes as candidates for future causal activity regulation studies.

2. Methods

2.1. Overall Procedures. Based on the available literature, nine genes with direct or indirect association (through functional relevance or GWAS) to physical activity were investigated: actinin 2 (*Actn2*, [27]), actinin 3 (*Actn3*, [27]), calsequestrin 1 (*Casq1*, [28]), dopamine receptor 2 (*Drd2*, [29]), leptin receptor (*Lepr*, [13, 30]), melanocortin 4 receptor (*Mc4r*, [31]), myostatin (*Mstn*, [27]), 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (*Papss2*, [32]), and glucose transporter 4 (*Glut4*—aka *Slc2a4*, [33]). Two methods were used to investigate these genes. Initially, published databases were interrogated to identify regional haplotype differences indicating potential genomic variation in the candidate gene between the high- and low-active mouse strains. Second, mRNA expression was measured in both naïve and running wheel-exposed mice of both strains.

2.2. Method One: Interval-Specific Haplotype Comparisons. Haplotypes of the nine candidate genes were compared within and between the high- and low-active mouse strains to identify potential genetic structural differences that could contribute to phenotypic variation. Initial haplotype analysis was conducted using the dense single nucleotide polymorphism (SNP) map from Perlegen Inc. (Mountain View, CA) (≈8.3 million SNPs). The Perlegen database utilized sequence data from 55 inbred strains of mice to predict haplotypes using pairwise comparisons between mouse strains. The specific chromosomal location of each target gene was determined using the NCBI GENE database (http://www.ncbi.nlm.nih.gov/gene/) and then inserted into the haplotype block viewer [20]. The haplotype viewer provided a binary determination of whether the haplotype and any SNPs, if present, were similar or dissimilar between the strains. Following the recent dismantling of the Perlegen online mouse haplotype viewer, the haplotype data were subsequently reverified using the Mouse Phylogeny Viewer (http://msub.csbio.unc.edu/ [34]).

2.3. Method Two: Gene Expression Determination. We had previously identified C3H/HeJ inbred mice as low-active and C57L/J inbred mice as high-active [10] with the C57L/J mice running, on average, 271% farther on a daily basis than the low-active C3H/HeJ mice. At eight weeks of age, four C57L/J and four C3H/HeJ mice (Jackson Labs, Bar Harbor, ME) were housed individually in cages with a 450 mm circumference solid surface running wheel (Ware Manufacturing, Phoenix, AZ) interfaced with a magnetic sensor and computer odometer (Sigma Sport BC600, St. Charles, IL) that counted revolutions of the running wheel and total time the mouse ran. Each cage computer was calibrated (as per manufacturer's instructions) for the circumference of the cage wheel allowing for measurement of distance (km) and time (min) the animals ran on the wheel, with subsequent calculation of speed (m/min). After one week of adaptation to the wheel, the activity of each mouse was monitored every 24 hours beginning at 63 days of age (9 weeks) for seven consecutive days. Each day the wheels were checked to insure that they turned freely. These methods have been validated for repeatability [35]. Subsequently, due to concerns that wheel exposure would cause training-induced gene expression changes, a separate group of high-active and low-active mice (n = 12, 3 ♂ and 3 ♀ of each strain) were housed with locked (i.e., nonturning) wheels from 8 to 10 weeks of age. Mice of respective activity groups were housed in the same room of the university vivarium with 12 h light/dark cycles (see discussion), with temperature and humidity maintained at 19–21°C and 50–60%, respectively. Food (Harland Tekland 8604 Rodent Diet, Madison, WI) and water were provided *ad libitum*. Mice were weighed on a weekly basis. At 10 weeks of age, the mice were anesthetized with 2–4% isofluorine for body composition testing and subsequently euthanized. The nucleus accumbens and the soleus muscle were harvested and flash frozen in liquid nitrogen and then stored at −80°C for later analysis. Body composition was analyzed in the naïve animals prior to tissue harvesting, using the Lunar Piximus DEXA (dual-energy X-ray absorptiometry) instrument (Fitchberg, WI). All procedures were approved by the University of North Carolina Charlotte and Texas A&M University Institutional Animal Care and Use Committees.

Target gene transcript expression was measured by quantitative real-time polymerase chain reaction (RT-qPCR) as reported previously, with minor modifications [25]. Total RNA was isolated from nucleus accumbens and soleus tissue using the Qiagen RNeasy mini kit (Qiagen, Valencia, CA). Immediately following the elution step, DNA was removed with a DNA-*free* kit (Ambion, Austin, TX). RNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA) and in naïve animals quality of RNA was determined by an Agilent 2100 Bioanalyzer (Santa Clara, CA). RNA samples with RIN quality values >7.5 were included in RT-qPCR assays. RNA was reverse transcribed using iScript Reverse Transcription Supermix for RT-PCR (Bio-Rad Laboratories, Hercules, CA). Then

RT-qPCR was conducted using SsoFast Probes Supermix with ROX (Bio-Rad Laboratories), along with predesigned PrimeTime RT-qPCR Assays (Integrated DNA Technologies, (IDT), Coralville, IA) and 2 μL cDNA to detect the transcript sequence of interest. All reactions were run in duplicate. RT-qPCR reactions were run on an Applied Biosystems 7900HT Fast Real-Time PCR System (Carlsbad, CA). A fivefold RNA dilution series was utilized to determine efficiency of each qPCR assay. Amplification data were analyzed with Sequence Detection Software v. 2.2.2 (Applied Biosystems). Expression was normalized to an endogenous control (18S ribosomal RNA (*RN18S*; IDT)) using methods described by Pfaffl [36]. A gene expression ratio was calculated that is positively related to expression level and takes the efficiency of each assay into consideration. Briefly, gene expression ratio (GER) = target gene efficiency$^{(\text{CT target reference} - \text{CT target gene})}$/control gene efficiency$^{(\text{CT control reference} - \text{CT control gene})}$. The reference value (calibrator) used for a given gene was the average Ct of all samples (in both strains) for that gene. Efficiency was calculated using the slope of the standard curve ($10^{(-1/\text{slope})}$).

Actn2, *Casq1*, *Glut4*, *Lepr*, and *Mstn* expression levels were measured in both the nucleus accumbens (central) and soleus (peripheral) tissue of animals exposed to running wheels. Based on evidence in the literature and results from the wheel-exposed animals, expressions of *Actn3*, *Actn2*, *Casq1*, *Glut4*, *Lepr*, and *Mstn* were assayed in the soleus of the naïve animals, while *Drd2*, *Mc4r*, *Papss2*, and *Lepr* were measured in the nucleus accumbens of the naïve animals.

2.4. Statistics. Gene expression data were checked for normality using a two-sided F test (JMP 10.0, SAS Institute, Cary, NC). If the expression ratio was not normal ($P < 0.05$), the expression data were analyzed by Chi-square nonparametric approaches. Normally distributed expression ratios were compared by a pooled t-test (if variances were equal) or Student's t-test (if variances were not equal). Alpha values were set *a priori* at 0.05. In all analyses, expression values that were greater than 2.5 standard deviations away from the mean were considered outliers and eliminated from the dataset. If differential expression was observed, data were subsequently analyzed for sex differences.

3. Results

No difference in body weight was observed between strains for mice exposed to the running wheel (23.7 g ± 2.6 g C57L/J versus 23.3 g ± 3.6 g C3H/HeJ, mean ± SD; $P = 0.93$) or naïve animals (24.0 g ± 2.6 g C57L/J versus 24.4 g ± 2.1 g C3H/HeJ; $P = 0.43$). In naïve animals, percent body fat was not different between the strains (12.7% ± 1.6% C57L/J versus 14.5% ± 1.8% C3H/HeJ; $P = 0.09$).

Differential haplotypes were exhibited across the entire transcribed region of *Actn2*, *Casq1*, *Drd2*, *Lepr*, and *Papss2*, as reflected by a number of SNPs in each gene (Table 1). The strains exhibited similar haplotype patterns for *Mstn*, *Glut4*, *Mc4r*, and *Actn3* (i.e., no differential SNPs). *Casq1* ($P = 0.0003$) and *Mstn* ($P = 0.002$) transcript expression in the soleus was found to be different between the high-and low-active mice exposed to a running wheel (Table 2; Figure 1), while there were no differences observed in *Actn2* ($P = 0.55$), *Glut4* ($P = 0.20$), or *Lepr* ($P = 0.85$). However, when these genes were evaluated in the soleus between strains of naïve animals, differences in expression of *Casq1* and *Mstn* were not observed ($P = 0.40$ and $P = 0.27$, resp.). No differential expression was observed in any of the genes evaluated in the nucleus accumbens (*Actn2*, $P = 0.13$; *Casq1*, $P = 0.64$; *Glut4*, $P = 0.58$; *Lepr*, $P = 0.72$; *Mstn*, $P = 0.37$; Table 2) in animals exposed to the running wheels.

In naïve animals, gene expression results indicated no differential expression between high- and low-active animals for any of the genes in the soleus (*Actn2*, $P = 0.58$; *Actn3*, $P = 0.58$; *Casq1*, $P = 0.40$; *Glut4*, $P = 0.22$; *Lepr*, $P = 0.82$; *Mstn*, $P = 0.27$; Table 2). No difference was seen between strains in *Drd2* ($P = 0.06$), *Lepr* ($P = 0.18$), *Mc4r* ($P = 0.08$), or *Papss2* ($P = 0.40$) in the nucleus accumbens (Table 2). Gene expression differences between sexes were not observed in either strain.

4. Discussion

As an extension of quantitative genetic approaches that have been used to investigate the genetic control of physical activity, several genes have been suggested to be associated with activity regulation with little or no supporting physiological evidence for their involvement. This study's purpose was to investigate whether nine putative candidate genes had interval-specific haplotype structure variability and were actually expressed differentially between high- and low-active mice. Although differential gene expression is not the only determinant of whether a gene is a candidate gene, it is one line of evidence suggesting that a gene may be involved in regulation of a particular phenotype. We found that prior exposure to a running wheel, in and of itself, caused changes in gene expression, demonstrating a training effect. Thus, as our goal was to investigate innate differences in gene expression between strains with varying activity levels, expression was subsequently measured in naïve mice. Interestingly, although these strains of mice have distinctively diverse levels of activity, none of the genes evaluated were differentially expressed between naïve high- and low-active mice in the nucleus accumbens or soleus. The majority of genes evaluated in this study were chosen from genome-wide association studies utilizing genomic DNA, which does not correspond to transcript levels. Therefore, differential expression between phenotypes should not necessarily be expected from genotype association studies alone. While not ruling these genes out as potential regulators of physical activity, our data provides evidence that differences in activity are not due to variability in transcript abundance in this model. Likewise, given that there are no SNPs located in protein-coding regions for any of the genes evaluated genomic variability between the strains in these genes does not account for phenotypic differences between strains. Thus, while association and functional relevance provide two lines of evidence, we suggest that further functional validation of these genes is necessary, possibly including investigation of post-transcriptional modification and differences in

Table 1: SNP variation between high- and low-active mice.

Gene	Sequence accession no.	Chromosome no.	SNP position		Nucleotide change		Region
			On chromosome	In gene	C3H/HeJ	C57L/J	
Actn2	NC_000079	1	12269977	551	T	C	intron 1
			12277437	8011	G	A	intron 1
			12282994	13568	C	T	intron 1
			12290237	20811	G	A	intron 1
			12299638	30212	A	T	intron 2
			12300030	30604	A	C	intron 2
			12301365	31939	G	A	intron 4
			12336213	66787	C	T	intron 18
			12336314	66888	G	A	intron 18
Casq1	NC_000067	1	172213506	3612	T	G	intron 3
			172213681	3787	A	G	intron 3
Drd2	NC_000075	9	49344757	4095	G	A	5′ UTR
			49353453	12791	A	G	5′ UTR
			49367545	26883	T	C	5′ UTR
			49372928	32266	G	A	5′ UTR
			49372964	32302	C	T	5′ UTR
			49373405	32743	A	T	5′ UTR
			49376079	35417	A	G	5′ UTR
			49376164	35502	T	C	5′ UTR
			49396183	55521	G	A	intron 1
Lepr	NC_000070	4	101802391	84984	C	T	intron 17
			101802709	85302	T	G	intron 17
Papss2	NC_000085	19	32607198	11483	T	C	intron 1
			32607669	11954	G	A	intron 1
			32613744	18029	C	T	intron 1
			32626984	31269	C	T	intron 1
			32633562	37847	T	C	intron 1
			32649103	53388	A	C	intron 7
			32653353	57638	G	A	intron 8
			32665695	69980	C	A	3′ UTR

Haplotype and SNP data were obtained from the Mouse Phylogeny Viewer (http://msub.csbio.unc.edu/). C57L/J are high-active mice and C3H/HeJ are low-active mice.

regulatory mechanisms as additional lines of support for the gene's candidacy in relation to any phenotype regulation.

It has been well established that genetic background is a significant regulator of daily physical activity in both humans and mice, with little input from common environmental influences [5–15, 23, 37–39]. In spite of the mounting evidence confirming genetic control of physical activity, little is known about the actual regulatory mechanisms, including the identity of the responsible genes. Identification of potential candidate genes has been primarily through speculated functional relevance and/or location within an identified quantitative trait locus, with little or no functional validation. More often than not, further examination of potential candidate genes has indicated that use of QTL location/perceived physiological relevance results in a large number of false positive quantitative trait genes (QTG). Indeed, the early promise of discovering QTG from QTL has had limited success, with some authors reporting less than a 1% success rate in finding QTG in QTL [16]. Flint et al. [16] also suggest that candidate genes derived from most QTL studies account for very small phenotypic effects. Therefore, the small effects of putative candidate genes associated with QTL, combined with sequence variance and position of the QTL relative to the coding region of the gene, make determining the actual causative gene and function using traditional quantitative genetic approaches extremely difficult.

For example, De Moor et al. [32] found novel SNPs in the *Papss2* gene region related to activity levels in humans, suggesting *Papss2* was associated with leisure time exercise behavior. *Papss2* produces a sulfonation enzyme that modifies macronutrients and exogenous compounds and is expressed in many tissues including skeletal muscle and brain [32]. In our mouse model, however, we found no differences in *Papss2* expression between our high- and low-active mice in the nucleus accumbens, a region of the brain that has been suggested as a primary site of activity regulation [25, 40]. *Papss2* was not expressed at observable amounts in the soleus of our mice using the methods employed, although this may

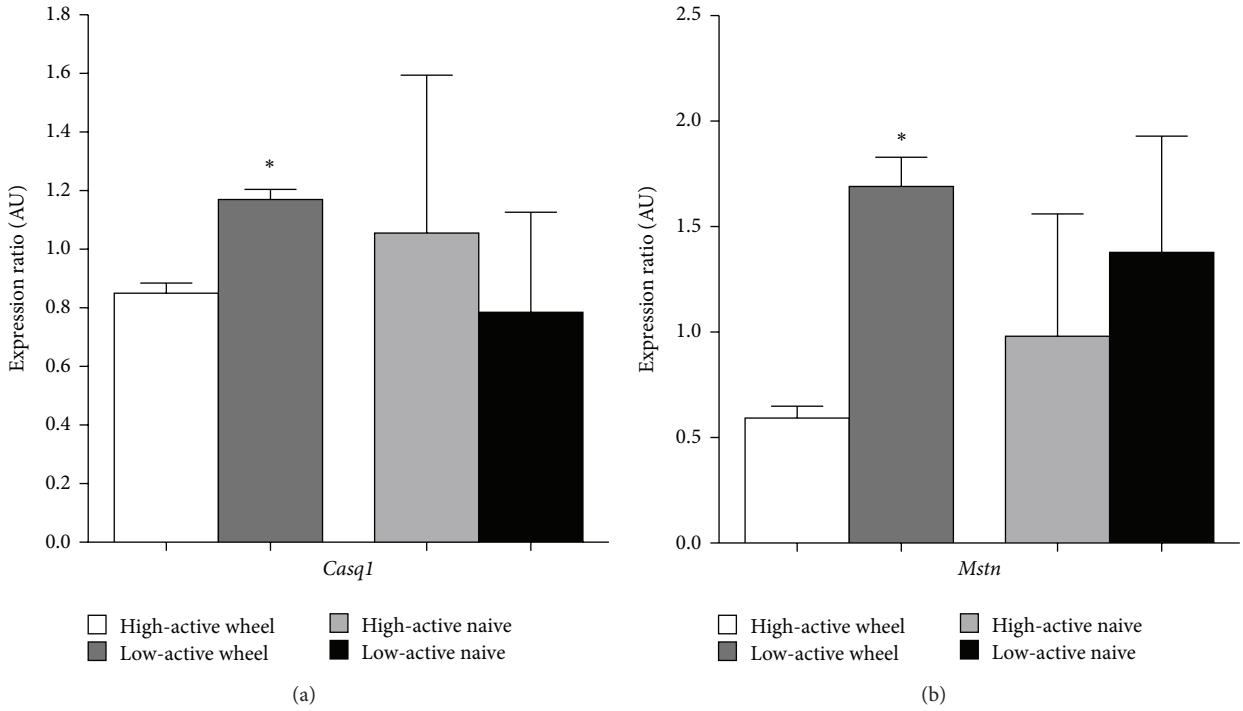


Figure 1: Expression of *Casq1* (a) and *Mstn* (b) in soleus muscle. In both panels, comparisons made between strains within activity state (high-active versus low-active). * Significantly different from wheel-exposed high-active mice ($P < 0.05$). Values are mean ± SD. AU, arbitrary units.

Table 2: Gene expression ratios.

	Gene	Tissue	Expression ratio (AU)		P value
			C57L/J (high-active)	C3H/HeJ (low-active)	
Wheel exposed	*Actn2*	sol	1.1 ± 0.32	0.95 ± 0.22	0.55
	Casq1	sol	0.85 ± 0.04	1.17 ± 0.04	0.0003*
	Glut4	sol	0.94 ± 0.07	1.08 ± 0.13	0.2
	Lepr	sol	1.0 ± 0.27	1.05 ± 0.26	0.85
	Mstn	sol	0.59 ± 0.05	1.7 ± 0.14	0.002*
	Actn2	NA	0.14 ± 0.12	0.44 ± 0.53	0.13
	Casq1	NA	0.16 ± 0.14	0.12 ± 0.05	0.64
	Glut4	NA	0.17 ± 0.10	0.2 ± .07	0.58
	Lepr	NA	1.77 ± 2.47	1.33 ± 0.73	0.72
	Mstn	NA	0.12 ± 0.07	0.22 ± 0.22	0.37
Naïve	*Actn2*	sol	1.27 ± 0.96	1.33 ± 1.1	0.58
	Actn3	sol	1.25 ± 0.94	1.35 ± 1.57	0.58
	Casq1	sol	1.05 ± 0.54	0.79 ± 0.40	0.4
	Glut4	sol	1.22 ± 0.56	0.89 ± 0.19	0.22
	Lepr	sol	0.94 ± 0.66	1.03 ± 0.40	0.82
	Mstn	sol	0.98 ± 0.58	1.38 ± 0.55	0.27
	Drd2	NA	1.69 ± 0.85	0.80 ± 0.47	0.06
	Lepr	NA	0.35 ± 0.09	0.26 ± 0.10	0.18
	Mc4r	NA	0.88 ± 0.19	1.21 ± 0.36	0.08
	Papss2	NA	1.17 ± 0.33	0.97 ± 0.48	0.4

Gene expression ratio was calculated from Pfaffl [36]. Values are described as mean ± SD; "sol" indicates soleus; "NA" nucleus accumbens. * Indicates differential expression of gene between strains.

have been due to the small quantities of RNA available to use in the reverse transcription reaction. Interestingly, all of the SNPs found in De Moor's work were located in intron 1 of *Papss2*. Likewise, we found five SNPs in intron 1 of *Papss2* between our strains of mice (Table 1); however, a BLAST comparison of the human and mouse gene sequence shows that none of the SNPs identified seem to match between species. Intronic SNPs are spliced out of the mRNA, therefore not affecting sequence of the mature transcript. Intronic sequence variance would only impact transcript levels through alteration of miRNA sequences or by location in the promoter region. None of these modes of regulation are currently presented in the literature for *Papss2*. As DNA sequence variation does not have a causal relationship with transcript abundance, we should not be surprised that our results differ from those of De Moor's et al. [32].

Unraveling the regulatory mechanisms of voluntary activity is further complicated by a variety of genetic mechanisms contributing to transcriptional regulation. Therefore, it is critical that potential candidate genes be examined thoroughly before they become entrenched in the literature as "causative" of a phenotype. With only 2% of the human genome actually coding for proteins, it is not surprising that mechanisms other than structural gene variation contribute to differences in phenotype. Regulatory regions of noncoding sequences may be contributing to regulation of voluntary physical activity through a variety of mechanisms (e.g., miRNA, siRNA, and ribosomal binding proteins [41]). These regulatory mechanisms have not been fully characterized and may be contributing to activity regulation as we have previously suggested [10]. We have shown that other genetic mechanisms such as epistasis (gene interactions) and pleiotropy (one gene has multiple effects) can affect physical activity regulation [23, 42, 43]. *Glut4* was selected as a putative candidate gene for inherent physical activity regulation based on QTL association [21, 23, 27] and from functional relevance [33]. *Glut4* functions to move glucose across the plasma membrane of cells, is found in skeletal muscle, and is induced by insulin or exercise [33]. Tsao and colleges [33] observed that mice with *Glut4* overexpression ran four times further than controls. *Glut4* was found to be close to the "mini-muscle" gene region [21] as well as near a QTL exhibiting significant epistasis for distance run [23]. Considering these previous physical and functional experiments of the role of *Glut4* in physical activity, we expected to see differential expression between our inherently high- and low-active strains of mice. However, like *Papss2*, we observed no differences in expression. It is possible that *Glut4* may function through epistasis with other genes; thus differential expression of *Glut4* itself would not be detected.

Considering the multitude of mechanisms contributing to gene regulation, it is not unreasonable that the only differential gene expression observed between strains in this study was due to a training effect. It is well known that a variety of perturbations can influence gene expression, such as repeated exercise bouts altering transcript levels in skeletal muscle and brain tissue [44, 45]. While we had not previously shown alteration in brain gene expression after running wheel activity [25], it is not surprising that even a minimal exposure to wheel running (seven days) produced changes in some of the skeletal muscle genes measured (*Mstn* and *Casq1*). The literature is ambiguous for *Mstn* expression changes in skeletal muscle with endurance exercise training, showing variable results depending on species, training mode, and time elapsed after exercise session, amongst other factors [46, 47]. *Casq1* protein levels have been shown to decrease in the soleus with endurance training by Kinnunen and Mänttäri [48], which is comparable to the gene expression results seen in our high-active mice. These observations highlight the need to use naïve animals when investigating inherent gene expression differences.

From our gene expression results we can conclude that differences in inherent variation in activity levels are not due to differences in transcript abundance of the genes investigated. Additionally, we propose that expression differences seen in *Mstn* and *Casq1* in the wheel-exposed animals did not arise through genomic structural differences. Our interstrain haplotype results indicated that five of the nine genes (*Actn2*, *Casq1*, *Drd2*, *Lepr*, and *Papss2*) contained SNPs, although none of the SNPs were located in coding regions. *Drd2* contains 5′ UTR SNPs; however, no known regulatory regions were found at these locations. None of the SNPs determined in this study were found to have obvious mechanisms of variation.

There are limitations that warrant consideration in this study, beginning with the tissues assessed, the number of strains evaluated, and the inability to compare between wheel treatments. Only slow-twitch oxidative muscle fiber was evaluated in this study without consideration of fast-twitch fibers. Previous studies [10] have shown that the average daily duration of activity in the high-active C57L/J mice was lengthy (351.1 ± 61.6 mins/day) suggesting that the slow-twitch fibers would be the primary locomotor muscles used; however, the genes we evaluated might be expressed differently in fast-twitch fibers. For instance, while Kinnunen et al. [48] found *Casq1* protein levels to decrease in soleus fibers, *Casq1* was increased in fast-twitch EDL muscle with endurance training. Additionally, while the nucleus accumbens was removed with the utmost care [26], it is possible that surrounding portions of hypothalamus were dissected along with the nucleus accumbens, leading to variability in expression levels. We do not expect this to be the case however as variability of the expression ratios of the naïve animals (as to not account for any variability caused by training adaptation) is consistent between genes in this study. Gene expression variability in the nucleus accumbens was also similar to that seen by Knab et al. [25]. Therefore, as the nucleus accumbens is considered the central reward center and a potential site of activity regulation [25, 40], we believe that our results reflect true differences in gene expression. Furthermore, it should be noted that only two strains of mice were evaluated in this study. It is possible that the mechanisms controlling activity in these two strains are specific to only those strains. While there are no direct data regarding this point, studies from our lab have shown that physical activity-QTL derived using two strain intercross methods (i.e., positional cloning approaches) [12] differs from physical activity-QTL derived using multiple strain,

genome-wide association approaches [10]. Thus, it is possible that the potential candidate genes we examined in this study might be expressed differentially in other strains. Finally, it is worth mentioning that gene expression comparisons were not made between wheel-exposed and naïve animals due to variability between these groups. Given that differences between the wheel-running animals and the naïve animals were not our primary hypothesis, as well as the fact that the animals were housed at different locations (wheel-exposed animals were housed at UNC-Charlotte, while naïve animals were housed at Texas A&M) which has been known to cause different phenotypic responses [49], gene expression comparisons between wheel-exposed mice and naïve mice may possibly lead to an inaccurate depiction of the physiological differences between these groups.

In conclusion, results showed augmented gene expression of *Casq1* and *Mstn* in the soleus of low-active mice that were exposed to a running wheel. In addition, we found that exposure to a running wheel resulted in differences in transcript abundance in and of itself, implying a training effect and highlighting the need to measure gene expression in naïve mice when studying naïve genetic regulation. None of the nine suggested activity-related candidate genes were differentially expressed between inherently high- and low-active mice in soleus or nucleus accumbens. Five genes have genomic structural differences (*Actn2*, *Casq1*, *Drd2*, *Lepr,* and *Papss2*); however, no SNPs were found in coding regions nor were any associations made between any 3′ UTR SNPs and known miRNA targets. Thus, the SNPs we found do not indicate an obvious mechanism of variation. As the understanding of genetic regulation continues to mature, it is clear that considering genomic structural variation solely, as suggested by association studies, is not adequate to establish a gene's candidacy for a regulatory role and that information regarding transcriptional expression, transcriptional regulatory mechanisms, and proteomic data is needed to establish solid genetic candidates for further causal investigations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Michelle Dawes performed assays, analyzed results, and drafted the paper and figures. Trudy Moore-Harrison designed real-time experiments, conducted real-time assays, and wrote/edited the first version of the paper. Alicia T. Hamilton designed experiments, conducted qPCR assays, and edited the paper. Tyrone Ceaser helped design and implement haplotype comparison analyses and edited the paper. Kelli J. Kochan assisted with assays. Kelli J. Kochan and Penny K. Riggs assisted with results. J. Timothy Lightfoot was primarily responsible for conception, design of research, analysis of results, project financial support development, and paper preparation.

Acknowledgments

The project described was supported by a National Institutes of Health Grant (NIAMS AR050085—J. T. Lightfoot) and by a Texas A&M start-up fund (Lightfoot), as well as student support from the Whole Systems Genomics Initiative at Texas A&M. The authors would like to thank the Vivarium staffs of both Texas A&M and UNC Charlotte. Additionally, the authors wish to thank David Ferguson, Emily Schmitt, and Analisa Jimenez for assistance with data collection.

References

[1] C. K. Roberts and R. J. Barnard, "Effects of exercise and diet on chronic disease," *Journal of Applied Physiology*, vol. 98, no. 1, pp. 3–30, 2005.

[2] B. R. Belcher, D. Berrigan, K. W. Dodd, B. A. Emken, C.-P. Chou, and D. Spruijt-Metz, "Physical activity in US youth: effect of race/ethnicity, age, gender, and weight status," *Medicine and Science in Sports and Exercise*, vol. 42, no. 12, pp. 2211–2221, 2010.

[3] R. P. Troiano, D. Berrigan, K. W. Dodd, L. C. Mâsse, T. Tilert, and M. Mcdowell, "Physical activity in the United States measured by accelerometer," *Medicine and Science in Sports and Exercise*, vol. 40, no. 1, pp. 181–188, 2008.

[4] T. Rankinen and C. Bouchard, "Invited commentary: physical activity, mortality, and genetics," *American Journal of Epidemiology*, vol. 166, no. 3, pp. 260–262, 2007.

[5] M. F. W. Festing, "Wheel activity in 26 strains of mouse," *Laboratory Animals*, vol. 11, no. 4, pp. 257–258, 1977.

[6] A. M. Joosen, M. Gielen, R. Vlietinck, and K. R. Westerterp, "Genetic analysis of physical activity in twins," *American Journal of Clinical Nutrition*, vol. 82, no. 6, pp. 1253–1259, 2005.

[7] J. M. Kaprio, M. Koskenvuo, and S. Sarna, "Cigarette smoking, use of alcohol and leisure-time activity among same-sexed adult male twins," in *Twin Research 3: Epidemiological and Clinical Studies*, L. Gedda, P. Parisi, and W. E. Nance, Eds., Progress in Clinical and Biological Research, pp. 37–46, Alan R. Liss, New York, NY, USA, 1981.

[8] D. S. Lauderdale, R. Fabsitz, J. M. Meyer, P. Sholinsky, V. Ramakrishnan, and J. Goldberg, "Familial determinants of moderate and intense physical activity: a twin study," *Medicine and Science in Sports and Exercise*, vol. 29, no. 8, pp. 1062–1068, 1997.

[9] I. Lerman, B. C. Harrison, K. Freeman et al., "Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains," *Journal of Applied Physiology*, vol. 92, no. 6, pp. 2245–2255, 2002.

[10] J. T. Lightfoot, L. Leamy, D. Pomp et al., "Strain screen and haplotype association mapping of wheel running in inbred mouse strains," *Journal of Applied Physiology*, vol. 109, no. 3, pp. 623–634, 2010.

[11] J. T. Lightfoot, M. J. Turner, M. Daves, A. Vordermark, and S. R. Kleeberger, "Genetic influence on daily wheel running activity level," *Physiological Genomics*, vol. 19, pp. 270–276, 2004.

[12] J. T. Lightfoot, M. J. Turner, D. Pomp, S. R. Kleeberger, and L. J. Leamy, "Quantitative trait loci for physical activity traits in mice," *Physiological Genomics*, vol. 32, no. 3, pp. 401–408, 2008.

[13] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *Plos One*, vol. 1, no. 1, article e22, 2006.

[14] J. H. Stubbe, D. I. Boomsma, and E. J. C. De Geus, "Sports participation during adolescence: a shift from environmental to genetic factors," *Medicine and Science in Sports and Exercise*, vol. 37, no. 4, pp. 563–570, 2005.

[15] J. G. Swallow, T. Garland Jr., P. A. Carter, W.-Z. Zhan, and G. C. Sieck, "Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*)," *Journal of Applied Physiology*, vol. 84, no. 1, pp. 69–76, 1998.

[16] J. Flint, W. Valdar, S. Shifman, and R. Mott, "Strategies for mapping and cloning quantitative trait genes in rodents," *Nature Reviews Genetics*, vol. 6, no. 4, pp. 271–286, 2005.

[17] K. DiPetrillo, X. Wang, I. M. Stylianou, and B. Paigen, "Bioinformatics toolbox for narrowing rodent quantitative trait loci," *Trends in Genetics*, vol. 21, no. 12, pp. 683–692, 2005.

[18] D. J. Good, C. A. Coyle, and D. L. Fox, "Nhlh2: a basic helix-loop-helix transcription factor controlling physical activity," *Exercise and Sport Sciences Reviews*, vol. 36, no. 4, pp. 187–192, 2008.

[19] J. S. Rhodes and T. Garland Jr., "Differential sensitivity to acute administration of Ritalin, apormorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior," *Psychopharmacology*, vol. 167, no. 3, pp. 242–250, 2003.

[20] K. A. Frazer, E. Eskin, H. M. Kang et al., "A sequence-based variation map of 8.27 million SNPs in inbred mouse strains," *Nature*, vol. 448, no. 7157, pp. 1050–1053, 2007.

[21] J. Hartmann, T. Garland, R. M. Hannon, S. A. Kelly, G. Muñoz, and D. Pomp, "Fine mapping of "mini-muscle," a recessive mutation causing reduced hindlimb muscle mass in mice," *Journal of Heredity*, vol. 99, no. 6, pp. 679–687, 2008.

[22] S. A. Kelly, D. L. Nehrenberg, J. L. Peirce et al., "Genetic architecture of voluntary exercise in an advanced intercross line of mice," *Physiological Genomics*, vol. 42, no. 2, pp. 190–200, 2010.

[23] L. J. Leamy, D. Pomp, and J. T. Lightfoot, "An epistatic genetic basis for physical activity traits in mice," *Journal of Heredity*, vol. 99, no. 6, pp. 639–646, 2008.

[24] D. L. Nehrenberg, S. Wang, R. M. Hannon, T. Garland, and D. Pomp, "QTL underlying voluntary exercise in mice: interactions with the "mini muscle" locus and sex," *Journal of Heredity*, vol. 101, no. 1, pp. 42–53, 2009.

[25] A. M. Knab, R. S. Bowen, A. T. Hamilton, A. A. Gulledge, and J. T. Lightfoot, "Altered dopaminergic profiles: implications for the regulation of voluntary physical activity," *Behavioural Brain Research*, vol. 204, no. 1, pp. 147–152, 2009.

[26] A. M. Knab, R. S. Bowen, A. T. Hamilton, and J. T. Lightfoot, "Pharmacological manipulation of the dopaminergic system affects wheel-running activity in differentially active mice," *Journal of Biological Regulators and Homeostatic Agents*, vol. 26, no. 1, pp. 119–129, 2012.

[27] J. T. Lightfoot, "Can you be born a couch potato? The genomic regulation of physical activity," in *Exercise Genomics*, L. S. Pescatello and S. M. Roth, Eds., Molecular and Translational Medicine, pp. 45–72, Humana Press, New York, NY, USA, 2011.

[28] R. M. Murphy, N. T. Larkins, J. P. Mollica, N. A. Beard, and G. D. Lamb, "Calsequestrin content and SERCA determine normal and maximal Ca^{2+} storage levels in sarcoplasmic reticulum of fast—and slow-twitch fibres of rat," *Journal of Physiology*, vol. 587, no. 2, pp. 443–460, 2009.

[29] R. L. Simonen, T. Rankinen, L. Pérusse et al., "A dopamine D2 receptor gene polymorphism and physical activity in two family studies," *Physiology and Behavior*, vol. 78, no. 4-5, pp. 751–757, 2003.

[30] L. Richert, T. Chevalley, D. Manen, J.-P. Bonjour, R. Rizzoli, and S. Ferrari, "Bone mass in prepubertal boys is associated with a Gln223Arg amino acid substitution in the leptin receptor," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 11, pp. 4380–4386, 2007.

[31] G. Cai, S. A. Cole, N. Butte et al., "A quantitative trait locus on chromosome 18q for physical activity and dietary intake in Hispanic children," *Obesity*, vol. 14, no. 9, pp. 1596–1604, 2006.

[32] M. H. M. De Moor, Y.-J. Liu, D. I. Boomsma et al., "Genome-wide association study of exercise behavior in Dutch and American adults," *Medicine and Science in Sports and Exercise*, vol. 41, no. 10, pp. 1887–1895, 2009.

[33] T.-S. Tsao, J. Li, K. S. Chang et al., "Metabolic adaptations in skeletal muscle overexpressing GLUT4: effects on muscle and physical activity," *The FASEB Journal*, vol. 15, no. 6, pp. 958–969, 2001.

[34] H. Yang, J. R. Wang, J. P. Didion et al., "Subspecific origin and haplotype diversity in the laboratory mouse," *Nature Genetics*, vol. 43, no. 7, pp. 648–655, 2011.

[35] A. M. Knab, R. S. Bowen, T. Moore-Harrison, A. T. Hamilton, M. J. Turner, and J. T. Lightfoot, "Repeatability of exercise behaviors in mice," *Physiology and Behavior*, vol. 98, no. 4, pp. 433–440, 2009.

[36] M. W. Pfaffl, "A new mathematical model for relative quantification in real-time RT-PCR," *Nucleic Acids research*, vol. 29, no. 9, article e45, 2001.

[37] S. Carlsson, T. Andersson, P. Lichtenstein, K. Michaëlsson, and A. Ahlbom, "Genetic effects on physical activity: results from the Swedish twin registry," *Medicine and Science in Sports and Exercise*, vol. 38, no. 8, pp. 1396–1401, 2006.

[38] L. Perusse, A. Tremblay, C. Leblanc, and C. Bouchard, "Genetic and environmental influences on level of habitual physical activity and exercise participation," *American Journal of Epidemiology*, vol. 129, no. 5, pp. 1012–1022, 1989.

[39] J. G. Swallow, P. A. Carter, and T. Garland Jr., "Artificial selection for increased wheel-running behavior in house mice," *Behavior Genetics*, vol. 28, no. 3, pp. 227–237, 1998.

[40] A. M. Knab and J. T. Lightfoot, "Does the difference between physically active and couch potato lie in the dopamine system?" *International Journal of Biological Sciences*, vol. 6, no. 2, pp. 133–150, 2010.

[41] E. Szostak and F. Gebauer, "Translational control by 3′-UTR-binding proteins," *Briefings in Functional Genomics*, vol. 12, no. 1, pp. 58–65, 2012.

[42] L. J. Leamy, D. Pomp, and J. T. Lightfoot, "Genetic variation in the pleiotropic association between physical activity and body weight in mice," *Genetics Selection Evolution*, vol. 41, no. 1, article 41, 2009.

[43] L. J. Leamy, D. Pomp, and J. T. Lightfoot, "Epistatic interactions of genes influence within-individual variation of physical activity traits in mice," *Genetica*, vol. 139, no. 6, pp. 813–821, 2011.

[44] A. Allen and C. Messier, "Plastic changes in the astrocyte GLUT1 glucose transporter and beta-tubulin microtubule protein following voluntary exercise in mice," *Behavioural Brain Research*, vol. 240, pp. 95–102, 2013.

[45] V. G. Coffey and J. A. Hawley, "The molecular bases of training adaptation," *Sports Medicine*, vol. 37, no. 9, pp. 737–763, 2007.

[46] A. Matsakas, A. Friedel, T. Hertrampf, and P. Diel, "Short-term endurance training results in a muscle-specific decrease

of myostatin mRNA content in the rat," *Acta Physiologica Scandinavica*, vol. 183, no. 3, pp. 299–307, 2005.

[47] T. Schiffer, S. Geisler, B. Sperlich, and H. K. Strüder, "MSTN mRNA after varying exercise modalities in humans," *International Journal of Sports Medicine*, vol. 32, no. 9, pp. 683–687, 2011.

[48] S. Kinnunen and S. Mänttäri, "Specific effects of endurance and sprint training on protein expression of calsequestrin and SERCA in mouse skeletal muscle," *Journal of Muscle Research and Cell Motility*, vol. 33, no. 22, pp. 123–130, 2012.

[49] J. C. Crabbe, D. Wahlsten, and B. C. Dudek, "Genetics of mouse behavior: interactions with laboratory environment," *Science*, vol. 284, no. 5420, pp. 1670–1672, 1999.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 601368, 15 pages
http://dx.doi.org/10.1155/2013/601368

Review Article

Do Telomeres Adapt to Physiological Stress? Exploring the Effect of Exercise on Telomere Length and Telomere-Related Proteins

Andrew T. Ludlow,[1] Lindsay W. Ludlow,[2] and Stephen M. Roth[3]

[1] *Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX 75309, USA*
[2] *Department of Applied Physiology and Wellness, Southern Methodist University, Dallas, TX 75206, USA*
[3] *Department of Kinesiology, School of Public Health, University of Maryland, College Park, MD 20742-2611, USA*

Correspondence should be addressed to Stephen M. Roth; sroth1@umd.edu

Received 13 October 2013; Accepted 26 November 2013

Academic Editor: J. Timothy Lightfoot

Aging is associated with a tissue degeneration phenotype marked by a loss of tissue regenerative capacity. Regenerative capacity is dictated by environmental and genetic factors that govern the balance between damage and repair. The age-associated changes in the ability of tissues to replace lost or damaged cells is partly the cause of many age-related diseases such as Alzheimer's disease, cardiovascular disease, type II diabetes, and sarcopenia. A well-established marker of the aging process is the length of the protective cap at the ends of chromosomes, called telomeres. Telomeres shorten with each cell division and with increasing chronological age and short telomeres have been associated with a range of age-related diseases. Several studies have shown that chronic exposure to exercise (i.e., exercise training) is associated with telomere length maintenance; however, recent evidence points out several controversial issues concerning tissue-specific telomere length responses. The goals of the review are to familiarize the reader with the current telomere dogma, review the literature exploring the interactions of exercise with telomere phenotypes, discuss the mechanistic research relating telomere dynamics to exercise stimuli, and finally propose future directions for work related to telomeres and physiological stress.

1. Introduction

Broadly, aging is defined as the accumulation of cellular damage that results in a loss of cellular and organismal fitness. Aging is marked by a substantial decrease in the regenerative potential of several cell types, including immune cells and skeletal muscle cells [1, 2]. Both genetic and environmental factors dictate the rate of tissue regeneration and the balance between accumulation and removal of cellular damage. Accumulation of unrepaired cellular damage and a lack of tissue regeneration via cell replication result not only in aging-related phenotypes (grey hair, wrinkled skin, etc.), but also in several age-related diseases such as Alzheimer's disease, cardiovascular disease, type II diabetes, and sarcopenia [3, 4]. Aging impacts the genome in several ways. For example, aging modifies the structure-function relationship of the genome through accumulation of mutations, changing epigenetic profiles (both changes in DNA methylation patterns and histone modification) and altered telomere dynamics [5, 6]. How physical activity and exercise training can modify the age-associated genomic changes is beginning to be explored and thus far has produced exciting results.

Interestingly, all of the aforementioned age-related diseases are modified by physical activity. Further, recent evidence indicates that telomere length is also associated with several age-related diseases, and that telomere length and the suite of proteins that maintain telomere length are altered by changes in physical activity level [7]. Thus, the purpose of this review is to describe the basic biological implications of telomeres and telomere shortening, to explain the function of the suite of telomere-associated proteins, and to review the recent literature involving telomere length and telomere-associated proteins as they are affected by exercise training or physical activity. We further discuss the potential

mechanisms of how exercise may cause beneficial adaptations in the telomere length maintenance system.

2. Human Telomere Biology

Recently, short telomeres have become a widely accepted molecular/cellular hallmark of aging [8]. Telomeres are repetitive DNA sequences ($5'$-TTAGGG$_n$-$3'$) at the ends of linear chromosomes [9]. With each cell division, telomeres shorten by about 60 base pairs due to the inability of DNA polymerase to fully replicate the chromosome end, a phenomena referred to as the end-replication problem (Figure 1; [10, 11]). In this sense, telomeres act as a mitotic clock that "records" the number of divisions a cell has undergone. When telomeres shorten to a critical length, the chromosome ends are recognized as DNA-double-strand breaks by the DNA damage response system [12–15]. Therefore, the second function of the telomere is to prevent a DNA damage response at the chromosome end through maintenance of a sufficient length, subsequently solving the "end-protection problem" [13, 14]. Thus, critically short telomeres in aged cells may be recognized as DNA double-strand breaks, ultimately causing the cell to enter senescence (Figure 1; [14, 16–21]). An increase in senescent cells, which may be observed in response to critically shortened telomeres, is a major part of tissue dysfunction with aging and is associated with age-related phenotypes (Figure 1; [22–24]). While senescence may occur via nontelomeric mechanisms, accumulation of replicative senescent cells (i.e., telomere-driven senescence) in tissues significantly contributes to the aging process.

Repeated cell division is one mechanism for reduced telomere length; however, other mechanisms also contribute to the total rate of telomere shortening in cells [25]. For example, chronic exposure to DNA damaging agents (e.g., UV, oxidative stress, and inflammation) may result in a sudden loss of large amounts of telomere DNA [26]. Therefore, telomeres are shortened by a combination of chronic, gradual shortening due to repeated cell division and acute, stochastic shortening of a few or single telomeres in a cell caused by DNA damaging agents.

Certain cell types, even with continuous cell division, are able to maintain their telomeres despite the end-replication problem. In highly mitotic cells (i.e., germline cells, stem cells, specialized subsets of immune cells, and cancer cells), the gradual telomere shortening due to the end-replication problem is overcome by the enzyme telomerase (Figure 1; [10]). In cells with telomerase, telomere length is maintained with each cell division and thus these cells are essentially immortal and able to undergo many population doublings without the accumulation of short telomeres (Figure 1). Therefore, the present review will focus not only on telomere length, but also on the suite of telomere-binding proteins in immune cells, skeletal muscle, and other tissues impacted by physical activity or exercise.

2.1. Proteins Involved in the Regulation of Human Telomere Length: Telomerase and Shelterin. Telomerase is a ribonucleoprotein that consists of two central components: a protein reverse transcriptase component (TERT) and an RNA template (TERC) [6, 27, 28]. From a functional standpoint, telomerase is thought to be preferentially recruited to short telomeres [29–33]. Current dogma is that telomerase activity is confined to developing cells, adult stem cells, germline cells, and subsets of immune cells and that most somatic tissues have low or undetectable telomerase; thus, telomerase in healthy adult tissues is tightly regulated (Figure 2). However, in cancer tissues telomerase activity is dysregulated, and about 85% of cancers/tumor cells have detectable telomerase activity, thus allowing cancer cells to maintain their telomeres despite regular cell division (Figure 2; [34]). In addition to enzymatic activity, telomerase processivity (i.e., a special property of telomerase that describes the ability of telomerase to successively add TTAGGG repeats to telomere ends) depends on several factors, the most important being the proteins of the telomere binding protein complex called shelterin.

Shelterin acts as both a positive and negative regulator of telomere length [13, 35] and as a negative regulator of telomerase enzyme activity [36]. Shelterin protein components bind double- and single- stranded telomere DNA and regulate telomere length by controlling the access of telomerase to telomeres [36]. In addition, shelterin also helps to solve the end-protection problem by masking the ends of chromosomes from being recognized as DNA double-strand breaks and by preventing DNA damage sensing kinases from accessing telomere ends (Figure 2; [13]). Lastly, shelterin folds the telomere DNA into a three-dimensional structure called a T-loop [37], which is thought to both control access of telomerase to telomeres and to package the telomeres into condensed heterochromatic DNA.

Shelterin consists of six proteins: telomere-repeat binding factors (TRF) 1 and 2, protection of telomeres (POT1), RAP1, TPPI, and TIN2 (Figure 2; [12, 13, 38]). TRF1 and TRF2 bind to the double-stranded portion of telomeres and dictate the state of the telomere end in either an open (telomerase accessible) or closed (telomerase inaccessible) state [19]. TRF1 and TRF2, along with their interacting proteins, RAP1 and TIN2, are important in the regulation of telomere length, as shown from gain and loss of function studies [14, 19, 39]. POT1 binds to the single-stranded portion of telomeres and is important in preventing telomere end recognition as damaged DNA and also in controlling access of telomerase to telomeres [36]. In addition, POT1 and its interacting partner TPPI have been shown to control the processivity and recruitment of telomerase to telomere ends [40]. Importantly, altering the function and/or abundance of any of the telomere-binding proteins affects telomere length regulation and function (i.e., prevention of a DNA damage response at telomeres). Thus, if physiological stress is able to alter either the function or the abundance of these important proteins, this may provide a mechanism by which exercise regulates telomere length.

Recent evidence has indicated that the environment can impact telomeres and the suite of proteins (i.e., telomerase, shelterin) that are related to telomere maintenance. Physiological stressors such as lifestyle choices and psychological stress have been shown to influence telomere length and telomerase enzyme activity [7, 41–45]. This review will focus on physiological stress in the form of physical exercise and

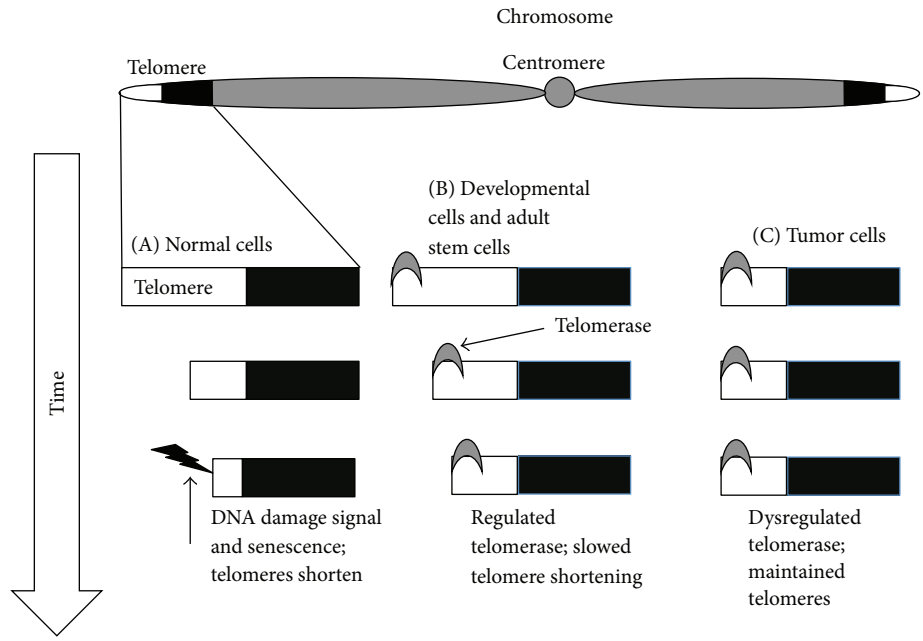


Figure 1: Common telomere/telomerase dogma across cell types in humans. Telomeres are located on the ends of linear chromosomes. Over time (i.e., with increased numbers of cell divisions), telomeres shorten due to a number of end-processing events; thus, short telomeres are associated with chronological age and a number of age-related diseases. (A) Telomeres function to mask the ends of chromosomes from being recognized by a cell's DNA damage response system. When telomeres reach a certain length, they are no longer masked and the cell recognizes the ends of the chromosome as damaged DNA. When the DNA damage signal is initiated, the cell arrests and enters telomere-induced senescence. This occurs in adult human cells lacking the enzyme telomerase, which maintains and elongates telomeres by using reverse transcriptase activity to add telomere repeats to the ends of chromosomes. (B) During development and in certain adult stem cells, telomerase is expressed and slows telomere shortening in these cells, thus maintaining the pool of cells available in a presenescent state. (C) In 85% of tumor cells, telomerase is dysregulated and allows cancer cells to be immortal and divide indefinitely since they do not undergo telomere-driven senescence.

the signals that may impact telomere biology in immune and skeletal muscle tissues.

3. Environmental Effects on Telomere Biology: Exercise and Physical Activity

Regular physical activity and exercise training (both resistance exercise and endurance exercise) are known to reduce the risk of developing many age-related chronic diseases such as cardiovascular disease, certain cancers, type II diabetes, and sarcopenia [7]. Exercise-related improvements in function at the whole body level are well-known, but the cellular, molecular, and genetic underpinnings are only beginning to be elucidated [46, 47]. While several groups have focused on how genetic variation influences both the response to exercise and the propensity to engage in exercise, few groups have investigated the impact of exercise on the genome structure itself (e.g., telomeres). Previous literature has described the association of various environmental stressors and lifestyle factors with telomere length, including psychological stress [44, 45, 48], comprehensive lifestyle changes (exercise and psychological stress counseling) [49, 50], diet [51, 52], body mass [53], socioeconomic status [54], and smoking status [55]. Since regular physical activity and exercise are well known environmental stressors with beneficial health outcomes (i.e., increased antioxidant gene expression, reduced inflammation, etc.), several groups have investigated the role of physical activity and exercise in human telomere biology. The notion that exercise reduces the impact of aging is well established; however, how exercise directly impacts telomere length remains to be fully elucidated. Several groups have hypothesized that exercise may be slowing cellular aging by reducing the rate of age-associated telomere shortening. However, this hypothesis is complicated by several lines of evidence that have demonstrated telomere shortening in response to extreme amounts of exercise. Here, we review how telomere length responds to exercise in both humans and rodents, point out current controversial issues in the field, and discuss data that demonstrate that telomere length may respond to exercise in a tissue-specific fashion.

We performed a systematic review of the relevant literature using the NCBI PubMed database. The following search terms were used: telomere length and exercise (45 results), telomere length and physical activity (74 results), telomerase and exercise (21 results), and telomerase and physical activity (82 results; all results current as of November 15, 2013). Studies were included in the review if they met the following criteria: (1) measured telomere length (by any method); (2) determined physical activity status either by physiological measure (i.e., maximal oxygen consumption) or survey (i.e., determination of exercise history based on questionnaire).

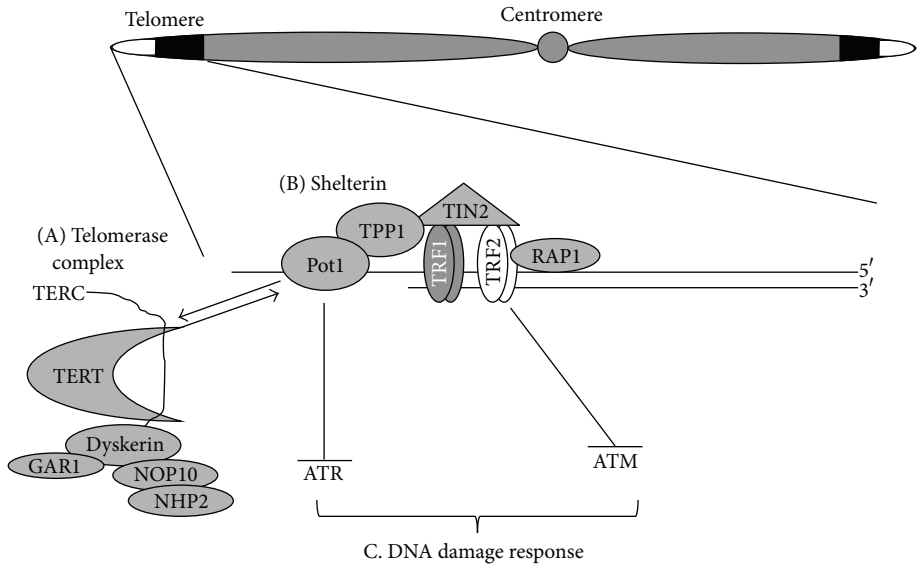


Figure 2: Telomere-related proteins. Telomere DNA sequences are bound by and interact with several proteins. These proteins and the enzyme telomerase function to regulate telomere length and prevent inappropriate recognition of telomere DNA by the DNA damage response machinery. (A) Telomerase is a ribonucleoprotein consisting of two core components: a catalytically active reverse transcriptase component, TERT and a noncoding RNA template, TERC. Together with several other cofactors such as dyskerin, GAR1, NOP10, and NHP2, telomerase functions to add telomere repeats to the ends of telomeres. (B) A complex of six proteins termed "shelterin" binds to telomere DNA in a tightly regulated stoichiometry and functions to regulate telomere length by preventing inappropriate telomere elongation by telomerase. Telomere repeat binding factors (TRFs) 1 and 2 bind to telomere double-stranded DNA and function to regulate telomere length and T-loop formation. (C) Shelterin also functions to prevent the DNA damage machinery from recognizing telomeres. Both POT1 and TRF2 prevent the telomere from being recognized by DNA damage kinases.

The final number of studies included in the review concerning telomere length and physical activity/exercise was 23 (summarized in Table 1).

3.1. Human Telomere Length Response to Chronic Exercise Training. Telomere length changes slowly (e.g., years) and for this reason very few well-controlled exercise training intervention studies have been performed in humans. The majority of the research on human telomere length and exercise has been done retrospectively on banked DNA samples from immune cells [7, 56]. Since the majority of the studies to date have not been performed on specific subsets of immune cells, or even isolated peripheral blood mononuclear cells (PBMCs), we will refer to immune cells (leukocytes) broadly in the following section (refer to Tables 1(a), 1(b), and 1(c) for details on cell types); this is a key limitation of the existing literature.

Multiple cross-sectional studies have described the associations between physical activity and/or exercise training and telomere length in immune cells, with three different relationships reported: a positive association, no association, and an inverted U relationship. In the inverted U relationship, sedentary individuals and extremely active individuals have shorter telomeres than moderately active individuals. The cross-sectional nature of these studies, their small sample size, variation in collection of exercise and physical activity data, methods of telomere length determination, cell types used for DNA extraction, and the various ages of the individuals in the study cohorts likely explain the discrepant results.

3.2. Studies Showing a Positive Relationship between Physical Activity and Telomere Length. Several studies have reported a positive association between physical activity and telomere length, in that active individuals have longer telomeres in immune cells compared to sedentary individuals [45, 57–60]. Cherkas et al. [61] reported a positive association between increasing physical activity and longer telomeres, with differences in telomere length equating to about 10 years of biological age difference between active and inactive subjects [61]. Other groups have confirmed or extended these results by showing that telomere length was longer in individuals with higher maximal oxygen consumption values compared to those with lower maximal oxygen consumption values [58, 62, 63]. Krauss et al. [64] found that individuals with low exercise capacity (as measured in METS) had a greater likelihood of having short telomeres compared to individuals with a greater exercise capacity and that this difference in telomere length was equivalent to about 4 years of biological age. Further, in a study of ultramarathon runners, longer telomeres were observed in the runners compared to sedentary age-matched individuals, with the difference approximately equal to 16 years of reduced biological age [65].

3.3. Studies Showing No Difference in Telomere Length between Active and Sedentary Individuals. Studying a group of marathon runners compared to sedentary age- and sex-matched individuals, Mathur et al. [66] found no association between maximal oxygen consumption or physical activity level and telomere length despite an extreme difference in

Table 1: (a) Studies showing a positive association of physical activity with telomere length. (b) Studies showing no association between physical activity and telomere length. (c) Studies showing an inverted U relationship between physical activity and telomere length.

(a)

Positive association of physical activity with telomere length				
Author	Study design	Subjects (*N*)	Tissue	Telomere length method
Cherkas et al. [61]	Cross-sectional	Twin cohort (2401)	Leukocytes-PBMCs	T/S qPCR
Werner et al. [63]	Cross-sectional	Young sedentary (26), young athletes (25), older sedentary (26), and older athletes (25)	Leukocytes	QFISH and T/S qPCR
Mirabello et al. [60]	Cross-sectional	Prostate cancer cases (612) versus age-matched controls (1049)	Leukocytes	T/S qPCR
Simpson et al. [59]	Longitudinal	Endurance trained men (9)	Sorted populations of PBMCs	T/S qPCR
Puterman et al. [45]	Cross-sectional	Postmenopausal women (63)	Leukocytes	T/S qPCR
LaRocca et al. [62]	Cross-sectional	Young sedentary (15), young athletes (10), older sedentary (15), and older athletes (17)	Leukocytes	Southern blot TRF
Krauss et al. [64]	Cross-sectional	Heart and Soul population (944)	Leukocytes	T/S qPCR
Kim et al. [58]	Cross-sectional	Postmenopausal women (44)	Leukocytes-PBMCs	T/S qPCR
Du et al. [57]	Cross-sectional	Nurse's health study (7,813)	Leukocytes	T/S qPCR
Osthus et al. [70]	Cross-sectional	Young sedentary (5), young athletes (5), older sedentary (5), and older athletes (5)	Skeletal muscle	T/S qPCR

Ref: reference. T/S qPCR: the ratio of telomere PCR value to single-copy gene value derived from quantitative PCR. TRF: terminal restriction fragment analysis. QFISH: quantitative fluorescence in situ hybridization with a telomere probe. *N*: number of subjects.

(b)

No association of physical activity with telomere length				
Author	Study design	Subjects (*N*)	Tissue	Telomere length method
Woo et al. [69]	Cross-sectional	65 years or older Chinese men and women (4000)	Leukocytes	T/S qPCR
Ornish et al. [50]	Longitudinal	Prostate cancer patients (30)	Leukocytes	T/S qPCR
Song et al. [68]	Cross-sectional	Diverse population (103)	Leukocytes	T/S qPCR
Mason et al. [67]	Randomized trial	Postmenopausal women (439)	Leukocytes	T/S qPCR
Mathur et al. [66]	Cross-sectional	Marathon athletes (17) versus matched individuals (15)	Lymphocytes and granulocytes	T/S qPCR
Kadi et al. [71]	Cross-sectional	Resistance trained strength athletes (7) versus active individuals (7)	Skeletal muscle	Southern blot TRF
Ponsot et al. [72]	Cross-sectional	Diverse population (42)	Skeletal muscle	Southern blot TRF

(b) Continued.

No association of physical activity with telomere length				
Author	Study design	Subjects (*N*)	Tissue	Telomere length method
Rae et al. [73]	Cross-sectional	Healthy endurance runners (18) versus sedentary age-matched (19)	Skeletal muscle	Southern blot TRF
Laye et al. [74]	Longitudinal	Marathon athletes (8)	Skeletal muscle and leukocytes	T/S qPCR

Ref: reference. "Diverse population" refers to a sample with a broad age range with both males and females of multiple races. T/S qPCR: the ratio of telomere PCR value to single-copy gene value derived from quantitative PCR. TRF: terminal restriction fragment analysis. *N*: number of subjects.

(c)

Inverted U relationship of physical activity with telomere length				
Author	Study design	Subjects (*N*)	Tissue	Telomere length method
Ludlow et. al. [43]	Cross-sectional	Population, 50–70-year-olds (69)	Leukocytes-PBMCs	T/S qPCR
Savela et al. [75]	Cross-sectional	Men (782)	Leukocytes	Southern blot TRF
Collins et al. [76]	Case-control	FAMS athletes (13) versus healthy endurance athletes (13)	Skeletal muscle	Southern blot TRF

Ref: reference. "Diverse population" refers to a sample with a broad age range with both males and females of multiple races. T/S qPCR: the ratio of telomere PCR value to single-copy gene value derived from quantitative PCR. TRF: terminal restriction fragment analysis. *N*: number of subjects. FAMS: fatigued athlete myopathic syndrome.

fitness. Several other studies have similarly reported no association between telomere length and physical activity level, but the age of the subjects, extent of physical activity, measurement of telomere length, and other uncontrolled factors (e.g., diet and psychological stress) likely contributed to the lack of association in these studies [50, 67–69].

3.4. Studies Showing an Inverted U Relationship between Activity and Telomere Length. A few studies have described an inverted U relationship between physical activity and telomere length where moderately active individuals exhibit longer telomeres compared to both sedentary and extremely active individuals. Ludlow et al. [43] showed that 50–70-year-old individuals in both the lowest (<990 kcal/wk) and highest quartiles (>3541 kcal/wk) of exercise-specific energy expenditure had shorter telomeres than individuals in the second quartile (991–2340 kcal/wk), even when controlling for age, gender, and body weight. Savela et al. [75] found that individuals who reported moderate levels of physical activity in midlife had a longer mean telomere length and also a smaller proportion of short telomeres compared to both low active and highly active individuals. Telomere lengths in a single cell are heterogeneous across chromosome ends and it is believed that the shortest telomere in a cell drives the induction of senescence; therefore, it is important to monitor the shortest telomere length in a population of cells [77, 78]. This is important because it takes into account the proportion of the shortest telomeres, which are likely the most important in dictating cellular fates, whereas previous reports only focused on mean telomere length.

Several factors must be considered when interpreting the mixed associations between telomere length and exercise/physical activity levels. Specifically, sampling bias, cell type, age of individuals when measures were made, and the timing of sample collection may all influence study outcomes. For instance, because circulating stem cells are released following exercise [79–81], if an active subject had recently completed an exercise bout, the peripheral blood would be biased by stem cells with longer telomeres. Thus, a 48 hr "washout" period or cell sorting techniques should be considered to prevent this type of sampling bias. Further, the inverted U phenomenon may be age dependent and only evident in older, highly active individuals, while in younger endurance athletes telomere length may be preserved even when extreme amounts of exercise are performed.

Another important factor that should be considered is the method of telomere length determination [82]. Telomere lengths in the above studies were mainly determined by two methods: terminal restriction fragment southern blot analysis or TRF and the telomere repeat copy number to single copy gene copy number ratio performed as a quantitative real-time PCR assay (qPCR). The TRF method is a southern blotting method and results in determination of mean telomere length from a smear of telomere signal [83]. Several biases may occur in this method including loss of short telomere signal and inclusion of subtelomeric DNA [82]. The qPCR method is a determination of relative telomere length that is dependent upon the ratio of telomere DNA content to chromosomal (single copy gene) DNA content within a given sample [84]. The qPCR method is highly correlated with the southern blot method, but certain differences and biases may arise. For example, the qPCR method is subject to wide variability between labs and sample preparations [85]. Nonetheless, both methods are valid for determining telomere length, but make comparisons between labs and

studies difficult. Thus, one must interpret the findings of studies with these methodological issues in mind.

While the current research findings are divergent, the overall consensus from these studies is that moderate levels of physical activity are associated with longer telomere length in immune cells. To expand upon these findings, future studies should focus on how moderate exercise can maintain the shortest telomeres and not just mean telomere length in leukocytes. Cross-sectional studies have provided several key insights, but the limitations of these types of studies have produced mixed results that may be biased by sample collection, cell type utilized, and telomere length measurement methodology.

Randomized control clinical exercise trials would eliminate some of the bias associated with cross-sectional studies and help to clearly define the effects of exercise (both acute and long-term training) on telomere length and telomere biology. However, to date few longitudinal studies investigating the relationship between telomere length and exercise have been performed in humans. Shin et al. [86] exercise trained women (treadmill walking and running) for 6-months and observed no change in immune cell telomere length from baseline. This study, though interesting, has several shortcomings including small sample size and short training duration, which make the data difficult to interpret. Other longitudinal studies have produced mixed results regarding the relationship between exercise and telomere length, with most studies being of short duration (i.e., less than 1 year) and reporting no change in telomere length [45, 50, 67].

Recently, Ornish et al. [49, 50] completed a trial with three-month and five-year follow-up time points investigating the influence of environmental factors on telomere biology. The trial included comprehensive lifestyle changes (i.e., psychological stress counseling, dietary modification, increased physical activity, and social support groups) in a group of older men. No change in telomere length was observed at the three-month time point [50]; however, at the five-year followup, longer telomere length in PBMCs was observed in the lifestyle intervention group compared to controls [49]. These data provide support for the idea that exercise, in combination with other lifestyle factors (i.e., stress reduction and dietary modifications) over a five-year period is able to slow cellular aging, as indicated by reduced telomere shortening. The studies described above provide the necessary preliminary data to pursue large-scale intervention studies of the effect of exercise alone on telomere biology.

Very few studies have investigated the effects of acute exercise (i.e., single or a few bouts of exercise) on telomere length. Endurance exercise, specifically marathon running, is a potent immune cell proliferative stress [87] and causes skeletal muscle remodeling (i.e., proliferation of muscle precursor cells [88]); thus, one could hypothesize that telomere length changes could occur following marathon running. To obtain insights into this hypothesis, Laye et al. [74] measured telomere length in individuals before and after they ran 7 marathons in 7 days. No change in either leukocyte telomere length or skeletal muscle telomere length was observed. These data indicate that in trained individuals, a massive amount of acute exercise is not sufficient to cause rapid proliferation-related or stochastic DNA damage-associated telomere shortening. Moreover, these data indicate that there is a gap in the literature concerning what would happen to immune cells or skeletal muscle cells from an untrained person exposed to similar physiological stress. Future studies will be needed to clarify the role of acute exercise on telomere dynamics.

The consensus from the above studies in immune cells is that telomere length decreases with age in sedentary individuals, longer telomeres are observed in individuals who are moderately active (threshold to be empirically determined), and extreme long-duration endurance training for an extended portion of one's lifetime may result in telomere shortening. These divergent responses are likely directly related to the antioxidant capacity of the immune cells and the proliferative demand placed on the progenitor cells by the exercise stimulus.

4. The Curious Case of Skeletal Muscle Telomere Biology in Humans

Skeletal muscle has unique telomere biology when compared to other tissues. Skeletal muscle consists of a syncytium of multinucleated muscle fibers that are postmitotic; thus, telomere length should remain stable in this population of nuclei, with the rare exception of DNA damaging stimuli [89]. In addition to myonuclei, single-nucleated populations of cells, of which the best described are satellite cells, also populate skeletal muscle [90]. Satellite cells are muscle precursor cells (i.e., adult stem cells) that are quiescent unless induced to divide by external stressors, such as contraction-induced or injury-induced muscle damage [90]. When induced to divide, satellite cells divide asymmetrically, with one daughter cell incorporating into the damaged muscle fiber and the other daughter cell returning to replenish the satellite cell pool [90]. Skeletal muscle telomere dogma states that when a muscle precursor cell is induced to divide and incorporate into the muscle fiber, the new nuclei will have the shortest telomeres in that fiber owing to the fact that they originate from precursor cells that had divided prior to becoming incorporated [89, 91–93]. With increased regenerative pressures on skeletal muscle, a greater number of nuclei with short telomeres would be present and thus the muscle fiber in total would have shortened telomeres, as is the case for some muscular dystrophies (i.e., Duchenne's muscular dystrophy; [94]).

Muscle contraction, such as heavy-resistance-type exercise, is known to cause injury to skeletal muscle and thus is a stimulus for satellite cell proliferation. Recently, long-duration and high-intensity endurance exercise was shown to cause satellite cell replication in skeletal muscle [95]. Since exercise can result in muscle damage and proliferation of satellite cells, and telomere length shortens with cell division, exercise may cause telomere shortening in skeletal muscle tissue of highly active individuals. This provides support for the inverted U hypothesis for the relationship between exercise and telomere length, with extreme exercise resulting in cellular damage. Using the logic that exercise

may be a proliferative stress to skeletal muscle, several groups have investigated the role that physical activity (endurance training and resistance exercise in particular) may play in skeletal muscle telomere biology (Table 1).

Two studies by the same group have investigated the effects of long-term endurance training and found that exercise may cause telomere shortening in skeletal muscle of chronically trained individuals. Though the sample size was small, Collins et al. [76] investigated skeletal muscle telomere length by comparing healthy athletes to chronically overtrained athletes with fatigued-myopathic syndrome (FAMS) who were matched for age and training volume. Despite similar training volumes the symptomatic group had reduced athletic performance, decreased ability to tolerate high-volume training, and excessive muscular fatigue during exercise [76]. All of these symptoms may be indicative that the FAMS athletes had a reduced capacity to repair skeletal muscle damage. The FAMS athletes had shorter telomeres compared to healthy athletes, indicating that the overtrained athletes may have induced greater proliferative stress on satellite cells compared to the healthy athletes. In another study, Rae et al. [73] characterized skeletal muscle telomeres from a large number of healthy endurance trained individuals compared to sedentary individuals. They observed no difference between groups for mean telomere length, but in the endurance-trained group, telomere length was inversely correlated to years and hours of training [73]. This indicates that endurance training could be a replicative stressor to satellite cells in lower limb skeletal muscle or that long-term exercise resulted in other telomere shortening stressors such as excess ROS. One limitation of this study was the quantification of minimal telomere lengths from TRF gels; since the shortest telomeres are likely lost using this method, the authors may have actually underestimated the number of short telomeres in the athletes. A different method that determines the proportion of short telomeres in a sample would provide clarification on the effect of endurance exercise on skeletal muscle telomere length. Importantly, future research should determine how different frequencies, intensities, and durations of exercise result in different proliferative demands on skeletal muscle as indicated by a shift in the proportion of short telomeres.

Since heavy-resistance exercise is a well-known muscle damaging and satellite cell proliferative stimulus, researchers have investigated the effect of chronic resistance training on telomere length in skeletal muscle. Kadi et al. [71] compared long-term competitive weight lifters to healthy age-matched active subjects. There was not an overt difference in telomere length between the resistance-trained and healthy active individuals; however, there was a negative correlation between individual records (i.e., heaviest weight lifted in a particular exercise) and minimal telomere length, indicating that a heavier load was correlated with shorter telomeres in skeletal muscles and was potentially a regenerative stress on the muscle [71]. The authors hypothesized that the shorter telomeres were due to greater satellite cell proliferation resulting from the contraction-induced muscle fiber damage. These data from skeletal muscle are similar to the inverted U phenomena observed in immune cells, in that moderate levels of endurance training or resistance training may maintain or not change telomere length, while extreme exercise levels may result in telomere shortening potentially due to increased cellular proliferation.

Not all data in skeletal muscle indicate that telomere shortening occurs with physical activity. Ponsot et al. [72] investigated telomere length in healthy physically active older men and women and observed that telomere length (both mean and minimum) was similar in active and sedentary individuals, leading to the conclusion that moderate physical activity is not a proliferative stress on skeletal muscle tissue. Two limitations of this study were that the subjects were only in two specific age groups (i.e., young versus old) and not across the age spectrum, and the activity levels were low and not representative of the full activity spectrum. These data indicate that moderate activity levels likely do not cause skeletal muscle damage, do not result in an excess proliferative demand, and do not shorten telomeres. Thus, moderate activity would appear to maintain telomere length with age. These data provide support for the inverted U hypothesis, that if the exercise stimulus is not causing cellular damage, telomere length should be maintained. Building on these data, a recent, small cross-sectional study compared skeletal muscle telomere lengths in young active and sedentary and older active and sedentary individuals. Osthus et al. [70] observed that telomere length in the older active group was longer than that in the older inactive group and that there was a positive correlation between maximal oxygen consumption and telomere length. These data seem to conflict with the current literature in that all other studies have either reported no difference or a small decrease in skeletal muscle telomere length in active individuals. Further, these data would seem to indicate that telomeres were elongated (either by telomerase or another mechanism) in the muscles of active individuals since typically telomere length would be constant with age in skeletal muscle due to its low turnover rate. Thus, these controversial data need to be confirmed in a larger sample size and with a longitudinal study.

In summary, the data for how exercise and physical activity influence telomere biology in human skeletal muscle are mixed and need clarification with longitudinal experiments. The effect of exercise on skeletal muscle telomere length is likely directly linked to the proliferative demand the exercise places upon the skeletal muscle. Thus, the more damaging the exercise stimulus (either endurance- or resistance-type exercise) to the muscle fibers and the greater the proliferative demand upon the muscle stem cells, the faster the rate of skeletal muscle telomere shortening. Furthermore, the longer the exercise duration, both in terms of length of individual exercise bouts and years of training an individual performs, the greater the observed decrease in telomere length. Another potential mechanism to consider is the antioxidant capacity of the skeletal muscle, which could be overwhelmed by contraction-induced reactive oxygen species (ROS) and result in damage to telomere DNA and the stochastic loss of telomere length, as has been shown in other tissues exposed to ROS [96–100]. Furthermore, given the recent findings that exercise may actually elongate telomeres in skeletal muscle from active individuals, mechanisms for how this could be

occurring (e.g., telomerase activity) should be explored. The relationship between exercise, physical activity, and skeletal muscle warrants further investigation, especially considering the recent controversial findings that telomere length in skeletal muscle may shorten with age at a similar rate to other proliferative tissues such as immune cells [101].

Are Telomere Lengths Synchronized across Tissues? Telomere length in mammalian species is tissue-specific [102, 103]. The standard model in the field is that telomeres in proliferative tissues (e.g., immune cells, intestinal epithelial cells) shorten with age [11], while in low-turnover tissues such as skeletal muscle [89], telomere length is constant over time; thus, telomere length is directly linked to proliferative history of the tissue. This indicates that the rate of telomere shortening is tissue-specific and directly dependent upon proliferative demands and rate of damage accumulation and repair in each tissue. In contrast to this long-held hypothesis, a recent report showed that the rate of telomere shortening in human lymphocytes was similar to the rate of telomere shortening in skeletal muscle and other low turnover tissues [101]. Daniali et al. [101] collected tissues (skin, immune cells, fat, and skeletal muscle) from 87 individuals across the age span 19–77 years and measured telomere length. The major finding of their study was that the rate of age-dependent telomere shortening was similar across the tissues studied despite different replicative dynamics of leukocytes, skeletal muscle, skin, and fat. As opposed to previous studies, Daniali et al. [101] observed skeletal muscle shortening with age, an unexpected finding that they attributed to their large sample size. This indicates that telomere lengths across tissues may be "synchronized" and that the rate of leukocyte telomere length shortening predicts the rate of skeletal muscle telomere shortening with age.

The data from the skeletal muscle telomere and exercise literature seem to directly conflict with Daniali et al.'s [101] data. According to the "synchrony hypothesis," telomere length in the peripheral blood cells should predict telomere length in other tissues; however, the majority of the literature has not documented an age-related change in skeletal muscle telomere length, while age-related decreases in immune cell telomere length have been widely reported [61, 104, 105]. Moreover, several reports have documented the potential for endurance exercise to significantly shorten telomeres in skeletal muscle while maintaining telomere lengths in other tissues [42, 43, 63, 73, 76]. Reconciliation of whether or not telomere length responds similarly across tissues (as would be expected from the telomere synchrony hypothesis), or if telomere length responds to exercise in a tissue-specific fashion, is an important area of future research.

5. Telomerase and Shelterin Response Exercise in Humans

Few reports have described the effects of physical activity, exercise training, or acute exercise on telomerase, shelterin, and other telomere-associated proteins [63, 74]. Of those studies, most have compared immune cells between chronically trained individuals and sedentary individuals. The results of these studies have been mixed, showing either a slight increase or no difference in telomerase activity or expression of shelterin components in the trained groups. There are no studies that we are aware of investigating the effects of a single bout of endurance exercise on telomerase activity in immune cells.

Strenuous exercise is a known proliferative stress for immune cells [106]. Mitogen stimulation of immune cells increases telomerase activity in T cells [107], suggesting that telomerase activity may be increased in long-term exercise-trained individuals in order to maintain telomere length following the repeated proliferative stress of strenuous exercise. Ludlow et al. [43] described a potential gene-environment interaction between physical activity level and a TERT promoter polymorphism that is associated with telomere length and telomerase enzyme activity [108, 109]. Individuals in the highest quartile of physical activity and carrying a specific TERT promoter genotype (rs2735940, C-1327T, TT genotype) were observed to have greater telomerase enzyme activity in PBMCs, thus demonstrating an association between physical activity level, TERT genotype, and telomerase activity [43]. These data were collected from a small cohort, but the association is interesting in that not only did those individuals with the particular genotype exhibit greater telomerase activity, but they also had PBMCs with short telomeres. Moreover, telomerase has been shown to be recruited preferentially to the shortest telomeres [77, 110]. Thus, if telomerase is preferentially recruited to the shortest telomeres in cells of exercise-trained individuals, exercise may prevent the induction of senescence by maintaining the shortest telomeres in these immune cells, thereby slowing an aging phenotype.

In support of this hypothesis, Werner et al. [63] showed that older athletes have immune cell telomerase enzyme activity similar to younger individuals and greater than age-matched sedentary individuals. These data support the hypothesis that telomerase may be part of the adaptive response to exercise training and could be a biomarker of improved physical health [43, 50]. In addition, while Laye et al.'s [74] study did not find a change in telomere length following 7 marathons in 7 days, they did observe increased gene expression of both DNA damage repair proteins and shelterin components in skeletal muscle and immune cells. Taken together these data indicate that exercise training is associated with a telomere-protective phenotype in both leukocytes and skeletal muscle; however, the adaptive mechanisms surrounding telomerase and shelterin may be different between tissues and depend upon the training status and age of the individuals. Future studies will be needed to clarify the exact mechanisms of how exercise results in a telomere-protective environment in specific tissues.

6. Mouse Telomere Length Response to Exercise

Since human telomeres are slow to shorten (i.e., over many years), groups have turned to model organisms, such as rodents, to explore the relationship between exercise and

telomere dynamics. However, rodents require special considerations when studying telomere biology. In this section, we discuss the positives, negatives, and caveats to using rodents for telomere-related studies and also describe the work that has been completed using mice as model organisms in this area of investigation.

6.1. Mouse Telomere Biology Review and Subsequent Caveats to Using Rodents. Though the biology of telomere mechanics is similar between humans and rodents, human telomeres are typically only 7–15 kilobases in length [18], while rodent telomeres are much longer (20 to 50 kilobases and up to 150 kilobases depending on inbreeding status and strain; [111]). Furthermore, humans and other short telomere mammals utilize telomere length as a tumor-suppressive mechanism, while long telomere mammals, such as rodents, do not [102]. Another important difference to consider in rodents is that the majority of somatic tissues are telomerase positive and that the mouse TERT gene is regulated differently than the human TERT gene [112]. Despite these differences, age-related telomere shortening does occur and the shelterin proteins are conserved in rodents, thus making studying the response to environmental stressors in rodents relevant to human biology. To reduce the long telomere bias that rodent studies introduce, rodents with shorter telomeres have been employed, with the most common models being strains with naturally occurring short telomeres (e.g., wild-derived inbred strains such as *mus musculus cataneous*, CAST/Ei) or models where gene disruption of telomerase components, mTERT and mTERC, has been performed [113–115].

Naturally occurring shorter telomere rodents, such as CAST/Ei mice, are typically wild derived, more recently inbred, and have telomere lengths (15–20 kb) much closer to those of humans (7–15 kb) [42, 111, 116]. Several studies have used the CAST/Ei animals in an attempt to more accurately model the response of telomeres and telomere length regulating proteins to environmental stress (e.g., exercise) and oxidative stress [42, 111, 116].

Another way to induce shorter telomere lengths in rodents is by knocking out either the protein catalytic subunit (mTERT) or the RNA component (mTERC) of telomerase. These models are unique in that the first generation of knockout animals retain wild-type telomere lengths while offspring generations three through six display shorter telomere lengths and aging phenotypes compared to age-matched wild-type controls [114]. In addition, the telomerase genetic manipulations have been developed on the CAST/Ei background, potentially providing a model that is even more similar to human telomere biology [115]. Thus, several rodent models are available to study the effects of environmental stressors on telomere length and telomere biology, but special caution must be used when interpreting and extrapolating the results to humans.

6.2. Effect of Exercise Training on Telomere Length in Rodent Models. To date only two groups have investigated the effect of long-term exercise training on telomere length in rodent models [41, 42, 63, 117]. Werner et al. [117] investigated the effect of 6-months of exercise training on telomere length in leukocytes and heart muscle of C57BL/6 mice (long telomeres; ~50 kb), as well as in several knockout and transgenic models, in order to delineate a mechanism of telomere protection in response to exercise. In both tissues following either 3 weeks or 6 months of voluntary wheel running activity, no effect on telomere length was observed compared to controls. To test if age-related telomere shortening was occurring in these animals the authors aged sedentary animals for 18 months and were able to detect telomere shortening in both leukocytes and left ventricular heart muscle. Thus, these first important analyses highlight the need for longer-duration-studies to delineate the effect of exercise on telomere length in rodents.

Ludlow et al. [42] performed a similar study but in the CAST/Ei mouse strain. Three groups of animals were investigated: 8-week-old sedentary, one-year-old sedentary, and one-year-old animals that had access to a voluntary running wheel for 44 weeks. Telomere length was assessed in liver, heart, and skeletal muscle tissues. Significant age-related telomere shortening in the heart and liver of the one-year old animals was attenuated by voluntary wheel running. In skeletal muscle, significant telomere shortening was observed in the chronic exercise group compared to both the sedentary young animals and the one-year-old animals. These data indicate that exercise not only affects telomere biology in leukocytes but also in cardiac muscle, skeletal muscle and liver, albeit with tissue-specific effects. These tissue-specific responses are likely related to differences in the proliferative demands placed on the individual tissues by the exercise stimulus, as well as differences in antioxidant capacity between the tissues. Future research is needed to determine if proliferation and oxidative stress are responsible for these tissue-specific responses.

6.3. Effect of Exercise on Telomere Length-Maintaining Proteins in Rodent Models. Several groups have investigated how exercise may alter the expression of telomere-related proteins in rodent tissue. Werner et al. [63, 117] performed a thorough series of experiments to elucidate how exercise produced adaptations in cells that made them more resistant to environmental stressors, specifically investigating the role of telomeres and telomere-related proteins in left ventricular, aortic, vascular, and immune tissues. Investigating short-term training effects (21 days of voluntary wheel running) in C57BL/6 mice, they observed that TERT protein and TRF2, Ku70, and Ku80 mRNA expression levels were increased compared to sedentary controls. These data indicate that short-term exercise training in rodents is associated with increases in both telomere length and senescence protective expression profiles. To determine if the effects of exercise for 21 days were dependent upon TERT protein, TERT knockout animals were given access to a running wheel for three weeks. It was observed that the effects of exercise on TRF2, p16, Chk2, and p53 were not present in the exercised knockout animals compared to wild-type exercised animals [63, 117]. These data provide evidence that TERT protein may be needed for the beneficial adaptation of exercise on telomere-related proteins, indicating that in rodent tissues TERT may

have extratelomeric functions, such as acting as a transcription factor or part of a chromatin remodeling complex [118]. These data provide substantial evidence that exercise and physical activity can result in a cellular environment that is protective against shortened telomeres and subsequently protective against aging phenotypes in heart, vascular, and immune cells.

In a different strain of mice (CAST/Ei), Ludlow et al. [42] observed tissue-specific responses of shelterin and telomerase to 44 weeks of voluntary wheel running. In skeletal muscle, exercise resulted in shortened telomeres, but increased telomerase enzyme activity. In addition, there was an age-associated increase in skeletal muscle TRF1 protein levels that was attenuated by exercise. In cardiac muscle, exercise attenuated the age-related reduction in shelterin gene expression, while in liver tissue no significant changes were observed. These data indicate that while exercise is beneficial to all three tissues, the adaptive response of telomere length regulating proteins is tissue-specific.

Overall these data indicate that telomere-binding proteins and telomerase adapt to exercise training in a tissue-specific fashion. Some tissues upregulate telomerase enzyme activity, while other tissues seem to alter the expression of shelterin components and DNA damage response and repair genes. These data indicate that exercise likely results in tissue-specific adaptation of the telomere maintenance pathways; however, regardless of tissue-specific effects, it is important to note that exercise results in a phenotype that is teloprotective in most tissues studied to date.

7. Signaling Mechanisms Associated with the Adaptive Response of Telomere Length-Regulating Proteins

Elucidating the mechanisms of how telomere length is preserved or lost following exercise is important for understanding how telomeres respond to physiological stressors. To date most mechanistic studies on exercise have focused on the stress response and growth/cellular proliferation pathways. Only three studies have investigated exercise-specific signaling mechanisms associated with altered telomere biology, all of which have been performed in rodent cells and tissues [41, 63, 117]. TERT, IGF-1, eNOS, and AKT were identified as being important in signal transduction of the exercise-induced telomere protective phenotype [63, 117]. In addition, p38MAPK was shown to regulate the gene expression of *Trf1* following acute exercise in rodent skeletal muscle [41]. These data indicate that more work is needed to fully elucidate the signaling mechanisms of exercise-induced telomere protection and point to the stress response and growth/cellular proliferation pathways as high-priority candidates for future studies.

8. Future Directions

Exploration of the effect that physiological stressors such as exercise and exercise training have on the structure-function relationship of the genome is fertile ground. Researchers should consider the following five directions of importance for future research: (1) determining whether or not telomere length shortens at equal rates across somatic tissues in response to exercise training; (2) if an inverted U relationship exists between physical activity and telomere length; (3) the tissue-specific functional consequences of short telomeres in trained versus untrained individuals; (4) the role of oxidative stress and inflammation during and following exercise and the effects on telomere biology; and (5) the specific pathways (e.g., stress response, growth, and proliferation) that cause the adaptation and response of telomerase and shelterin to exercise and how these adaptations result in altered telomere length.

9. Conclusions

Numerous studies have implicated a telomere-protective phenotype induced by moderate levels of physical activity, indicating an important cellular adaptation that may slow the onset of symptoms or prevent certain age-related diseases. In contrast, several lines of evidence in both immune cells and skeletal muscle indicate that telomeres may actually shorten in response to long-term high-intensity endurance training. As such, the tissue-specific response of telomeres should be investigated, with specific consideration given to the proliferative demands placed on the tissue by the exercise stimulus and the antioxidant capacity of the individual tissues. Understanding how telomeres adapt on a tissue-specific basis and if immune cells are predictive of the adaptive response of other tissues is a necessary next step in this field. Additionally, determination of the type, time, intensity, and frequency of exercise that results in an excess proliferative demand on immune and skeletal muscle tissues and results in loss of telomere length is important. A multidisciplinary approach must be taken to tackle these important questions and to further solidify telomere length as a useful biomarker in monitoring the long-term effects of environmental and physiological stressors, such as exercise training.

Conflict of Interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

Andrew T. Ludlow was supported by UTSWMC NCI Cell Biology Department Training Grant (CA12433404).

References

[1] S. T. Arthur and I. D. Cooley, "The effect of physiological stimuli on sarcopenia, impact of Notch and Wnt signaling on impaired aged skeletal muscle repair," *International Journal of Biological Sciences*, vol. 8, no. 5, pp. 731–760, 2012.

[2] H. Geiger and Y. Zheng, "Cdc42 and aging of hematopoietic stem cells," *Current Opinion in Hematology*, vol. 20, no. 4, pp. 295–300, 2013.

[3] D. Kipling, "Telomeres, replicative senescence and human ageing," *Maturitas*, vol. 38, no. 1, pp. 25–38, 2001.

[4] T. B. L. Kirkwood, "Molecular gerontology," *Journal of Inherited Metabolic Disease*, vol. 25, no. 3, pp. 189–196, 2002.

[5] J.-P. Issa, "Age-related epigenetic changes and the immune system," *Clinical Immunology*, vol. 109, no. 1, pp. 103–108, 2003.

[6] J. W. Shay and W. E. Wright, "Role of telomeres and telomerase in cancer," *Seminars in Cancer Biology*, vol. 21, no. 6, pp. 349–353, 2011.

[7] A. T. Ludlow and S. M. Roth, "Physical activity and telomere biology: exploring the link with aging-related disease prevention," *Journal of Aging Research*, vol. 2011, Article ID 790378, 2011.

[8] C. López-Otín, M. A. Blasco, L. Partridge et al., "The hallmarks of aging," *Cell*, vol. 153, no. 6, pp. 1194–1217, 2013.

[9] E. H. Blackburn, "Structure and function of telomeres," *Nature*, vol. 350, no. 6319, pp. 569–573, 1991.

[10] A. G. Bodnar, M. Ouellette, M. Frolkis et al., "Extension of lifespan by introduction of telomerase into normal human cells," *Science*, vol. 279, no. 5349, pp. 349–352, 1998.

[11] C. B. Harley, A. B. Futcher, and C. W. Greider, "Telomeres shorten during ageing of human fibroblasts," *Nature*, vol. 345, no. 6274, pp. 458–460, 1990.

[12] T. De Lange, "Shelterin: the protein complex that shapes and safeguards human telomeres," *Genes and Development*, vol. 19, no. 18, pp. 2100–2110, 2005.

[13] T. De Lange, "How telomeres solve the end-protection problem," *Science*, vol. 326, no. 5955, pp. 948–952, 2009.

[14] T. De Lange, "How shelterin solves the telomere end-protection problem," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 75, pp. 167–177, 2010.

[15] W. E. Wright and J. W. Shay, "Telomere-binding factors and general DNA repair," *Nature Genetics*, vol. 37, no. 2, pp. 116–118, 2005.

[16] M. Armanios, "Telomeres and age-related disease: how telomere biology informs clinical paradigms," *The Journal of Clinical Investigation*, vol. 123, no. 3, pp. 996–1002, 2013.

[17] M. A. Blasco, "Telomere length, stem cells and aging," *Nature Chemical Biology*, vol. 3, no. 10, pp. 640–649, 2007.

[18] C. M. Kong, X. W. Lee, and X. Wang, "Telomere shortening in human diseases," *The FEBS Journal*, vol. 280, no. 14, pp. 3180–3193, 2013.

[19] A. Smogorzewska, B. Van Steensel, A. Bianchi et al., "Control of human telomere length by TRF1 and TRF2," *Molecular and Cellular Biology*, vol. 20, no. 5, pp. 1659–1668, 2000.

[20] W. E. Wright and J. W. Shay, "Cellular senescence as a tumor-protection mechanism: the essential role of counting," *Current Opinion in Genetics and Development*, vol. 11, no. 1, pp. 98–103, 2001.

[21] A. J. Cesare, M. T. Hayashi, L. Crabbe et al., "The telomere deprotection response is functionally distinct from the genomic DNA damage response," *Molecular Cell*, vol. 51, no. 2, pp. 141–155, 2013.

[22] T. Tchkonia, Y. Zhu, J. van Deursen et al., "Cellular senescence and the senescent secretory phenotype: therapeutic opportunities," *The Journal of Clinical Investigation*, vol. 123, no. 3, pp. 966–972, 2013.

[23] S. Tumpel and K. L. Rudolph, "The role of telomere shortening in somatic stem cells and tissue aging: lessons from telomerase model systems," *Annals of the New York Academy of Sciences*, vol. 1266, pp. 28–39, 2012.

[24] Y. Wang, N. Sharpless, and S. Chang, "p16INK4a protects against dysfunctional telomere-induced ATR-dependent DNA damage responses," *The Journal of Clinical Investigation*, vol. 123, no. 10, pp. 4489–4501, 2013.

[25] A. J. Sfeir, W. Chai, J. W. Shay, and W. E. Wright, "Telomere-end processing: the terminal nucleotidesof human chromosomes," *Molecular Cell*, vol. 18, no. 1, pp. 131–138, 2005.

[26] L. Bendix, P. B. Horn, U. B. Jensen, I. Rubelj, and S. Kolvraa, "The load of short telomeres, estimated by a new method, Universal STELA, correlates with number of senescent cells," *Aging Cell*, vol. 9, no. 3, pp. 383–397, 2010.

[27] J. W. Shay and W. E. Wright, "Telomeres and telomerase in normal and cancer stem cells," *FEBS Letters*, vol. 584, no. 17, pp. 3819–3825, 2010.

[28] K. Collins, "The biogenesis and regulation of telomerase holoenzymes," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 7, pp. 484–494, 2006.

[29] E. Cusanelli, C. A. Romero, and P. Chartrand, "Telomeric noncoding RNA TERRA is induced by telomere shortening to nucleate telomerase molecules at short telomeres," *Molecular Cell*, vol. 51, no. 6, pp. 780–791, 2013.

[30] J. Fakhoury, D. T. Marie-Egyptienne, J.-A. Londoño-Vallejo, and C. Autexier, "Telomeric function of mammalian telomerases at short telomeres," *Journal of Cell Science*, vol. 123, no. 10, pp. 1693–1704, 2010.

[31] A. Bianchi and D. Shore, "Increased association of telomerase with short telomeres in yeast," *Genes and Development*, vol. 21, no. 14, pp. 1726–1730, 2007.

[32] R. E. Hector, R. L. Shtofman, A. Ray et al., "Tellp preferentially associates with short telomeres to stimulate their elongation," *Molecular Cell*, vol. 27, no. 5, pp. 851–858, 2007.

[33] G. Cristofari, E. Adolf, P. Reichenbach et al., "Human telomerase RNA accumulation in Cajal bodies facilitates telomerase recruitment to telomeres and telomere elongation," *Molecular Cell*, vol. 27, no. 6, pp. 882–889, 2007.

[34] N. W. Kim, M. A. Piatyszek, K. R. Prowse et al., "Specific association of human telomerase activity with immortal cells and cancer," *Science*, vol. 266, no. 5193, pp. 2011–2015, 1994.

[35] J. A. Stewart, M. F. Chaiken, F. Wang, and C. M. Price, "Maintaining the end: roles of telomere proteins in end-protection, telomere replication and length regulation," *Mutation Research*, vol. 730, no. 1-2, pp. 12–19, 2012.

[36] J. Nandakumar and T. R. Cech, "Finding the end: recruitment of telomerase to telomeres," *Nature Reviews*, vol. 14, no. 2, pp. 69–82, 2013.

[37] J. D. Griffith, L. Comeau, S. Rosenfield et al., "Mammalian telomeres end in a large duplex loop," *Cell*, vol. 97, no. 4, pp. 503–514, 1999.

[38] I. Diala, N. Wagner, F. Magdinier et al., "Telomere protection and TRF2 expression are enhanced by the canonical Wnt signalling pathway," *EMBO Reports*, vol. 14, no. 4, pp. 356–363, 2013.

[39] B. Van Steensel and T. De Lange, "Control of telomere length by the human telomeric protein TRF1," *Nature*, vol. 385, no. 6618, pp. 740–743, 1997.

[40] J. Nandakumar, C. F. Bell, I. Weidenfeld et al., "The TEL patch of telomere protein TPP1 mediates telomerase recruitment and processivity," *Nature*, vol. 492, no. 7428, pp. 285–289, 2012.

[41] A. T. Ludlow, L. C. J. Lima, J. Wang et al., "Exercise alters mRNA expression of telomere-repeat binding factor 1 in skeletal muscle via p38 MAPK," *Journal of Applied Physiology*, vol. 113, no. 11, pp. 1737–1746, 2012.

[42] A. T. Ludlow, S. Witkowski, M. R. Marshall et al., "Chronic exercise modifies age-related telomere dynamics in a tissue-specific fashion," *The Journals of Gerontology*, vol. 67, no. 9, pp. 911–926, 2012.

[43] A. T. Ludlow, J. B. Zimmerman, S. Witkowski, J. W. Hearn, B. D. Hatfield, and S. M. Roth, "Relationship between physical activity level, telomere length, and telomerase activity," *Medicine and Science in Sports and Exercise*, vol. 40, no. 10, pp. 1764–1771, 2008.

[44] E. S. Epel, E. H. Blackburn, J. Lin et al., "Accelerated telomere shortening in response to life stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 49, pp. 17312–17315, 2004.

[45] E. Puterman, J. Lin, E. Blackburn, A. O'Donovan, N. Adler, and E. Epel, "The power of exercise: buffering the effect of chronic stress on telomere length," *PLoS ONE*, vol. 5, no. 5, Article ID e10837, 2010.

[46] F. W. Booth and M. J. Laye, "The future: genes, physical activity and health," *Acta Physiologica*, vol. 199, no. 4, pp. 549–556, 2010.

[47] S. M. Roth, T. Rankinen, J. M. Hagberg et al., "Advances in exercise, fitness, and performance genomics in 2011," *Medicine and Science in Sports and Exercise*, vol. 44, no. 5, pp. 809–817, 2012.

[48] E. S. Epel, J. Lin, F. H. Wilhelm et al., "Cell aging in relation to stress arousal and cardiovascular disease risk factors," *Psychoneuroendocrinology*, vol. 31, no. 3, pp. 277–287, 2006.

[49] D. Ornish, J. Lin, J. M. Chan et al., "Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study," *The Lancet Oncology*, vol. 14, no. 11, pp. 1112–1120, 2013.

[50] D. Ornish, J. Lin, J. Daubenmier et al., "Increased telomerase activity and comprehensive lifestyle changes: a pilot study," *The Lancet Oncology*, vol. 9, no. 11, pp. 1048–1057, 2008.

[51] S. García-Calzón, A. Gea, C. Razquin et al., "Longitudinal association of telomere length and obesity indices in an intervention study with a Mediterranean diet: the PREDIMED-NAVARRA trial," *International Journal of Obesity*, 2013.

[52] V. Boccardi, A. Esposito, M. R. Rizzo et al., "Mediterranean diet, telomere maintenance and health status among elderly," *PLoS ONE*, vol. 8, no. 4, 2013.

[53] M. Du, J. Prescott, M. C. Cornelis et al., "Genetic predisposition to higher body mass index or type 2 diabetes and leukocyte telomere length in the Nurses' Health Study," *PLoS ONE*, vol. 8, no. 2, 2013.

[54] B. L. Needham, N. Adler, S. Gregorich et al., "Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999–2002," *Social Science & Medicine*, vol. 85, pp. 1–8, 2013.

[55] A. M. Valdes, T. Andrew, J. P. Gardner et al., "Obesity, cigarette smoking, and telomere length in women," *The Lancet*, vol. 366, no. 9486, pp. 662–664, 2005.

[56] A. Aviv, A. M. Valdes, and T. D. Spector, "Human telomere biology: pitfalls of moving from the laboratory to epidemiology," *International Journal of Epidemiology*, vol. 35, no. 6, pp. 1424–1429, 2006.

[57] M. Du, J. Prescott, P. Kraft et al., "Physical activity, sedentary behavior, and leukocyte telomere length in women," *American Journal of Epidemiology*, vol. 175, no. 5, pp. 414–422, 2012.

[58] J. H. Kim, J. H. Ko, D. C. Lee et al., "Habitual physical exercise has beneficial effects on telomere length in postmenopausal women," *Menopause*, vol. 19, no. 10, pp. 1109–1115, 2012.

[59] R. J. Simpson, C. Cosgrove, M. M. Chee et al., "Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans," *Exercise Immunology Review*, vol. 16, pp. 40–55, 2010.

[60] L. Mirabello, W.-Y. Huang, J. Y. Y. Wong et al., "The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer," *Aging Cell*, vol. 8, no. 4, pp. 405–413, 2009.

[61] L. F. Cherkas, J. L. Hunkin, B. S. Kato et al., "The association between physical activity in leisure time and leukocyte telomere length," *Archives of Internal Medicine*, vol. 168, no. 2, pp. 154–158, 2008.

[62] T. J. LaRocca, D. R. Seals, and G. L. Pierce, "Leukocyte telomere length is preserved with aging in endurance exercise-trained adults and related to maximal aerobic capacity," *Mechanisms of Ageing and Development*, vol. 131, no. 2, pp. 165–167, 2010.

[63] C. Werner, T. Fürster, T. Widmann et al., "Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall," *Circulation*, vol. 120, no. 24, pp. 2438–2447, 2009.

[64] J. Krauss, R. Farzaneh-Far, E. Puterman et al., "Physical fitness and telomere length in patients with coronary heart disease: findings from the heart and soul study," *PLoS ONE*, vol. 6, no. 11, Article ID e26983, 2011.

[65] J. Denham, C. P. Nelson, B. J. O'Brien et al., "Longer leukocyte telomeres are associated with ultra-endurance exercise independent of cardiovascular risk factors," *PLoS ONE*, vol. 8, no. 7, 2013.

[66] S. Mathur, A. Ardestani, B. Parker et al., "Telomere length and cardiorespiratory fitness in marathon runners," *Journal of Investigative Medicine*, vol. 61, no. 3, pp. 613–615, 2013.

[67] C. Mason, R. A. Risques, L. Xiao et al., "Independent and combined effects of dietary weight loss and exercise on leukocyte telomere length in postmenopausal women," *Obesity*, vol. 21, no. 12, pp. E549–E554, 2013.

[68] Z. Song, G. von Figura, Y. Liu et al., "Lifestyle impacts on the aging-associated expression of biomarkers of DNA damage and telomere dysfunction in human blood," *Aging Cell*, vol. 9, no. 4, pp. 607–615, 2010.

[69] J. Woo, N. Tang, and J. Leung, "No association between physical activity and telomere length in an elderly Chinese population 65 years and older," *Archives of Internal Medicine*, vol. 168, no. 19, pp. 2163–2164, 2008.

[70] I. B. Osthus, A. Sgura, F. Berardinelli et al., "Telomere length and long-term endurance exercise: does exercise training affect biological age? A pilot study," *PLoS ONE*, vol. 7, no. 12, 2012.

[71] F. Kadi, E. Ponsot, K. Piehl-Aulin et al., "The effects of regular strength training on telomere length in human skeletal muscle," *Medicine and Science in Sports and Exercise*, vol. 40, no. 1, pp. 82–87, 2008.

[72] E. Ponsot, J. Lexell, and F. Kadi, "Skeletal muscle telomere length is not impaired in healthy physically active old women and men," *Muscle and Nerve*, vol. 37, no. 4, pp. 467–472, 2008.

[73] D. E. Rae, A. Vignaud, G. S. Butler-Browne et al., "Skeletal muscle telomere length in healthy, experienced, endurance runners," *European Journal of Applied Physiology*, vol. 109, no. 2, pp. 323–330, 2010.

[74] M. J. Laye, T. P. J. Solomon, K. Karstoft, K. K. Pedersen, S. D. Nielsen, and B. K. Pedersen, "Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event," *Journal of Applied Physiology*, vol. 112, no. 5, pp. 773–781, 2012.

[75] S. Savela, O. Saijonmaa, T. E. Strandberg et al., "Physical activity in midlife and telomere length measured in old age," *Experimental Gerontology*, vol. 48, no. 1, pp. 81–84, 2012.

[76] M. Collins, V. Renault, L. A. Grobler et al., "Athletes with exercise-associated fatigue have abnormally short muscle DNA telomeres," *Medicine and Science in Sports and Exercise*, vol. 35, no. 9, pp. 1524–1528, 2003.

[77] M. T. Hemann, M. A. Strong, L.-Y. Hao, and C. W. Greider, "The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability," *Cell*, vol. 107, no. 1, pp. 67–77, 2001.

[78] Y. Zou, A. Sfeir, S. M. Gryaznov, J. W. Shay, and W. E. Wright, "Does a sentinel or a subset of short telomeres determine replicative senescence?" *Molecular Biology of the Cell*, vol. 15, no. 8, pp. 3709–3718, 2004.

[79] E. M. F. Van Craenenbroeck, C. J. Vrints, S. E. Haine et al., "A maximal exercise bout increases the number of circulating CD34+/KDR+ endothelial progenitor cells in healthy subjects. Relation with lipid profile," *Journal of Applied Physiology*, vol. 104, no. 4, pp. 1006–1013, 2008.

[80] M. R. Bonsignore, G. Morici, R. Riccioni et al., "Hemopoietic and angiogenetic progenitors in healthy athletes: different responses to endurance and maximal exercise," *Journal of Applied Physiology*, vol. 109, no. 1, pp. 60–67, 2010.

[81] S. Witkowski, M. M. Lockard, N. T. Jenkins, T. O. Obisesan, E. E. Spangenburg, and J. M. Hagberg, "Relationship between circulating progenitor cells, vascular function and oxidative stress with long-term training and short-term detraining in older men," *Clinical Science*, vol. 118, no. 4, pp. 303–311, 2010.

[82] G. Aubert, M. Hills, and P. M. Lansdorp, "Telomere length measurement-Caveats and a critical assessment of the available technologies and tools," *Mutation Research*, vol. 730, no. 1-2, pp. 59–67, 2012.

[83] R. K. Moyzis, J. M. Buckingham, L. S. Cram et al., "A highly conserved repetitive DNA sequence, (TTAGGG)(n), present at the telomeres of human chromosomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 18, pp. 6622–6626, 1988.

[84] R. M. Cawthon, "Telomere measurement by quantitative PCR," *Nucleic acids research*, vol. 30, no. 10, p. e47, 2002.

[85] P. Correa, "The new era of cancer epidemiology," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 1, no. 1, pp. 5–11, 1991.

[86] Y.-A. Shin, J.-H. Lee, W. Song, and T.-W. Jun, "Exercise training improves the antioxidant enzyme activity with no changes of telomere length," *Mechanisms of Ageing and Development*, vol. 129, no. 5, pp. 254–260, 2008.

[87] D. C. Nieman, K. S. Buckley, D. A. Henson et al., "Immune function in marathon runners versus sedentary controls," *Medicine and Science in Sports and Exercise*, vol. 27, no. 7, pp. 986–992, 1995.

[88] M. J. Warhol, A. J. Siegel, W. J. Evans, and L. M. Silverman, "Skeletal muscle injury and repair in marathon runners after competition," *American Journal of Pathology*, vol. 118, no. 2, pp. 331–339, 1985.

[89] F. Kadi and E. Ponsot, "The biology of satellite cells and telomeres in human skeletal muscle: effects of aging and physical activity," *Scandinavian Journal of Medicine and Science in Sports*, vol. 20, no. 1, pp. 39–48, 2010.

[90] H. Yin, F. Price, and M. A. Rudnicki, "Satellite cells and the muscle stem cell niche," *Physiological Reviews*, vol. 93, no. 1, pp. 23–67, 2013.

[91] S. Decary, V. Mouly, C. Ben Hamida, A. Sautet, J. P. Barbet, and G. S. Butler-Browne, "Replicative potential and telomere length in human skeletal muscle: implications for satellite cell-mediated gene therapy," *Human Gene Therapy*, vol. 8, no. 12, pp. 1429–1438, 1997.

[92] V. Mouly, A. Aamiri, A. Bigot et al., "The mitotic clock in skeletal muscle regeneration, disease and cell mediated gene therapy," *Acta Physiologica Scandinavica*, vol. 184, no. 1, pp. 3–15, 2005.

[93] V. Renault, L.-E. Thornell, G. Butler-Browne, and V. Mouly, "Human skeletal muscle satellite cells: aging, oxidative stress and the mitotic clock," *Experimental Gerontology*, vol. 37, no. 10-11, pp. 1229–1236, 2002.

[94] S. Decary, C. Ben Hamida, V. Mouly, J. P. Barbet, F. Hentati, and G. S. Butler-Browne, "Shorter telomeres in dystrophic muscle consistent with extensive regeneration in young children," *Neuromuscular Disorders*, vol. 10, no. 2, pp. 113–120, 2000.

[95] S. Joanisse, J. B. Gillen, L. M. Bellamy et al., "Evidence for the contribution of muscle stem cells to nonhypertrophic skeletal muscle remodeling in humans," *FASEB Journal*, vol. 27, no. 11, pp. 4596–4605, 2013.

[96] S. Kawanishi and S. Oikawa, "Mechanism of telomere shortening by oxidative stress," *Annals of the New York Academy of Sciences*, vol. 1019, pp. 278–284, 2004.

[97] G. Saretzki and T. Von Zglinicki, "Replicative aging, telomeres, and oxidative stress," *Annals of the New York Academy of Sciences*, vol. 959, pp. 24–29, 2002.

[98] V. Serra, T. Grune, N. Sitte, G. Saretzki, and T. Von Zglinicki, "Telomere length as a marker of oxidative stress in primary human fibroblast cultures," *Annals of the New York Academy of Sciences*, vol. 908, pp. 327–330, 2000.

[99] T. Von Zglinicki, V. Serra, M. Lorenz et al., "Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor?" *Laboratory Investigation*, vol. 80, no. 11, pp. 1739–1747, 2000.

[100] C. Wang, D. Jurk, M. Maddick, G. Nelson, C. Martin-ruiz, and T. Von Zglinicki, "DNA damage response and cellular senescence in tissues of aging mice," *Aging Cell*, vol. 8, no. 3, pp. 311–323, 2009.

[101] L. Daniali, A. Benetos, E. Susser et al., "Telomeres shorten at equivalent rates in somatic tissues of adults," *Nature Communications*, vol. 4, 2013.

[102] N. M. V. Gomes, O. A. Ryder, M. L. Houck et al., "Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination," *Aging Cell*, vol. 10, no. 5, pp. 761–768, 2011.

[103] K. R. Prowse and C. W. Greider, "Developmental and tissue-specific regulation of mouse telomerase and telomere length," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 11, pp. 4818–4822, 1995.

[104] R. M. Cawthon, K. R. Smith, E. O'Brien, A. Sivatchenko, and R. A. Kerber, "Association between telomere length in blood and mortality in people aged 60 years or older," *The Lancet*, vol. 361, no. 9355, pp. 393–395, 2003.

[105] L. F. Cherkas, A. Aviv, A. M. Valdes et al., "The effects of social status on biological aging as measured by white-blood-cell telomere length," *Aging Cell*, vol. 5, no. 5, pp. 361–365, 2006.

[106] D. C. Nieman, "The immune response to prolonged cardiorespiratory exercise," *American Journal of Sports Medicine*, vol. 24, pp. S98–S103, 1996.

[107] K. Hiyama, Y. Hirai, S. Kyoizumi et al., "Activation of telomerase in human lymphocytes and hematopoietic progenitor cells," *Journal of Immunology*, vol. 155, no. 8, pp. 3711–3715, 1995.

[108] Y. Matsubara, M. Murata, K. Watanabe et al., "Coronary artery disease and a functional polymorphism of hTERT," *Biochemical and Biophysical Research Communications*, vol. 348, no. 2, pp. 669–672, 2006.

[109] Y. Matsubara, M. Murata, T. Yoshida et al., "Telomere length of normal leukocytes is affected by a functional polymorphism of hTERT," *Biochemical and Biophysical Research Communications*, vol. 341, no. 1, pp. 128–131, 2006.

[110] B. Britt-Compton, R. Capper, J. Rowson, and D. M. Baird, "Short telomeres are preferentially elongated by telomerase in human cells," *FEBS Letters*, vol. 583, no. 18, pp. 3076–3080, 2009.

[111] M. T. Hemann and C. W. Greider, "Wild-derived inbred mouse strains have short telomeres," *Nucleic Acids Research*, vol. 28, no. 22, pp. 4474–4478, 2000.

[112] I. Horikawa, Y. J. Chiang, T. Patterson et al., "Differential cis-regulation of human versus mouse TERT gene expression *in vivo* identification of a human-specific repressive element," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 51, pp. 18437–18442, 2005.

[113] E. H. Blackburn, "Cell biology: shaggy mouse tales," *Nature*, vol. 436, no. 7053, pp. 922–923, 2005.

[114] M. A. Blasco, H. W. Lee, M. Rizen et al., "Mouse models for the study of telomerase," *Ciba Foundation Symposium*, vol. 211, pp. 160–170, 1997.

[115] M. A. Strong, S. L. Vidal-Cardenas, B. Karim, H. Yu, N. Guo, and C. W. Greider, "Phenotypes in mTERT+/- and mTERT-/- mice are due to short telomeres, not telomere-independent functions of telomerase reverse transcriptase," *Molecular and Cellular Biology*, vol. 31, no. 12, pp. 2369–2379, 2011.

[116] V. Cattan, N. Mercier, J. P. Gardner et al., "Chronic oxidative stress induces a tissue-specific reduction in telomere length in CAST/Ei mice," *Free Radical Biology and Medicine*, vol. 44, no. 8, pp. 1592–1598, 2008.

[117] C. Werner, M. Hanhoun, T. Widmann et al., "Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis," *Journal of the American College of Cardiology*, vol. 52, no. 6, pp. 470–482, 2008.

[118] J.-I. Park, A. S. Venteicher, J. Y. Hong et al., "Telomerase modulates Wnt signalling by association with target gene chromatin," *Nature*, vol. 460, no. 7251, pp. 66–72, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 821678, 10 pages
http://dx.doi.org/10.1155/2013/821678

Review Article

Why Control Activity? Evolutionary Selection Pressures Affecting the Development of Physical Activity Genetic and Biological Regulation

J. Timothy Lightfoot

Huffines Institute of Sports Medicine and Human Performance, Health and Kinesiology Department, Texas A&M University, 356 Blocker Building, 4243 TAMU, College Station, TX 77843, USA

Correspondence should be addressed to J. Timothy Lightfoot; tlightfoot@hlkn.tamu.edu

Received 11 October 2013; Accepted 20 November 2013

Academic Editor: Jaakko Kaprio

The literature strongly suggests that daily physical activity is genetically and biologically regulated. Potential identities of the responsible mechanisms are unclear, but little has been written concerning the possible evolutionary selection pressures leading to the development of genetic/biological controls of physical activity. Given the weak relationship between exercise endurance and activity levels and the differential genomic locations associated with the regulation of endurance and activity, it is probable that regulation of endurance and activity evolved separately. This hypothesis paper considers energy expenditures and duration of activity in hunter/gatherers, pretechnology farmers, and modern Western societies and considers the potential of each to selectively influence the development of activity regulation. Food availability is also considered given the known linkage of caloric restriction on physical activity as well as early data relating food oversupply to physical inactivity. Elucidating the selection pressures responsible for the genetic/biological control of activity will allow further consideration of these pressures on activity in today's society, especially the linkages between food and activity. Further, current food abundance is removing the cues for activity that were present for the first 40,000 years of human evolution, and thus future research should investigate the effects of this abundance upon the mechanisms regulating activity.

1. Introduction

It has been a relatively short period of time since the first suggestions were made that physical activity may have a genetic control component [1]. Since that time, significant strides have been made in understanding the basis of both genetic and biological regulatory mechanisms of physical activity. Estimates of the amount of physical activity regulated by genetics are variable, with both human and animal studies suggesting that genetics is responsible for between 29% and 92% of activity [2, 3] with larger studies suggesting that this number is closer to 50% [4, 5]. Further, a deep and extensive set of studies have suggested that inherent biological pathways (e.g., sex steroids) have a marked control over physical activity [6–12]. Whether variability in this biological control of activity is controlled by genetics or an alternative biological mechanism is currently unclear, and thus this paper will refer to "genetic/biological" control. Thus, the purpose of this hypothesis paper is to propose a conceptual framework for considering why there might be genetic/biological regulation of physical activity and the potential selection pressures that drove the evolution of physical activity regulation. Additionally, as the scope of this paper is limited, the reader is referred to other reviews for a full discussion of the factors influencing the variability of physical activity heritability [13–17].

2. Exercise Endurance and Physical Activity Appear to Have Evolved Separately

It is generally accepted that *Homo sapiens* initially evolved the anatomical and physiological capability for endurance running approximately 40,000–50,000 years ago [18, 19]. However, it is unclear whether the genetic control of physical

activity is a derivation from the selected traits that allowed endurance running or whether physical activity evolved as a separate trait. As we have noted elsewhere [20], it is tempting to suggest that physiological characteristics that increase endurance (e.g., mitochondrial density, fiber type) might also be key components leading to higher voluntary physical activity levels, and thus both exercise endurance and physical activity would have evolved in lockstep with each other. However, two independent lines of evidence suggest that exercise capacity and activity levels did not evolve together.

First, correlations of exercise capacity and activity levels in humans generally suggest that while the relationship is positive, there is only a low to moderate association between activity and endurance in adults (r = 0.01 to 0.61; [21–24]). In particular, 13 studies reviewed by Lamb and Brodie [23] demonstrated that the wide variability in the relationship between physical activity and endurance is potentially due to the use of various populations and differing recall methods for activity, as well as the use of submaximal or other indirect measures of exercise capacity. In children, the literature is not much clearer, with the largest analysis of available data (n = 20 studies and 53 comparisons; [25]) noting that the median relationship between physical activity and exercise capacity in children and adolescents was extremely low (r^2 value < 0.03). Results from studies using more objective measures of physical activity in children have shown higher associations in 8- to 10-year-old children (r-values = 0.59–0.66; [26]) and 11–18-year-old children (r-values = 0.031–0.393; [27]). However, in early studies relating direct measures of exercise capacity and accelerometer-based measurements of activity in adults, the association between exercise capacity and physical activity appears to be relatively weak, with males showing no correlation (P = 0.41, r^2 = 0.017, n = 42, and age = 25.2 ± 5.7 years) and females showing a significant but weak correlation (P = 0.003, r^2 = 0.11, n = 76, and age = 23.6 ± 5.2 years; [28]). Thus, while there appears to be methodological constraints on the earlier data, recent data still shows a moderate association at best between exercise capacity and activity levels with these associations dropping precipitously when used to fit prediction equations (i.e., r^2 values). Animal models, which allow the objective measurement of activity and exercise capacity without many of the environmental influences and confounds present in human models, have supported the low/moderate correlation (r = 0.15–0.44) between exercise capacity and daily activity levels [29–32]. Thus, even though there are methodological concerns with the data, the predominant view from the literature is that while there is a positive relationship between activity level and exercise capacity, that relationship is weak and certainly cannot be used to predict either activity level or functional capacity.

A second line of developing evidence that suggests that physical activity and exercise capacity did not evolve together involves the actual genetic mechanisms that underlie inherent exercise capacity and inherent physical activity level. While delineation of actual genetic mechanisms for either phenotype is still lacking for both animals and humans, genomic loci (i.e., QTL) associated with various indices of exercise capacity and physical activity appear to be separate and distinct [20, 33–37]. These distinct QTL suggest that genetic regulation of these traits arises through differing pathways. Thus, while there may be pathways common to both phenotypes, the low/moderate association between activity and exercise capacity in humans and animals, as well as the differing genomic loci associated with each trait, suggests that the underlying physiological regulation of inherent exercise capacity and inherent activity level of mammals evolved separately.

3. Potential Selection Pressures for the Evolution of Physical Activity Regulation

The evolution of a physiological system is necessarily linked to genetic selection pressure [38], and the current literature is silent as to what selection pressure would have driven the evolution of systems to regulate physical activity. While hunter/gatherers were well known for having irregular, but sometimes extensive, hunting/foraging ranges [39, 40], their overall activity patterns were not uniform (e.g., [41]). Recent data have suggested that total daily energy expenditure demands (not corrected for body weight) of hunter/gatherers were not different than modern, western-based lifestyles [42, 43]. Further, comparison of energy expenditure by weight between pretechnology farmers and hunter-gatherer populations does not show significant differences in daily energy expenditures (Tables 1 and 2 and Figure 1). Whereas current Western populations show decreased energy expenditures when corrected by weight, it can be argued that the higher energy expenditure required by either hunting/gathering and/or pretechnology farming could have been a selection pressure driving the development of activity regulation. However, some investigators dispute that energy expenditure requirements have decreased [42, 43] which casts questions on the potential role that energy expenditure played in evolving activity regulation.

While it is unclear whether energy expenditure would have been a selection pressure in the evolution of physical activity control mechanisms, comparisons of required daily activity (i.e., duration of activity) in nontechnology dependent agricultural societies (Table 2) show that the activity levels exhibited by both males and females in these populations were at least 3-fold higher than activity levels shown in hunter/gatherer populations (Figure 2). For example, Panter-Brick [44, 45] characterized a Nepali agropastoralist community (the Tamang) living at 1,350 to 3,800 m that exhibited food self-sufficiency through manual farming and livestock rearing with little to no technology use. Using both direct observations and indirect respirometry, Panter-Brick observed that the men worked an average of 8.15 ± 0.9 hours/day, while the women worked 8.4 ± 0.8 hrs/day. More recently, Bassett and colleagues [46] measured physical activity levels in a North American labor-intensive, non-technological Amish farming community. In this population, Bassett and colleagues observed that the men averaged vigorous, moderate, or walking activity for 9.3 hrs/day and women averaged 6.9 hrs/day and only sat 3.3 hr/day (13% of the day) and

Table 1: Physical activity energy expenditures of various hunter/gatherer populations.

Populations	Sex	TEE	AEE	AEE/wt	Foraging range/day (km)	Weight (kg)
Ju/'hoansi (Africa)[a,b]	M	2178	903	19.52	14.9[a]	46.0
Ju/'hoansi (Africa)[a,b]	F	1770	600	14.52	9.10[a]	41.0
Ache (Paraguay)[b]	M	3327	1778	29.75	19.2	59.6
Ache (Paraguay)[b]	F	2626	1232	24.51	9.20	51.8
Hadza (Tanzania)[c]	M	2649	1476.9	29.0	11.4	50.9
Hadza (Tanzania)[c]	F	1877	822.5	18.9	5.8	43.4
Average hunter/gatherer (±SD)	M	2718 (578)	1386.0 (444.5)	26.1 (5.7)*	15.3 (5.4)	52.2 (6.9)*
Average hunter/gatherer (±SD)	F	2091 (466)	884.8 (320.6)	19.3 (5.0)*	7.5 (2.4)	45.4 (5.7)*
Average Western population[c]	M	3053 (464)	1366.3 (268.3)	16.9 (3.3)	4.2 (2.7)[d]	81 (11.1)
Average Western population[c]	F	2347 (360)	950.0 (177.1)	12.8 (2.4)	3.2 (2.2)[d]	74.4 (12.8)

TEE: total energy expenditure (kcal/day); RMR: resting metabolic rate (kcal/day); AEE: activity energy expenditure = TEE-RMR; AEE/wt: activity energy expenditure divided by weight (kcal/kg/d); data from [a][39]; [b][40]; [c][42]; [d]values calculated using average daily step counts for men and women [47] and average step lengths for men [48] and women [49]. *Significantly different $P < 0.05$ between hunter/gatherer and average western population. Values for average western population TEE, AEE, and AEE/wt used in statistical analysis derived from artificial dataset derived from means, standard deviations, and subject numbers as reported in [42].

Table 2: Physical activity energy expenditures of various agricultural populations.

Populations	Sex	TEE	AEE	AEE/wt	Weight (kg)
Tamang[a] (Nepal)	M	3164	1674.3	31.3	53.5
Tamang[a] (Nepal)	F	2382	1141.2	24.5	46.6
Devarishi Kuppam[b,c,j] (Tamil Nadu, India)	M	2860	1580.3	31.5	50.2
Devarishi Kuppam[b,c,j] (Tamil Nadu, India)	F	1984	902.3	20.6	43.8
Gambian[b,k] (Gambia)	M	2292	716.4	12.3	58.47
Gambian[b,d,e](Gambia)	F	2480	1178.45	23.73	49.7
Mossi[b,f] (Upper Volta)	M	2913	920.51	15.74	58.5
Mossi[b,g] (Upper Volta)	F	2603	822.55	16.25	50.6
Senegal[b,h] (Senegal)	M	2538	901.25	13.78	65.4
Senegal[b,h] (Senegal)	F	2573	1219.75	21.10	57.8
Amish[i] (Canada)	M	3100	1292.3	17.65	73.2
Amish[i] (Canada)	F	1850	304.04	4.85	62.6
Aymara[l] (Bolivia)	M	2329	1299.4	23.7	54.8
Aymara[l] (Bolivia)	F	2654	1184.2	24.4	48.6
Average farming populations (±SD)	M	2742 (357)	1197.9 (362.7)	20.8 (8.1)†	59.2 (7.8)†
Average farming populations (±SD)	F	2361 (318)	964.6 (329.3)	19.3 (7.0)†	51.4 (6.6)†

TEE: total energy expenditure (kcal/day; average between dry and wet season where available); RMR: resting metabolic rate (kcal/day); AEE: activity energy expenditure =TEE-RMR; AEE/wt: activity energy expenditure divided by weight in kcal/kg/d; data from [a][44, 45]; [b]reviewed by [45]; data from [c][50]; [d][51]; [e][52]; [f][53]; [g][54]; [h][55]; [i][46]: BMRs estimated using formula (3.5 mL/kg/min O_2) ∗ 4.9; [j][56]; [k][57] values derived from Ph.D. thesis [58]; [l][59]. †Significantly different $P < 0.05$ between farming and Western populations. Values for Western TEE, AEE, and AEE/wt used in statistical analysis derived from artificial dataset derived from means, standard deviations, and subject numbers as reported in [42]. No significant differences between hunter/gatherer and Farming populations.

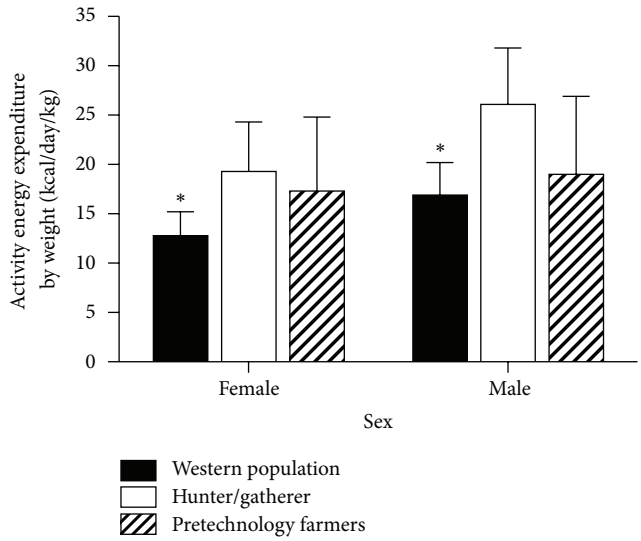


Figure 1: Activity energy expenditure by weight. The amount of energy expended on nonbasal activity and standardized by weight of population. Hunter/gatherer population estimates using Ju/'hoansi [39], Ache [40], and Hadza [42]. Pretechnology farmer values from populations in Figure 2 [39–41, 45, 46, 50, 54, 56, 60]. Western population data from [42]. *Significantly lower ($P < 0.05$) than Hunter/gatherer and Pretechnology farmers. There were no statistical differences between Hunter/gatherers and Pretechnology farmers. Values for Western AEE/wt used in statistical analysis derived from artificial dataset derived from means, standard deviations, and subject numbers as reported in [42].

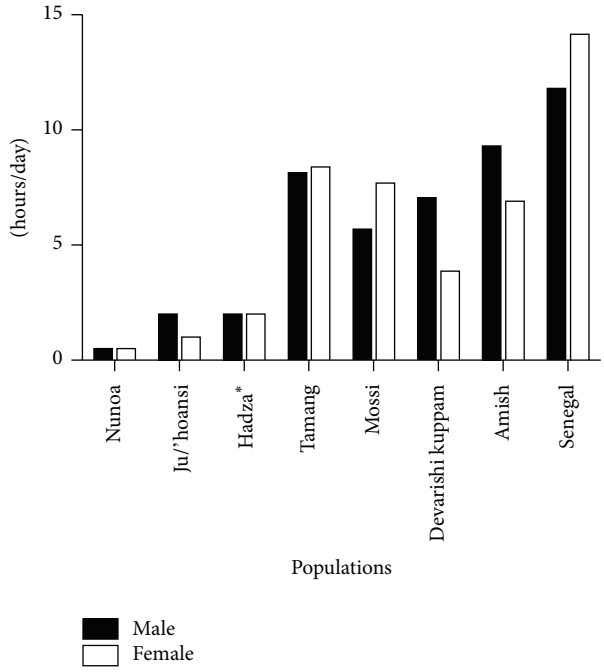


Figure 2: Total time spent in vigorous, moderate, or walking activity on a daily basis in hunter/gatherer (Nunoa, Ju/'hoansi, Hadza) or nontechnological agriculture-based populations (Tamang, Mossi, Devarishi Kuppam, Amish, and Senegali). Data from [39–41, 45, 46, 50, 54, 56, 60]. *Hadza activity time based on estimates from [41] which provide the only known total daily activity time estimates for this population.

2.8 hr/day (12% of the day), respectively (Figure 2). This extensive physical activity pattern was reflected in their total steps per day where the Amish men averaged 18,425 steps/day and the women averaged 14,196 steps/day. Given the extensive data from both Panter-Brick and Bassett's groups, as well as from other nontechnological farming populations (Table 2 and Figure 2), there is little doubt that non-technological subsistence farming required extensive, long-duration, and low-intensity physical activity on a daily basis.

The sustained agricultural activity requirement may not have required higher total daily energy expenditures than hunting/gathering (Figure 1), but the extensive time requirements that were 3–5-fold higher than hunting/gathering (Figure 2) would have required the physiological capability to complete lower intensities of exercise for much longer time frames than in hunting/gathering populations. The differing time requirements across which the energy was expended would have stressed different substrate systems—especially in farmers—favoring those individuals that could store and metabolize fats for longer duration activity. Thus, the ability to be physically active for long periods of the day and the requisite requirement to produce calories from fat stores could have been a significant genetic selection pressure in the development of biological/genetic control of physical activity. Further, in those early populations that adopted agriculture, individuals that were predisposed to higher levels of motivation and physical capability for daily activity would have been more successful and would have had a greater chance of reproductive success [61]. In essence, a farmer could not have been lazy and insure that his genes would be passed on to future generations because his family would not survive.

Whether the genetic selection pressure linked to the development of biological control of physical activity was energy expenditure or duration of activity, ultimately, both factors link back to the availability of food. While estimates of average hunter/gatherer foraging ranges can appear extensive (e.g., Table 1), hunter/gatherers did not range far and had reduced energy expenditure when food was close at hand. When food became difficult to get or the hunting/foraging ranges became lengthy, hunter/gatherers simply moved to more fertile sites where food was more abundant [39]. For farmers, because they were bound to a specific location, without physical activity, there was no food. In fact, food availability appears to have a direct causative effect on physical activity that is exhibited in both animal and human models, especially in the area of caloric restriction. Numerous studies report that short-term caloric restriction decreases rodent activity, but long-term caloric restriction actually increases physical activity (e.g., [62]). This same phenomenon appears in nonhuman primates (e.g., rhesus monkeys; [63]) with a concomitant increase in metabolic efficiency of movement. Further, it has been suggested that this caloric restriction-related hyperactivity also occurs in humans. Casper [64] hypothesized that, in the majority of anorexia nervosa (AN) patients that present hyperactivity (suggested to range from 38% to 70% of AN; [65, 66]), the increased activity is a result of the hypocaloric nature of AN, which differs from the lethargy seen in semistarvation

states. Casper suggested several potential physiological pathways that govern this human caloric restriction and related hyperactivity. For example, Casper [64] uniquely suggests that mutations in the "foraging" gene first found in drosophila (*dgcalpha1*; [67]) can increase foraging locomotion in fruit flies [67] and honey bees [68] and may be involved in the regulation of the increased activity in AN patients. Further, the gene homologous to *dgcalpha1* in rodents and humans is "guanylate cyclase 1, soluble, alpha 2" (*GUCY1A2*), which is one of the genes that encodes soluble guanylyl cyclase (sGC), the most sensitive receptor for nitric oxide [69]. Further, the mouse homolog of *GUCY1A2* (i.e. *Gucy1A2*) is located on Chrm. 9 downstream of a known physical activity-related QTL [20]. The involvement of *GUCY1A2*, or any other genetically-based mechanism regulating activity, would support Epling and Pierce's early speculation [70] that AN patients represent a natural selection of individuals who become active during food shortages, leading to an increased chance of food finding even at the risk of negative caloric balance. Garland and Kelly [38] also suggested that individuals with higher foraging behavior could be an example of a directed natural selection. Thus, in individuals with foraging behavior more suited to the available food supply, the alleles responsible for this higher locomotor activity may be favored more highly in reproduction [38].

Conversely, to our knowledge, there have been no direct studies designed to determine if excess caloric intake directly decreases activity in human and/or animal models. Indirectly, several studies suggest that, with overfeeding, physical activity levels decrease. For example, in an elegant study, Levine and colleagues [71] showed that overfeeding both lean and obese human subjects 1,000 kcal/day above their weight maintenance needs resulted in significant decreases in free-living walking in both groups. Schmidt et al. [72] directly measured spontaneous physical activity levels (i.e., NEAT) in obesity-prone and obesity-resistant individuals and observed decreases in spontaneous physical activity in the obesity-prone individuals three days after overfeeding (but not in obesity-resistant individuals). Anecdotally, it has been observed [73] that male baboons are markedly less active (e.g., reduced climbing, laying close to sugar-source) when their caloric intake was significantly increased through the availability of a sweetened beverage containing water, high fructose corn syrup, and artificial fruit flavoring [74]. Supporting these observations are indirect results strongly suggesting, in both adults and children, that decreased physical activity was driven by an increased adiposity as opposed to adiposity being an effect of decreased activity [75, 76]. Neither Ekelund and colleagues [75] or Metcalf et al. [76] proposed potential causative biological mechanistic ties between overfeeding and inactivity, instead preferring to speculate on potential biomechanical and physical discomfort of increased weight prohibiting activity. However, other meta-analyses and animal studies have shown no relationship between body mass and activity levels (e.g., [77–79]) suggesting that it is not body weight per se decreasing activity, but rather the increase in caloric intake. Supporting this indirect evidence of a tie between overfeeding and a decrease in physical activity is a potential mechanistic pathway. It has long been known that removal of sex hormones and subsequent reduction in testosterone or estrogen levels results in large decreases in activity (e.g., [7, 80]) that can be rescued with administration of testosterone and/or estrogen which is mediated primarily through androgenic receptor pathways [6]. Recently, Bouchard et al. [81] showed conclusively that overfeeding in humans significantly decreases androgenic production—especially in males. Therefore, hypothetically, this reduction in androgenic production from overfeeding could result in a reduction in physical activity through established pathways. Thus, while it is not currently known whether increased caloric availability decreases the drive for activity, there are tentative evidence and potential hypothetical mechanisms that strongly support further research into this question.

There are some significant limitations to the preceding discussion that should be considered in interpretation of these data. In particular, the quantification of daily activity levels in both human and animals continues to undergo refinement, and the limitations of older methods should be appreciated [82]. Thus, the use of older studies that used less than optimal methods of activity measurement, such as survey or observational methods, may need to be reconsidered. For example, much of the extant hunter/gatherer activity data is based on observational or estimated activity levels and can be open to question. An example of this limitation is the recent publication of direct measures of energy expenditures collected in a Hadza population by Pontzer et al. [42] which contradict earlier observations in the Hadza which noted marked swings and inconsistencies in Hadza activity patterns [41]. Further complicating the issue of valid activity measurement is the rapid diminishing of the opportunity to collect data on peoples that represent hunter/gatherer lifestyles. Lee, who is considered the leading expert on the Ju/'hoansi, has observed the creeping influence of Western lifestyle and the diminishment of hunting/gathering in the Ju/'hoansi is due to wide access to motorized transport, other food sources, and reduction in available foraging range [39]. Thus, if modern data were collected on the Ju/'hoansi, whether this data truly represented a Paleolithic hunter/gatherer lifestyle would be a fair question—as it is with the Hadza data of Pontzer and colleagues [42]. Therefore, it is important to use the best data available in populations that best represent the target populations and we have strived to do so in this paper.

As scientists work to understand the identities of the genetic and biological mechanisms that control physical activity, it is important to also work to develop an understanding of the evolutionary selection pressures that have led to these activity regulation mechanisms. At this point, it is unclear what the specific genetic selection pressures were that caused the development of genetic/biological regulation of activity, but there are suggestions that physical activity evolved separately from endurance capability (Figure 3). Further, while energy expenditure may be an attractive candidate for genetic selection pressure, data suggests that total daily energy expenditure has not significantly changed, but rather, the duration of daily activity required to procure food radically changed with the adoption of agriculture approximately 10,000 years ago. Additionally, the suggestions of an inverse link between caloric intake and physical activity would add

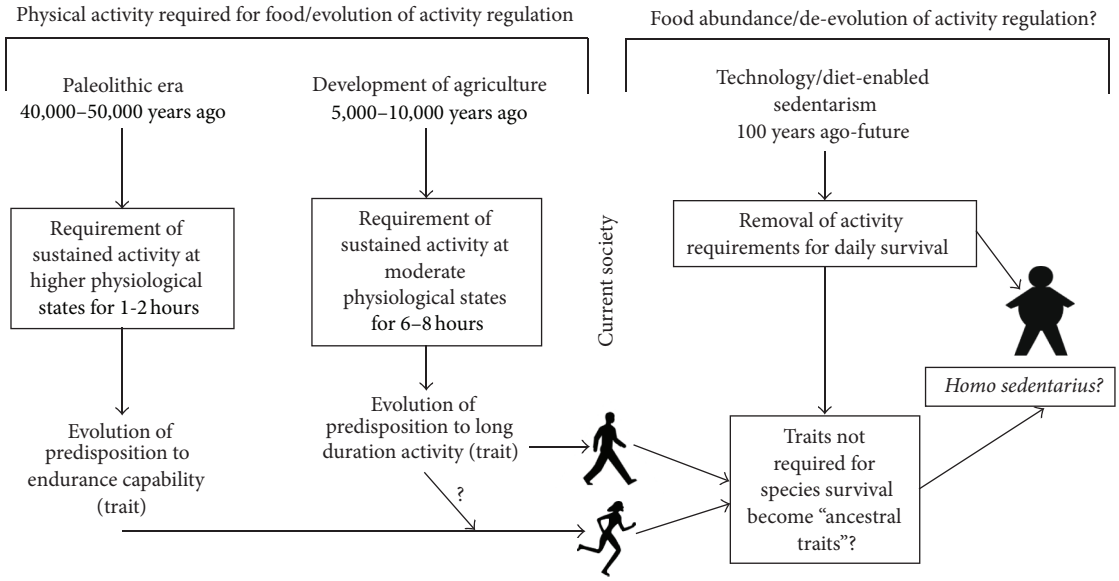


Figure 3: Potential selection pressures on activity regulation in humans and possible future effects of food abundance.

a strong biological cause/effect relationship that would both help explain evolution of genetic/biological regulation of activity and could further explain the precipitous declines in physical activity currently seen in most nations [83]. Again, the reader is cautioned that at this point in the maturity of the physical activity regulation literature, the above facts and hypotheses appear to provide the most probable—yet still hypothetical—explanation of the selection pressures influencing the evolution of physical activity regulation. Further studies directly addressing these hypotheses, especially those using animal models and experimental evolution models [38], may provide the best pathway toward conclusively establishing the evolutionary selection pressures on physical activity regulation.

4. Applications and Future Directions

While we have looked backward to discuss potential past causes/pressures that drove the evolution of physical activity in humans (Figure 3), it is imperative that we also look forward to consider potential areas of needed research, especially given the large health and economic consequences of the current downward trend of physical activity worldwide [83–86]. With the general acceptance of a continuing evolutionary change pattern in *Homo sapiens* amongst evolutionary biologists [87], it is interesting to speculate as to the effect of our current technology- and diet-enabled sedentarism on the genetic regulation of physical activity. As Zimmer noted in 2009 [88], predicting the outcome of evolution is difficult, especially human evolution where there are myriad factors influencing the selection of different traits. But as scientists, we should consider whether our current proclivity toward sedentarism—for example, Troiano and colleagues objectively observed less than 3.5% of adults in the United States were moderately active more than 30 mins per day [89]—will drive our evolution toward physiological mechanisms that allow us to remain inactive, yet healthy.

Theoretically, environment drives selection toward traits that increase reproductive fitness. For the first time in the history of *Homo sapiens*, we live in an era where our ability to be active or have high exercise capacity does not impact our ability to obtain food. Our current technology- and diet-enabled environment in most cases has removed the need to stay fit and be active on a daily basis. Most of us neither have to hunt and gather or grow our own food. However, since the majority of hypokinetic chronic diseases do not significantly impact health until long after the reproductive cycle of most humans has begun, as long as one can find a reproductive partner, the embracing of a technology- and diet-enabled sedentarism would not affect societal reproduction as a whole. Further, if it is assumed that the majority of individuals in a society embrace technology- and diet-enabled sedentarism, those individuals that are fit and active will become a smaller minority of the population and while potentially drawn to each other and finding health benefits in such a pairing, will find no reproductive advantage by daily exercise or activity. While the underlying genetic code that predisposes to a higher daily drive to be active will be transmitted to offspring, the environmental drive requiring the expression of that drive will be removed. Thus, in the long term, if our current technological- and diet-enabled sedentarism continues, while the mechanisms that predispose and regulate physical activity will be transmitted to our offspring, these mechanisms may fall into the category of ancestral genes that are no longer required for species survival as a whole [90]. Further, it will be interesting to observe whether genetic variants eventually evolve that enable *Homo sapiens* to physiologically deal with sedentarism—such as altered metabolic mechanisms to handle the increased fat and sugar loads characteristic of a modern diet. Whether and how *Homo sapiens* adapt and evolve for this new environment—perhaps into *Homo Sedentarius* (Figure 3)—will be an interesting topic of study and observation for years to come.

5. Summary and Conclusions

Evidence suggests that daily physical activity is significantly influenced by genetic mechanisms. However, these mechanisms and the actual site of physiological regulation of physical activity at this point are somewhat unclear. This paper's goal was to provide—given the current literature—a conceptual framework that can be used to guide future investigations targeting the delineation of the genetic regulation of physical activity. First, it is unclear as to what environmental selective pressure resulted in the evolution of genetic mechanisms to control physical activity. While it is tempting to speculate that the need for ancient hunters/gatherers to run/walk long distances may have been a selective pressure, daily activity above what was needed to provide food would have put strains on energy balance within the individual and impacted the collective tribe's food supply. The acceptance of widespread agriculture demanded longer periods of activity (generally at lower intensities) and thus suggests that the longer required periods of activity inherent in farming might have provided a selection pressure. Indeed, it is often noted that lazy farmers were dead farmers. Given the known tie between food availability and activity, especially in animals, it is possible that food availability was the underlying selection pressure for the evolution of activity-regulating mechanisms. Indeed, both hunter/gatherer populations and farming populations show a negative relationship between food availability and activity. If food was scarce, activity increased and if food was available, activity decreased. Thus, food availability becomes a factor in the reason to be active. Whether food availability was the actual selection pressure for evolving regulation of physical activity is unknown but could be potentially studied given the multiple available methods of experimentally invoking evolution (e.g., [38]). The value of continued research and thought regarding the selection pressures responsible for activity regulation is to consider how modern lifestyle and food availability may impact those regulatory mechanisms. With plentiful food for the majority of the Earth's population, the requirement for physical activity to provide sustenance is markedly reduced, and thus the requirement to be physically active does not impact the survival of the species. Therefore, in the future, it will be interesting to observe whether the removal of these potential selection pressures will affect not only physical activity levels, but also the regulation of physical activity in *Homo sapiens*.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Funding for much of the data underlying this paper was originally provided by NIH through RO1AR050085 and R15DK61635, as well as a start-up grant from Texas A&M University. Further, thanks go to D. Ferguson, M. Dawes, E. Schmitt, H. Vellers, and B. Collins for their comments and discussion of these ideas. Thanks also go to D. Epstein for the insightful comments on this paper.

References

[1] T. W. Rowland, "The biological basis of physical activity," *Medicine and Science in Sports and Exercise*, vol. 30, no. 3, pp. 392–399, 1998.

[2] A. M. C. P. Joosen, M. Gielen, R. Vlietinck, and K. R. Westerterp, "Genetic analysis of physical activity in twins," *American Journal of Clinical Nutrition*, vol. 82, no. 6, pp. 1253–1259, 2005.

[3] L. Perusse, A. Tremblay, C. Leblanc, and C. Bouchard, "Genetic and environmental influences on level of habitual physical activity and exercise participation," *American Journal of Epidemiology*, vol. 129, no. 5, pp. 1012–1022, 1989.

[4] J. T. Lightfoot, L. Leamy, D. Pomp et al., "Strain screen and haplotype association mapping of wheel running in inbred mouse strains," *Journal of Applied Physiology*, vol. 109, no. 3, pp. 623–634, 2010.

[5] J. H. Stubbe, D. I. Boomsma, J. M. Vink et al., "Genetic influences on exercise participation in 37.051 twin pairs from seven countries," *PLoS ONE*, vol. 1, no. 1, article e22, 2006.

[6] R. S. Bowen, D. P. Ferguson, and J. T. Lightfoot, "Effects of aromaase Inhibition on the physical activity levels of male mice," *Journal of Steroids & Hormonal Science*, vol. 1, no. 1, pp. 1–7, 2011.

[7] R. S. Bowen, A. M. Knab, A. T. Hamilton, J. R. McCall, T. L. Moore-Harrison, and J. T. Lightfoot, "Effects of supraphysiological doses of sex steroids on wheel running activity in mice," *Journal of Steroids & Hormonal Science*, vol. 3, no. 2, article 110, 2012.

[8] J. F. Gorzek, K. C. Hendrickson, J. P. Forstner, J. L. Rixen, A. L. Moran, and D. A. Lowe, "Estradiol and tamoxifen reverse ovariectomy-induced physical inactivity in mice," *Medicine and Science in Sports and Exercise*, vol. 39, no. 2, pp. 248–256, 2007.

[9] R. G. Hoskins, "The effect of castration on voluntary activity," *American Journal of Physiology*, vol. 72, pp. 324–330, 1925.

[10] M. A. Morgan, J. Schulkin, and D. W. Pfaff, "Estrogens and nonreproductive behaviors related to activity and fear," *Neuroscience and Biobehavioral Reviews*, vol. 28, no. 1, pp. 55–63, 2004.

[11] C. P. Richter, "Animal behavior and internal drives," *The Quarterly Review of Biology*, vol. 2, no. 3, pp. 307–343, 1927.

[12] J. R. Slonaker, "The effect of pubescence, oestruation and menopause on the voluntary activity in the albino rat," *American Journal of Physiology*, vol. 68, pp. 294–315, 1924.

[13] T. Garland Jr., H. Schutz, M. A. Chappell et al., "The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives," *Journal of Experimental Biology*, vol. 214, no. 2, pp. 206–229, 2011.

[14] J. T. Lightfoot, "Can you be born a couch potato? The genomic regulation of physical activity," in *Exercise Genomics*, L. S. Pescatello and S. M. Roth, Eds., pp. 45–72, Humana Press, New York, NY, USA, 2011.

[15] J. T. Lightfoot, "Current understanding of the genetic basis for physical activity," *Journal of Nutrition*, vol. 141, no. 3, pp. 526–530, 2011.

[16] T. Moore-Harrison and J. T. Lightfoot, "Driven to be inactive? The genetics of physical activity," *Progress in Molecular Biology and Translational Science*, vol. 94, pp. 271–290, 2010.

[17] R. S. Bowen, M. J. Turner, and J. T. Lightfoot, "Sex hormone effects on physical activity levels: why doesn't jane run as much as dick?" *Sports Medicine*, vol. 41, no. 1, pp. 73–86, 2011.

[18] D. M. Bramble and D. E. Lieberman, "Endurance running and the evolution of Homo," *Nature*, vol. 432, no. 7015, pp. 345–352, 2004.

[19] D. R. Carrier, "The energetic paradox of human running and hominid evolution," *Current Anthropology*, vol. 25, no. 4, pp. 483–495, 1984.

[20] J. T. Lightfoot, M. J. Turner, D. Pomp, S. R. Kleeberger, and L. J. Leamy, "Quantitative trait loci for physical activity traits in mice," *Physiological Genomics*, vol. 32, no. 3, pp. 401–408, 2008.

[21] K. L. Andersen, J. Ilmarinen, and J. Rutenfranz, "Leisure time sport activities and maximal aerobic power during late adolescence," *European Journal of Applied Physiology and Occupational Physiology*, vol. 52, no. 4, pp. 431–436, 1984.

[22] L. B. Andersen and J. Haraldsdottir, "Coronary heart disease risk factors, physical activity, and fitness in young Danes," *Medicine and Science in Sports and Exercise*, vol. 27, no. 2, pp. 158–163, 1995.

[23] K. L. Lamb and D. A. Brodie, "Leisure-time physical activity as an estimate of physical fitness: a validation study," *Journal of Clinical Epidemiology*, vol. 44, no. 1, pp. 41–52, 1991.

[24] D. R. Young and M. A. Steinhardt, "The importance of physical fitness versus physical activity for coronary artery disease risk factors: a cross-sectional analysis," *Research Quarterly for Exercise and Sport*, vol. 64, no. 4, pp. 377–384, 1993.

[25] J. R. J. Morrow and P. S. Freedson, "Relationship between habitual physical activity and aerobic fitness in adolescents," *Pediatric Exercise Science*, vol. 6, no. 4, pp. 315–329, 1994.

[26] A. V. Rowlands, R. G. Eston, and D. K. Ingledew, "Relationship between activity levels, aerobic fitness, and body fat in 8- to 10-yr-old children," *Journal of Applied Physiology*, vol. 86, no. 4, pp. 1428–1435, 1999.

[27] L. Aires, P. Silva, G. Silva, M. P. Santos, J. C. Ribeiro, and J. Mota, "Intensity of physical activity, cardiorespiratory fitness, and body mass index in youth," *Journal of Physical Activity and Health*, vol. 7, no. 1, pp. 54–59, 2010.

[28] T. Moore-Harrison, A. Hamilton, A. Knab et al., "The relationship between aerobic capacity, body composition, and physical activity among ethnic groups," in *Proceedings of the Integrative Biology of Exercise Meeting V.*, 2008.

[29] W. A. Friedman, T. Garland Jr., and M. R. Dohm, "Individual variation in locomotor behavior and maximal oxygen consumption in mice," *Physiology and Behavior*, vol. 52, no. 1, pp. 97–104, 1992.

[30] M. I. Lambert, C. van Zyl, R. Jaunky, E. V. Lambert, and T. D. Noakes, "Tests of running performance do not predict subsequent spontaneous running in rats," *Physiology and Behavior*, vol. 60, no. 1, pp. 171–176, 1996.

[31] I. Lerman, B. C. Harrison, K. Freeman et al., "Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains," *Journal of Applied Physiology*, vol. 92, no. 6, pp. 2245–2255, 2002.

[32] J. T. Lightfoot, M. J. Turner, M. Daves, A. Vordermark, and S. R. Kleeberger, "Genetic influence on daily wheel running activity level," *Physiological Genomics*, vol. 19, pp. 270–276, 2005.

[33] M. H. M. de Moor, Y.-J. Liu, D. I. Boomsma et al., "Genome-wide association study of exercise behavior in dutch and american adults," *Medicine and Science in Sports and Exercise*, vol. 41, no. 10, pp. 1887–1895, 2009.

[34] C. Bouchard, T. Rankinen, Y. C. Chagnon et al., "Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE Family study," *Journal of Applied Physiology*, vol. 88, no. 2, pp. 551–559, 2004.

[35] J. Rico-Sanz, T. Rankinen, T. Rice et al., "Quantitative trait loci for maximal exercise capacity phenotypes and their responses to training in the HERITAGE Family study," *Physiological Genomics*, vol. 16, pp. 256–260, 2004.

[36] J. T. Lightfoot, M. J. Turner, A. K. Knab et al., "Quantitative trait loci associated with maximal exercise endurance in mice," *Journal of Applied Physiology*, vol. 103, no. 1, pp. 105–110, 2007.

[37] S. M. Courtney and M. P. Massett, "Identification of exercise capacity QTL using association mapping in inbred mice," *Physiological Genomics*, vol. 44, no. 19, pp. 948–955, 2012.

[38] T. Garland Jr. and S. A. Kelly, "Phenotypic plasticity and experimental evolution," *Journal of Experimental Biology*, vol. 209, no. 12, pp. 2344–2361, 2006.

[39] R. B. Lee, *The Dobe Ju/'Hoansi*, Wadsworth, 3rd edition, 2003.

[40] W. R. Leonard and M. L. Robertson, "Nutritional requirements and human evoltuion: a bioenergetics model," *American Journal of Human Biology*, vol. 4, no. 2, pp. 179–195, 1992.

[41] J. Woodburn, "An introduction to hadza ecology," in *Man the Hunter*, R. Lee and I. DeVore, Eds., pp. 49–55, Aldine, Chicago, Ill, USA, 1968.

[42] H. Pontzer, D. A. Raichlen, B. M. Wood, A. Z. Mabulla, S. B. Racette, and F. W. Marlowe, "Hunter-gatherer energetics and human obesity," *PLoS ONE*, vol. 7, no. 7, Article ID e40503, 2012.

[43] K. R. Westerterp and J. R. Speakman, "Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals," *International Journal of Obesity*, vol. 32, no. 8, pp. 1256–1263, 2008.

[44] C. Panter-Brick, "Seasonality of energy expenditure during pregnancy and lactation for rural Nepali women," *American Journal of Clinical Nutrition*, vol. 57, no. 5, pp. 620–628, 1993.

[45] C. Panter-Brick, "Seasonal and sex variation in physical activity levels among agro-pastoralists in Nepal," *American Journal of Physical Anthropology*, vol. 100, no. 1, pp. 7–21, 1996.

[46] D. R. Bassett Jr., P. L. Schneider, and G. E. Huntington, "Physical activity in an old order Amish community," *Medicine and Science in Sports and Exercise*, vol. 36, no. 1, pp. 79–85, 2004.

[47] D. R. Bassett, H. R. Wyatt, H. Thompson, J. C. Peters, and J. O. Hill, "Pedometer-measured physical activity and health behaviors in U.S. adults," *Medicine and Science in Sports and Exercise*, vol. 42, no. 10, pp. 1819–1825, 2010.

[48] M. P. Murray, A. B. Drought, and R. C. Kory, "Walking patterns of normal men," *The Journal of Bone and Joint Surgery*, vol. 46, pp. 335–360, 1964.

[49] M. P. Murray, R. C. Kory, and S. B. Sepic, "Walking patterns of normal women," *Archives of Physical Medicine and Rehabilitation*, vol. 51, no. 11, pp. 637–650, 1970.

[50] S. Gillepsie and G. McNeill, *Food, Health and Survival in India and Developing Countries*, Oxford University Press, New Delhi, India, 1992.

[51] M. Lawrence and R. G. Whitehead, "Physical activity and total energy expenditure of child-bearing Gambian village women," *European Journal of Clinical Nutrition*, vol. 42, no. 2, pp. 145–160, 1988.

[52] J. Singh, A. M. Prentice, E. Diaz et al., "Energy expenditure of Gambian women during peak agricultural activity measured by the doubly-labelled water method," *British Journal of Nutrition*, vol. 62, no. 2, pp. 315–329, 1989.

[53] T. Brun, F. Bleiberg, and S. Goihman, "Energy expenditure of male farmers in dry and rainy seasons in Upper-Volta," *British Journal of Nutrition*, vol. 45, no. 1, pp. 67–75, 1981.

[54] F. M. Bleiberg, T. A. Brun, S. Goihman, and E. Gouba, "Duration of activities and energy expenditure of female farmers in dry and rainy seasons in Upper-Volta," *British Journal of Nutrition*, vol. 43, no. 1, pp. 71–82, 1980.

[55] K. B. Simondon, E. Benefice, F. Simondon, V. Delaunay, and A. Chahnazarian, "Seasonal variation in nutritional status of adults and children in rural Senegal," in *Seasonality and Human Ecology*, S. J. Ulijaszek and S. S. Strickland, Eds., pp. 166–183, Cambridge University Press, 1993.

[56] G. McNeill, J. P. W. Rivers, P. R. Payne, J. J. de Britto, and R. Abel, "Basal metabolic rate of Indian men: no evidence of metabolic adaptation to a low plane of nutrition," *Human Nutrition*, vol. 41, no. 6, pp. 473–483, 1987.

[57] A. E. Dugdale and P. R. Payne, "A model of seasonal changes in energy balance," *Ecology of Food and Nutrition*, vol. 19, no. 3, pp. 231–245, 1987.

[58] R. H. Fox, *A study of the energy expenditure of Africans engaged in various activities, with special references to some environmental and physiological factors which may influence the efficiency of their work [Ph.D. thesis]*, 1953.

[59] H. Kashiwazaki, Y. Dejima, J. Orias-Rivera, and W. A. Coward, "Energy expenditure determined by the doubly labeled water method in Bolivian Aymara living in a high altitude agropastoral community," *American Journal of Clinical Nutrition*, vol. 62, no. 5, pp. 901–910, 1995.

[60] P. W. Leslie, J. R. Bindon, and P. T. Baker, "Caloric requirements of human populations: a model," *Human Ecology*, vol. 12, no. 2, pp. 137–162, 1984.

[61] G. Cochran and H. Harpending, *The 10,000 Year Explosion: How Civilization Accelerated Human Evolution*, Basic Books/Perseus Books Group, New York, NY, USA, 2009.

[62] C. L. Goodrick, D. K. Ingram, and M. A. Reynolds, "Effects of intermittent feeding upon growth, activity, and lifespan in rats allowed voluntary exercise," *Experimental Aging Research*, vol. 9, no. 3, pp. 203–209, 1983.

[63] Y. Yamada, R. J. Colman, J. W. Kemnitz et al., "Long-term calorie restriction decreases metabolic cost of movement and prevents decrease of physical activity during aging in rhesus monkeys," *Experimental Gerontology*, vol. 48, no. 11, pp. 1226–1235, 2013.

[64] R. C. Casper, "The "drive for activity" and "restlessness" in anorexia nervosa: potential pathways," *Journal of Affective Disorders*, vol. 92, no. 1, pp. 99–107, 2006.

[65] A. H. Crisp, L. K. G. Hsu, B. Harding, and J. Hartshorn, "Clinical features of anorexia nervosa. A study of a consecutive series of 102 female patients," *Journal of Psychosomatic Research*, vol. 24, no. 3-4, pp. 179–191, 1980.

[66] C. Davis, S. H. Kennedy, E. Ravelski, and M. Dionne, "The role of physical activity in the development and maintenance of eating disorders," *Psychological Medicine*, vol. 24, no. 4, pp. 957–967, 1994.

[67] M. B. Sokolowski, H. S. Pereira, and K. Hughes, "Evolution of foraging behavior in drosophila by density-dependent selection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 14, pp. 7373–7377, 1997.

[68] Y. Ben-Shahar, A. Robichon, M. B. Sokolowski, and G. E. Robinson, "Influence of gene action across different time scales on behavior," *Science*, vol. 296, no. 5568, pp. 741–744, 2002.

[69] E. Martin, V. Berka, A.-L. Tsai, and F. Murad, "Soluble guanylyl cyclase: the nitric oxide receptor," *Methods in Enzymology*, vol. 396, pp. 478–492, 2005.

[70] W. F. Epling and W. D. Pierce, "Activity-based anorexia: a biobehavioral perspective," *International Journal of Eating Disorders*, vol. 7, no. 4, pp. 475–485, 1988.

[71] J. A. Levine, S. K. Mecrady, L. M. Lanningham-Foster, P. H. Kane, R. C. Foster, and C. U. Manohar, "The role of free-living daily walking in human weight gain and obesity," *Diabetes*, vol. 57, no. 3, pp. 548–554, 2008.

[72] S. L. Schmidt, K. A. Harmon, T. A. Sharp, E. H. Kealey, and D. H. Bessesen, "The effects of overfeeding on spontaneous physical activity in obesity prone and obesity resistant humans," *Obesity*, vol. 20, no. 11, pp. 2186–2193, 2012.

[73] A. G. Comuzzie, *Personal Communications*, 2013.

[74] P. B. Higgins, R. A. Bastarrachea, J. C. Lopez-Alvarenga et al., "Eight week exposure to a high sugar high fat diet results in adiposity gain and alterations in metabolic biomarkers in baboons (*Papio hamadryas* sp.)," *Cardiovascular Diabetology*, vol. 9, article 71, 2010.

[75] U. Ekelund, S. Brage, H. Besson, S. Sharp, and N. J. Wareham, "Time spent being sedentary and weight gain in healthy adults: reverse or bidirectional causality?" *American Journal of Clinical Nutrition*, vol. 88, no. 3, pp. 612–617, 2008.

[76] B. S. Metcalf, J. Hosking, A. N. Jeffery, L. D. Voss, W. Henley, and T. J. Wilkin, "Fatness leads to inactivity, but inactivity does not lead to fatness: a longitudinal study in children (EarlyBird 45)," *Archives of Disease in Childhood*, vol. 96, no. 10, pp. 942–947, 2011.

[77] A. E. Bauman, R. S. Reis, J. F. Sallis, J. C. Wells, R. J. F. Loos, and B. W. Martin, "Correlates of physical activity: why are some people physically active and others not?" *The Lancet*, vol. 380, no. 9838, pp. 258–271, 2012.

[78] J. T. Lightfoot, A. Hamilton, and T. Moore-Harrison, "Differential gene expression in high and low active animals," in *Proceedings of the Conference on Integrative Physiology of Exercise*, 2010.

[79] C. A. Macera, S. A. Ham, M. M. Yore et al., "Prevalence of physical activity in the United States: Behavioral Risk Factor Surveillance System, 2001," *Preventing Chronic Disease*, vol. 2, no. 2, article A17, 2005.

[80] C. P. Richter and G. B. Wislocki, "Activity studies on castrated male and female rate with testicular grafts, in correlation with histological of the grafts," *American Journal of Physiology*, vol. 86, no. 3, pp. 651–660, 1928.

[81] C. Bouchard, A. Tchernof, and A. Tremblay, "Predictors of body composition and body energy changes in response to chronic overfeeding," *International Journal of Obesity*, 2013.

[82] R. J. Shephard, "Limits to the measurement of habitual physical activity by questionnaires," *British Journal of Sports Medicine*, vol. 37, no. 3, pp. 197–206, 2003.

[83] Centers for Disease Control And Prevention, *United Nations Summit on Noncommunicable Diseases, September 19–20, 2011*, 2011.

[84] F. W. Booth, S. E. Gordon, C. J. Carlson, and M. T. Hamilton, "Waging war on modern chronic diseases: primary prevention through exercise biology," *Journal of Applied Physiology*, vol. 88, no. 2, pp. 774–787, 2000.

[85] D. Chenoweth and J. Leutzinger, "The economic cost of physical inactivity and excess weight in american adults," *Journal of Physical Activity and Health*, vol. 3, pp. 148–163, 2006.

[86] A. H. Mokdad, J. S. Marks, D. F. Stroup, and J. L. Gerberding, "Actual causes of death in the United States, 2000," *Journal of the American Medical Association*, vol. 291, no. 10, pp. 1238–1245, 2004.

[87] J. Hawks, E. T. Wang, G. M. Cochran, H. C. Harpending, and R. K. Moyzis, "Recent acceleration of human adaptive evolution," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 52, pp. 20753–20758, 2007.

[88] C. Zimmer, "On the origin of tomorrow," *Science*, vol. 326, no. 5958, pp. 1334–1336, 2009.

[89] R. P. Troiano, D. Berrigan, K. W. Dodd, L. C. Mâsse, T. Tilert, and M. Mcdowell, "Physical activity in the United States measured by accelerometer," *Medicine and Science in Sports and Exercise*, vol. 40, no. 1, pp. 181–188, 2008.

[90] N. Venkataraman, A. L. Cole, P. Ruchala et al., "Reawakening retrocyclins: ancestral human defensins active against HIV-1," *PLoS Biology*, vol. 7, no. 4, article e95, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 643575, 11 pages
http://dx.doi.org/10.1155/2013/643575

Review Article

Highlights from the Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength or FAMuSS Study

Linda S. Pescatello,[1] Joseph M. Devaney,[2] Monica J. Hubal,[2] Paul D. Thompson,[3] and Eric P. Hoffman[2]

[1] *Department of Kinesiology and Human Performance Laboratory, Neag School of Education, University of Connecticut, Gampel Pavilion Room 206, 2095 Hillside Road, U-1110, Storrs, CT 06269-1110, USA*
[2] *Children's National Medical Center, 111 Michigan Avenue, N.W., Washington, DC 20010-2970, USA*
[3] *Division of Cardiology, Hartford Hospital, 85 Jefferson Street, Hartford, CT 06106, USA*

Correspondence should be addressed to Linda S. Pescatello; linda.pescatello@uconn.edu

Received 5 October 2013; Accepted 20 November 2013

Academic Editor: J. Timothy Lightfoot

The purpose of the *Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength* study or FAMuSS was to identify genetic factors that dictated the response of health-related fitness phenotypes to resistance exercise training (RT). The phenotypes examined were baseline muscle strength and muscle, fat, and bone volume and their response to RT. FAMuSS participants were 1300 young (24 years), healthy men (42%) and women (58%) that were primarily of European-American descent. They were genotyped for ~500 polymorphisms and completed the Paffenbarger Physical Activity Questionnaire to assess energy expenditure and time spent in light, moderate, and vigorous intensity habitual physical activity and sitting. Subjects then performed a 12-week progressive, unilateral RT program of the nondominant arm with the dominant arm used as a comparison. Before and after RT, muscle strength was measured with the maximum voluntary contraction and one repetition maximum, while MRI measured muscle, fat, and bone volume. We will discuss the history of how FAMuSS originated, provide a brief overview of the FAMuSS methods, and summarize our major findings regarding genotype associations with muscle strength and size, body composition, cardiometabolic biomarkers, and physical activity.

1. Introduction

We are part of a multidisciplinary research team, the Exercise and Genetics Collaborative Research Group, that completed a large exercise genomics study entitled *Functional Single Nucleotide Polymorphisms (SNPs) Associated with Human Muscle Size and Strength* (FAMuSS NIH R01 NS40606-02) [1]. The primary aim of FAMuSS was to identify non-synonymous SNPs (i.e., SNPs leading to amino acid changes) that dictated baseline muscle size and strength, and the extent of the muscle size and strength response to resistance exercise training (RT). Other phenotypes examined were baseline fat and bone volume and the response of these phenotypes to RT, and baseline cardiometabolic biomarkers. We envisioned that FAMuSS findings would lead to a better understanding of physical health and well being as well as disease processes such as sarcopenia during aging, atrophy during weightlessness of space flight, sports performance, and the progression of neuromuscular disease.

To achieve our aims about 1300 young, healthy men (42%) and women (58%) (24 years, body mass index [BMI] 25 $kg \cdot m^{-2}$) primarily of European-American descent were recruited and genotyped for ~500 polymorphisms. Volunteers provided blood samples for determination of fasting baseline cardiometabolic biomarkers and genotyping. They completed the Paffenbarger Physical Activity Questionnaire [2] to assess energy expenditure and time spent in light, moderate, and vigorous intensity physical activity and sitting.

Subjects then performed a progressive, unilateral RT program of the nondominant arm with the dominant arm used as a comparison. Before and after RT, muscle strength was measured with the maximum voluntary contraction (MVC) and one repetition maximum (1RM), while magnetic resonance imagining (MRI) measured muscle, fat, and bone volume. To date there are over 30 FAMuSS publications. The purpose of this review is to highlight the FAMuSS findings by discussing the history of how FAMuSS originated, providing a brief overview of the FAMuSS methods, and summarizing our major findings regarding genotype associations with baseline muscle strength and size and body composition and the response of these phenotypes to RT, baseline fasting cardiometabolic biomarkers, and habitual physical activity levels.

2. The History of How FAMuSS Originated

Thomas A. Edison said, "Great Ideas Originate in the Muscles". The study of skeletal muscle is not a new idea but one that has often intrigued human curiosity. If we begin to understand the complexities behind basic skeletal muscle function (i.e., strength) then this knowledge would provide insight into understanding normal human body movement and the ability to stress that system via an intervention. The FAMuSS study was an attempt to understand the genetic causes behind the response of muscle to an external stimulus. The aims of the FAMuSS project were to utilize molecular biology to answer two simple questions: (1) can genetic variation explain differences in skeletal muscle size and strength; and (2) can genetic variation explain how skeletal muscle responds to RT? The purpose of FAMuSS was not an attempt to understand the mechanical reasons for skeletal muscle size or strength but to comprehend the biology that controls the muscular apparatus.

Skeletal muscle makes up 30% of the human body so that the genetics behind the strength/size of this organ deserves attention. The maximum strength capacity of skeletal muscle is manipulated by a multitude of factors including genetics that can act synergistically. However, the most influential stimulus in the response of muscle is RT, which effectively increases maximal isometric and dynamic muscle contraction strength. Additionally, muscle strength is a key determinant of an individual's functional capacity. Even with the critical importance of skeletal muscle in human health, little was known regarding the genetic factors influencing skeletal muscle size and strength and the response of this organ to environmental factors such as RT. Thus, a comprehensive study was needed to discover how genetics influence skeletal muscle size and strength among healthy individuals as the paradigm at that time was studying genetic variation and its effect on dysfunction and disease. Our approach was not to lessen the need for the study of SNPs and disease—but to add new information to this important body of knowledge.

The FAMuSS study was built on the early work of Thomis et al. [3] that showed the inheritance of arm strength and size before and after RT in 25 monozygotic and 16 dizygotic male twins. Muscle strength measured as 1RM showed a high degree of heritability (77% pre- and 81% post-RT). Similarly, handgrip strength among 257 male and 353 female twins between 59–70 years suggested that strength had a heritability of 65% and 30%, respectively [4]. Pérusse et al. [5] used a statistical procedure, path analysis, which allows the partition of transmissible variance into genetic and cultural components among 1630 nontwin, French-Canadians from 375 families and attributed 30% of the phenotypic variance in muscular strength in these families to genetic factors. These findings made the rationale for the FAMuSS study even stronger. The identification of genetic variants that play a role in the normal response of muscle to external stimuli such as RT would have impact on the sports world, but more importantly, would also provide insight into health and disease processes permitting the possibility of new therapeutics to treat neuromuscular disorders.

3. The FAMuSS Methods

3.1. Overview. FAMuSS methods have been described in detail [1] and will be briefly overviewed here. FAMuSS was conducted by the Exercise and Genetics Collaborative Research Group consisting of researchers and site coordinators from the University of Central Florida (TJ Angelopoulos), University of Massachusetts (PM Clarkson), West Virginia University (PM Gordon), Dublin City University (NM Moyna), University of Connecticut (LS Pescatello), Central Michigan University (P Visich), Florida Atlantic University (RF Zoeller), Yale University (TB Price), Hartford Hospital (PD Thompson and RL Seip), and the Children's National Medical Center (EP Hoffman, PI, and JM Devaney). The institutional review boards from the 10 institutions involved in FAMuSS approved the study protocol. Study volunteers were recruited to complete a 12-week progressive, unilateral RT program to improve the strength and size of elbow flexor and extensor muscles in the nondominant arm with the dominant arm used as a comparison. Muscle strength was measured as biceps MVC and 1RM and muscle size by MRI of the biceps cross-sectional area. We used MRI to also measured fat and bone volume. Prior to RT, investigators obtained a blood sample for determination of a fasting cardiometabolic profile and DNA extraction, and subjects completed the Paffenbarger Physical Activity Questionnaire to assess habitual physical activity [2].

3.2. Subjects. Subjects were excluded if they took corticosteroids, anabolic steroids, antihypertensive or antilipidemic medications, diuretics, Depo-Provera contraceptive injection, Clenbuterol, Rhinocort nasal inhaler, lithium, or nonsteroidal anti-inflammatory medications. They were also excluded if they took dietary supplements to enhance muscle strength and size or weight; had chronic medical conditions; had metal implants in the arms, eyes, head, brain, neck, or heart; consumed >2 alcoholic drinks per day; performed RT or other physical activity involving repetitive arm use within the past year; and/or were seeking to gain or lose weight or had a weight change >5 lb in the past 3 months. Furthermore, subjects were instructed not to alter their habitual physical

activity, lifestyle, or dietary habits, or otherwise gain or lose weight during the study. Upon enrollment we measured body weight and height to calculate BMI. To ensure weight maintenance, body weight was measured throughout the study.

3.3. Physical Activity Assessment. Habitual physical activity phenotypes were obtained from the Paffenbarger Physical Activity Questionnaire [2]. The derived phenotypes were energy expended (kcal·wk^{-1}) and time spent (hr·wk^{-1}) in light, moderate, and vigorous intensity physical activity as well as walking, stair climbing, participation in sports and recreational activities, and sitting. A total physical activity index (kcal·wk^{-1}) was also calculated.

3.4. Muscle Strength Testing. We assessed muscle strength with the MVC and 1 RM in the trained (nondominant) and untrained (dominant) elbow flexor muscles before and after RT.

3.5. MRI Assessment of Muscle, Fat, and Bone Volume. MRI assessment of both upper arms (trained and untrained) was performed before and after RT. Fifteen 16 mm contiguous axial slices from each arm were taken from each arm independently. Scans for both arms were taken by Fast Spoiled Gradient Recalled and Fast Spin Echo with TE 1.8/TR 200 msec. We used Rapidia (INFINITT Inc, Seoul, Korea) for the volumetric analysis of the MRI images. Volume measures were taken using an anatomical landmark (metaphyseal-diaphyseal junction of the humerus) as our starting point and assayed the six 1 cm slices proximal to it.

3.6. Resistance Exercise Training Program. RT was performed unilaterally in the nondominant arm. Subjects attended supervised RT sessions twice weekly at least 48 hours apart for 12 weeks. The program consisted of five exercises designed primarily to train the elbow flexors and secondarily to train the elbow extensors for balance. At the start of RT, subjects performed three sets of 12 repetitions at 65–75% of 1RM. At week five sets were reduced to eight repetitions at 75–82% 1RM and at week 10 to six repetitions at 83–90% 1RM. Subjects took 2 seconds each for the concentric and eccentric phase of each repetition with a recovery between sets of 2 minutes.

3.7. Fasting Blood Sampling and Analyses

Cardiometabolic Biomarkers. Prior to RT, fasting blood samples were drawn and serum was separated by centrifugation at 1110 g for 10 min and frozen for further analysis of the cardiometabolic biomarkers by Quest Diagnostics. Cardiometabolic biomarkers included glucose, insulin, total cholesterol, low-density lipoprotein cholesterol [LDL], high-density lipoprotein cholesterol [HDL], and triglyceride levels. The homoeostasis model assessment (HOMA) was then calculated [6]. *DNA*. In addition, blood was drawn into vacutainer tubes containing ethylenediamine tetraacetic acid. These tubes were sent to Children's National Medical Center where DNA was extracted using Puregene kits (Gentra Systems, Inc., Minneapolis, MN). Genotyping in the FAMuSS study was completed using TaqMan allele discrimination assays that employed the 5′ nuclease activity of Taq polymerase. Both alleles were detected simultaneously using allele-specific oligonucleotides labeled with different fluorophores (VIC and FAM) and genotypes were determined automatically by the ratio of the two fluorophores used. For each SNP examined, a Taqman assay was used to genotype the 1300 samples. Therefore, we generated 650,000 genotypes for the 500 SNPs that were part of the FAMuSS study. Data were processed using SDS v2.3 software. All gels were called by two investigators, and if any disagreement in genotyping was found, the genotyping was repeated.

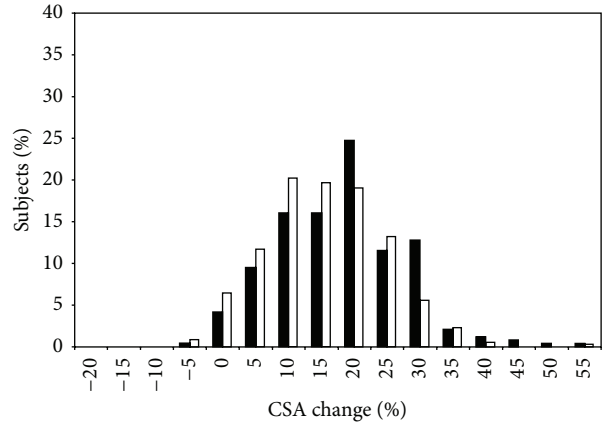


Figure 1: Biceps cross-sectional area. Histogram of biceps cross-sectional area changes (relative to baseline) within each gender for the trained arm. Black bars denote responses of men, while white bars denote responses of women. Reprinted with permission [7].

4. FAMuSS Findings: Muscle Strength and Size

In 2005, Hubal et al. [7] published the results of the unilateral RT program on muscle size and strength in the FAMuSS cohort, highlighting the high degree of variability across all subjects given a standardized RT program. Across 485 subjects (342 women and 243 men), RT resulted in modest size gains and moderate (isometric) to high (dynamic) strength gains. Men averaged significantly higher absolute and relative size gains than women in the trained arm (Figure 1), while no significant changes were seen in the untrained arm. Size gains ranged from −2 to +59% of baseline muscle volume with similar distribution of responses in relative size gains between men and women. While absolute gains in strength (both dynamic and isometric strength) were greater in men, women greatly outpaced men for relative strength gains (64% to 40% for dynamic strength by 1 RM; 22% to 16% for MVC; $P < 0.001$) (Figure 2). Distribution parameters for strength gains indicated a strong clustering of men around the mean, while women ranged in a more normal distribution pattern, indicating that more women were higher or lower responders than men.

The high degree of variation that we observed in muscle size and strength responses to RT that were sex specific

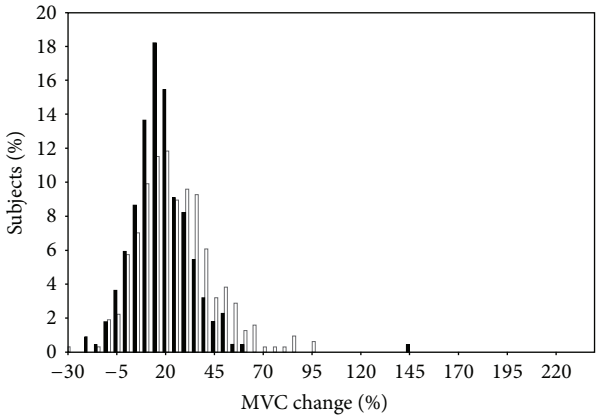


Figure 2: Isometric strength test. Histogram of isometric strength changes (relative to baseline) within each gender for the trained arm. Black bars denote responses of men, while white bars denote responses of women. Reprinted with permission [7].

allowed us to test for various factors that influenced these phenotypes at baseline and in response to RT. While genetic modifiers of muscle strength and size were a priority for FAMuSS, other factors were also explored, such as sex [7], age [8], and BMI [9, 10]. However, the primary focus of this section is to summarize the findings regarding genetic influences on muscle size and strength at baseline and following RT. Twin and other genetic studies have reported wide-ranging estimates of heritability for baseline human muscle size ($h^2 \sim 45$–90%) and strength ($h^2 \sim 30$–85%) in a large part dependent upon the population and muscle group studied [11–13]. In addition to genetic influences on the development of muscle strength and size, there are other factors (i.e., training protocols, diet, etc.) that can modify the adaptive response of muscle to exercise training, such that even wider estimates of heritability are seen for hypertrophy and strength gains ($h^2 \sim 35$–85%) [11–13].

To date, the FAMuSS group has published results for 17 genes specifically tested for association with muscle strength or size that are summarized in Table 1 [14–26]. These genes can be categorized according to their biological functions, including muscle structural elements, growth factors, and inflammatory factors. Examples of structural genes include *ACTN3* (actinin, alpha 3) [14] and *BMP2* (bone morphogenetic protein 2) [15]; growth factors include *GDF8* [growth differentiation factor 8 (myostatin, *MSTN*)] [20], *FST* (follistatin) [20], and *IGF1* (insulin-like growth factor 1) [21]; and inflammatory factors include *CCL2* [chemokine (C-C motif) ligand 2] [17], *IL15* (Interleukin 15) [23], *IL15Rα* (interleukin 15 receptor, alpha) [23], and *SPP1* (osteopontin or secreted phosphoprotein 1) [18], among others. A few genes from other biological function families (mainly related to blood flow and angiogenesis) have also been investigated that include *ACE* (angiotensin I converting enzyme) [19] and *NOS3* (nitric oxide synthase 3) [16].

One structural gene variation that has garnered much attention is the common R577X (rs1815739) mutation in *ACTN3* [27], a premature stop codon that essentially eliminates ACTN3 protein expression in individuals with the XX (nonancestral) genotype. The ACTN3 protein is a sarcomeric actin-anchor expressed exclusively in Type II muscle fibers. The loss of this protein has been associated with athletic performance, such that the frequency of the mutation is underrepresented in elite power athletes and overrepresented in endurance athletes [28]. While animal knockout models have suggested compensatory upregulation of the similar alpha actinin 2 (*Actn2*) gene and possible alterations in aerobic energy pathway elements [29, 30], these are yet to be confirmed in human studies.

A myriad of studies have examined the effect of *ACTN3* R577X genotype on athletic parameters, with widely varying results. Many of these studies suffer from having inadequate sample sizes and are often done in subjects that have varied exercise-training experiences, which can greatly overshadow subtle genotype effects. We reported significant sex-specific findings for muscle strength, but not size, among 602 subjects (247 men; 355 women) from FAMuSS [14]. Women with the XX genotype had lower baseline MVC but greater increases in dynamic strength as compared to women with the RX genotype. In addition, among women, *ACTN3* accounted for 2.2% of variability in baseline MVC and 1.8% of the variability in 1RM gain.

The FAMuSS group has reported genetic associations in several growth related genes in relation to both baseline and posttraining muscle traits [15, 20, 21]. Skeletal muscle growth and protein synthesis are controlled by several key signaling pathways, such as the phosphatidylinositol-3-kinase (PI3 K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway. IGF1 positively controls this pathway via initiation of signaling at the cell surface, affecting protein synthesis rates. Conversely, *MSTN* and its related genes are negative regulators of muscle growth via inhibition of the PI3 K/AKT/mTOR signaling pathway. Animal models in which *MSTN* expression is greatly reduced exhibit gross muscle hypertrophy [31].

In the FAMuSS cohort, Kostek et al. [21] found associations of one particular promoter mutation in *IGF1* (rs35767) with muscle measures. Caucasian women with the CT genotype had greater baseline dynamic strength compared to the two other genotype groups.

In another study, Kostek et al. [20] found ethnicity-specific associations between *MSTN* (rs1805086) and muscle traits. A small group of African Americans showed greater baseline MVC in those with the G allele ($N = 15$) as compared to subjects with the AA genotype ($N = 8$), while no associations were found among Caucasians ($N = 645$). They also found associations between *FST* (rs722910) and strength measures among African Americans but not Caucasians. Finally, Devaney et al. [15] described associations between muscle size and a common polymorphism in *BMP2* (rs15705), which is known to inhibit myogenic differentiation [32]. Following RT, subjects with the CC genotype ($N = 10$) had significantly greater muscle volume gains compared to A allele carriers ($N = 179$), with 3.9% of trait variation explained by *BMP2*. Devaney et al. [15] noted that reporter assays specific for each allele showed that the C allele disrupted a posttranslational regulatory motif, possibly resulting in reduced inhibition, thereby allowing more muscle growth.

Table 1: FAMuSS findings: genetic loci associated with muscle size and strength at baseline and in response to resistance training.

Gene	Name	Reference
ACE	Angiotensin I converting enzyme	[19]
ACTN3	Actinin, alpha 3	[14]
ANKRD6	Ankyrin repeat domain 6	[24]
BMP2	Bone morphogenetic protein 2	[15]
CCL2	Chemokine (C-C motif) ligand 2	[17]
CCR2	Chemokine (C-C motif) receptor 2	[17]
CNTF	Ciliary neurotrophic factor	[26]
FST	Follistatin	[20]
GDF8/MSTN	Growth differentiation factor 8/myostatin	[20]
IGF1	Insulin-like growth factor 1	[21]
IL15	Interleukin 15	[23]
IL15Rα	Interleukin 15 receptor, alpha	[23]
LEP	Leptin	[25]
LEPR	Leptin receptor	[25]
NOS3	Nitric oxide synthase 3	[16]
RETN	Resistin	[22]
SPP1	Osteopontin or secreted phosphoprotein 1	[18]

BMP2 can be considered both a growth-related gene and an inflammation-related gene, based on its role in the transforming growth factor beta (TGFB) pathway. Inflammation and growth pathways have substantial overlap, fitting with the idea that postdevelopmental growth is in large part modulated by environmental stimuli such as exercise. Exercise that evokes the inflammatory system, such as loading muscles with lengthening (eccentric) muscle actions, often produces the greatest size gains [33].

Other key inflammatory genes investigated in FAMuSS for associations with muscle strength and size include *IL-15*, *IL15Rα*, and *SPP1*. Pistilli et al. [23] reported associations between *IL15Rα* (rs2228059) and baseline muscle size in men but not women. This report also showed various relationships among *IL15* or *IL15Rα* and strength gains, including *IL15* (rs1057972) with strength gains in men and *IL15Rα* (rs2296135) with strength gains in women. Most recently, Hoffman et al. [34] reported a stronger association than is normally observed for exercise genomic studies between *SPPI* (rs28357094) and muscle size gains in women but not men [35]. The G allele was associated with increased size gains in women following RT, explaining a relatively high 5% of variance in the response.

In conclusion, despite the relatively strong association Hoffman et al. [34] observed with muscle size gains in women with *SPP1* after RT, in general, single variants explained minor trait variability percentages in baseline muscle size and strength and the response of these phenotypes to RT in the FAMuSS study [35]. Although it is possible that interactions between multiple genetic loci could have accounted for more trait variability [36, 37]. These genotype-phenotype associations were also often sex specific. From a clinical standpoint, genetic modifiers of muscle size and strength are already being studied in relation to management of various myopathies. For example, *SPP1* is a known modifier of disease severity in Duchenne muscular dystrophy [18]. Further studies into genetic influences on muscle size and strength (and their response to exercise training) will inform treatment options given an individual's genetic background, an example of personalized medicine. While these studies often involve muscle at pathological ends of the muscle size and strength spectra, FAMuSS findings provide a very valuable window into variant influences in "normal" (i.e., nonpathological) subjects. In addition, athletes will also seek advantage over their opponents using genomic medicine techniques such as exon-skipping to restore dystrophin expression in Duchenne muscular dystrophy that increases "natural" muscle size and strength possibly improving performance [38].

5. FAMuSS Findings: Body Composition and Cardiometabolic Biomarkers

As part of the FAMuSS study, body composition measurements were made such as BMI and MRI-dictated volumes of subcutaneous fat and bone of the upper arms before and after RT. In addition, before RT, measures of fasting glucose, insulin, total cholesterol, LDL, HDL, and triglyceride levels were made, and the HOMA was calculated [6]. To date, the FAMuSS group has published results for 33 genes specifically tested for association with measures of body composition at baseline and in response to RT and baseline cardiometabolic biomarkers that are summarized in Table 2 [22, 23, 39–43]. Some of these genes were also examined for skeletal muscle phenotypes pre- and post-RT that were described in the previous section and habitual physical activity that will be described in the next section so that only the findings relating to body composition and cardiometabolic biomarkers will be discussed in this section.

Table 2: FAMuSS findings: genetic loci tested for association with body composition at baseline and in response to resistance training and cardiometabolic biomarkers at baseline*.

Gene	Name	Reference
AKT1	V-akt murine thymoma viral oncogene homolog 1	[39]
ANGPT3	Angiopoietin-like 3	[40]
BCL7B	B-cell CLL/lymphoma 7B	[40]
BMP2	Bone morphogenetic protein 2	[40]
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1	[40]
CDKN2A/2B	Cyclin-dependent kinase inhibitor 2A and 2B	[40]
CDKN2BAS/CDNKN2B-AS1 (*ANRIL*)	CDKN2B-AS1 CDKN2B antisense RNA 1	[40]
CILP2	Cartilage intermediate layer protein 2	[40]
FTO	Fat mass and obesity associated	[42]
GALNT2	UDP-N-acetyl-alpha-D-galactosame:polypeptide N-acetylgalactosaminyltransferase 2	[40]
GNPDA2	Glucosamine-6-phosphate deaminase 2	[42]
HHEX	Hematopoietically expressed homeobox	[40]
HNF1A	HNF1 homeobox A	[40]
IGFBP2	Insulin-like growth factor binding protein 2	[40]
IL15	Interleukin 15	[23]
IL15Rα	Interleukin 15 receptor, alpha	[23]
INSIG2	Insulin-induced gene 2	[41]
KCTD10	Potassium channel tetramerisation domain containing 10	[40]
KCTD15	Potassium channel tetramerisation domain containing 15	[42]
KCNJ11	Potassium inwardly rectifying channel, subfamily J, member 11	[40]
MC4R	Melanocortin-4 receptor	[42]
MRAS	Muscle RAS oncogene homolog	[40]
MTCH2	Mitochondrial carrier 2	[42]
NEGR1	Neuronal growth regulator 1	[42]
PPARα	Peroxisome proliferator-activated receptor alpha	[43]
PPARG2	Peroxisome proliferator-activated receptor gama	[40]
RETN	Resistin	[22]
SH2B1	SH2B adaptor protein 1	[42]
SLC30A8	Solute carrier family 30, member 8	[40]
SORT1	Sortilin 1	[40]
TCF7L2	Transcription factor 7-like 2	[40]
TMEM18	Transmembrane protein 18	[42]
TRIB1	Tribbles pseudokinase 1	[40]

*Bolded genes were significantly associated with the phenotypes of interest.

The metabolic syndrome is considered to be a pre-diabetic state, with elevated values for three out of five of the following cardiometabolic risk factors: blood pressure, waist circumference, blood glucose, triglycerides, and HDL [44]. The metabolic syndrome predisposes people to diabetes mellitus and cardiovascular disease. The FAMuSS study collected these cardiometabolic biomarkers among young, healthy adults, an optimal time for the implementation of lifestyle interventions to prevent disease progression [44, 45] as well as avoid the confounding effects of aging and its associated comorbidities on heritability [35].

Pistilli et al. [22] examined the influence of *RETN* (Resistin), a gene that has a potential role in inflammatory processes and metabolic diseases such as obesity, diabetes mellitus, and cardiovascular disease [46] on measures of body composition at baseline and in response to RT. *RETN* variants (rs34124816, rs1862513, rs3219177, rs3745367, and rs3219178) were strongly associated with muscle strength and muscle, bone, and fat volume phenotypes in men and women, but only when stratified by a BMI $\geq 25\,\mathrm{kg{\cdot}m^{-2}}$, and they explained a relatively strong proportion of the variance in these phenotypes ranging from 7% to 12%. This study is

evidence of the complex interactions that can exist among genes and measures of body composition [35].

BMP2 (bone morphogenetic protein 2) regulates the differentiation of pluripotent mesenchymal cells and inhibits myogenesis. In addition, high BMP2 levels promote osteogenesis or chondrogenesis and low levels promote adipogenesis. The interrelationships of muscle, fat, and bone cell deposition are key factors in both normal morphologic variation and a variety of medical conditions including the metabolic syndrome, vascular calcification, and osteoporosis [47, 48]. Devaney et al. [15] discovered sex-specific associations with *BMP2* (rs15705) and baseline subcutaneous fat volume and the response of subcutaneous fat and bone volume to RT. In addition, *BMP2* explained 2–4% of the variability in these phenotypes.

Due to rapid advances in field of genomics, new genetic tools became available while FAMuSS was being conducted. This work stemmed from two important achievements: (1) the completion of the Human Genome Project and (2) provision of an initial catalogue of human genetic variation and a haplotype map (HapMap, http://hapmap.ncbi.nlm.nih.gov/) [49]. These two important achievements coupled with the rapid improvements in genotyping technology and analysis led to genome wide association studies (GWAS). The FAMuSS study sought to leverage GWAS studies for variants that were identified to be associated with BMI and type 2 diabetes mellitus. We utilized the FAMuSS study to determine if these GWAS-identified variants would be associated with baseline adiposity, bone, and skeletal muscle phenotypes and the response of these phenotypes to RT as measured by MRI. In addition, we asked the question "do GWAS variants associated with BMI, lipid phenotypes, type 2 diabetes mellitus, and other cardiometabolic risk factors and diseases affect how an individual responds to exercise?".

Orkunoglu-Suer et al. [41] examined one of the first variants identified using GWAS that was associated with obesity as denoted by BMI, *INSIG2* (insulin-induced gene 2) (rs7566605), for association with baseline subcutaneous fat volume and the response of this phenotype to RT. They found sex-specific associations with *INSIG2* and subcutaneous fat volume at baseline and in response to RT that accounted for <1–2.3% of the variance in subcutaneous fat volume.

GWAS was utilized to identify eight SNPs associated with BMI that highlighted a possible neuronal influence on the development of obesity [50]. These variants were *FTO* (fat mass and obesity associated) (rs9939609), *GNPDA2* (glucosamine-6-phosphate deaminase 2) (rs10938397), *KCTD15* (potassium channel tetramerisation domain containing 15) (rs11084753), *MC4R* (melanocortin-4 receptor) (rs17782313), *MTCH2* (mitochondrial carrier 2) (rs10838738), *NEGR1* (neuronal growth regulator 1) (rs2815752), *TMEM18* (transmembrane protein 18) (rs6548238), and *SH2B1* (SH2B adaptor protein 1) rs7498665). Orkunoglu-Ser et al. [42] found sex-specific associations with *MC4R* (rs17782313) and BMI; *TMEM18* (rs6548238) and baseline subcutaneous fat volume; and *FTO* (rs9939609) and *SH2B1* (rs7498665) and the response of subcutaneous fat volume to RT. Collectively, these variants explained <1-2% of the variance in these body composition phenotypes.

The first gene examined in FAMuSS for associations with cardiometabolic phenotypes was *PPARα* (peroxisome proliferator-activated receptor alpha) that is involved in adipocyte differentiation and lipid and lipoprotein metabolism [43]. Studies in mice have shown that PPARα-deficient animals were unable to metabolize lipids and develop late onset obesity even when kept on a stable diet [51, 52]. Uthurralt et al. [43] examined one of the most studied *PPARα* variants, a missense SNP in exon five that results in the amino acid substitution, leucine 162 valine (L162V; rs1800206). Uthurralt et al. [43] found European-American men with the V allele had higher baseline triglyceride levels and arm subcutaneous fat volume and lower HDL and tended to increase arm subcutaneous fat volume following RT compared to men with the LL genotype. The strength of the association with triglycerides was noteworthy with the V allele accounting for 4% of the variance.

IL-15 has influence on muscle-to-adipose tissue pathways as well as lipid and glucose metabolism [53]. Pistilli et al. [23] examined associations among genetic variants in *IL-15* and its receptor *IL-15Rα* with baseline cardiometabolic biomarkers and skeletal muscle, subcutaneous fat, and bone phenotypes at baseline and in response to RT. Sex-specific associations were found with *IL-15* and baseline total cholesterol (rs1589241), LDL (rs1589241), HOMA (rs1589241), BMI (rs1057972), glucose (rs1057972), and triglycerides (rs2228059) levels. In addition, men showed associations with *IL-15* and *IL-15α* and baseline total bone volume (rs2296135) and cortical bone volume (rs2228059) as well as measures of muscle volume (rs2228095) and strength (rs1057972).

Converging lines of evidence suggested that AKT1 (V-akt murine thymoma viral oncogene homolog 1) was a major mediator of the responses to insulin, IGF1, and glucose. In addition, AKT1 has a key role in the regulation of muscle cell hypertrophy and atrophy. Devaney et al. [39] sought to validate associations with *AKT1* and metabolic syndrome phenotypes found in FAMuSS within three other study populations [Strong Heart Study (SHS) (n = 2,134; 55.5 ± 7.9 years), Dynamics of Health, Aging and Body Composition (Health ABC) (n = 3,075; 73.6 ± 2.9 years), and Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) (n = 175; 40–65 years)]. They found that a three-SNP (rs1130214, rs10141867, and rs33926946) *AKT1* haplotype (i.e., a specific combination of neighboring alleles that tend to be inherited together) associated with fasting glucose levels among women in FAMuSS and with other metabolic phenotypes among women and men in the other three study populations. This study was an early attempt by the FAMuSS study investigators to functionally validate genetic associations that were previously discovered, for the validation of phenotype-genotype associations is an important prerequisite to better understand disease risk and provide therapeutic interventions that are often lacking in the field of exercise genomics [36].

Devaney et al. [40] analyzed 20 GWAS-identified SNPs associated with cardiometabolic risk factors in younger populations that included FAMuSS and a cohort of 6th grade children (Cardiovascular Health Intervention Program; CHIP). They established that the 1p13.3 LDL locus (rs646776) (near

SORT1, sortilin 1) was associated with LDL in both of these young populations. The variance accounted for by *SORT1* was considerably higher in these young populations (2.5%–4.1%) compared to older subjects from GWAS studies (1%).

In summary, the FAMuSS study discovered and validated numerous loci for associations with measures of body composition and cardiometabolic biomarkers. The genetic variants we examined explained <1–12% of the variance in the phenotypes examined suggesting these traits are highly polygenic with many loci contributing to a very small proportion of the variation. Furthermore, many of the genotype-phenotype associations were sex specific. More recently, the FAMuSS study began to mine GWAS studies to find, explore, and in some instances validate the impact of identified loci on a younger population that represents a critical period for therapeutic intervention as well as minimize the confounding effects of age on these phenotypes. In this way, the variance accounted for by genotype was higher in FAMuSS than GWAS that typically involve older subjects.

6. FAMuSS Findings: Physical Activity

Examining genetic variants that associate with habitual physical activity termed *physical activity genetics* was not a primary purpose of FAMuSS. Nonetheless, FAMuSS presented us with the opportunity to contribute to a growing body of literature showing the effect-mediation genetic variants associated with physical activity may have on chronic diseases and health conditions such as type 2 diabetes mellitus [54, 55], hypertension [36], and in the case of FAMuSS, overweight and obesity [41, 42, 56–59].

Over 67% of Americans are overweight to obese [60]. Physical inactivity is a major contributor to overweight and obesity as 74% of Americans do not meet the physical activity recommendations for weight maintenance [61]. It is of interest that the control of voluntary movement resides in similar central neural pathways as energy intake, emphasizing the role of the central nervous system in the regulation of energy expenditure and intake, and ultimately weight control [50, 59]. The redundancies in the etiology and control of physical activity and obesity led us to test the hypothesis that genetic variants associated with obesity will associate with physical activity phenotypes derived from the Paffenbarger Physical Activity Questionnaire among the FAMuSS cohort.

The 11 genes reported to be associated with obesity phenotypes [25, 42, 50, 56, 58, 62, 63] that were tested for specific association with physical activity in FAMuSS are summarized in Table 3. Our work has revealed genotype differences in physical activity energy expenditure that ranged from about 500 to 2000 $\text{kcal}\cdot\text{wk}^{-1}$ that were dependent upon BMI, sex, and intensity or the level of physical exertion. These genotype differences have public health importance, equating to a potential weight gain or loss of 7–29 $\text{lb}\cdot\text{yr}^{-1}$. Furthermore, genotype accounted for ~1–5% of the variance in physical activity phenotypes substantiating the polygenetic influence on physical activity, and the large amount of heritability that remains unaccounted for [37].

Understanding the interactions between genetic variants associated with obesity and physical activity will provide insight into the causes and treatments of overweight and obesity. Our vision is that this research may eventually have important implications for a personalized approach to the prescription of physical activity for the treatment of overweight and obesity. For example, prescribing physical activity to reduce weight or maintain weight loss will be more effective if clinicians are able to create a unique prescription that targets the type or amount of physical activity an individual prefers to engage in based upon their genetic makeup. The vision is that such a personalized exercise prescription based upon this genetic information would facilitate physical activity adoption and adherence for that person [16, 24, 25, 35, 41, 42]. Nonetheless, due to the significant challenges in identifying genes and their regulatory factors that may influence overweight and obesity and their interactions with physical activity, a personalized approach for the prescription of physical activity for the treatment of this major public health epidemic is not evident for the immediate future.

Table 3: FAMuSS findings: genetic loci associated with habitual physical activity.

Gene	Name	Reference
ANKRD6	Ankyrin repeat domain 6	[24]
FTO	Fat mass and obesity associated	[42]
GHRL	Ghrelin	[64]
KCTD15	Potassium channel tetramerisation domain containing 15	[42]
LEP	Leptin	[25]
LEPR	Leptin receptor	[25]
MC4R	Melanocortin-4 receptor	[42]
NEGR1	Neuronal growth regulator 1	[42]
NOS3	Nitric oxide synthase 3	[16]
SH2B1	SH2B adaptor protein 1	[42]
TMEM18	Transmembrane protein 18	[42]

7. Take-Home Messages, Future Directions, and Conclusions

The FAMuSS study was an attempt to understand the genetic causes behind the response of muscle to an external stimulus, RT. The aims of the FAMuSS project were to utilize molecular biology to answer two questions: (1) can genetic variation explain differences in skeletal muscle size and strength? and (2) can genetic variation explain how skeletal muscle responds to RT? To date, the FAMuSS group has published results for 17 genes tested for association with muscle strength or size that have been categorized according to their biological functions that include muscle structural elements, growth factors, and inflammatory factors (Table 1). In general, single variants explained minor trait variability in baseline muscle size and strength and the response of these phenotypes to RT, indicating a polygenetic influence on these complex phenotypes, and many of these muscle size and strength genotype associations were sex specific. Moreover, FAMuSS findings have provided a very valuable window into

variant influences in "normal" (i.e., nonpathological) young, healthy subjects.

In addition to its primary purpose, the FAMuSS group has published results for 33 genes tested for association with measures of body composition and their response to RT and baseline cardiometabolic biomarkers (Table 2) as well as 11 obesity genes tested for association with habitual physical activity levels (Table 3). The genetic variants that emerged from these analyses explained <1–12% of the variance in the phenotype examined, once again suggesting these traits are highly polygenic with many loci contributing a very small proportion of the variation, and these phenotype-genotype associations were often sex specific. More recently, the FAMuSS study mined GWAS studies to find, explore, and in some instances validate the impact of GWAS-identified loci on body composition and cardiometabolic biomarkers among a young population. In this way, the variance accounted for by genotype was higher in FAMuSS than GWAS involving older subjects partially due to the confounding effects of age being less in younger populations.

One persistent effect modifier of FAMuSS findings has been sex differences in the various phenotypes examined at rest and in response to RT. Hubal et al. [7] provided a detailed analysis of the variance of muscle strength and size responses in men and women to the 12 wk unilateral RT program, noting similar distributions in size gains (though men gained slightly more muscle volume than women in the trained arm) (Figure 1), but more variability in strength gains in women (as well as greater relative strength gains) (Figure 2). This increased variance in women for strength gains could account for some of the sex differences in the various phenotypes found in FAMuSS, as more high or low responders in a population could denote greater genetic influences. It is also possible that the greater amounts of androgens in men could account for a larger percentage of variation in responses, lowering the variance left to be accounted for by genetic factors. Furthermore, the response of a phenotype is often a function of baseline values that also varied by sex in FAMuSS [65]. In any case, sex-specific findings in genetic association studies are rather common, stemming from the large effect that biological sex has on a wide variety of phenotypes [35].

Our vision when we began FAMuSS was that with the identification of genetic variants that play a role in the normal response of muscle to external stimuli such as RT we would be able to better inform the sports world to maximize athletic performance and, more importantly, provide insight into disease processes—permitting the possibility of new therapeutics to treat neuromuscular disorders and other diseases and health conditions via a personalized medicine approach. What we have come to realize is that the journey to establish a personalized medicine approach to the treatment of disease that may also include a personalized approach to exercise prescription as lifestyle therapy was far more complex than anyone envisioned in 2001. Knowing what we have learned from the FAMuSS study and with the rapid advancement of technology since FAMuSS began in 2001, if we were to perform FAMuSS Part 2, we would (1) perform a GWAS and/or whole exome sequencing; (2) use an "interomic" approach to also measure the transcriptome, proteome, and metabolome at baseline and in response to RT to better capture gene expression; and (3) use bioinformatics combining quantitative with systems biology to conduct pathway analyses to elucidate mechanisms for the heritable factors and phenotype associations that emerge. Such an "interomic" bioinformatic approach would require a multidisciplinary team that has expertise in quantitative and systems biology as well as exercise physiology and preventive medicine. Nonetheless, FAMuSS has and will continue to have an important role in the journey to establish a personalized medicine approach to prevent, treat, and control disease as well as the development of new and more effective therapeutic options that will eventually be able to be prescribed on a more individualized basis.

Conflict of Interests

The authors have no conflict of interests to declare regarding the publication of this paper.

Acknowledgments

The authors acknowledge the hard work and dedication of the other members of the Exercise and Genetics Collaborative Research Group consisting of FAMuSS researchers and site coordinators from the University of Central Florida (TJ Angelopoulos), University of Massachusetts (PM Clarkson), West Virginia University (PM Gordon), Dublin City University (NM Moyna), Central Michigan University (P Visich), Florida Atlantic University (RF Zoeller), Yale University (TB Price), and Hartford Hospital (RL Seip). The authors thank these investigators and the other authors of the 30+ manuscripts that have been published from the FAMuSS study to date, many of which are referenced in this paper. The authors would also like to acknowledge Dr. Heather Gordish-Dressman from Children's National Medical Center for her expertise and patience when working with the authors on the statistical analyses of the FAMuSS data set, and the many graduate students that assisted with data collection. Last, the authors dedicate this manuscript to the honor and memory of their dear colleague and friend, Dr. Priscilla Clarkson. This paper is supported by Functional Single Nucleotide Polymorphisms (SNPs) Associated with Human Muscle Size and Strength (FAMuSS) NIH R01 NS40606-02; 2001–2006.

References

[1] P. D. Thompson, N. Moyna, R. Seip et al., "Functional polymorphisms associated with human muscle size and strength," *Medicine and Science in Sports and Exercise*, vol. 36, no. 7, pp. 1132–1139, 2004.

[2] R. S. Paffenbarger Jr., A. L. Wing, and R. T. Hyde, "Physical activity as an index of heart attack risk in college alumni," *American Journal of Epidemiology*, vol. 108, no. 3, pp. 161–175, 1978.

[3] M. A. I. Thomis, G. P. Beunen, M. van Leemputte et al., "Inheritance of static and dynamic arm strength and some of its determinants," *Acta Physiologica Scandinavica*, vol. 163, no. 1, pp. 59–71, 1998.

[4] T. Reed, R. R. Fabsitz, J. V. Selby, and D. Carmelli, "Genetic influences and grip strength norms in the NHLBI twin study males aged 59–69," *Annals of Human Biology*, vol. 18, no. 5, pp. 425–432, 1991.

[5] L. Pérusse, G. Lortie, C. Leblanc, A. Tremblay, G. Thériault, and C. Bouchard, "Genetic and environmental sources of variation in physical fitness," *Annals of Human Biology*, vol. 14, no. 5, pp. 425–434, 1987.

[6] D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner, "Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man," *Diabetologia*, vol. 28, no. 7, pp. 412–419, 1985.

[7] M. J. Hubal, H. Gordish-Dressman, P. D. Thompson et al., "Variability in muscle size and strength gain after unilateral resistance training," *Medicine and Science in Sports and Exercise*, vol. 37, no. 6, pp. 964–972, 2005.

[8] J. Lowndes, R. L. Carpenter, R. F. Zoeller et al., "Association of age with muscle size and strength before and after short-term resistance training in young adults," *The Journal of Strength & Conditioning Research*, vol. 23, no. 7, pp. 1915–1920, 2009.

[9] M. D. Peterson, D. Liu, H. Gordish-Dressman et al., "Adiposity attenuates muscle quality and the adaptive response to resistance exercise in non-obese, healthy adults," *International Journal of Obesity*, vol. 35, no. 8, pp. 1095–1103, 2011.

[10] L. S. Pescatello, B. K. Kelsey, T. B. Price et al., "The muscle strength and size response to upper arm, unilateral resistance training among adults who are overweight and obese," *The Journal of Strength & Conditioning Research*, vol. 21, no. 2, pp. 307–313, 2007.

[11] A. M. Costa, L. Breitenfeld, A. J. Silva, A. Pereira, M. Izquierdo, and M. C. Marques, "Genetic inheritance effects on endurance and muscle strength: an update," *Sports Medicine*, vol. 42, pp. 449–458, 2012.

[12] L.-J. Tan, S.-L. Liu, S.-F. Lei, C. J. Papasian, and H.-W. Deng, "Molecular genetic studies of gene identification for sarcopenia," *Human Genetics*, vol. 131, no. 1, pp. 1–31, 2012.

[13] M. A. Thomis and J. Aerssens, "Genetic variation in human muscle strength—opportunities for therapeutic interventions?" *Current Opinion in Pharmacology*, 2012.

[14] P. M. Clarkson, J. M. Devaney, H. Gordish-Dressman et al., "ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women," *Journal of Applied Physiology*, vol. 99, no. 1, pp. 154–163, 2005.

[15] J. M. Devaney, L. L. Tosi, D. T. Fritz et al., "Differences in fat and muscle mass associated with a functional human polymorphism in a post-transcriptional BMP2 gene regulatory element," *Journal of Cellular Biochemistry*, vol. 107, no. 6, pp. 1073–1082, 2009.

[16] M. A. Guidry, M. A. Kostek, T. J. Angelopoulos et al., "Endothelial nitric oxide synthase (NOS3) +894 G > T associates with physical activity and muscle performance among young adults," *ISRN Vascular Medicine*, vol. 2012, Article ID 901801, 7 pages, 2012.

[17] B. T. Harmon, E. F. Orkunoglu-Suer, K. Adham et al., "CCL2 and CCR2 variants are associated with skeletal muscle strength and change in strength with resistance training," *Journal of Applied Physiology*, vol. 109, no. 6, pp. 1779–1785, 2010.

[18] E. Pegoraro, E. P. Hoffman, L. Piva et al., "SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy," *Neurology*, vol. 76, no. 3, pp. 219–226, 2011.

[19] L. S. Pescatello, M. A. Kostek, H. Gordish-Dressman et al., "ACE ID genotype and the muscle strength and size response to unilateral resistance training," *Medicine and Science in Sports and Exercise*, vol. 38, no. 6, pp. 1074–1081, 2006.

[20] M. A. Kostek, T. J. Angelopoulos, P. M. Clarkson et al., "Myostatin and follistatin polymorphisms interact with muscle phenotypes and ethnicity," *Medicine and Science in Sports and Exercise*, vol. 41, no. 5, pp. 1063–1071, 2009.

[21] M. C. Kostek, J. M. Devaney, H. Gordish-Dressman et al., "A polymorphism near IGF1 is associated with body composition and muscle function in women from the Health, Aging, and Body Composition Study," *European Journal of Applied Physiology*, vol. 110, no. 2, pp. 315–324, 2010.

[22] E. E. Pistilli, H. Gordish-Dressman, R. L. Seip et al., "Resistin polymorphisms are associated with muscle, bone, and fat phenotypes in white men and women," *Obesity*, vol. 15, no. 2, pp. 392–402, 2007.

[23] E. E. Pistilli, J. M. Devaney, H. Gordish-Dressman et al., "Interleukin-15 and interleukin-15Rα SNPs and associations with muscle, bone, and predictors of the metabolic syndrome," *Cytokine*, vol. 43, no. 1, pp. 45–53, 2008.

[24] K. N. van Deveire, S. K. Scranton, M. A. Kostek et al., "Variants of the ankyrin repeat domain 6 gene (ANKRD6) and muscle and physical activity phenotypes among European-derived American adults," *The Journal of Strength & Conditioning Research*, vol. 26, pp. 1740–1748, 2012.

[25] S. Walsh, C. J. Haddad, M. A. Kostek et al., "Leptin and leptin receptor genetic variants associate with habitual physical activity and the arm body composition response to resistance training," *Gene*, vol. 510, pp. 66–70, 2012.

[26] S. Walsh, B. K. Kelsey, T. J. Angelopoulos et al., "CNTF 1357 G $\rightarrow$ A polymorphism and the muscle strength response to resistance training," *Journal of Applied Physiology*, vol. 107, no. 4, pp. 1235–1240, 2009.

[27] K. N. North and A. H. Beggs, "Deficiency of a skeletal muscle isoform of α-actinin (α-actinin-3) in merosin-positive congenital muscular dystrophy," *Neuromuscular Disorders*, vol. 6, no. 4, pp. 229–235, 1996.

[28] N. Yang, D. G. MacArthur, J. P. Gulbin et al., "ACTN3 genotype is associated with human elite athletic performance," *American Journal of Human Genetics*, vol. 73, no. 3, pp. 627–631, 2003.

[29] D. G. Macarthur, J. T. Seto, S. Chan et al., "An Actn3 knockout mouse provides mechanistic insights into the association between α-actinin-3 deficiency and human athletic performance," *Human Molecular Genetics*, vol. 17, no. 8, pp. 1076–1086, 2008.

[30] D. G. MacArthur, J. T. Seto, J. M. Raftery et al., "Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans," *Nature Genetics*, vol. 39, no. 10, pp. 1261–1265, 2007.

[31] A. C. McPherron and S.-J. Lee, "Double muscling in cattle due to mutations in the myostatin gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 23, pp. 12457–12461, 1997.

[32] N. Yamamoto, S. Akiyama, T. Katagiri, M. Namiki, T. Kurokawa, and T. Suda, "Smad1 and smad5 act downstream of intracellular signalings of BMP-2 that inhibits myogenic differentiation and induces osteoblast differentiation in C2C12 myoblasts," *Biochemical and Biophysical Research Communications*, vol. 238, no. 2, pp. 574–580, 1997.

[33] P. M. Clarkson and M. J. Hubal, "Exercise-induced muscle damage in humans," *American Journal of Physical Medicine and Rehabilitation*, vol. 81, no. 11, pp. S52–S69, 2002.

[34] E. P. Hoffman, H. Gordish-Dressman, V. D. McLane et al., "Alterations in osteopontin modify muscle size in females in both humans and mice," *Medicine & Science in Sports & Exercise*, vol. 45, pp. 1060–1068, 2013.

[35] L. S. Pescatello and S. M. Roth, *Exercise Genomics*, Springer, New York, NY, USA, 2011.

[36] G. I. Ash, J. D. Eicher, and L. S. Pescatello, "The promises and challenges of the use of genomics in the prescription of exercise for hypertension: the 2013 update," *Current Hypertension Reviews*, vol. 9, pp. 130–147, 2013.

[37] T. A. Manolio, F. S. Collins, N. J. Cox et al., "Finding the missing heritability of complex diseases," *Nature*, vol. 461, no. 7265, pp. 747–753, 2009.

[38] T. Yokota, S. Takeda, Q.-L. Lu, T. A. Partridge, A. Nakamura, and E. P. Hoffman, "A renaissance for antisense oligonucleotide drugs in neurology: exon skipping breaks new ground," *Archives of Neurology*, vol. 66, no. 1, pp. 32–38, 2009.

[39] J. M. Devaney, H. Gordish-Dressman, B. T. Harmon et al., "AKT1 polymorphisms are associated with risk for metabolic syndrome," *Human Genetics*, vol. 129, no. 2, pp. 129–139, 2011.

[40] J. M. Devaney, P. D. Thompson, P. S. Visich et al., "The 1p13.3 LDL (C)-associated locus shows large effect sizes in young populations," *Pediatric Research*, vol. 69, no. 6, pp. 538–543, 2011.

[41] F. E. Orkunoglu-Suer, H. Gordish-Dressman, P. M. Clarkson et al., "INSIG2 gene polymorphism is associated with increased subcutaneous fat in women and poor response to resistance training in men," *BMC Medical Genetics*, vol. 9, article 117, 2008.

[42] F. E. Orkunoglu-Suer, B. T. Harmon, H. Gordish-Dressman et al., "MC4R variant is associated with BMI but not response to resistance training in young females," *Obesity*, vol. 19, no. 3, pp. 662–666, 2011.

[43] J. Uthurralt, H. Gordish-Dressman, M. Bradbury et al., "PPARα L162V underlies variation in serum triglycerides and subcutaneous fat volume in young males," *BMC Medical Genetics*, vol. 8, article 55, 2007.

[44] National Cholesterol Education Program (U.S.), "Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults, third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III)," Final Report, The Program, Washington, DC, USA, 2002.

[45] A. V. Chobanian, G. L. Bakris, H. R. Black et al., "Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure," *Hypertension*, vol. 42, no. 6, pp. 1206–1252, 2003.

[46] D. R. Schwartz and M. A. Lazar, "Human resistin: found in translation from mouse to man," *Trends in Endocrinology and Metabolism*, vol. 22, no. 7, pp. 259–265, 2011.

[47] I. R. Reid, "Relationships among body mass, its components, and bone," *Bone*, vol. 31, no. 5, pp. 547–555, 2002.

[48] J.-S. Shao, J. Cai, and D. A. Towler, "Molecular mechanisms of vascular calcification: lessons learned from the aorta," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 7, pp. 1423–1430, 2006.

[49] K. A. Frazer, D. G. Ballinger, D. R. Cox et al., "A second generation human haplotype map of over 3. 1 million SNPs," *Nature*, vol. 449, pp. 851–861, 2007.

[50] C. J. Willer, E. K. Speliotes, R. J. Loos et al., "Six new loci associated with body mass index highlight a neuronal influence on body weight regulation," *Nature Genetics*, vol. 41, pp. 25–34, 2009.

[51] P. Costet, C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau, "Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis," *The Journal of Biological Chemistry*, vol. 273, no. 45, pp. 29577–29585, 1998.

[52] F. Djouadi, C. J. Weinheimer, J. E. Saffitz et al., "A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor α-deficient mice," *Journal of Clinical Investigation*, vol. 102, no. 6, pp. 1083–1091, 1998.

[53] J. M. Argilés, F. J. López-Soriano, and S. Busquets, "Therapeutic potential of interleukin-15: a myokine involved in muscle wasting and adiposity," *Drug Discovery Today*, vol. 14, no. 3-4, pp. 208–213, 2009.

[54] T. O. Kilpeläinen, T. A. Lakka, D. E. Laaksonen et al., "Physical activity modifies the effect of SNPs in the SLC2A2 (GLUT2) and ABCC8 (SUR1) genes on the risk of developing type 2 diabetes," *Physiological Genomics*, vol. 31, no. 2, pp. 264–272, 2007.

[55] T. O. Kilpeläinen, T. A. Lakka, D. E. Laaksonen et al., "SNPs in PPARG associate with type 2 diabetes and interact with physical activity," *Medicine and Science in Sports and Exercise*, vol. 40, no. 1, pp. 25–33, 2008.

[56] C. Bouchard, "The biological predisposition to obesity: beyond the thrifty genotype scenario," *International Journal of Obesity*, vol. 31, no. 9, pp. 1337–1339, 2007.

[57] M. S. Bray, "Implications of gene-behavior interactions: prevention and intervention for obesity," *Obesity*, vol. 16, no. 3, pp. S72–S78, 2008.

[58] M. S. Bray, J. M. Hagberg, L. Pérusse et al., "The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update," *Medicine and Science in Sports and Exercise*, vol. 41, no. 1, pp. 35–73, 2009.

[59] J. T. Lightfoot, "Current understanding of the genetic basis for physical activity," *Journal of Nutrition*, vol. 141, no. 3, pp. 526–530, 2011.

[60] K. M. Flegal, D. Carroll, B. K. Kit, and C. L. Ogden, "Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010," *The Journal of the American Medical Association*, vol. 307, no. 5, pp. 491–497, 2012.

[61] J. E. Donnelly, S. N. Blair, J. M. Jakicic et al., "Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults," *Medicine and Science in Sports and Exercise*, vol. 41, no. 2, pp. 459–471, 2009.

[62] T. Fall and E. Ingelsson, "Genome-wide association studies of obesity and metabolic syndrome," *Molecular and Cellular Endocrinology*, vol. 382, no. 1, pp. 740–757, 2014.

[63] O. Ukkola, E. Ravussin, P. Jacobson et al., "Role of Ghrelin polymorphisms in obesity based on three different studies," *Obesity Research*, vol. 10, no. 8, pp. 782–791, 2002.

[64] T. Kilpeläinen, T. Lakka, D. Laaksonen et al., "Interaction of single nucleotide polymorphisms in ADRB2, ADRB3, TNF, IL6, IGF1R, LIPC, LEPR, and GHRL with physical activity on the risk of type 2 diabetes mellitus and changes in characteristics of the metabolic syndrome: the Finnish Diabetes Prevention Study," *Metabolism*, vol. 57, no. 3, pp. 428–436, 2008.

[65] L. S. Pescatello and J. M. Kulikowich, "The aftereffects of dynamic exercise on ambulatory blood pressure," *Medicine and Science in Sports and Exercise*, vol. 33, no. 11, pp. 1855–1861, 2001.