International Journal of Hypertension

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Editorial Board

Contents

Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 634798, 2 pages
http://dx.doi.org/10.1155/2013/634798

Editorial

Hypertension and Cardiometabolic Risk Factors

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Received 10 June 2013; Accepted 10 June 2013

Despite the availability of nonpharmacological approaches and pharmacological therapies for hypertension, blood pressure control rates are disappointing all over the world [1]. Among several considerations, hypertension is usually associated with metabolic disorders, especially obesity, diabetes, and dyslipidemia, which may contribute to a greater difficulty of lowering blood pressure. In fact, the significant association between systemic hypertension and other cardiometabolic risk factors is well recognized, such as an elevated body mass index, waist circumference, fasting glucose, triglycerides, and a reduced HDL cholesterol, even not reaching criteria for diagnosis of metabolic syndrome. Individually, each one of these metabolic disorders is associated with adverse cardiovascular outcomes.

This special issue presents some papers related to these cardiometabolic risk factors and their involvement mainly in prognosis and treatment of hypertensive patients. Firstly, a very low diastolic blood pressure, with a threshold value of 70 mmHg, was associated with increased all-cause mortality. It has been discussed that blood pressure level is the most important factor in cardiovascular risk evaluation even in hypertensive subjects with important metabolic disorders. The linear relationship between blood pressure and cardiovascular mortality starting from 115/75 mmHg is well known [2]. Nevertheless, cardiovascular events have also been observed after insistent lowering of blood pressure, at least in patients with coronary artery disease [3].

Sleep disorders have been increasingly studied in recent years. Obstructive sleep apnea is related to cardiovascular disease and metabolic dysfunction, but sleep duration is a parameter not commonly considered in this association. Indeed, short sleep duration has been previously associated with an increased likelihood for hypertension [4]. In a paper of this collection, interactions of deficient sleep with race/ethnicity were noted even after adjusting to metabolic factors such as body mass index and diabetes. This was an interesting finding considering that insulin resistance exacerbated by renin angiotensin system (RAS) activation might be a mechanism involved in the link between sleep disorders and metabolic syndrome. The causal relationship of RAS and oxidative stress with vascular inflammation was documented in this special issue in a study with an experimental model of metabolic syndrome. In this protocol, a RAS inhibitor and an antioxidant agent were able to attenuate metabolic factors and systolic blood pressure in spontaneously hypertensive rats submitted to fructose administration.

The interaction between hypertension and diabetes seems to be the principal concern to the cardiovascular system. The mechanisms that may increase cardiovascular risk in diabetic hypertensive patients are not completely understood. Oxidative stress and low-grade inflammation resulting in endothelial dysfunction as the first process of atherosclerosis were reviewed in this special issue. These factors may be also relevant in patients with longer duration of type 1 diabetes.

In another paper, a cross-sectional study carried out in 20 Brazilian cities demonstrated that less than one quarter of treated type 1 diabetic patients achieved the target values for systolic and diastolic blood pressure. These data indicate that a more aggressive approach of this population is extremely necessary also in developing countries.

Nutritional factors involved in the treatment of hypertensive subjects are also discussed in this special issue. Many dietary components, especially sodium, potassium, calcium, and magnesium, are reviewed based on the available evidence. Restriction of daily sodium intake is recommended in several guidelines about hypertension. However, low salt intake has resulted in cardiovascular mortality in some particular groups of patients including diabetic subjects [5]. Potassium, calcium, and magnesium supplementations are not indicated as a usual practice for hypertensive subjects, but natural source of these micronutrients is the mainstay of the Dietary Approaches to Stop Hypertension (DASH) plan. Also, as a nonpharmacological approach, two original papers in this collection demonstrated the beneficial effects of dark chocolate consumption on endothelial function in specific populations: stage 1 hypertensive subjects with excess body weight and younger hypertensive individuals with low cardiovascular risk who already present endothelial dysfunction. It has been reported that these favorable outcomes are associated with reasonably high amount of polyphenol in dark chocolate.

Lastly, in a study that enrolled patients with uncontrolled hypertension and criteria for metabolic syndrome, moxonidine therapy resulted in blood pressure reduction and improvement of blood pressure control rates, especially in younger individuals. The pharmacological treatment of hypertension in the context of metabolic syndrome presents many challenges in the daily clinical practice due to several mechanisms involved in this medical condition including the sympathetic nervous system (SNS) hyperactivation. Thus, moxonidine, a selective agonist at the imidazoline receptor subtype 1 which determines a decrease in SNS activity, may be beneficial in this circumstance.

Undoubtedly, hypertension is very commonly associated with other metabolic chronic conditions. In fact, more than 80% of hypertensive patients present one or more concomitant metabolic risk factors. This clinical condition has a growing global prevalence, clearly related to the modern lifestyles characterized by lack of physical activity resulting in overweight or obesity. Hopefully, we believe that this special issue can help to better understand the interaction between raised blood pressure levels and cardiometabolic factors, pointing out the involvement of insulin resistance and RAS/SNS activation as linking factors and also discussing some therapeutic options for this population.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 276564, 8 pages
http://dx.doi.org/10.1155/2013/276564

Research Article

Prevalence of 10-Year Risk of Cardiovascular Diseases and Associated Risks in Canadian Adults: The Contribution of Cardiometabolic Risk Assessment Introduction

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Received 30 November 2012; Accepted 10 April 2013

Academic Editor: Mario Fritsch Neves

Background. Cardiovascular disease (CVD) is the leading cause of death in adult Canadians. Cardiometabolic risk (CMR) derived from 10-year risk of cardiovascular diseases and metabolic syndrome (MetS) needs to be evaluated in Canadian adults. *Objective*. To determine CMR among Canadian adults by sociodemographic and lifestyle characteristics. *Subjects and Methods*. Data from the Canadian Health Measures Survey (CHMS), Cycle 1, 2007–2009, was used. Framingham Risk Score (FRS) was implemented to predict 10-year risk of CVD, and metabolic syndrome was identified using the most recent criteria. The 10-year risk of CVD was multiplied by 1.5 in individuals with MetS to obtain CMR. Data were weighted and bootstrapped to be able to generalize the results nationally. *Results and Conclusion*. CMR gave more accurate estimation of 10-year risk of CVD in Canadian adults from 30 to 74 years than using only FRS. The 10-year risk of CVD in Canadian adults significantly increased when CMR was taken into account from 8.10% to 9.86%. The CVD risk increased by increase in age, decrease in education, and decrease in physical activity and in smokers. Canadians with medium risk of CVD consumed significantly less fruit and vegetable juice compared to Canadians with low risk. No other dietary differences were found.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death in Canadian men and women [1]. In 2008, CVD accounted for 29% of all deaths in Canada (28.0% in males and 29.7% in females) [2]. Over $20.9 billion is spent each year in Canada for physician services, hospital costs, lost wages, and decreased productivity related to heart disease and stroke [2].

Population-based strategies in assessing the risk of CVD and cost-effective interventions would play a fundamental role in decreasing rates of morbidity and mortality from CVD. The Framingham Risk Score (FRS), developed through Framingham Heart Study in 1971 to 1974, is the most commonly recommended assessment tool for evaluating 10-year risk of CVD in the United States [3]. The National Cholesterol Education Program (Adult Treatment Panel III) recommends using FRS to assess 10-year risk of CVD [4]. The FRS has been validated for the Canadian population by a committee of clinicians and researchers [3]. However, increasing evidence has indicated that CVD risk algorithms such as FRS may underestimate an individual's actual risk and postpone the initiation of appropriate intervention probably due to not considering waist circumference and triglyceride [5, 6].

Metabolic syndrome (MetS) is a clustering of five chronic disease risk factors, including abdominal obesity, dyslipidemia (elevated triglycerides (TG) and reduced high-density lipoprotein cholesterol (HDL-C) level), hypertension, and elevated fasting plasma glucose (FPG) [7]. MetS is considered to be the main contributor to CVD and diabetes [7, 8]. Regardless of ethnic diversity, the risk of CVD doubles with MetS; also, the risk of diabetes increases fivefold [9–12]. According to our recent data from Canadian Health Measures Survey (CHMS), 2007–2009, 18.31% of Canadians aged from 12 to 79 y had MetS [13]. However, an increase in relative risk of CVD cannot be used to evaluate absolute

risk. Furthermore, reported relative risk of CVD and its association with MetS are not comparable between studies as not all studies take into consideration potential confounders.

The new approach on assessing "cardiometabolic risk (CMR)" or "global cardiometabolic risk" considers the factors that go beyond the traditional risk factors [5, 6]. In 2009, the Canadian Cardiometabolic Risk Working Group suggested that CMR represents the comprehensive catalogue of factors related to CVD and Type 2 diabetes [5, 6]. Therefore, in evaluating CMR, both MetS and 10-year risk of CVD are considered. The calculation of FRS followed by the evaluation of the presence or the absence of MetS helps identify individuals whose risk might be underestimated. Therefore, in order to evaluate CMR, both the risk factors of MetS and the risk factors used to calculate 10-year risk of CVD are considered. This novel approach, CMR, has been used to identify the risk of CVD at the individual level [5, 6]; however, to our knowledge no nationally representative study has used this method to evaluate the risks at the population level. Moreover, it is not understood if calculating CMR could possibly change the prevalence of the 10-year risk of CVD at a population level.

Different dietary patterns impact cardiometabolic components in different ways. The Dietary Approaches to Stop Hypertension (DASH) diet or Mediterranean diet are inversely associated with cardiometabolic abnormalities [14–16]. Similarly, coronary heart disease is positively associated with the Western diet as opposed to the prudent diet [17]. Our recent findings from CHMS indicate that Canadians with MetS consumed less dairy products, sugar sweetened beverages, and dietary fat, but had a greater intake of diet soft drinks [13]. The dietary behavior of Canadians at different levels of CVD risk has yet to be explored.

The first objective of the present study was to determine the 10-year risk of CVD in Canadian adults and potential changes at population level when CMR is taken into account. The second objective was to determine 10-year risk of CVD in Canadian adults by different sociodemographic characteristics. Further, we determined the dietary intakes of Canadians in risk categories of 10-year risk of CVD.

2. Subjects and Methods

2.1. Study Population. Data from the Canadian Health Measures Survey (CHMS), Cycle 1, 2007–2009, conducted by Statistics Canada in partnership with Health Canada and Public Health Agency of Canada, was used. CHMS is a nationally representative survey collecting health indicators among a sample of approximately 5,500 Canadians aged 6 to 79 y (representative of 96.3% Canadians through multi-stage sampling strategy). The survey consists of two stages: the first stage is self-reported data collection through interviews, and the second stage consists of taking direct physical measurements at Mobile Examination Centers (MEC). Individuals living on reserves or in other aboriginal settlements in the provinces, remote areas, institutional residents, and full-time members of the Canadian Forces were excluded from the survey. The sampling weights, provided by Statistics Canada, were calculated by multiplying the selection weights for collection sites and the selection weights for dwellings (obtained from 2006 Census of Canada), adjusted for non-response [18]. The final individual weight was obtained after converting the household weights followed by adjustment for nonresponse at the interview stage and the MEC stage. For the purpose of our study, we excluded individuals who were either under the age of 30 years or over the age of 74 years, as well as nonfasting subjects, pregnant women, and those with confirmed diagnosis of heart disease. The final number of respondents for the current study was 1,293.

2.2. Indicators Needed to Estimate 10-Year Risk of CVD. To estimate the 10-year risk of CVD, categorical charts and tables based on FRS were used [19]. Separate score sheets were developed for each sex according to age, blood pressure, total cholesterol (Tchol), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) categories. The points assigned for each risk factor are based on the value for the β-coefficient of the proportional hazards regressions [19]. Further, we factored the presence or absence of diabetes and smoking status (smoker versus nonsmoker) in our estimations.

2.3. Creating Variables of Interest Using CHMS Dataset. Using either LDL-C or Tchol is optional in assessing CVD risk [3]. LDL-C could be found in only fasted CHMS subsample; whereas, Tchol was available in both fasted and nonfasted subsample. For the current study we used fasted subsample and we evaluated both LDL-C and Tchol. Daily smokers, occasional smokers, or those who stopped smoking less than a year ago were classified as smokers. To recognize individuals with undiagnosed diabetes, Glycated Hemoglobin (HbA1c) $\geq$ 6.5% was used as the criteria [20]. To obtain the total number of diabetics, cases of self-reported diabetes were added to the individuals with undiagnosed diabetes. Other variables such as age, HDL-C, and systolic and diastolic blood pressure were used to obtain the risk score [19]. All variables were categorized according to the corresponding cutoffs defined in FRS [3]. We reported 10-year risk of CVD using two approaches: (1) 10-year risk of CVD by implementing LDL-C and (2) 10-year risk of CVD by implementing Tchol. Both LDL-C and Tchol cut-points are specified in FRS.

2.4. Calculating CMR. To include the CMR in the estimation of 10-year CVD risk, we first identified individuals with MetS. We applied the most recent unified definition established in 2005 by the International Diabetes Federation (IDF), in collaboration with American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), for adults. The presence of at least three of the following five metabolic risk factors constitutes a diagnosis of MetS: abdominal obesity (shows the cut-points used in the current study) [21], elevated TG level (1.7 mmol/L), reduced HDL-C level (1.0 mmol/L in males; 1.3 mmol/L in females), elevated blood pressure (BP) (systolic $\geq$ 130 and/or diastolic $\geq$ 85 mm Hg), and elevated FPG level ($\geq$5.6 mmol/L). We followed IDF's recommendation by using ethno-specific cutoffs for waist

measurement [21]. Individuals who have already been diagnosed as hypertensive, diabetic, or those who were using antihypertensive drugs were also included. In the next step, the 10-year CVD risk among individuals with MetS was multiplied by 1.5 [6].

2.5. Prevalence of CMR by Different Level of Sociodemographic Characteristics. The age- and sex-specific groups in our analysis were males and females aged 30–34 y, 35–39 y, 40–44 y, 45–49 y, 50–54 y, 55–59 y, 60–64 y, 54–69, and 70–74 y. Four levels of education based on the highest level achieved by any member of the household were defined as less than secondary school graduation, secondary school graduation, and some postsecondary, or postsecondary graduation. Four economic status levels were based on the total household income and the number of individuals in the household. Only two ethnic groups, that is, White and non-White, were created due to few numbers of non-White individuals in various ethnic groups in that category. Physical activity was measured in CHMS using a questionnaire which calculated the total daily leisure time energy expenditure (EE) values (kcal/kg/day) during leisure time activities. Respondents were subsequently categorized into "active" (EE $\geq$ 3), "moderate" (1.5 $\leq$ EE $<$ 3), or "inactive" (0 $\leq$ EE $<$ 1.5) physical activity.

2.6. Dietary Assessment in Three Levels of Risk. In CHMS, usual dietary intake was collected through a semiquantitative food-frequency questionnaire. Dietary intake was collected based on the frequency of daily, weekly, monthly, or yearly consumption. Food groups in CHMS were defined as follows: meat and fish (e.g., red meat, organs, hotdogs, sausage or bacon, seafood, eggs, beans, nuts), grains, fruit and vegetable (e.g., hot/cold cereal, white bread, brown bread, any kind of rice, any kind of pasta, fruit, and vegetable including potato), milk and dairy product (e.g., milk, cottage cheese, yogurt, and ice cream), dietary fat (regular-fat salad dressing or mayonnaise, regular-fat potato chips, tortilla chips, and corn chips), water and soft drink (e.g., regular soft drink, sport drink, fruit drink, diet soft drink, and fruit/vegetable juice). The questions for each section were only on the frequency of consumption without quantifying the amount of intake. The respondents were asked to state the frequency of consumption per day, week, month, or year. Responses "I don't know" and "Refused to answer" were not included [22]. With the exception of the section on water and soft drink consumption, all other dietary intake questions were included for the first time for CHMS. Water and soft drink consumption questions were derived from the National Population Health Survey, NPHS, Cycle 6 [23]. The verification of questionnaire responses was conducted at the end of completing data collection at each site by reviewing and adjusting using notes recorded by interviewers. The food groups used in this study are consistent with what was used in CHMS. For the purpose of the current study all dietary consumption data was converted into a daily frequency of consumption (times/day).

Based on the FRS scores, we categorized participants in three levels of risk: low, medium, and high [4]. The low number of individuals at high risk level resulted in wide confidence intervals for estimates. Therefore, we only compared individuals at medium risk with individuals at low risk for dietary differences.

2.7. Data Analysis. To meet the first objective, the 95% confidence intervals (CI) of 10-year risk of CVD and CMR estimates were compared. No overlap in 95% CIs of the estimates was considered significant statistical difference at 0.05 [24]. For the second objective, we report the difference in 10-year risk of CVD by sex, age, and each sociodemographic characteristic using independent sample t-test or one-way ANOVA at sample level. At the population level, data was weighted and bootstrapped and comparisons across groups were performed using 95% CIs overlap.

To examine possible differences between dietary intakes across three levels of 10-year risk of CVD (Objective 3), the same method of using 95% weighted CIs overlap was implemented.

Data manipulation, cleaning, and creation of new variables were done using PASW Statistics 19. All statistical analyses were conducted by STATA/SE 11, StataCorp. As per Statistics Canada's recommendation, all analyses were weighted and bootstrapped in order to be representative of the Canadian population. The degrees of freedom of 11 in CHMS Cycle 1 were limited due to sampling structure. Alpha was set at 0.05.

3. Results

3.1. 10-Year Risk of CVD in Canadian Adults. The CHMS participants in our study ($n = 1,293$) represent 17, 250,853 Canadians aged 30–74 y. Overall, the risk of CVD in Canadian adults using LDL-C indicator and Tchol was 8.10% ± 8.89 (CI: 7.33–8.87) and 7.70% ± 8.75 (CI: 7.07–8.32), respectively. The corresponding risk when CMR was accounted for was 9.86% ± 12.93 (CI: 8.71–11.01) using LDL-C indicator and 9.41% ± 12.66 (CI: 8.43–10.39) using Tchol indicator. Using LDL-C or Tchol for either CMR or 10-year risk of CVD did not show any significant difference.

The 10-year CVD risk was significantly greater using CMR compared to the 10-year risk of CVD based on only FRS (using Tchol indicator). However, the estimate was not significantly greater when LDL-C was used as an indicator. In the current study, the risk of CVD considering different sociodemographic characteristics or dietary intake was reported using CMR with LDL-C indicator.

3.2. Sociodemographic Characteristics of 10-Year Risk of CVD in Canadian Adults. The risk of CVD was not significantly different across sex groups (Table 1). CVD risk increased significantly by increase in age and decrease in the levels of education and physical activity (Table 1). Individuals from the highest-income level households were significantly less at risk compared to those from upper-middle income families. In addition, the risk was greater in smokers and White Canadians compared to nonsmokers and non-White Canadians, respectively ($P < 0.05$) (Table 1).

Table 1: Weighted estimates of 10-year risk of CVD or cardiometabolic risk (CMR) by sociodemographic characteristics of Canadians aged from 30 to 74 years Canadian Health Measures Survey, Cycle 1, 2007–2009 (n = 1,293).

Characteristics	10-year risk of CVD, percent (SE[†])	Confidence intervals (CIs)
Sex		
Male	8.72 (0.43)	7.77–9.67
Female	10.92 (1.07)	8.56–13.28
Age[**1]		
(1) 30–34 years	1.95 (0.10)	1.72–2.17
(2) 35–39 years	2.95 (0.40)	2.07–3.84
(3) 40–44 years	5.42 (0.59)	4.11–6.73
(4) 45–49 years	6.02 (0.39)	5.15–6.88
(5) 50–54 years	11.91 (2.21)	7.03–16.79
(6) 55–59 years	14.32 (1.80)	10.34–18.30
(7) 60–64 years	19.83 (2.07)	15.26–24.40
(8) 65–69 years	20.68 (0.94)	18.60–22.75
(9) 70–74 years	24.68 (2.93)	18.21–31.14
Education level[**2]		
Less than secondary school graduation	19.35 (3.69)	11.20–27.49
Secondary school graduation	14.85 (1.52)	11.49–18.20
Some postsecondary	8.76 (0.78)	7.04–10.49
Postsecondary graduation	8.50 (0.44)	7.52–9.47
Income level[**3]		
Lowest income	11.10 (3.04)	4.39–17.81
Lower-middle income	12.06 (1.72)	8.26–15.86
Upper-middle income	11.34 (0.81)	9.53–13.14
Highest income	8.10 (0.49)	7.01–9.20
Physical activity[**4]		
Inactive	10.80 (0.78)	9.06–12.54
Moderately active	9.66 (0.66)	8.19–11.13
Active	7.46 (0.52)	6.30–8.63
Alcohol		
Never drink	10.74 (2.42)	5.41–16.08
Ever drink	9.81 (0.48)	8.74–10.87
Ethnicity[*]		
Non-White	7.32 (0.89)	5.35–9.30
White	10.32 (0.53)	9.15–11.49
Smoking[*]		
Nonsmokers	8.87 (0.32)	8.15–9.60
Smokers	13.18 (1.52)	9.83–16.53

[†]SE: standard error.
[**]Significant ($P < 0.05$), 95% confidence interval overlap.
[*]Significant ($P < 0.05$), independent t-test.
[1]According to 95% confidence interval, the risk of CVD in age groups 2 and 3 was significantly different. Age groups 5, 6, 7, 8, and 9 were significantly different from age groups 1, 2, 3, and 4. In addition, age group 9 and 8 were significantly different from age group 5.
[2]According to 95% confidence interval, the risk of CVD was significantly less among Canadians with some postsecondary education or postsecondary graduation compared to Canadians with less than secondary school graduation or secondary school graduation.
[3]The risk of CVD was significantly less among Canadians with highest income compared to upper-middle income.

3.3. Dietary Intake and 10-Year Risk of CVD. Most Canadian adults were in the low CVD risk level (93.4%) (Figure 1). The only significant association was observed in fruit and vegetable juice intake; Canadians at medium risk of CVD consumed less amounts of fruit and vegetable juice compared to their counterparts at low risk (Table 2).

4. Discussion

Overall the 10-year risk of CVD in Canadian adults accounted for 9.86% considering individuals identified with MetS. The risk of CVD increased with age or by a decrease in the level of education (Table 1). The CVD risk was greater among

Table 2: Dietary consumption among Canadians aged from 30 to 74 years at different level of 10-year risk of CVD, Canadian Health Measures Survey, Cycle 1, 2007–2009 (n = 1,293).

Food and beverages times/day[1]	Low 10-year CVD risk (n = 1190)	Medium 10-year CVD risk (n = 84) Mean of intake (SE[2]) CIs[3]	High 10-year CVD risk (n = 19)
Meat and fish			
Red meat, organs, hotdogs, sausage or bacon, seas foods, eggs, beans, and nuts	1.81 (0.13)	1.31 (0.08)	1.95 (0.42)
	1.50–2.11	1.12–1.49	1.01–2.89
Grains, fruit, and vegetable			
Hot/cold cereal, white bread, brown bread, rice, and pasta (grains)	2.87 (0.13)	3.76 (1.24)	3.35 (1.84)
	2.56–3.18	1.02–6.50	−0.71–7.41
Fruit and vegetable	3.78 (0.08)	3.65 (0.18)	3.95 (0.45)
	3.59–3.98	3.25–4.05	2.94–4.96
Milk and dairy products			
Milk, cottage cheese, and yoghurt or ice cream	1.52 (0.05)	1.59 (0.18)	1.44 (0.22)
	1.41–1.63	1.17–2.00	0.94–1.95
Dietary fat			
Regular-fat salad dressing or mayonnaise and regular-fat potato chips, tortilla chips, or corn chips	0.42 (0.02)	0.37 (0.07)	0.37 (0.08)
	0.38–0.47	0.21–0.53	0.18–0.55
Water and soft drinks			
Regular soft drink, sport drink, and fruit drink (sugar-sweetened beverages)	0.39 (0.05)	0.22 (0.05)	0.15 (0.06)
	0.28–0.50	0.11–0.34	0.01–0.29
Diet soft drink	0.16 (0.01)	0.31 (0.11)	0.39 (0.17)
	0.12–0.20	0.06–0.55	0.01–0.77
Fruit and vegetable juice*	0.71 (0.03)	0.43 (0.09)	1.46 (0.78)
	0.64–0.79	0.23–0.63	−0.26–3.19

[1] Frequency of consumption.
[2] SE: standard error.
[3] Confidence intervals.
* Significant ($P < 0.05$), based on 95% confidence interval between low risk versus medium risk.

upper-middle income households compared to higher-income households. Additionally, active Canadians with EE ≥ 3 had significantly lower risk compared to their inactive counterparts with 0 ≤ EE < 1.5. Canadians with medium risk of CVD significantly consumed less fruit and vegetable juice compared to Canadians with low risk of CVD.

The introduction to the concept of cardiometabolic risk (CMR) and its risk factors which are not included in traditional risk assessment tools such as FRS, as well as the guidelines for identification and management, was published in a position paper by the Canadian cardiometabolic risk Working Group, in 2009 [6]. The authors believe that traditional risk assessment tools solely underestimated the absolute risk of CVD at an individual level. According to this concept, the absolute CVD risk can be obtained from the algorithms validated by the observational cohort studies (e.g., FRS) taking into account individuals identified with MetS [6]. MetS imparts a relative increase in risk of CVD about 1.5- to 2-fold.

Evaluating the 10-year risk of CVD is usually conducted in prospective studies. Our study and one study among American adults [25] used a cross-sectional design which might be considered as a limitation. The estimate of 9.86% of 10-year CVD risk among Canadian adult population in the current study was similar to American and European prospective studies [26, 27]. Approximately 9.05% of the 12,089 Black and White middle-aged individuals, participated in Atherosclerosis Risk in Communities (ARIC) study after 11 years of follow-up, developed ischemic stroke and coronary heart disease (CHD) [26]. The corresponding prevalence in the European cohorts of the seven-country study, comprised of men aged 40–59 y after 10 years of follow-up, accounted for 9.69% for fatal or nonfatal CVD [27]. Additionally, the earlier study among Americans indicated 11.41% incidence of CHD after 12 years of follow-up through Framingham Heart Study which shows a decrease in the incidence of CVD among Americans over time [28]. Therefore, since the reports from prospective studies were similar to our finding, it might be possible to use the estimate of 10-year risk of CVD through cross-sectional studies which are more feasible than prospective studies.

Our finding suggests including CMR in assessing the risk of CVD not only at individual level but also at population level. The similar estimates using Tchol and LDL-C indicate

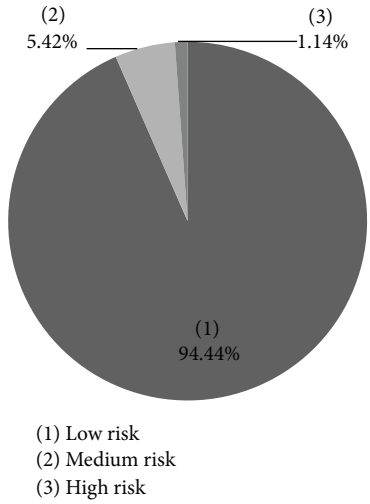

Figure 1: 10-year risk of CVD in Canadian adults, 30–74 y, Canadian Health Measures Survey (CHMS), cycle 1, 2007–2009.

the importance of simple measure of Tchol in this assessment at population level. Assessing CMR by using a simple measure of Tchol allows avoiding the underestimation of CVD risk in individuals with MetS. Our study showed only a small overlap in the 95% confidence interval of the two estimates when LDL-C was used, which likely relates to the small sample size.

Older age was considered as one of the strong factors in elevating the risk of CVD, as observed in our study. The greater CVD risk in older adults is not surprising as this group usually has higher prevalence of most CVD defining components compared to other adults. A similar finding was seen among American adults 20 years and older who participated in a cross-sectional sample of 5,440 in the National Health and Nutrition Examination Surveys (NHANES) 1999–2004 [29]. According to NHANES the prevalence of metabolic abnormalities increased with age among all individuals. In this study they used elevated blood pressure, elevated triglyceride and glucose levels, insulin resistance, systemic inflammation, and decreased HDL-C level as cardiometabolic abnormalities which were comparable to our study.

Physical activity in the current study was based on the energy expenditure during leisure time. Specifically, the significant difference in CVD risk between active and inactive individuals was similar to the finding of a Multiple Risk Factor Intervention Trial [30]. Among 12,138 middle-aged men measuring self-selected leisure-time physical activity (LTPA), combined fatal and nonfatal major CHD events were 20% lower with high activity as compared with low LTPA [30]. Moreover, better cardiometabolic characteristics such as lower heart rate, lower body mass index, and a higher HDL-C level or lower risk of CHD have been observed in more active women from two cohort studies than less active ones [31, 32]. Although Lee et al. 2001 [32] indicated that vigorous intensity of physical activity had a significant impact on reducing the risk of CHD compared to the lowest intensity, regular walking independently predicted lower risk [32]. The significant role of physical activity is more evident when the impact has still remained after adjustment for potential confounders such as smoking status, diet, and alcohol use in most studies. Physical activity independently, regardless of obesity, could contribute to the development of CHD in women [33].

In our study, risk of CVD was about 1.5 times greater in smokers than nonsmokers. In general, there is a nonlinear relation between the risk of CVD and the number of cigarettes smoked daily [34, 35]. The mechanisms that cause acute cardiovascular events in smokers include increased hypercoagulability leading to thrombosis, endothelial dysfunction, and development of chronic inflammatory state by an increase in white cells and CRP values [36]. In studies evaluating the impact of physical activity and risk of CVD, the interaction between physical activity and smoking has been reported [31, 32]. Among American women from Women's Health Study, physical activity was inversely associated with CHD rate in current and past smokers but not in nonsmokers [32]. Additionally, subjects from Framingham Offspring Study who were more active tended to smoke fewer cigarettes [31]. On the other hand, the impact of physical activity and smoking on the risk of CVD cannot be reported hierarchically; that is, each has its own strong impact.

Others report that current rather than childhood socioeconomic status has more influence on most cardiometabolic risk factors: physical inactivity, HDL-C, triglycerides, postload glucose, fibrinogen, and smoking [37]. Individuals in households with high level of education and income had lower risk of CVD in the current study. Education had a greater impact on the risk of CVD; that is, a decreasing trend in the CVD risk was observed by raising the level of education. The greater impact of education might be explained by its unchangeable virtue (occupation or income to). Thus, education is the most frequent measure used to evaluate the impact of socioeconomic status and cardiometabolic abnormalities [38]. According to a review by Kaplan and Keil, 1993, during 40 years of study there has been a consistent inverse relation between cardiovascular disease, primarily coronary heart disease, and many of the indicators of socioeconomic status [38]. Therefore, it would be necessary to control the impact of socio-economic status while evaluating the association between cardiometabolic risk and potential variables.

The dietary intake was compared only between individuals with medium risk of CVD and individuals with low risk of CVD, due to the small number categorized as high risk. The only significant difference in the dietary consumption was found in fruit and vegetable juice intake, with medium risk group having a decreased intake of fruit and vegetable juice. This finding is likely due to the adherence of people with diagnosed disease such as diabetes to a special diet [39]. In our study on MetS among Canadian population using CHMS data, we found that diabetics significantly consumed less fruit and vegetable juice compared to individuals with no diagnosis of diabetes [13]. Diabetics are reported to pay more attention to food labels, especially to the sugar information, than those without diabetes [39]. Diabetics in the Multiethnic Cohort Study (MEC) had lower consumption

of juice (>10% difference) compared to those without diabetes [40]. The Canadian Diabetes Association recommends that diabetics: "have vegetables and fruit more often than juice" [41].

Limitations. The approach of determining the absolute risk considering relative factor of about 1.5 to 2 in individuals with MetS is not fully validated in longitudinal studies, rather it is based on studies reporting that the presence of the metabolic syndrome is associated with a 1.5–2 increased risk, above and beyond Framingham Risk Score. According to Guize et al., 2007, the prevalence of MetS or the risk of all-cause mortality is different using different combinations of MetS diagnostic criteria [42]. However, this finding needs further investigation considering different factors such as ethnicity, lifestyle factors, and underlying disease. To be able to include all indicators, we had to use the fasted CHMS subsample. This along with the exclusion criteria made the sample size of the study smaller than the whole CHMS sample. Further cycles of CHMS in upcoming years are needed to compare the dietary intake between three levels of CVD risk with a greater sample size, especially in high risk group. Although CHMS collected usual dietary intake through semiquantitative questionnaires, the quantity of the consumption is not provided to obtain an exact measure of dietary intake. To generalize data to the Canadian population, we used the specific weights provided in CHMS for fasted subsample. Moreover, since CHMS has provided fasted subsample, we were able to compare the prevalence of 10-year risk of CVD using either LDL-C (present in fasted subsample) or Tchol (present in both fasted and nonfasted subsample).

5. Conclusion

The 10-year risk of CVD in Canadian adults population aged from 30 to 74 years significantly changed when we considered CMR (8.10% versus 9.86%, resp.). In fact, the absolute CVD risk obtained from the validated traditional risk assessment tools might underestimate the 10-year risk of CVD in Canadian adult population. Compared to prospective studies, using cross-sectional study may also give similar estimation of the 10-year risk of CVD. The 10-year risk of CVD was greater in smokers, individuals with low physical activity, or with low level of education. These factors need to be controlled when evaluating the association between cardiometabolic risk and potential factors. Dietary intake across three levels of 10-year risk of CVD showed a significant difference in fruit and vegetable juice intake between low and medium risk of CVD, with individuals at medium risk having less intake. However, larger studies are needed to evaluate the difference in dietary patterns among individuals at different levels of CVD risk.

Conflict of Interests

Solmaz Setayeshgar, Susan J. Whiting, and Hassanali Vatanparast have no conflict of interests.

Acknowledgments

The authors acknowledge Research Data Centre at the University of Saskatchewan for allowing access to CHMS data. Support for this paper is obtained from University of Saskatchewan.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 436502, 7 pages
http://dx.doi.org/10.1155/2013/436502

Research Article

Linking Sleep to Hypertension: Greater Risk for Blacks

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Received 8 October 2012; Accepted 6 February 2013

Academic Editor: Antonio Felipe Sanjuliani

Background. Evidence suggests that insufficient sleep duration is associated with an increased likelihood for hypertension. Both short (<6 hours) and long (>8 hour) sleep durations as well as hypertension are more prevalent among blacks than among whites. This study examined associations between sleep duration and hypertension, considering differential effects of race and ethnicity among black and white Americans. *Methods*. Data came from a cross-sectional household interview with 25,352 Americans (age range: 18–85 years). *Results*. Both white and black short sleepers had a greater likelihood of reporting hypertension than those who reported sleeping 6 to 8 hours. Unadjusted logistic regression analysis exploring the race/ethnicity interactions between insufficient sleep and hypertension indicated that black short (<6 hours) and long (>8 hours) sleepers were more likely to report hypertension than their white counterparts (OR = 1.34 and 1.37, resp.; $P < 0.01$). Significant interactions of insufficient sleep with race/ethnicity were observed even after adjusting to effects of age, sex, income, education, body mass index, alcohol use, smoking, emotional distress, diabetes, coronary heart disease, and stroke. *Conclusion*. Results suggest that the race/ethnicity interaction is a significant mediator in the relationship between insufficient sleep and likelihood of having a diagnosis of hypertension.

1. Background

In the last two decades, great strides have been made in identifying health disparities in minority populations [1, 2]. Studies have shown an alarming difference in the prevalence of chronic illnesses between blacks and whites, particularly in those that cooccur as in the metabolic syndrome. Epidemiologic data from the National Health and Nutrition Examination Survey (NHANES) have shown that blacks have a greater prevalence of three of the metabolic syndrome's five criteria: hypertension (black: 42.5%, white: 29.1%), diabetes (blacks: 14.6%, whites: 9.9%), and obesity (blacks: 44.1%; whites: 32.4%) [3, 4]. Recognition of these health disparities has led to several important trials targeting reduction of health disparities in these areas. In the context of sleep medicine, little has been done to address metabolic diseases associated with short sleep among racial/ethnic minority groups.

The observation of a gradual decline in population sleep time along with a concomitant increase in the prevalence of hypertension over the past 20 years provided the impetus to examine whether various racial/ethnic groups might be differentially affected [5, 6]. Recent data has shown that individuals of the black race/ethnicity have a higher prevalence of both short and long sleep durations, relative to their white counterparts [7, 8]. In an earlier investigation, we showed that blacks were twice as likely to report short sleep compared with individuals of the white race/ethnicity [9]. Indeed, the fact that short sleep duration is linked to greater mortality and that it is more prevalent among blacks suggests greater mortality risks associated with short sleep in that population [10, 11].

It is uncertain whether disparities in sleep duration are determined by lifestyle choices or by a greater prevalence of

untreated sleep apnea among blacks [9]. Nonetheless, it is evident from recent findings that sleep restriction among blacks might put them at a greater risk for metabolic diseases associated with short sleep [12–14]. The present study investigated whether blacks reporting short or long sleep durations are at a greater likelihood for hypertension, relative to their white counterparts independently of known medical covariates.

2. Methods

2.1. Participants. A total of 25,352 Americans (age range: 18–85 years) who participated in the 2009 National Health Interview Survey (NHIS) provided sociodemographic and subjective data as well as data regarding physician-diagnosed chronic conditions for the present analysis. They estimated their habitual sleep duration. Analysis focused on race/ethnicity effects on associations between sleep duration and hypertension. Final weights were applied to all analyses to adjust for the use of complex design in the NHIS.

2.2. Procedures. NHIS is an ongoing, cross-sectional, in-person household interview survey conducted annually by the National Center for Health Statistics of the Centers for Disease Control and Prevention. The NHIS uses a multistage area probability design sampling a noninstitutionalized representative of US civilian population. Probability samples of the adult population of all 50 states and the District of Columbia were obtained. The final sample was characterized by a response rate of 69%. As the response rate was relatively low, we compared demographic characteristics between responders and nonresponders, finding no significant differences. Details on sample design can be found in the Design and Estimation for the National Health Interview Survey [15].

During face-to-face interviews conducted by trained interviewers from the US Census Bureau, volunteers provided sociodemographic data and information about physician-diagnosed chronic conditions. Self-reported diseases were defined based on the answer "yes" to the question "Have you ever been told by a doctor or other health professional that you have (disease or condition)?" Information was also obtained on the mood of the participants within the last 30 days prior to the interview, for example, feelings of sadness, hopelessness, worthlessness, and poor effort. Using this information, a depression severity score was generated which was a composite score estimated using the K-6 scaling system [16]. Information about the feelings of sadness, hopelessness, worthlessness, and poor effort were used to generate a score ranging from 0 to 24. Scores ≥13 indicated a greater degree of depression—this group was defined as having emotional stress [17]. Information on both weight and height was also collected by the interviewers—this was used to generate BMI. Body mass index was dichotomized as either normal weight (BMI $\leq 25\,kg/m^2$) or overweight/obese (BMI $\geq 25\,kg/m^2$) for descriptive purposes. Chronic conditions included hypertension, coronary heart disease, diabetes, and stroke. Participants also estimated habitual sleep duration (using full hour units, i.e., 5 hours, 6 hours, 7 hours, etc.); no information on specific sleep disorders was elicited during the interview. Participants could only report sleep time in one-hour increments and in whole number units with instructions to round 30 minutes (1/2 hour) or more UP to the next whole hour and dropping 29 or fewer minutes. Habitual sleep duration was coded as either short sleep duration (<6 hours/night) or long sleep (>8 hours/night) with a reference of 6–8 hours/night representing sufficient sleep duration [18]. These cut-off points were chosen based on previous research showing health risk associated with short and long sleep durations [19, 20]. Race/ethnicity was classified according to the revised 1997 US Office of Management and Budget standards for race ethnicity which government agencies follow.

Surveys were conducted using computer-assisted personal interviewing (CAPI), which utilizes a computer program for data collection that guides the interviewer through the questionnaire. The interviewer enters survey responses directly into the computer. The program determines through a computer algorithm whether data entered by the user match against all possible responses to specific questions; the program also checks for consistency against other data collected during the interview and saves the responses into a survey data file [21].

2.3. Statistical Analysis. Frequency and measures of central tendency were used to describe the sample. In preliminary analyses, Pearson and Spearman correlations were used to explore relationships between variables of interest; only factors showing a P value <0.05 were considered in the final regression model [22]. ANOVA was used for group mean comparisons, and Chi-square test was employed to assess differences in categorical variables. The association between sleep duration and hypertension was assessed by logistic regression: adjusted and unadjusted analyses. Hypertension was considered the dependent variable in this analysis (a binary measure where the participants had self-reported hypertension versus no hypertension), sleep duration was assessed as a categorical variable, and participants were categorized into the aforementioned groups. Univariate analyses were first carried out followed by multivariate analyses. In these analyses, adjustment was made for sociodemographic factors (age, gender, education, income, and obesity), risk factors (smoking, alcohol use, and physical inactivity), and medical comorbidities (emotional distress, coronary heart disease, diabetes, and stroke). The factors included in the final and most parsimonious model were selected based on presence of a known relationship with sleep disturbances from the extant literature and our preliminary analysis. Statistically significant variables with a P value <0.01 on univariate analysis were carried forward in a stepwise multivariate logistic regression analysis. Race/ethnicity was tested as a stand alone variable in our initial models. We then tested for possible effect modification by race/ethnicity of the association between sleep duration and self-reported hypertension by interaction models as outlined in Jaccard [23]. All analyses were performed with SPSS, version 20.0, using sampling weights published for the respective cohort of NHIS.

3. Results

A total of 25,352 Americans (age range: 18–85 years) enrolled in the 2009 NHIS provided complete data for this analysis. Of the sample, 82% self-reported their race/ethnicity as white and 18% as black. Among the respondents, the average age was 46.3 years and 52.0% were female.

Sociodemographic and health characteristics of study participants are provided in Table 1. Overall, blacks were younger than their white counterparts; blacks were more likely to be female, less likely to have finished high school, considerably less likely to report income of greater than 35,000, less likely to be of normal weight (BMI <25), less likely to report alcohol use and smoking, more likely to report sedentary lifestyle, and reported greater levels of emotional distress. Black participants were more likely to report a diagnosis of diabetes and hypertension, whereas whites had a higher prevalence of heart disease. Of the sample, 29% of white participants and 35% of black participants reported that they have been told by a healthcare professional that they have hypertension. Blacks tended to report more insufficient sleep while a significant percentage of whites reported sleeping 6–8 hours ($P < 0.001$).

As shown in Table 2, logistic regression analysis among black and white participants showed that short sleepers had a higher likelihood of reporting a diagnosis of hypertension compared with those sleeping 6–8 hours (OR = 1.21, 95% CI = 1.04–1.41). In our model, alcohol use and physical activity were statistically insignificant. Age and sex did not have a significant influence, but race/ethnicity and medical comorbidities had significant contributions in explaining observed odds ratios. Obesity and diabetes were the main drivers of the relationship in both short and long sleep durations.

Table 3 presents adjusted and unadjusted odds ratios of the likelihood of reporting hypertension by testing interactions of race/ethnicity with insufficient sleep durations. In our hierarchical model exploring black and white race/ethnicity, we observed significant interactions of race and ethnicity with both short and long sleep in all models. Black short and long sleepers were more likely to report hypertension compared with respondents reporing habitual sleep duration of 6–8 hours (see Table 3). Education and obesity were significant contributors to the interaction of insufficient sleep with race/ethnicity. Further adjustments for medical factors indicated marginally yet statistically significant relationships for long sleep. The results of the final regression model for each confounder are presented in Table 4. Post-hoc analysis using continuous BMI and categorization of overweight and/or obese in dummy coded variable did not change the overall model results with respect to significance or magnitude (not shown).

4. Discussion

Recent evidence indicates that individuals sleeping abnormally less or more than the population average sleep duration are at greater risk of cardiovascular disease [24, 25]. The main finding of our analyses is that adult short and long sleepers had a greater likelihood of hypertension and the relationship varied by race/ethnicity. Blacks who reported sleeping habitually <6 hours or >8 hours per night were characterized by a higher likelihood of hypertension compared with their white counterparts. Of note, the race/ethnicity effect was independent of known sociodemographic and medical confounders for long sleep.

Our finding of greater likelihood of hypertension among insufficient sleepers is consistent with previous data suggesting that insufficient sleep durations predicted increased odds of incident hypertension among American adults, both age related and unrelated. In addition, they are also consistent with recent epidemiologic studies that have shown associations between sleep duration and hypertension [26, 27].

Our investigation, with a representative adult population of the United States, examined the potential effects of race/ethnicity on associations between sleep duration and hypertension. These findings are consistent with the CARDIA study, an investigation examining cross-sectional and longitudinal associations between objectively measured sleep duration and blood pressure in middle-aged adults. In the CARDIA study, sleep duration partially mediated the larger increases in blood pressure that are associated with African-American race, particularly when diastolic blood pressure was considered [28]. While our analyses focused on black and white Americans, we should note that reduced sleep time may contribute to the severity of hypertension in other racial groupings [29]. It has been shown that short sleep has been independently associated with hypertension among young and middle-aged Korean adults [30].

Prevalence of hypertension and its subsequent sequelae have been shown to be higher among blacks relative to whites [31, 32]. Coding single equally Chi polymorphisms, albeit rare and inadequately understood, have been identified to influence essential hypertension [33]. Recent literature has shed light on the impact of race/ethnicity through social and environmental elements in the epidemiological study of hypertension [34]. Among the pre-hypertensive cohorts from the REGARDS study, blacks were at higher risk for both hypertension and prehypertension than whites with conventional risk factors such as obesity and excess alcohol consumption acting as lead modifiers [35]. In our investigation, obesity was also a significant contributor in our models. Investigators from the multicenter Multi-Ethnic Study of Atherosclerosis (MESA) attempted a novel approach beyond the conventional risk factors to study neighborhood characteristics such as violence and disorder to examine racial/ethnic differences among hypertensives. They found that neighborhood stressors contributed significantly to the positive association between blacks and hypertension [36].

To date, there are inconclusive explanations for the increased prevalence of hypertension among insufficient sleepers. Thus, further investigations aiming at better understanding of the mechanistic link is warranted. Probable mechanisms elucidating increased blood pressure among patients with short sleep may be linked to sustained activation of the sympathetic nervous system [37, 38]. Another possible explanation relates to the process or the dipping phenomenon occurring during slow-wave sleep [39]. Although studies in sleep-deprived animals [40] and humans [41] have shown

Table 1: Characteristics of adult participants from the National Health Interview Survey (NHIS). Stratified by race/ethnicity; *P* value obtained from λ square.

	Black	White	*P* value
Participants (*N*; %)	4571; 18.0	20781; 82.0	
Age (mean)	43.3	46.8	
Female sex (%)	55.6	51.1	<0.001
Education; ≥HS (%)	89.2	92.1	<0.001
Income; ≥35,000 (%)	53.1	71.1	<0.001
Body mass index (%; normal weight; ≤25 kg/m^2) (%)	27.9	35.7	<0.001
Alcohol use (%)	71.3	82.7	<0.001
Smoking (%)	35.5	44.7	<0.001
Physical activity (%)	52.0	61.9	0.008
Emotional distress (%)	3.5	2.5	<0.001
Diabetes (%)	12.1	8.8	<0.001
Heart disease (%)	6.4	8.4	0.004
Stroke (%)	3.3	2.7	0.045
Short sleepers (<6 hours) (%)	12.3	7.3	<0.001
Long sleepers (>8 hours) (%)	11.1	10.0	<0.001
Hypertension (%)	35.3	28.5	<0.001

Table 2: Multivariate-adjusted logistic regression analysis indicating odds ratios (ORs) associated with the presence of hypertension between short and long sleep durations in the selected population. In the model, short sleep was defined as sleep durations <6 hours and in Model B; long sleep was defined as sleep durations >8 hours; reference sleep was 6 to 8 hours.

Variables	Short sleep (<6 hours)				Long sleep (>8 hours)			
	P	OR	95% CI		*P*	OR	95% C.I.	
			Lower	Upper			Upper	Lower
Sleep duration (categorized)	0.001	1.21*	1.04	1.41	0.834	1.02	0.88	1.18
Age	<0.001	1.06	1.06	1.06	<0.001	1.06	1.05	1.06
Sex (reference: male)	0.001	0.87	0.87	0.96	<0.001	0.89	0.81	0.97
Race (reference: white)	<0.001	1.68*	1.47	1.91	<0.001	1.74*	1.53	1.97
Income (reference: <$35,000)	0.004	0.90	0.81	0.99	0.10	0.94	0.85	1.03
Education (reference: high school)	0.001	0.83	0.71	0.95	0.12	1.13*	0.99	1.30
Obesity (reference: nonobese)	<0.001	2.38*	2.17	2.61	<0.001	2.45	2.23	2.69
Alcohol (reference: never)	0.34	1.07	0.93	1.22	0.51	1.03	0.91	1.16
Smoking (reference: never)	0.001	1.12*	1.02	1.22	<0.001	1.17*	1.07	1.28
Activity (reference: no physical activity)	0.74	1.02	0.92	1.12	0.93	1.02	0.93	1.13
Emotional Distress (reference: none)	<0.001	1.83*	1.35	2.48	<0.001	1.97*	1.43	2.70
Diabetes (reference: none)	<0.001	3.15*	2.69	3.70	<0.001	3.40*	2.95	3.93
Coronary heart disease (reference: none)	<0.001	2.06*	1.76	2.42	<0.001	2.03*	1.74	2.36
Stroke (reference: none)	<0.001	2.08*	1.58	2.73	<0.001	1.97*	1.48	2.62

*Variables contributing significantly to the relationship.

a strong physiological association between sleep and hypertension, more clinical trials need to be performed to delineate the underlying pathophysiology. Previous research has shown that long sleep is associated with hypertension [42], obesity [43], and stroke [44], and long sleep may actually be more detrimental to health than short sleep. Underlying mechanisms that could be responsible for the effect of long sleep, as suggested in previous analyses, include sleep fragmentation and photoperiodic abnormalities, which could all lead to increased blood pressure levels [45]. Studies have found higher prevalence of systemic hypertension among those with sleep fragmentation possibly related to transient elevation mainly due to frequent arousal and transient increase in BP and sympathetic activity due to these arousals. The observed association of long sleep with hypertension needs to be corroborated by future studies before definitive conclusions can be reached. It is also evident that both long and short sleep durations are associated with elevated markers of inflammation, abnormal lipid profile, insulin resistance, increased BMI, and diabetes mellitus, all independent predictors of hypertension.

We surmise that sleep duration may be a risk factor in the development of hypertension in addition to insulin resistance [46], obesity [47, 48], and diabetes [49, 50]. Among

Table 3: Multivariate-adjusted hierarchal logistic regression analysis indicating odds ratios (ORs) associated with the presence of hypertension based on interactions between short/long sleep duration and black and white race/ethnicity. Model adjustments were Model 1 adjusted for age, and sex; Model 2 adjusted for Model 1 + education, income, alcohol use, smoking, physical activity, and body mass index; Model 3 adjusted for Model 2 + emotional distress, diabetes, coronary heart disease, and stroke.

Variables	Short sleep (<6 hours)				Sufficient Sleep (6–8 hours)	Long sleep (>8 hours)			
	P	OR	95% CI			*P*	OR	95% CI	
			Upper	Lower				Lower	Upper
Sleep∗Race									
Unadjusted	<0.001	1. 34[¥]	1.02	1.75	1.0 (Referent)	<0.001	1.37[¥]	1.07	1.75
Model 1[a]	<0.001	1.30[¥]	0.97	1.74	1.0 (Referent)	<0.001	1.12[¥]	0.83	1.50
Model 2[b]	<0.001	1.08[¥]	0.81	1.45	1.0 (Referent)	<0.001	1.01	0.74	1.38
Model 3[c]	<0.001	0.95	0.71	1.28	1.0 (Referent)	<0.001	1.03	0.74	1.43

[¥]Values significant than 1.0.
[1]Reference category is white for race/ethnicity and habitual sleep duration of 6 to 8.

Table 4: Multivariate-adjusted logistic regression analysis indicating odds ratios (ORs) for the presence of hypertension associated with short/long sleep duration among black and white participants.

Variables	Short sleep (<6 hours)				Long sleep (>8 hours)			
	P	OR	95% CI		*P*	OR	95% CI	
			Lower	Upper			Lower	Upper
Sleep duration∗race/ethnicity (categorized)	0.001	0.95	0.71	1.28	<0.001	1.03	0.74	1.45
Age	<0.001	1.05	1.02	1.21	<0.001	1.04	1.01	1.09
Sex (reference: male)	0.001	0.87	0.80	0.96	0.01	0.89	0.81	0.97
Income (reference: <$35,000)	0.004	0.90	0.82	1.00	0.17	0.94	0.85	1.03
Education (reference: high school)	0.001	1.21*	1.05	1.39	0.08	1.13*	0.99	1.30
Obesity (reference: nonobese)	<0.001	2.38*	2.17	2.62	<0.001	2.45*	2.23	2.69
Alcohol (reference: never)	0.34	1.07	0.93	1.22	0.68	1.03	0.91	1.16
Smoking (reference: never)	0.001	1.12*	1.02	1.22	<0.001	1.17*	1.07	1.28
Activity (reference: no physical activity)	0.74	1.02	0.93	1.12	0.62	1.02	0.93	1.13
Emotional distress (reference: None)	<0.001	1.83*	1.35	2.48	<0.001	1.97*	1.43	2.70
Diabetes (reference: none)	<0.001	3.15*	2.69	3.70	<0.001	3.41*	2.95	3.93
Coronary heart disease (reference: none)	<0.001	2.06*	1.76	2.42	<0.001	2.03*	1.74	2.36
Stroke (reference: none)	<0.001	2.09*	1.59	2.74	<0.001	1.97*	1.48	2.62

*Variables contributing significantly to the relationship.

racial/ethnic groups, African-American adults have the highest rates (44%) of hypertension [51] and blacks have greater prevalence of short sleep and sleep disturbance compared with whites [7, 9, 52]. While the exact causes of sleep loss were not ascertained in these studies, conceivably it might result from untreated sleep-related disorders such as sleep apnea, which is known to affect blacks disproportionately [11]. In sum, findings of our study suggest that individuals reporting insufficient sleep durations and who are characterized by a diagnosis of hypertension may constitute a vulnerable population, requiring optimal clinical management to reduce cardiovascular sequelae from hypertension.

Our study has some notable limitations. One such limitation relates to our inability to verify reported sleep durations and clinical diagnoses. However, previous self-reported data from blacks and whites in a smaller cohort showed no significant differences between self-reported and actigraphic sleep durations [53]. Another important limitation concerns the unavailability of data regarding the presence of sleep apnea, which would have provided some explanation of the associations between short sleep and hypertension. There is also the possibility of potential of misclassification because of undiagnosed hypertension by healthcare professionals. Our analysis did not adjust for other important factors such as sleep apnea, insomnia, and excessive daytime sleepiness or fatigue, which have adverse effects on sleep duration; such data were not available in the NHIS data.

Our study explores the relationship between race and ethnicity and hypertension and does not establish any cause and effect relationships between race/ethnicity, sleep durations, and likelihood of reporting hypertension or exclude reverse causality because of the cross-sectional design.

It is of interest to examine whether our observations could be replicated in other nationally representative data and clinical trials. Notwithstanding these limitations, our results provide some insight as to the importance of race/ethnicity in understanding associations between sleep durations and likelihood of hypertension.

5. Conclusions

There is a greater likelihood of hypertension for blacks with insufficient sleep duration compared with whites. Assessment of sleep duration should be performed in all hypertensives, especially among those of the black race/ethnicity.

Disclosure

All coauthors meet the criteria for authorship, including acceptance of responsibility for the scientific content of the paper. They have seen and agreed on the contents of the paper and there is no financial conflict or conflicts of interests to report. They certify that the submission is the original work and is not under review at any other publication.

Acknowledgments

This research was supported by funding from the NIH (R25HL105444, R01HL095799, and R01MD004113).

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 698940, 12 pages
http://dx.doi.org/10.1155/2013/698940

Review Article

A Review of Nutritional Factors in Hypertension Management

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Received 1 January 2013; Accepted 15 March 2013

Academic Editor: Mario Fritsch Neves

Hypertension is a major health problem worldwide. Its attendant morbidity and mortality complications have a great impact on patient's quality of life and survival. Optimizing blood pressure control has been shown to improve overall health outcomes. In addition to pharmacological therapies, nonpharmacological approach such as dietary modification plays an important role in controlling blood pressure. Many dietary components such as sodium, potassium, calcium, and magnesium have been studied substantially in the past decades. While some of these nutrients have clear evidence for their recommendation, some remain controversial and are still of ongoing study. Dietary modification is often discussed with patients and can provide a great benefit in blood pressure regulation. As such, reviewing the current evidence will be very useful in guiding patients and their physician and/or dietician in decision making. In this review article of nutritional factors in hypertension management, we aim to examine the role of nutritional factors individually and as components of whole dietary patterns.

1. Introduction

In adults aged 18 years and older, hypertension is defined as a systolic blood pressure (SBP) equal or more than 140 mmHg and/or diastolic blood pressure (DBP) equal or more than 90 mmHg based on the mean of 2 or more properly measured seated blood pressure readings on each of 2 or more office visits by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC) [1]. According to the 2012 World Health Statistics report released by the World Health Organization (WHO), hypertension affects approximately 24.8% of the global population with the range from 19.7% to 35.5% in different regions [2]. It is one of the most common diseases that lead to office visits or hospitalizations and a major risk factor for stroke, congestive heart failure (CHF), myocardial infarction (MI), peripheral vascular disease, and overall mortality. Many of those with hypertension are undiagnosed, and of those detected, about two-thirds are suboptimally controlled [1]. Early treatment can improve blood pressure and its complications significantly [1]. Therapeutic options include diet and lifestyle changes (including weight loss, smoking cessation, and increased physical activity), antihypertensive drugs, and surgery in special situations.

Clinical and population-based studies show that several components of the diet such as sodium, potassium, calcium, magnesium, fiber, and fish oil affect blood pressure, and modification of these nutritional factors provide an important strategy to control blood pressure especially in the prehypertensive stage (SBP 120–139 mmHg and/or DBP 80–89 mmHg) or stage I hypertension (SBP 140–159 mmHg and/or DBP 90–99 mmHg). The role of these dietary factors singly or in combination in blood pressure regulation and to what extent each contributes has been a subject of research for many decades, and despite this, it remains controversial. Modifying one's diet can be a difficult and significant change

in life, and many patients oppose this or fail despite several attempts. Given how frequently clinicians have to provide patients with this recommendation and the potential impact on the overall outcome in patient health, it is of great benefit to study the current evidence in nutritional approach to hypertension management. In this paper, we aim to review the role of each individual dietary factor as well as whole dietary patterns.

2. Sodium

The relationship between sodium intake and blood pressure changes has been a topic of discussion for decades. Hypertension is predominantly observed in societies with average sodium chloride intake >100 mmol/day and very rare in populations consuming <50 mmol/day [3, 4]. However, the link between salt and hypertension is still an ongoing debate. The concept of salt sensitivity versus salt resistance originated from studies demonstrating heterogeneous blood pressure responses to changes in sodium intake. These changes were observed both in hypertensive and normotensive subjects [5–8]. Till date, there is no uniformed definition of salt sensitivity due to great variations in published studies regarding study protocols, techniques, duration, and magnitude of sodium intake and blood pressure changes. However, the most commonly used method was introduced by Weinberger [9]. Based on mean arterial blood pressure response to sodium load by intravenous administration of 2 liters of normal saline and sodium depletion by a 10 mmol sodium diet and 3 doses of oral furosemide (40 mg each), the authors arbitrarily classified salt sensitivity as a decrease in mean arterial pressure ≥10 mmHg and salt resistance as a decrease ≤5 mmHg when comparing the 2 blood pressure measurements.

As a brief review of physiology, sodium homeostasis is maintained by glomerular filtration and tubular reabsorption. Sixty-five to seventy-five percent of sodium reabsorption is mediated by neurohumoral hormone in the proximal (angiotensin II and norepinephrine) and collecting tubules (aldosterone, atrial natriuretic peptide), whereas 30–35% occurs in the loop of Henle and distal tubules and this is flow dependent. A parallel adaptation in renal tubular activity in response to alterations in glomerular filtration of sodium keeps little variation in urinary sodium excretion [10]. This explains a steady range of sodium level even in cases of advanced kidney failure.

Salt sensitivity has been found to have a higher prevalence in certain populations: older age [11, 12], blacks [12], insulin resistance [13, 14], microalbuminuria [15], chronic kidney disease (CKD) [16], and low renin level [17, 18]. Recent animal and molecular studies have suggested the contribution of certain genetic polymorphisms to the development of salt sensitive hypertension via different mechanisms: affecting renal tubular Na+K+ATPase activity [19, 20], decreasing dopamine receptor function in renal proximal tubule [21], or altering endothelin receptor activity [22]. Salt sensitivity has been reported to be an independent prognostic factor for increased risk of left ventricular hypertrophy [23], cardiovascular events [24], and cumulative mortality [25], regardless of blood pressure.

Dietary sodium restriction is strongly advocated as a lifestyle behavioral change for prevention and treatment of hypertension and consequently cardiovascular morbidity and mortality by several professional organizations [1, 26–28]. Despite an abundance of studies on its efficacy, results are conflicting and naturally provoke questions on the benefits of continuing to advocate this intervention to patients [29, 30].

Evidence from several clinical studies shows that a reduction in sodium intake leads to modest to large reduction in blood pressure in normotensive and hypertensive participants [31–36] as well as decreased risk of cardiovascular events [37–39].

The INTERSALT study, a population-based study across 52 centers from 32 countries, examined the relationship between sodium excretion and blood pressure among over 10,000 participants. Sodium excretion was highly variable (0.2–242 mmol/24 hour (hr)), with 4 centers having very low levels (0.2–51.3 mmol/24 hr). A significant positive association between sodium excretion and blood pressure in individuals was reported overall. However, with exclusion of data from the 4 centers with the lowest excretion, no relation could be found [4].

The Dietary Approaches to Stop Hypertension (DASH)-sodium trial randomized subjects to either the DASH diet (rich in fruits, vegetables, and low-fat dairy and reduced in saturated and total fat) or a control diet (typical United States (US) diet which is high in fat and low in fruits, vegetables, and dairy products) with participants consuming a graded sodium intake (high, intermediate, low) within each group during the study period. The study demonstrated significant dose-response decreases in SBP and DBP overall and blunting of the age-related increase in blood pressure with sodium restriction. The DASH diet and sodium restriction each lowered blood pressure substantially, but the effect was greater when combined [35, 36].

Investigators on a prospective followup study of participants in the trials of hypertension prevention phase I and II for 10–15 years found a 30% and 20% risk reduction of cardiovascular disease (MI, stroke and coronary revascularization) and cardiovascular death, respectively, but the reduction in death was not statistically significant [37].

In contrast, several studies demonstrate harmful effects of lower salt intake on important clinical outcomes including MI [40], all-cause and cardiovascular mortality [41, 42], sympathetic hormones [43, 44], fasting plasma glucose and insulin levels [44, 45], cholesterol [43, 44, 46], and in addition, higher CHF readmissions and brain natriuretic peptide levels [47, 48]. Higher mortality [49, 50] and development of end stage renal disease (ESRD) [49] have also been reported with lower sodium intake among diabetics.

All together, despite several years and high magnitude of population-based studies and clinical research, the evidence supporting the recommendation of dietary sodium restriction should be interpreted with caution.

Given that salt restriction is not a "one size fits all" approach, differentiating individuals with salt sensitive and salt resistant hypertension would be beneficial. However, subjecting all hypertensive patients to salt sensitivity testing as in research protocols seems inconvenient and unfeasible.

Thus, the question appears to be how physicians can clinically determine salt sensitivity. To address this query, a couple of studies have suggested ambulatory, however, indirect methods in evaluating salt responsiveness. De la Sierra and coinvestigators reported that patient's performance on ambulatory blood pressure monitoring could be a useful tool to assess salt sensitivity [51]. In their study, salt sensitive patients exhibited a nondipper profile on both low salt and high salt diets, while salt resistant subjects with high salt intake had increased blood pressure during sleep with no significant changes in the 24-hr blood pressure. Galletti et al. found that in salt sensitive patients, there was a strong correlation between 24-hr urinary sodium excretion and blood pressure changes [52]. In another study, involving 89 Caribbean Hispanic hypertensive patients, plasma renin level was used to evaluate patient's response to antihypertensive therapy [53]. Sixty-two percent of the patients had low renin essential hypertension and responded preferentially to monotherapy with hydrochlorothiazide or calcium channel blockers. A phase 4 randomized study testing Fludrocortisone administration in identifying salt sensitivity is currently underway [54]. These methods need to be validated by larger studies before being considered for routine tests in evaluating and/or guiding treatment in hypertensive patients.

Currently, based on available evidence, the WHO strongly recommends the restriction of daily sodium intake to less than 2000 mg (or 5000 mg salt) [55], while the AHA advises lower than 1500 mg sodium (or 3800 mg salt) per day [28].

3. Potassium

The association of potassium and blood pressure has been described in many studies. Potassium intake was found to be inversely related to both DBP and SBP in a population-based study including 685 men and women who were predominantly Caucasian in Southern California, United States of America (USA) [56]. Similar result was illustrated in the Rotterdam Study [57], a big population-based study in which 3239 participants older than 55 years old were included. Patients with an increase in potassium intake of 1000 mg/day had a 0.9 mmHg lower SBP and a 0.8 mmHg lower DBP. In a study by Krishna and Kapoor, potassium depletion was shown to be associated with a decrease in sodium excretion, plasma renin activity, and plasma aldosterone concentrations and an increase of 7 mmHg in SBP and 6 mmHg in DBP [58].

Several interventional studies have shown the positive effects of potassium supplementation on blood pressure reduction. Cappuccio and MacGregor reviewed 19 clinical trials in which oral potassium supplements significantly lowered SBP (mean of −5.9 mmHg, 95% confidence internal (CI), −6.6 to −5.2 mmHg) and DBP (mean of −3.4 mmHg, 95% CI, −4.0 to 2.8 mmHg) [59]. A meta-analysis consisting of 27 potassium trials in adults with a minimum of 2 weeks duration also demonstrated a change in blood pressure with increased potassium intake: a mean of −2.42 mmHg (95% CI, −3.75 to −1.08 mmHg) in SBP and −1.57 mmHg (95% CI, −2.65 to −0.50 mmHg) in DBP pressure [60]. Dickinson and colleagues used stricter inclusion criteria and only selected 5 randomized controlled trials for their meta-analysis published in the Cochrane Database in 2006. The authors did not find a statistical significant effect of potassium supplementation on blood pressure. However, the argument of small number of subjects, short duration of followup, and substantial heterogeneity of these trials was discussed as a possible explanation for the finding of this meta-analysis [61].

The benefit of potassium intake on blood pressure reduction appears to be greater in patients with hypertension [59, 60], longer duration of supplementation [59], and concurrent high intake of sodium [62].

The magnitude of blood pressure reduction seems small in number but might be translated into a benefit in mortality from complications of hypertension. In a study of 6 different populations, the relative increase in 25-year mortality risk due to coronary heart disease was 1.17 (95% CI, 1.14 to 1.20) per 10 mmHg increase in SBP and 1.13 (95% CI, 1.10 to 1.15) per 5 mmHg increase in DBP. After adjustment for within-subject variability in blood pressure, this relative risk was 1.28 [63]. A 5 mmHg decrease in DBP was reported to be associated with less than one-third of strokes [64, 65].

It is worth mentioning that some protective effects of high potassium intake on cardiovascular health might be independent of blood pressure. An analysis of sodium and potassium intake and mortality among US adults based on data from the third National Health and Nutrition Examination Survey (NHANES) showed that higher sodium intake was associated with increased all-cause mortality, while higher potassium intake seemed to be associated with lower mortality. Of note, this finding was independent of sex, age, hypertension, physical activity, and body mass index (BMI) [66]. In a 12-year prospective study, a 10 mmol increase in daily potassium intake was associated with a 40% reduction in the risk of stroke-associated mortality. This protective effect of potassium did not differ by other dietary variables and known cardiovascular risk factors (age, sex, blood pressure, blood cholesterol level, obesity, fasting blood glucose level, and cigarette smoking) [67].

The antihypertensive effect of potassium supplementation might be from various mechanisms: (1) natriuresis by inhibiting sodium reabsorption in the proximal renal tubules [68] and suppressing renin secretion [69], (2) normalization of the plasma level of digitalis like substance [70], (3) increased urinary volume excretion [70], (4) smooth muscle relaxation [71, 72] by increasing nitric oxide production [73] and/or by stimulating the rectifier K(+) channels resulting in potential membrane hyperpolarization and subsequently vasodilation [74], (5) suppression of free radical formation [75], and (6) protection against vascular injury in salt sensitive hypertension [76]. Which one of these mechanisms plays a predominant role in the reduction of blood pressure and/or cardiovascular mortality is not quite clear at this time.

Based on available data, the Institute of Medicine has recommended a potassium intake of 4700 mg (120 mmol) a day as adequate intake for all adults [77]. A similar amount of daily potassium consumption was also suggested by the American Heart Association (AHA) in 2006 to achieve the potential benefit of blood pressure reduction [28]. The 2003 WHO/International Society of Hypertension statement on

management of hypertension supported an increased dietary potassium intake although a threshold was not specified [78]. However, the suggested daily intake of potassium might be lower in patients who are prone to developing hyperkalemia such as those with impaired renal excretion of potassium from CKD, CHF, adrenal insufficiency, and medications use (angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB), potassium-sparing diuretics, trimethoprim, cyclosporine, heparin, etc.) These patients need to be monitored frequently.

In reality, the average intake of dietary potassium in many countries is relatively lower than recommended. As in a report from the third NHANES 1988–1994 study, estimated mean potassium intakes of adults in the USA ranged from 2900 to 3300 mg in men and 2200 to 2400 mg in women [28]. The NHANES data from 2003 to 2008, the time span during which the above recommendations were issued, revealed that less than 2% of adults and approximately 5% of men in the USA met the recommendations for potassium intake (i.e., at least 4700 mg/day) [79]. In European countries, the average daily potassium intake in adults was reported to be below 4700 mg. The number varied from 3200 mg to 4000 mg/day in Finland [80] or from 2655 mg to 3371 mg/day in the United Kingdom [81]. In China, the mean intake of potassium was 1950 mg/day in the urban and 1830 mg/day in the rural diet [82].

Foods rich in potassium are vegetables, fruit, dairy products, nuts, and so forth. Natural source of potassium is preferable. Currently, pharmacological potassium supplementation is not recommended as a method to obtain the advised daily intake of potassium.

4. Calcium

The relationship between calcium intake and hypertension is a complex and difficult one to isolate largely because of the interaction with other nutrients in the diets and difficulty in reliably collecting calcium intake data and important unmeasured confounding variables.

Despite an abundance of studies on the effect of dietary or supplemental calcium on blood pressure, the evidence on its benefit is inconclusive and remains controversial. An inverse relationship between dietary calcium intake and blood pressure has been reported in many studies [83–89]. Similarly, supplementation with 1000 mg calcium/day has been demonstrated to lead to a decrease in blood pressure, although results were inconsistent and mainly among hypertensives [90–92]. In contrast, other studies report minimal to no effect of dietary calcium or supplementation on blood pressure [93–99]. In addition, contradictory results have been deduced from analysis of the same data as exemplified by the Nurses' Health Study [85, 94] and the NHANES [89, 100–103]. The differences in study results may be explained by a variety of factors including heterogeneity of study participants, flaws in study design, limitations in measurement of blood pressure and calcium intake, short duration of studies, different analytical methods, as well as collinearity with other dietary factors such as magnesium, fiber, protein, and potassium.

Two meta-analyses assessed the relationship between dietary calcium supplementation and blood pressure. The authors found small reductions in SBP (1-2 mmHg), and results were even smaller and insignificant for DBP [104, 105].

Similar results were reported in another meta-analysis by van Mierlo et al., effect on SBP of −1.86 mmHg (95% CI, −2.91 to −0.81 mmHg) and DBP of −0.99 mmHg (95% CI, −1.61 to −0.37 mmHg), however, the impact on SBP was larger in people with relatively low calcium intake of ≤800 mg/day [106]. A Cochrane review published in 2006 examined the efficacy of oral calcium supplementation as a treatment for hypertension. Only 13 randomized controlled trials with a total of 485 participants with at least 8 weeks duration were included. A statistically significant reduction in SBP (−2.5 mmHg, 95% CI, −4.5 to −0.6 mmHg), but not DBP (−0.8 mmHg, 95% CI, −2.1 to 0.4 mmHg), was found. Many of the trials were of poor quality, and the authors concluded that there was insufficient evidence to recommend calcium supplementation as a treatment for hypertension [107].

Currently, the evidence on the benefit of calcium supplementation in prevention or treatment of hypertension is weak; therefore, there is no justification to increase the intake of calcium above the recommended dietary allowance of 1000–1300 mg/day based on age and gender. Foods rich in calcium are mainly dairy products (preferably low fat) such as milk, cheese, and yogurt.

Proposed mechanisms by which calcium intake regulates blood pressure include alteration in intracellular calcium which in turn affects vascular smooth muscle contraction [108], effect of calcium metabolism and regulatory hormones [109–111], increased natriuresis [112–114], and modulation of the function of the sympathetic nervous system [113]. Interestingly, among people consuming low levels of calcium in their diets, high salt intake is associated with higher blood pressure levels [115–117], and it is suggested that the hypertensive effect of a high sodium intake may be mitigated by increasing dietary calcium [109, 112, 118, 119]. Resnick also published extensively on the interlinking of the renin-aldosterone system, calcium regulation, and salt sensitivity in modulating blood pressure responses to salt loading, calcium supplementation, and calcium channel blockers [120–125]. He suggested that these models may provide a targeted approach to identifying and treating hypertensives with calcium supplementation or calcium channel blockers based on their serum renin level and salt sensitivity [126–128].

5. Magnesium

Magnesium deficiency has been found to result in increased blood pressure in single studies [129, 130]. However, a meta-analysis looking at 29 observational studies points to a negative correlation between dietary magnesium intake and blood pressure [131].

The evidence of a causal association between magnesium supplementation and blood pressure reduction was weak in a meta-analysis including 12 randomized trials with followup ranging from 8 to 26 weeks [132]. In another meta-analysis with 20 studies, although a dose-response pattern was found, magnesium intake only resulted in a small overall reduction

in blood pressure, a mean of −0.6 mmHg (95% CI, −2.2 to 1.0 mmHg) for SBP and −0.8 mm Hg (95% CI, −1.9 to 0.4 mmHg) for DBP [133].

On the basis of these data, the relationship between magnesium and hypertension seems inconsistent and not convincing. Currently, magnesium supplementation is not recommended as a means of hypertensive treatment.

6. Alcohol

A standard drink in the USA is equal to 14 g (6 ounces (oz)) of pure alcohol. This amount is present in 12 oz of regular beer, 5 oz of wine (12% alcohol), 8 oz of malt liquor, and 1.5 oz of 80-proof distilled spirits [134]. This serving size can be varied in different countries. In Britain or Australia, a unit of alcohol contains 8 to 10 g [135], while in Japan, a drink contains 19.75 g of alcohol. In this paper, we refer "a drink" to the US serving size. Otherwise, the amount of alcohol will be specified.

Moderate alcohol intake, defined as a maximum of 2 alcoholic drinks/day in men and 1 alcoholic drink/day in women and lighter-weight persons, is supported by the AHA 2006 scientific statement of hypertension management [28]. In a prospective cohort study involving 11,711 men with preexisting hypertension, individuals who consumed a moderate amount of alcohol tended to have a decreased risk of MI. After adjustment for measurement error, BMI and dietary variables, the hazard ratio for participants with MI per 12.5 g/day increment of alcohol intake was 0.68 (95% CI, 0.46 to 1.00) [136].

On the other hand, heavy drinking, generally referred to as any amount of alcohol use above the moderate level, is associated with a higher risk of hypertension in a dose-dependent manner. This has been demonstrated in various populations: Japanese men [137], US women [138], and both men and women of different races [139]. The risk seems more pronounced in individuals with a smaller BMI [137]. In the INTERSALT study, compared to nondrinkers, men who drank 300–499 mL alcohol/week had higher SBP/DBP, on average 2.7/1.6 mmHg. Women who drank at least 300 mL/week had blood pressures higher by 3.9/3.1 mmHg than nondrinkers [140]. Alcohol reduction was associated with a fall in blood pressure [141]. Available data supports that moderate alcohol consumption should be recommended as one of the components of hypertensive therapy.

7. Fiber

Fiber is the indigestible portion from plant-based food. How exactly fiber might affect blood pressure is not entirely understood. An inverse association between blood pressure and fiber intake has been described. Among 30,681 predominantly white US male health professionals, 40–75 years old, those with a fiber intake of less than 12 g/day were at higher risk of developing hypertension compared to those taking more than 24 g/day, relative risk 1.57 (95% CI, 1.20 to 2.05) [88]. This relationship was independent of other nutrients including sodium, potassium, calcium, and magnesium. When the results of 24 randomized controlled trials were evaluated, fiber supplementation with an average dose of 11.5 g/day modestly reduced SBP by –1.13 mmHg (95% CI, −2.49 to 0.23 mmHg) and DBP by –1.26 mmHg (95% CI, −2.04 to −0.48 mmHg). It seemed to have greater effect in populations with hypertension and in those older than 40 years of age [142]. Similarly, a large randomized trial failed to show a significant decrease in blood pressure with a high fiber diet [143]. According to the 2006 scientific statement from the AHA, there is insufficient evidence to recommend increased dietary fiber intake alone for the reduction of blood pressure [28].

8. Omega-3 Polyunsaturated Fatty Acid (Fish Oil)

Daily dietary fish consumption as a part of weight reduction regimens has shown benefits in blood pressure fall [144]. A body of evidence demonstrated the association between concentrated Omega-3 polyunsaturated fatty acid extracted from fish, hence usually known as "fish oil", and blood pressure reduction [145, 146]. This effect might have a dose limit, as it was reported that fish oil did not change mean blood pressure in the subjects who ate fish three or more times a week as part of their usual diet, or in those who had a baseline concentration of plasma phospholipid omega-3 fatty acids above 175.1 mg/L [146]. Of note, these studies employed large doses of Omega-3 polyunsaturated fatty acid (at least 3 g/day), which raises a concern of safety and side effects in view of long-term use.

Mercury is a chemical element of which fish living in contaminated water can contain a high level in their body. Some cross-sectional studies have mentioned the possibility of increasing blood pressure caused by mercury. Would this go against the above findings of a positive correlation between daily fish meal and blood pressure reduction? Recently, two large prospective cohort studies on 6045 US men and women, do not support any effects of methylmercury on the risk of incident hypertension [147].

9. Garlic

Garlic is one of the most commonly used natural herbs. Its role in hypertension has also been explored by researchers. In patients with baseline elevated blood pressure, compared with placebo, garlic significantly reduced SBP by 16.3 mmHg (95% CI, 6.2 to 26.5 mmHg) and DBP by 9.3 mmHg (95% CI, 5.3 to 13.3 mmHg) [148]. The effect was repeatedly illustrated in another meta-analysis [149]. Despite the potential advantage, the problem with nonstandardization in product preparation, formulas, and study heterogeneity does not allow a conclusive message regarding garlic use in hypertensive patients. It might be beneficial. However, how much, how long, and which products to use are not clearly identified.

10. Products with Potential Harms

10.1. Caffeine. Being the main ingredient in stimulant drinks, caffeine is found in coffee, tea, sodas, and many energy drinks. Acutely, caffeine can elevate blood pressure in nonhabitual

caffeine users [150], whereas little to no effect was seen in habitual coffee drinkers [151, 152]. Chronic coffee drinkers who have hypertension might not need to change their habit based on the available data. Nevertheless, it can potentially be harmful for irregular coffee drinkers.

There is evidence that other ingredient than caffeine might be responsible for the stimulating effect of coffee on blood pressure. An increase in blood pressure was observed in decaffeinated coffee drinkers [153]. The use of caffeine alone did not exert an elevated blood pressure in healthy volunteers in a study [152].

10.2. Licorice. Licorice has been long used as a flavoring agent in chewing tobacco, candies, spices, and as a medical product in some gastrointestinal and upper respiratory disorders. It contains glycyrrhetinic acid which inhibits 11-beta-hydroxysteroid dehydrogenase enzyme type 2 isoform, allowing cortisol to bind to the mineralocorticoid receptors creating a status of mineralocorticoid excess and subsequently blood pressure elevation. The use of licorice can be potentially dangerous in hypertensive individuals [154].

11. Whole Dietary Pattern

11.1. DASH Diet. The DASH diet is a diet rich in fruits and vegetables (4-5 servings/day) and low-fat dairy products (2-3 servings/day) and includes whole grains, poultry, fish, and nuts. This diet is rich in potassium, magnesium, calcium, dietary fiber, and protein and has reduced fat (total and saturated) and cholesterol (<25%), red meat, sweets, and sugar-containing beverages.

Two controlled clinical trials established its efficacy in lowering blood pressure [35, 155]. The initial DASH trial [155] enrolled 459 untreated participants with prehypertension and stage I hypertension and randomly assigned them to one of 3 groups (1) a control group which consumed a typical US diet which was low in fruits, vegetables, and dairy products and had a high fat content, (2) a diet rich in fruits and vegetables, or (3) the DASH diet sodium intake and body weight were held constant during the study period of eight weeks. Compared with the control group, blood pressure decreased by 5.5/3.0 mmHg and 2.8/1.1 mmHg in the DASH diet and fruits-and-vegetables diet, respectively. This reduction was higher among the subset of hypertensives at 11.4/5.5 mmHg compared with 3.5/2.1 mmHg for those without hypertension consuming the DASH diet. In addition, the reduction in blood pressure began within two weeks and was sustained for the next six weeks.

The DASH-sodium trial [35] was a crossover trial in which 412 subjects were randomized to either the control diet or DASH diet and 3 levels of sodium intake (low: 1.2 g/day, intermediate: 2.3 g/day, and high: 3.5 g/day) within each diet arm for four weeks. Participants had SBP between 120 to 159 mmHg and DBP between 80 to 95 mmHg. Similar to the earlier study, the DASH diet significantly lowered blood pressure independent of sodium intake. With each diet, reducing the sodium intake significantly decreased blood pressure, and these effects persisted among those with and without hypertension, as well as across the different races and sex. The combination of the DASH diet and low sodium intake had the greatest impact, reducing SBP by 11.5/5.7 mmHg and 7.1/3.1 mmHg among hypertensives and those without hypertension, respectively, compared with the control diet and high sodium intake. The level of reduction among hypertensives is comparable to that obtained with drug monotherapy.

African Americans with hypertension derive the most benefit from the blood pressure lowering effect of the DASH diet [156], however they may be less likely to adhere to the diet [157, 158]. Furthermore, the increase in blood pressure with aging can be reduced by adopting the DASH diet with sodium restriction [36, 159].

Other potential benefits of the DASH diet include reduction in cardiovascular morbidity [160, 161], mortality [162], CHF events [163, 164], and cardiovascular risks factors [165–167] as well as prevention of type 2 diabetes [168].

The DASH and low sodium diet has been endorsed by professional organizations including the AHA [169], the JNC [1], the American Association of Clinical Endocrinologists [170], and the Canadian Hypertension Education Program [27] for the prevention and treatment of hypertension.

Barriers to compliance with the DASH diet include cost, availability, accessibility, lack of information, and cultural dietary preferences. The US Department of Health and Human Services published a guidebook, *Your Guide to Lowering your Blood Pressure with DASH*, which is a great resource to guide patients in preparation of meals in accordance with the DASH diet [171].

The PREMIER trial studied 810 adults with SBP ranging from 120 to 159 mmHg and DBP 80 to 95 mmHg [172]. Patients were divided into 3 intervention groups: one-time advice, "established" behavioral intervention based on the established recommendations, and "established plus DASH." Food was prepared by participants and they were followed up by telephone interview. The results showed that compared with the group receiving advice only, the 2 groups with behavioral interventions had significant decrease in the prevalence of hypertension and a higher percentage of optimal blood pressure at 6 month followup. However, there was no statistically significant difference between them.

11.2. Vegetarian Diet. Early observation has described lower blood pressure in vegetarians compared to patients on regular diets [173]. It seemed that sodium intake in vegetarian diet did not contribute a significant effect to blood pressure changes [174]. Recent studies also demonstrated that vegans do have lower SBP and DBP and less likely to use antihypertensive medications. In a study, for vegans, the odds ratio of hypertension compared with omnivores was 0.37 (95% CI, 0.19 to 0.74) [175]. Vegetarian diet with increased intake of fruit and vegetables, polyunsaturated vegetable margarines, and oils, fiber, calcium, and magnesium and decreased intake of protein in mild untreated hypertensive patients resulted in a fall of 5 mmHg in SBP. This diet improved blood pressure without a change in urinary sodium or potassium excretion or body weight [176]. Rouse and colleagues included 59 healthy normotensive patients in a randomized controlled

study in which they were randomized to a control group (omnivorous diet) and one of 2 experimental groups (omnivorous and/or lacto-ovo-vegetarian diet). Mean SBP dropped by 5 to 6 mmHg and DBP dropped by 2 to 3 mmHg in the group on vegetarian diet after adjustment for age, obesity, heart rate, weight change, and blood pressure before dietary change. Blood pressure rose substantially in subjects who reverted to the omnivorous diet [177].

It appears that a vegetarian diet might reduce blood pressure by several factors: increased vegetables, fiber and fruit intake, low protein, and so forth.

12. Conclusion

Dietary modification has important therapeutic roles in blood pressure control. Strong evidence supports the recommendation of a diet containing high potassium, moderate alcohol, and high fiber intake. As a whole, a DASH pattern diet rich in fruits, vegetables, low-fat dairy products, whole grains, nuts, and fish with reduced amount of red meat, fat, sugar-sweetened food and beverages, and/or a vegetarian diet, which is also high in vegetables and fruits and low in animal protein should be considered. The use of pharmacological supplements to achieve these dietary goals is not advised. Sodium restriction is strongly recommended by professional organizations, however, this needs to be carefully interpreted and individualized given the evidence of associated potential harmful effects and its unequal efficacy in various patient populations. The recommendation is not quite definite in terms of increased intake of calcium, magnesium, fish oil, and garlic. Irregular coffee drinkers and licorice consumers face a possibility of inducing hypertension; thus, these habits need to be avoided in patients at risk.

Established nutrition recommendations are proven to be helpful in reducing blood pressure and thus hypertension-related complications and overall mortality. However, in view of the heterogeneity in risk factors, patient features, and pathogenesis of hypertension, the approach should be individualized and discussed in detail between the patient and their physician and/or dietician, based on the understanding of each patient's distinct disease characteristics.

The importance of dietary modification in hypertension management has been substantially studied for past decades with encouraging findings. Additional studies are warranted to further explore the role of these nutrient factors in preventing and treating this condition in special populations.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 178780, 5 pages
http://dx.doi.org/10.1155/2013/178780

Research Article

Low Diastolic Blood Pressure as a Risk for All-Cause Mortality in VA Patients

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Received 1 January 2013; Revised 2 March 2013; Accepted 3 March 2013

Academic Editor: Agostino Virdis

Background. A paradoxical increase in cardiovascular events has been reported with intensively lowering diastolic blood pressure (DBP). This J-curve phenomenon has challenged the aggressive lowering of blood pressure, especially in patients with coronary artery disease. *Objective*. Our objective was to study the effects of low DBP on mortality and determine a threshold for which DBP should not be lowered beyond. *Methods*. We evaluated a two-year cross-section of primary care veteran patients, from 45 to 85 years of age. Receiver operating characteristics (ROC) were employed to establish an optimal cut-off point for DBP. Propensity-score matching and multivariate logistic regression were used to control for confounders. All-cause mortality was the primary outcome. *Results*. 14,270 patients were studied. An ROC curve found a threshold value of DBP 70 mmHg had the greatest association with mortality ($P < 0.001$). 49% of patients had a DBP of 70 mmHg or less. Using a propensity-matched multivariate logistic regression, odds ratio for all-cause mortality in subjects with a DBP less than 70 mmHg was 1.5 (95% CI 1.3–1.8). *Conclusions*. Reduction of DBP below 70 mmHg is associated with increased all-cause mortality. Hypertension guidelines should include a minimum blood pressure target.

1. Introduction

Hypertension affects 29% of male and 25% of female adults worldwide [1]. Its impact on mortality has improved with advances in detection and treatment, yet the U.S. mortality rate still lies at 14.3 per 1,000 people per year [2]. Mortality gaps in hypertensive versus nonhypertensive patients persist due to its occurrence with other appendages of the metabolic syndrome, thus treatment advances. As treatment has expanded, so has its intensity. Recently the question has frequently been raised as to potential harms associated with aggressive treatment of hypertension [3]. A systematic review of aggressive versus standard blood pressure targets did not find any benefit in total mortality when blood pressure is lowered less than 140/90 mmHg [4].

The incidence in cardiovascular events, including mortality, increases with extremes in blood pressure. The paradoxical increase in events at lower blood pressures has been represented by a J-shaped or U-shaped curve. The J-curve phenomenon has been researched since 1979 [5] and has been amplified with individual trials, post hoc analyses, and systemic reviews in support of this finding. Not all patients appear to be equally affected by the J-curve, if at all. Patient with established coronary artery disease (CAD) and diabetes are the most affected by an overcorrection of blood pressure [6]. Elevation in systolic blood pressure has been a more important predictor of mortality than diastolic blood pressure (DBP) [7]. On the other hand, low DBP in patients treated for hypertension has been associated with increased risk of cardiovascular disease [8, 9].

This study evaluates the concept of the J-curve in DBP across a large primary care population with a high prevalence of CAD, diabetes, and hypertension. We sought to determine a threshold for which DBP should not be lowered beyond. We also evaluated low DBP as an independent risk factor for all-cause mortality.

2. Materials and Methods

2.1. Study Design. A cross-sectional study of predominantly male patients at the VA Central California Healthcare System aged from 45 to 85 years was conducted over a 2-year period. Data were collected from the electronic medical record and provided demographic information, vital signs, comorbid diagnoses, and medications. The study was approved by the VA Northern California Health Care System Institutional Review Board.

2.2. Patient Population. All patients at least 45 years of age or older with a minimum of one outpatient encounter with recorded blood pressure were included in the study.

2.3. Variables. The primary outcome, death from any cause, was used as the dependent variable. Blood pressure (BP) was collected at the time of an ambulatory encounter. Pulse pressure was calculated as systolic BP minus diastolic BP. Covariates were included that were thought to contribute to the overall risk of mortality as well as variables that might alter a patient's blood pressure goals. These included age, body mass index, and the presence of comorbidities including CAD, hypertension, diabetes, cerebrovascular disease, and chronic kidney disease. The investigators felt that these conditions were the most likely to contribute to the cardiovascular causes of death and were important potential confounding variables in this study. CAD was defined by ICD9 code, by a history of myocardial infarction with abnormal electrocardiogram or troponin elevation, coronary artery bypass graft surgery, percutaneous coronary intervention, or coronary stent placement. Data were also gathered on the classes of blood pressure medications used to treat the patients.

2.4. Statistical Analysis. Grouping was based on a cut-off point for DBP. Receiver operating characteristic curve was used to determine the optimal cut-off point for DBP as a continuous scale against death. Baseline characteristics were compared between the two groups with the use of the chi-square test and the independent samples t-test. Interval likelihood ratios were calculated for each interval for DBP against all-cause mortality along with 95% confidence interval (CI). Multivariate logistic regression (LR) assessed for independence of low DBP as a risk for all-cause mortality.

Propensity score matching was conducted using DBP grouping as above mentioned for the dependent variable. Each study subject received a propensity score based on the presence of selected covariates. Covariates included age, comorbidities including CAD, hypertension, diabetes, chronic kidney disease, cerebrovascular disease, total number of antihypertensive medications, and individual medication classes. Finally, subjects were matched based on their scores, looking for the closest match.

Statistical analyses were carried out with SPSS Statistics Software for Windows, Version 20.0 (IBM Corp., Armonk, NY). Propensity score matching was carried out using R Project for Statistical Computing, Version 2.12.0 (R Development Core Team, Vienna, Austria) along with the SPSS R Essentials plug-in.

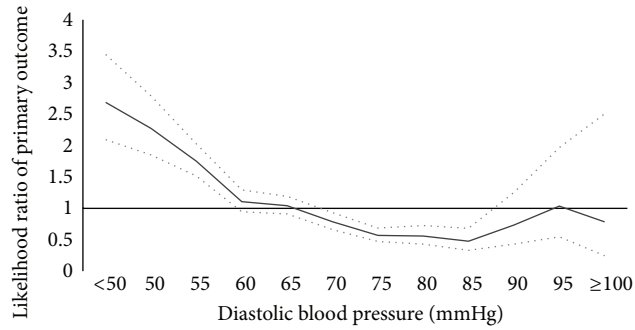

Figure 1: Interval likelihood ratios of all-cause mortality against a range of diastolic blood pressure. (Upper and lower 95% confidence intervals denoted by dotted lines.)

3. Results

A total of 14,270 patients were included in the study. The mean age of study population was 67 years with 96% male patients. The prevalence of comorbid conditions was as follows: hypertension 66.7%, diabetes 29.5%, CAD 19.5%, stroke 9.3%, and chronic kidney disease 6.9%.

Interval likelihood ratios of all-cause mortality as a function of DBP were calculated and are shown graphically (Figure 1). The lower 95% CI was greater than one for values of a DBP ranging 55 mmHg and less. The only values in the interval likelihood ratio that achieved a 95% CI less than one were DBP ranges from 70 to 85 mmHg. Those values greater than 85 mmHg did not achieve statistical significance. Eighteen percent of patients had an ambulatory DBP of 60 mmHg or below, while 49% had a reading of 70 mmHg or less.

A receiver operating characteristic curve found that the threshold value of DBP with the greatest specificity and sensitivity for mortality was 70 mmHg ($P < 0.001$). Patients were grouped according to DBP less than 70 mmHg and those at or above 70. Baseline characteristics of these two groups were significantly different with respect to age, comorbidities, and use of antihypertensive medications (Table 1). To adjust for such differences, all subjects were assigned a propensity score and then matched based on that score. The results were a closely paired group of 8856 patients with small differences between the groups.

After matching, the comorbidities were similar in the two groups with the exceptions of diabetes (31.7% versus 29.7%, P 0.04) and CAD (22.2% versus 18.8%, $P < 0.001$), with more patients having diabetes and CAD in the group with lower DBP. Medication use by class was similar in both groups except for a trend in increased use of loop diuretics in the low DBP group (7.5% versus 6.4%, P 0.037). There was no difference in the total number of antihypertensive medications in the two groups (Table 2). A multivariate logistic regression was used to adjust for low DBP, pulse pressure, and CAD as potential confounders and assess for independence in their association with all-cause mortality. Other recorded comorbidities were also included. Among the comorbid conditions, low DBP, along with pulse pressure,

Table 1: Characteristics of patients, according to diastolic blood pressure before matching*.

Characteristic	Patients with DBP <70 (N = 6175)	Patients with DBP ≥70 (N = 8095)	P value
Age (yr)	70.7 ± 9.6	64.6 ± 9.5	<0.001
Comorbidities			
Hypertension	4275 (69.2)	5243 (64.8)	<0.001
Diabetes	2201 (35.6)	2006 (24.8)	<0.001
Chronic kidney disease	595 (9.6)	393 (4.9)	<0.001
Cerebrovascular disease	717 (11.6)	607 (7.5)	<0.001
Coronary artery disease	1623 (26.3)	1164 (14.4)	<0.001
Systolic blood pressure	121 ± 15	133 ± 15	<0.001
Diastolic blood pressure	61 ± 6	79 ± 7	<0.001
Pulse pressure	59 ± 14	54 ± 14	<0.001
Number of antihypertensives	1.2 ± 1.2	1.0 ± 1.1	<0.001
Number of antihypertensives			<0.001
0	2182 (35.3)	3483 (43)	
1	1697 (27.5)	2228 (27.5)	
2	1409 (22.8)	1598 (19.7)	
3	684 (11.1)	606 (7.5)	
4	169 (2.7)	156 (1.9)	
5	31 (0.5)	22 (0.3)	
6	3 (0)	2 (0)	
Medications by class			
Beta blocker	1732 (28)	1828 (22.6)	<0.001
Alpha blocker	1110 (18)	885 (10.9)	<0.001
ACE-I/ARB	2531 (47.1)	2569 (36)	<0.001
Calcium channel blocker	1032 (16.7)	957 (11.8)	<0.001
Thiazide diuretic	1402 (22.7)	2000 (24.7)	0.006
Loop diuretic	635 (10.3)	358 (4.4)	<0.001
All-cause mortality	617 (10)	399 (4.9)	<0.001

DBP: diastolic blood pressure; ACE-I: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers.
*Values reported as N (%) or means ± SD.

Table 2: Characteristics of patients after matching on propensity score*.

Characteristic	Patient with DBP <70 (N = 4428)	Patients with DBP ≥70 (N = 4428)	P value
Age (yr)	68.9 ± 9.7	67.8 ± 9.4	<0.001
Comorbidities			
Hypertension	3001 (67.8)	2940 (66.4)	0.168
Diabetes	1403 (31.7)	1314 (29.7)	0.040
Chronic kidney disease	321 (7.2)	294 (6.6)	0.259
Cerebrovascular disease	425 (9.6)	424 (9.6)	1.000
Coronary artery disease	985 (22.2)	832 (18.8)	<0.001
Systolic blood pressure	125 ± 14	127 ± 12	<0.001
Diastolic blood pressure	62 ± 6	77 ± 5	<0.001
Pulse pressure	62 ± 14	50 ± 12	<0.001
Number of antihypertensives	1.1 ± 1.1	1.1 ± 1.1	0.064
Number of antihypertensives			0.123
0	1693 (38.2)	1722 (38.9)	
1	1193 (26.9)	1263 (28.5)	
2	985 (22.2)	937 (21.2)	
3	433 (9.8)	386 (8.7)	
4	100 (2.3)	107 (2.4)	
5	22 (0.5)	11 (0.2)	
6	2 (0)	2 (0)	
Medications by class			
Beta blocker	1149 (25.9)	1094 (24.7)	0.179
Alpha blocker	681 (15.4)	643 (14.5)	0.257
ACE-I /ARB	1913 (43.2)	1828 (41.3)	0.067
Calcium channel blocker	675 (15.2)	634 (14.3)	0.220
Thiazide diuretic	1029 (23.2)	1038 (23.4)	0.821
Loop diuretic	334 (7.5)	284 (6.4)	0.037
All-cause mortality	367 (8.3)	244 (5.5)	<0.0001

DBP: diastolic blood pressure; ACE-I: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers.
*Values reported as N (%) or means ± SD.

CAD, chronic kidney disease, and cerebrovascular disease were each associated with all-cause mortality after controlling for each comorbid condition. Neither diabetes nor hypertension achieved statistical significance (Table 3). The odds ratio for all-cause mortality in subjects with a DBP less than 70 mmHg was 1.34 (95% CI 1.11–1.61) (Table 3).

4. Discussion

This study of primary care veterans offers additional insight into the relationship between low-DBP and mortality. This study utilizes a large sample size to evaluate the effects of the J-curve in DBP and to define a lower threshold for DBP. In our study population, the risk of death at various diastolic pressures was not continuous but followed the J-shared curve that has been established previously [10].

The majority of studies looking at harm with aggressive BP lowering have not been consistent in defining a limit to which BP should not be lowered beyond. The data in this study suggest that the benefit of lower DBP is limited to the range of 70–85 mmHg, with a nonstatistically significant

Table 3: Multivariate analysis of comorbidities on all-cause mortality.

Variable	Odds ratio (95% CI)	*P* value
DBP <70 mmHg	1.34 (1.11–1.61)	0.002
Pulse pressure	1.01 (1.00–1.02)	0.001
Coronary artery disease	1.52 (1.26–1.84)	<0.001
Chronic kidney disease	2.88 (2.28–3.64)	<0.001
Cerebrovascular disease	1.63 (1.28–2.06)	<0.001
Hypertension	0.91 (0.75–1.10)	0.328
Diabetes	1.04 (0.86–1.25)	0.688

DBP: diastolic blood pressure; CI: confidence interval.

trend between 60–65 mmHg. Any DBP value less than 60 mmHg increases the likelihood of all-cause mortality.

When assessing comorbid conditions for confounding biases, the multivariate logistic regression model identified low DBP (defined as less than 70 mmHg) as an individual risk factor for mortality, after adjusting for pulse pressure, CAD, chronic kidney disease, or cerebrovascular disease. Pulse pressure was included as a potential cofounder due to its association with cardiovascular disease and its unique relationship to DBP: decreases in diastolic blood pressure result in increased pulse pressure. Increased pulse pressure has been reported to increase the risk of developing diabetes [11], lead to progression of kidney disease [12], and confer a higher risk for CAD [13]. In this population, pulse pressure had no significant association with mortality after adjusting for DBP and other comorbidities (OR 1.01, 95% CI 1.00–1.02).

Low DBP was not independent of hypertension or diabetes, which suggests that a low DBP may only be harmful as a consequence of antihypertensive therapy or in patients with diabetes. Current literature on aggressive treatment of hypertension among diabetics has failed to show any benefit on mortality [14]. It was startling to learn that 18% of our entire study population had an ambulatory DBP of 60 mmHg or less. Notably, nearly half of the participants had a DBP of 70 or less. These findings underscore a lack of awareness amongst physicians regarding the paucity of evidence showing benefit in aggressive BP lowering and in particular the potential harms associated with it.

Owing to the debatable nature of the clinical significance of the J-curve [15–17], major societal guidelines have not previously given due recognition to the phenomenon. While the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure has been the gold standard for nearly a decade [7], it fails to address the question as to the potential harms with lowering blood pressure beyond a certain threshold. Likewise, indications and targets for aggressive blood pressure control have not been well defined. This report also defines the relationship between blood pressure and cardiovascular events as linear and independent of other factors. While this is likely true for patients without existing cardiovascular disease, patients with atherosclerotic CAD and LVH have a more narrow range for which autoregulation of the coronary arterial pressure can occur [18]. Different from the coronary circulation, which is mostly dependent on diastole for perfusion, the cerebral vasculature depends mostly on systolic BP [19] which allows it to tolerate a wider range of mean arterial pressures. Studies have differed in the clinical impact of low DBP on stroke [9, 19, 20]. Our study found that mortality due to low DBP was independent of a history of stroke. These results diverge from prior studies. A post hoc analysis reveals that the prevalence of CAD was 35% in patients with a history of cerebrovascular disease, showing significant overlap in these diseases. This suggests that individualization of BP goals should be tailored to comorbid conditions.

The growing body of evidence for J-shaped relationships between blood pressure and cardiovascular outcomes has led to the revision of guidelines from the European Society of Hypertension [21]. This represents a significant action towards broad recognition of the J-curve. The Joint National Committee is currently in progress in their draft of blood pressure guidelines for their 8th report. One of the questions we hope it will be addressed is how low blood pressure should be reduced.

5. Limitations

As with all retrospective studies we were limited by unidentified or incompletely documented potential confounders. The trend towards increased CAD in the group with DBP <70 mmHg, even after matching, poses a particular confounder in the relationship between low DBP and all-cause mortality, though this was accounted for using a multivariate logistical regression model. This bias remains a concern for nearly all trials of BP treatment, that the group which requires the most vigilant treatment could also be the group that possesses the greatest pretreatment cardiovascular risk profile [22]. Hence, we cannot conclude whether the low DBP was due to underlying heart disease, which also confers higher mortality. The study design also does not allow us to find causation, only association between low blood pressure and mortality.

Another limitation is that while this was a large population, it was also a specific population. The Veterans Administration medical record does not routinely record ethnicity as part of patient demographics. While younger veteran groups are more ethnically diverse, World War II and Korean War veterans are more than 88% Caucasian.

Finally, our dataset lacks intervals and averages on the recording of BP, and we are limited to the final reading at the time of data collection. It is not possible to consider trends in BP over time with this limitation.

6. Conclusions

While treatment of hypertension reduces mortality due to cardiovascular events, reduction of DBP below 70 mmHg is associated with increased all-cause mortality in this male predominant study population with significant comorbidities. The relationship between DBP and mortality follows a J-shaped curve. Avoidance of DBP less than 70 mmHg may be advisable in the management of hypertension, although prospective studies are warranted in more representative

patient populations. Our findings suggest the need of a shift in paradigm with the guidelines including a minimum as well as a maximum BP target. BP therapeutic goals should also be individualized to the patient's comorbid conditions.

Abbreviations

BP: Blood pressure
CAD: Coronary artery disease
CI: Confidence interval
DBP: Diastolic blood pressure.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

The authors would like to thank Dr. Jocelyn Fong and Mr. Sean McFarland for their assistance with data retrieval and verification.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 985087, 6 pages
http://dx.doi.org/10.1155/2013/985087

Clinical Study

Characterisation of Hypertensive Patients with Improved Endothelial Function after Dark Chocolate Consumption

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Received 27 November 2012; Revised 1 February 2013; Accepted 6 February 2013

Academic Editor: Antonio Felipe Sanjuliani

Recent findings indicate an inverse relationship between cardiovascular disease and consumption of flavonoids. We aimed to identify clinical and vascular parameters of treated hypertensive who present beneficial effects of dark chocolate for one-week period on vascular function. Twenty-one hypertensive subjects, aged 40–65 years, were included in a prospective study with measurement of blood pressure (BP), brachial flow-mediated dilatation (FMD), peripheral arterial tonometry, and central hemodynamic parameters. These tests were repeated after seven days of eating dark chocolate 75 g/day. Patients were divided according to the response in FMD: responders ($n = 12$) and nonresponders ($n = 9$). The responder group presented lower age (54 ± 7 versus 61 ± 6 years, $P = 0.037$), Framingham risk score (FRS) (2.5 ± 1.8 versus $8.1 \pm 5.1\%$, $P = 0.017$), values of peripheral (55 ± 9 versus 63 ± 5 mmHg, $P = 0.041$), and central pulse pressure (PP) (44 ± 10 versus 54 ± 6 mmHg, $P = 0.021$). FMD response showed negative correlation with FRS ($r = -0.60$, $P = 0.014$), baseline FMD ($r = -0.54$, $P = 0.011$), baseline reactive hyperemia index (RHI; $r = -0.56$, $P = 0.008$), and central PP ($r = -0.43$, $P = 0.05$). However, after linear regression analysis, only FRS and baseline RHI were associated with FMD response. In conclusion, one-week dark chocolate intake significantly improved endothelial function and reduced BP in younger hypertensive with impaired endothelial function in spite of lower cardiovascular risk.

1. Introduction

Hypertension notably contributes to the worldwide cardiovascular morbidity and mortality. Hypertensive disease seems to have a complex association with endothelial dysfunction, a phenotypical alteration of the vascular endothelium that precedes the development of adverse cardiovascular events and anticipates future cardiovascular risk [1]. Several studies have confirmed the connection between hypertension and abnormal endothelial function in the peripheral, coronary, and renal circulation, suggesting an important mechanism, whereby hypertension promotes the development and progression of vascular disease [1–4]. Given that endothelial dysfunction may be reversible, early detection of this disorder may have therapeutic and prognostic implications [5, 6].

Lifestyle modifications, including dietary habits, have substantial effects on risk factors for cardiovascular disease such as hypertension [7]. Epidemiological evidence demonstrates that a diet rich in fruits and vegetables promotes heart and vascular health [8–10]. These beneficial effects have been largely ascribed to their content in flavonoids. These compounds are synthesized in many edible plants and remain present when plants are processed to foods. Grapes, wine, cocoa and chocolate, teas, and soy are among the most important sources of flavonoids in the human diet. A significant number of studies have been carried out in humans, analyzing the effect of foods rich in flavonoids on

the presence and progression of risk factors associated with cardiovascular disease. Cocoa derived products have been thoroughly studied and demonstrated to be efficient improving endothelial function and decreasing blood pressure (BP) [11–14].

Some individuals may have positive health benefits when chocolate is ingested in moderation as part of a balanced diet [15]. Thus, the aim of this study was to identify clinical and vascular parameters of treated hypertensive patients who present beneficial effects of dark chocolate on vascular function for one-week period.

2. Materials and Methods

2.1. Study Population. Twenty-one hypertensive patients recruited from Pedro Ernesto Hospital on stable drug therapy over at least 4 weeks, aged 40–65 years, were included in a prospective study. Exclusion criteria were evidence of secondary hypertension, body mass index $\geq 35\,Kg/m^2$, coronary artery disease, kidney or thyroid disease, hormone replacement therapy, diabetes or impaired tolerance glucose, severe dyslipidemia (LDL-cholesterol ≥ 4.14 mmol/L and/or triglycerides ≥ 3.39 mmol/L), use of lipid-lowering drugs, using restricted diets (vegetarian, carbohydrate restriction), allergy to chocolate or cocoa, and use of nutritional supplements (vitamins, minerals) for up to seven days before the study beginning. The protocol was approved by the local Ethics Committee Research (2415-CEP/HUPE), and all patients gave written informed consent. This study was registered in Clinicaltrials.gov (NCT01314924).

2.2. Flow-Mediated Dilation (FMD) of Brachial Artery. After overnight fasting, without smoking or drinking coffee for a least 12 h before tests, patients were examined in the supine position in a dark, quiet, and air-conditioned room (22–24°C). A linear-array transducer operating at 10 MHz was used to acquire longitudinal images of the right brachial artery. A standard BP cuff was positioned around the right arm, 5 cm above the antecubital fossa. After obtaining baseline images of end-diastolic diameter of the brachial artery, the cuff was inflated to 50 mmHg above systolic BP for 5 minutes. Longitudinal images of the brachial artery were captured continuously for 30, 60, and 90 seconds after cuff deflation to document the vasodilator response. FMD was calculated as the percentage change in diameter from the baseline value to the peak value after cuff release.

2.3. Study Design. Volunteer patients meeting the selection criteria underwent clinical and laboratory evaluation. For laboratory tests and vascular assessment, patients were asked to fast for eight to twelve hours. At the first visit, patients were instructed to maintain the habitual physical activity, usual diet, but avoiding consumption of foods rich in flavonoids (apples, grapes, wine, tea, and chocolate) during the intervention. All patients then received 75 g of dark chocolate with 70% cocoa, rich in flavonoids, daily for seven days.

After 1-week consumption of dark chocolate and reexamination, patients were divided according to the response in brachial FMD into responders (RESP) when there was at least an increase of 1% in FMD and nonresponders (N-RESP) when this improvement in FMD was not observed.

Total polyphenol content of the chocolate used in this study was measured by spectrophotometry in the Laboratory of Pharmacology at our institution. We found 42.7 mg of polyphenols/g of dark chocolate, resulting in 3202 mg of polyphenols in the total daily dose.

2.4. Clinical and Laboratory Analyses. Office brachial systolic and diastolic BP were measured in the sitting position using a validated electronic device (HEM-705CP, Omron Healthcare Inc., IL, USA) with an appropriate cuff size. Three consecutive readings, one minute apart, were obtained, and the average value was used as the clinic BP.

Routine biochemical assessment included fasting glucose and lipid profile (total cholesterol, HDL-cholesterol, triglycerides, and LDL-cholesterol calculated by Friedewald formula). The Framingham risk score was estimated for each patient [16].

2.5. Peripheral Arterial Tonometry (PAT). Endothelial function was also assessed by PAT using the Endo-PAT2000 device (Itamar Medical Ltd., Caesarea, Israel). PAT is a noninvasive technique, to assess peripheral microvascular endothelial function by measuring changes in digital arterial pulse volume during reactive hyperemia [5].

Probes were placed on index finger of each hand, and a BP cuff was placed on nondominant (study arm), while the contralateral arm served as a control. Continuous recording of pulsatile blood volume responses from both hands was initiated. After a 10 min equilibration period, the BP cuff on the study arm was inflated to 60 mmHg above systolic pressure. The changes in arterial tone were elicited by a standard 5 min occlusion of the brachial artery. The cuff was then deflated to induce reactive hyperemia, while PAT recording was continued. Reactive hyperemia index (RHI) was analysed by a computer in an operator-independent manner.

2.6. Central Hemodynamic Parameters. Radial artery applanation tonometry and pulse wave analysis were carried out to derive central BP and other parameters using dedicated system SphygmoCor v.7 (SphygmoCor, AtCor Medical Inc., USA). This method generates central aortic pressure waveforms from the radial pressure waveform using a previously validated transfer function. The central pressure waves were analyzed to identify the outgoing and reflected components and to calculate the augmentation index (Aix), that is, the proportion of the central pulse pressure that is attributable to pulse wave reflection which is calculated as augmentation pressure (AP).

2.7. Sample Size Calculation. One goal of the proposed study is to test the null hypothesis that the mean difference (or change) within pairs is zero. The criterion for significance (alpha) has been set at 0,050. The test is 2 tailed, which means that an effect in either direction will be interpreted. With the

proposed sample size of 20 pairs of cases, the study will have power of 80% to yield a statistically significant result.

2.8. Statistical Analysis. The results were expressed as mean ± standard deviation (SD) or as percentages when appropriate. In the intragroup analysis, continuous variables obtained before and after chocolate consumption were compared by paired t-test with a confidence interval of 95%, and the value of $P < 0.05$ was considered statistically significant. Student's t-test for independent samples was used for intergroup analysis. Pearson coefficient was obtained in correlation tests between continuous variables. Multiple linear regression, stepwise model, was done considering FMD response as dependent variable and those significantly correlated to this parameter as independent variables. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL) version 18.0.

3. Results

Twenty-four eligible patients were identified for this study. One patient was excluded due to high levels of fasting glucose, and another 2 patients failed to complete the intervention period. Thus, 21 individuals completed the study of whom 16 (76%) were female. Baseline characteristics and vascular profile of the study population are given in Table 1. A moderate consumption (twice a week) of alcohol was reported by 38% of patients. Regarding regular physical activity, only 4 patients (19%) were physically active on a regular basis.

Data on drug therapy show that classes of antihypertensive drugs used by patients were thiazide diuretics (95%), angiotensin receptor blocker (10%), angiotensin converting enzyme inhibitor (5%), and calcium channel antagonist (5%). From the total sample, 81% were on monotherapy (76% on thiazides), and 19% were on a combination of two drugs.

The clinical and lipid profiles of responders and nonresponders are shown in Table 1. Mean age and Framingham risk score were significantly lower among responders. Who also had greater impairment of endothelial function evidenced by decreased baseline brachial FMD and RHI. In addition, responder group presented significantly lower brachial and aortic pulse pressure than nonresponders (Table 2).

Besides improvement in FMD, the responder group presented significant changes in clinical parameters such as systolic (140 ± 13 versus 131 ± 10 mmHg, $P < 0.05$) and diastolic (85 ± 7 versus 82 ± 8 mmHg, $P = 0.05$) BP and in mean arterial pressure (103 ± 9 versus 98 ± 9 mmHg, $P < 0.01$).

FMD response was inversely correlated with Framingham risk score ($r = -0.60$, $P = 0.014$), baseline FMD ($r = -0.54$, $P = 0.011$), baseline RHI ($r = -0.56$, $P = 0.008$; Figure 1), and aortic pulse pressure ($r = -0.43$, $P = 0.050$). However, after multiple linear regression analysis, only the Framingham risk score and baseline RHI were independently associated with FMD response.

4. Discussion

This study demonstrated that treated hypertensive subjects responding to 7-day dark chocolate intakes with improved endothelial function and reduced blood pressure were younger and had less cardiovascular risk than nonresponders.

In contrast to our study, other protocols commonly included only naive hypertensive patients with never-treated raised BP levels or a washout period with discontinuation of drug therapy before the intervention with chocolate [11, 17–19]. Both groups had similar baseline blood pressure and metabolic profiles, but responders had lower baseline FMD and RHI than nonresponders, much as reported by Grassi et al. [18] who described baseline FMD around 8%. Most studies show lower values than these observed in the present study [11, 17, 19], suggesting that those with compromised vascular function are more responsive to chocolate. Moreover, the higher brachial and aortic pulse pressure among nonresponder patients might indicate increased vascular stiffness in these subjects and could be associated with resistance to favorable effects of cocoa.

Nonresponders were older and had higher cardiovascular risk scores than responders as reportedv previously in patients of similar ages to those in our study [21]. Recently, Muniyappa et al. also found a significant improvement of 2.3% in endothelial function in hypertensive patients without drug therapy after consumption of a cocoa drink for fifteen days [19]. The consumption of 100 g of dark chocolate showed benefits in vascular function in healthy subjects after acute consumption of 3 hours, reflected by 1.43% improvement in FMD and 7.8% reduction in augmentation index. These findings also confirmed that chocolate can be beneficial to vascular health [22]. The results of our study showed no improvement in central hemodynamic parameters after consumption of dark chocolate. However, the group that did not respond in FMD, although presenting a good baseline endothelial function, presented significantly greater values of central and peripheral pulse pressure than those in responder group. These findings could indicate increased vascular stiffness among nonresponder patients. The small decrease in FMD after consumption of dark chocolate in nonresponder group was not clinically significant because it remained in the normal range of a good endothelial function. On the other hand, the responder group had a significant increase in FMD which was also clinically relevant. Patients in this group varied from 8.4% (before chocolate) to 16.6% (after chocolate) in FMD, demonstrating a clear improvement in endothelial function.

To date, the gold standard noninvasive technique for measuring endothelial function in clinical studies is the FMD of the brachial artery [23, 24]. It has been recently shown that PAT, which can detect abnormalities in the amplitude of the wave digital pulse, is significantly associated with FMD [25], and we used both methods. However, neither group showed effects on RHI. This discrepancy might reflect the short intervention period. Positive effects are reported on PAT after 5-day cocoa supplementation, probably reflecting increased nitric oxide bioavailability with cocoa [26].

Table 1: Baseline characteristics of study population and responder (RESP) and nonresponder (N-RESP) groups.

Parameters	Total sample ($n = 21$)	RESP ($n = 12$)	N-RESP ($n = 9$)	P value
Age, years	57 ± 8	54 ± 8	61 ± 6*	0.037
Framingham risk score, %	5.3 ± 4.7	2.5 ± 1.8	8.1 ± 5.1*	0.011
Body mass index, Kg/m^2	28.2 ± 4.5	29.7 ± 5.2	26.1 ± 2.9	0.065
Systolic BP, mmHg	141 ± 11	140 ± 12	142 ± 8	0.718
Diastolic BP, mmHg	82 ± 9	85 ± 7	79 ± 9	0.147
Pulse pressure, mmHg	58 ± 9	55 ± 9	63 ± 5	0.041
Mean arterial pressure, mmHg	101 ± 9	103 ± 8	100 ± 9	0.417
Total cholesterol, mmol/L	5.56 ± 1.21	5.30 ± 0.77	5.84 ± 1.52	0.355
HDL-cholesterol, mmol/L	1.24 ± 0.36	1.27 ± 0.31	1.21 ± 0.41	0.771
LDL-cholesterol, mmol/L	3.46 ± 0.72	3.31 ± 0.67	3.62 ± 0.77	0.411
Triglycerides, mmol/L	1.56 ± 1.13	1.41 ± 0.53	1.70 ± 1.55	0.594
Fasting glucose, mmol/L	4.72 ± 0.5	4.72 ± 0.61	4.67 ± 0.39	0.864
Flow-mediated dilation, %	10.7 ± 5.5	8.4 ± 5.9	13.8 ± 2.8*	0.023
Reactive hyperemia index, units	2.03 ± 0.71	1.70 ± 0.63	2.47 ± 0.51**	0.006
Augmentation pressure, mmHg	15 ± 7	15 ± 7	16 ± 5	0.869
Augmentation index, %	32 ± 11	34 ± 12	29 ± 9	0.343
Aortic systolic BP, mmHg	130 ± 15	128 ± 18	133 ± 11	0.550
Aortic pulse pressure, mmHg	48 ± 10	44 ± 10	54 ± 6*	0.021

Results are expressed as mean ± SD. BP: blood pressure; HDL: high density lipoprotein; LDL: low-density lipoprotein. * $P < 0.05$ and ** $P < 0.01$ versus responder group.

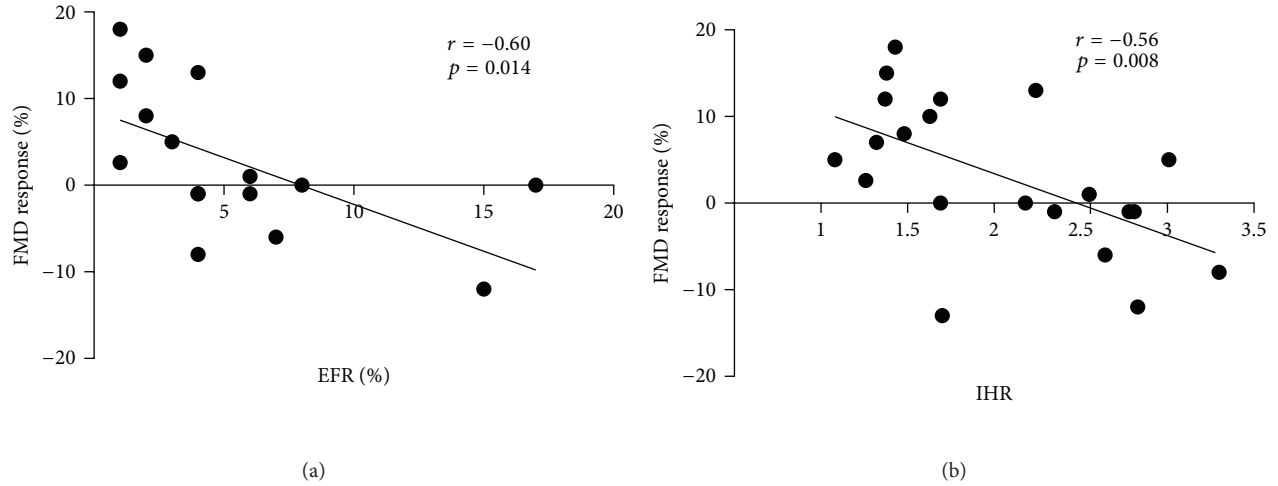
Table 2: Clinical and vascular parameters in responder (RESP) and nonresponder (N-RESP) groups before and after chocolate consumption.

Parameters	RESP group ($n = 12$)			N- RESP group ($n = 9$)		
	Before chocolate	After chocolate	P	Before chocolate	After chocolate	P
Weight (Kg)	78.8 ± 11.9	78.3 ± 11.4	0.185	71.2 ± 16.1	71.5 ± 16.3	0.403
SBP (mmHg)	140 ± 13	131 ± 10	0.015	142 ± 8	142 ± 10	1.000
DBP (mmHg)	85 ± 7	82 ± 8	0.050	79 ± 9	83 ± 10	0.276
PP (mmHg)	55 ± 9	48 ± 5	0.084	63 ± 5	60 ± 7	0.313
MAP (mmHg)	103 ± 9	98 ± 9	0.007	100 ± 9	102 ± 10	0.368
FMD (%)	8.4 ± 5.9	16.6 ± 8.2	<0.001	13.8 ± 2.8	11.3 ± 4.2	0.030
RHI (units)	1.70 ± 0.63	1.87 ± 0.54	0.134	2.47 ± 0.51	2.17 ± 0.42	0.124
AP (mmHg)	15 ± 7	11 ± 5	0.092	16 ± 5	13 ± 10	0.323
Aix (%)	34 ± 13	28 ± 12	0.209	29 ± 9	26 ± 12	0.299
Aortic SBP (mmHg)	128 ± 19	121 ± 9	0.249	133 ± 11	131 ± 18	0.732
Aortic PP (mmHg)	44 ± 10	36 ± 7	0.079	53 ± 6	46 ± 13	0.127

Results are expressed as mean ± SD. SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; FMD: flow-mediated dilation; RHI: reactive hyperemia index; AP: augmentation pressure; Aix: augmentation index.

Our responders also showed reductions in blood pressure after eating chocolate as others have with untreated hypertension and in longer studies [11, 17, 20]. Furthermore the reductions being reported, though relatively small, are clinically significant, since it has been found that a reduction of 3 mmHg in systolic BP can reduce relative risk of death from stroke by 8%, from cardiovascular disease in general by 5%, and overall all-cause mortality by 4% [27]. In our study, the responder group also demonstrated a reduction in aortic systolic blood pressure and pulse pressure, although not reaching statistical significance. This result may suggest a less prominent effect of dark chocolate on vascular stiffness parameters.

In a recent prospective study, Buijsse et al. investigated the effects of chocolate consumption on BP and incidence of cardiovascular disease after 8-year followup and found chocolate eating reduced cardiovascular risk, largely due to the reductions in blood pressure [28]. A new meta-analysis supports the findings for benefit with chocolate consumption [14], which may reflect increased polyphenol intakes which likely improves vascular health through increased nitric oxide bioavailability, inhibition of angiotensin-converting enzyme stimulating the production of vasodilator factors, and improvement of insulin sensitivity which are the most studied pathways to explain the effect of epicatechin, a monomer of polyphenols, on vascular function [13, 29–32].



(a) (b)

Figure 1: Negative correlation of flow-mediated dilation (FMD) response with Framingham risk score (a) and with baseline reactive hyperemia index (b).

Another recent meta-analysis showed chocolate usage was associated with reductions in risk of cardiovascular disease by 31%, of diabetes by 37%, and of stroke by 29% [33], and protection is greater with dark than other chocolates [34].

Our study had some limitations, especially concerning the sample size and lack of a control group. Nevertheless, the findings are in line with other recent observations. There is as yet no way to identify those who will benefit from dark chocolate consumption, but the present study provides data that could be examined in larger randomized controlled studies for the selection of subjects likely to benefit from increased intakes of dark chocolate.

5. Conclusion

In conclusion, the intake of dark chocolate significantly improved endothelial function and reduced blood pressure in some individuals who were younger hypertensive patients with impaired endothelial function in spite of lower cardiovascular risk.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

This paper is supported by Fundação de Amparo à Pesquisa no Estado do Rio de Janeiro (FAPERJ).

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 565263, 8 pages
http://dx.doi.org/10.1155/2013/565263

Research Article

Prevalence, Awareness, and Treatment of Hypertension in Patients with Type 1 Diabetes: A Nationwide Multicenter Study in Brazil

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Received 13 November 2012; Accepted 24 January 2013

Academic Editor: Mario Fritsch Neves

Objective. This study evaluated the prevalence, awareness, and type of treatment for hypertension in Brazil in patients with type 1 diabetes (T1D). *Methods*. This was a cross-sectional, multicenter study that was conducted from December 2008 to December 2010 in 28 public clinics located in 20 Brazilian cities. *Results*. A total of 3,591 patients were studied, 56% female, average age 21.2 ± 11.7 years, with a median duration of diabetes 9.6 ± 8.1 years. Blood pressure levels were available for a total of 3,323 patients and 689 (19.2%) patients were hypertensive. Hypertensive patients were older, exhibited longer duration of diabetes, and had higher body mass index (BMI), total cholesterol, triglycerides, and LDL-C values ($P < 0.001$, for all comparisons), but only 370 (53.7%) received treatment. Patient awareness of hypertension was documented in 453 (65.5%) patients. However, only 76 (22.9%) of the treated patients attained the target systolic (sBP) and diastolic blood pressures (dBP). *Conclusions*. Our results demonstrate that a large number of T1D patients with hypertension do not receive appropriate treatment; few of the treated T1D patients achieved the target sBP and dBP values. Greater attention should be paid to blood pressure evaluation, hypertension diagnosis, and treatment of T1D patients in Brazil.

1. Introduction

Type 1 diabetes mellitus (T1D) is a chronic disease that carries a great risk of morbidity and mortality, as a result of the microvascular and macrovascular complications that reduce an affected individual's quality of life and life expectancy [1]. Progress in diabetes management in recent decades has improved the survival rates among T1D patients, although life expectancy remains lower for these individuals compared to nondiabetic subjects of equal age [2].

Diabetes has emerged as a major health problem in societies in which noncommunicable diseases are the most common causes of disability and death [1, 2]. Furthermore, diabetes treatment has become a large financial burden because of the increased associated direct and indirect costs [3].

The occurrence of hypertension in T1D patients is directly correlated with the presence of microvascular complications, primarily nephropathy and retinopathy and the progression of these chronic complications [4, 5]. There is strong evidence, relating to the efficacy and cost effectiveness of treatment, to support blood pressure control in T1D and T2D patients, as well as the nondiabetic population, for reducing levels of morbidity and mortality [6–8]. Target blood pressure levels have been described in many guidelines, including the American Diabetes Association (ADA) [9], American Heart Association (AHA) [10], and Brazilian Diabetes Society (BDS) [11]. However, a large gap remains between the recommendations for blood pressure control and the values that have been described in most observational T1D [12–14] and T2D [15] studies.

Previous studies on the prevalence, awareness, treatment type, and control of hypertension have examined nondiabetic populations or T2D patients [16, 17] but rarely T1D patients. The Coronary Artery Calcification in type 1 Diabetes Study (CACT1) [12] demonstrated a higher rate of hypertension among T1D (43%) patients compared to nondiabetic subjects (15%) but observed a similar rate of hypertension awareness between T1D subjects (53%) and controls (45%). Furthermore, the EURODIAB study demonstrated a hypertension prevalence of 24% among T1D patients and less than half of these patients were aware of this condition. Only 42.2% of the T1D patients in this study received treatment, and only 26.7% of the treated T1D patients attained the established blood pressure targets [13].

The results of these studies emphasize the difficulties associated with the treatment of hypertensive T1D patients in routine clinical care and the need for improved treatment quality.

The absence of national data on the prevalence, awareness, type of treatment, and control of hypertension in T1D patients led the Brazilian Type 1 Diabetes Study Group (BrazDiab1SG) to conduct this study, seeking to provide current and reliable data on the topic with regard to the ADA guidelines.

2. Research Design and Methods

This was a multicenter, cross-sectional, and observational study that was conducted between December 2008 and December 2010 in 28 public secondary (ambulatory outpatient clinics) and tertiary care level clinics (ambulatory outpatient clinics in university hospitals), located in 20 cities in four Brazilian geographic regions (north/northeast, midwest, southeast and south). The detailed data collection methods have been described previously [18]. Briefly, all patients received health care from the National Brazilian Health Care System (NBHCS). All eligible participating centers possessed a diabetes clinic with at least one endocrinologist. Each clinic provided data from a minimum of 50 consecutive outpatients with an initial diagnosis of T1D who regularly attended the clinic. The inclusion criteria consisted of a diagnosis of T1D by a physician that was based on the typical clinical presentation, including variable degrees of weight loss, polyuria, polydipsia, and polyphagia, and the need for continuous insulin use since T1D diagnosis. All patients were diagnosed between 1960 and 2010.

The following variables were assessed in each interview during the clinical visit: current age, age at diagnosis, duration of diabetes (y), height (m), weight (kg), mean blood pressure (systolic and diastolic in mmHg from three consecutive measurements in one day using a standard clinical sphygmomanometer), modality of diabetic treatment, comorbidities, frequency of SBGM, and smoking status. The levels of glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides on the last clinical visit were obtained from medical records. The screenings for retinopathy, using fundoscopy; nephropathy, according to microalbuminuria; and foot examinations in patients with diabetes duration equal or greater than five years were noted when these procedures were performed within one year of the study assessment.

Demographic, educational, and economic data were also obtained. Patients with diabetes for less than five years were not included in the analysis of diabetic chronic microvascular complications ($n = 1,160$, 32.3%). Each local center's ethics committee approved the study (the appendix). The Brazilian Diabetes Society (BDS) monitored and reviewed all study-related documents and approved all amendments and publications. Each center's coordinator reviewed the chart form prior to final approval.

The following ADA goals for adequate metabolic and clinical control [11] were adopted by the BrazDiab1SG: HbA1c < 7.5% for T1D patients of 13 to 19 years of age, HbA1c < 8% for T1D patients of six to twelve years of age, HbA1c > 7.5% and < 8.5% for T1D for patients less than 6 years of age, and HbA1c < 7% for adult T1D patients; systolic blood pressure (sBP) < 130 mmHg; diastolic blood pressure (dBP) < 80 mmHg; body mass index (BMI) < 25 kg/m^2; FPG < 130 mg/dL (7.2 mmol/L); total cholesterol < 200 mg/dL (5.2 mmol/L); HDL cholesterol > 40 mg/dL for men (1.1 mmol/L) and > 50 mg/dL (1.3 mmol/L) for women; LDL cholesterol < 100 mg/dL (2.6 mmol/L); non-HDL cholesterol < 130 mg/dL (3.30 mmol/L); and triglycerides < 150 mg/dL (1.7 mmol/L).

Hypertension in adults was defined as sBP ≥ 140 mmHg and/or dBP ≥ 90 mmHg, measured during the last clinical visit [8] or was self-reported, while hypertension in children and adolescents was defined as a sBP or dBP ≥ 95th percentile, according to the patient's age, sex,missimg and height [19] with the measurements taken during the clinical visit. Patient awareness of hypertension in adults was based on patient self-reporting of any prior hypertension diagnosis that was made by a health practitioner on at least two separate occasions. Patients who received angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) for the treatment of micro- or macroalbuminuria and those who were not hypertensive were not included in the hypertensive group ($n = 197$, 7.5%).

Microalbuminuria and clinical nephropathy were defined according to the ADA recommendations [9]. Overweight adults were defined as those with a BMI ≥ 25 kg/m^2, and obesity was defined as a BMI ≥ 30 kg/m^2 [9]. Overweight children and adolescents were defined as those with a BMI

≥ the 85th percentile, and obesity was defined as a BMI ≥ the 95th percentile, according to the patient's age and gender [20].

HbA1c values obtained in the last clinical visit and the corresponding measurement methods were collected from the patients' medical charts. HbA1c measurements were obtained for 3,099 patients (86.2%), using methods that were certified by the National Glycohemoglobin Standardization Program (NGSP); of these, 1,766 patients (51.3%) were evaluated using high-performance liquid chromatography, whereas 1,601 patients (46.6%) were evaluated using turbidimetry. Measurements of HbA1c obtained using methods that were not certified by the NGSP, missing data, and HbA1c measurements obtained more than one year before the study assessment were excluded from the glycemic control analyses (n = 494, 13.8%). FPG, triglycerides, HDL, and total cholesterol were measured using enzymatic techniques. LDL levels were calculated using Friedewald's equation [21]. BMI (kg/m^2) was determined by dividing an individual's weight (kg) by the square of his\her height (m^2). Current smoking was defined as smoking more than one cigarette per day at the time of the interview. Patients younger than 13 years of age were considered children (toddlers, preschoolers, or grade-schoolers), patients ≥ 13 years and ≤ 18 years were deemed adolescents, and patients > 18 years were considered adults [9].

2.1. Statistical Analysis. A detailed description of the study sample calculation has been given previously [18]. Briefly, the study sample represented the distribution of T1D cases across four geographic regions in Brazil. The proportion of cases from each region was estimated using the overall population distribution reported in the 2000 Brazilian Institute of Geography and Statistics Population Census (IBGE); 38.8%, 31.7%, 23.0%, and 6.6% of the population was distributed in the southeast, north/northeast, south, and midwest regions, respectively [22]. These data were combined with the national estimates of the prevalence of diabetes, which were derived from a 1988 survey, to determine the minimum number of patients to be studied in each region [23]. Recruitment in each region of the country enrolled > 95% of the estimated number of T1D patients for the region. Economic status was defined according to the Brazilian Economic Classification Criteria [24]. This classification also takes into account education level, which is categorized as illiterate/incomplete primary education, complete primary education/incomplete secondary education, complete secondary education/incomplete high school, complete high school/some college, or complete college education. The following classes of economic status were considered for this analysis: high, middle, low, and very low [24].

Data are presented as the means (± SD) for continuous variables and as counts (relative frequencies) for discrete variables. For analyzing blood pressure data, the mean from three consecutive measurements in a single day was used. Comparisons between numeric variables were performed using independent two-sided t-tests and two-sided z-tests for discrete variables with a normal approximation to the binomial distribution. An unadjusted Pearson's correlation coefficient was calculated when indicated. A multiple logistic regression (Forward-Wald) was performed with hypertension (yes/no) as the dependent variable. The following independent variables were included: race (Caucasian or non-Caucasian based on self-reporting), age, BMI, geographic region, gender, urine albumin excretion rate, and economic status. The Nagelkerke R square value was also calculated for this analysis. All of the analyses were performed using SPSS version 16.0 (Statistical Package of Social Sciences, Chicago, IL, USA). Odds ratios with 95% confidence intervals (CI) were performed when indicated. A two-sided P value less than 0.05 was considered significant.

Table 1: Demographic data of the studied population.

Variable	
Age, years	21.2 ± 11.7
Gender, female (%)	2,010 (56.0)
Age at diagnosis, years	10.0 (<1 to 44)
Age at diagnosis, years (%)	
0–4.9	667 (18.5)
5–9.9	961 (26.8)
10–14.9	941 (26.2)
≥15	1,022 (28.5)
Diabetes duration, years	9.6 ± 8.1
Diabetes duration, years (%)	
0–4.9	672 (18.7)
5–9.9	961 (26.8)
10–14.9	941 (26.2)
≥15	1,017 (28.3)
Level of care, n (%)	
Secondary	995 (27.7)
Tertiary	2,596 (72.3)
Geographic region (%)	
Southeast	1,424 (39.7)
North/northeast	1,113 (31)
South	820 (22.8)
Mid-west	234 (6.5)

The data are presented as counts (percentage), means ± SD, or medians (minimum/maximum). *African-Brazilians, Mulattos, Asians, and Native Indians; **Data were available for 3,434 patients.

3. Results

The clinical and demographic data for the study population are shown in Table 1. The majority of the patients evaluated were less than 30 years old (n = 1,077, 30%).

Due to missing data, in the total population of 3,591 patients, 268 (7.5%) could not be classified as either hypertensive or normotensive. Among the 3,323 (92.5%) T1D patients evaluated, a total of 689 (19.2%) of the studied patients were considered hypertensive, 236 (6.6%) were based on actual blood pressure measurements, and 453 (12.6%) were based on a history of or treatment for hypertension (self-reported). Hypertension was more frequent in adults than in children or adolescents (n = 562 (31.3%) versus n = 127 (8.3%), respectively, $P < 0.001$). Patients with hypertension were also

Table 2: Demographic, clinical, and laboratory data for the presence of hypertension in the studied population.

Variables	Hypertension*		*P* value
	Yes (%)	No (%)	
n (%)	689 (19.2)	2,634 (73.4)	—
Age, years	30.5 ± 12.8	19.7 ± 10.4	<0.001
Age at diagnosis of diabetes, years	14.8 ± 9.1	11.2 ± 7.6	0.19
Gender, female *n* (%)	318 (21.9)	1,136 (78.1)	0.19
Duration of diabetes, years	15.7 ± 9.6	8.5 ± 7.0	<0.001
Race, *n* (%)			<0.001
Caucasian	353 (18.2)	1,585 (81.8)	
Non-Caucasian	336 (24.3)	1,049 (75.7)	
Economic status, *n* (%)**			0.02
High	62 (26.6)	171 (73.4)	
Medium	174 (23.2)	575 (76.8)	
Low	208 (18.9)	890 (81.1)	
Very low	242 (21.3)	896 (78.6)	
BMI (Kg/m^2)	24.0 ± 4.7	21.4 ± 3.9	<0.001
Overweight/obesity, *n* (%)	276 (40.5)	767 (29.5)	<0.001
Fasting glycemia (mg/dL)	179.7 ± 107.1	182.5 ± 105.7	0.5
HbA1c (%)	9.3 ± 2.4	9.3 ± 2.3	0.8
HbA1c < 7%, *n* (%)	77 (12.8)	294 (13.0)	0.7
sBP (mmHg)	128.4 ± 20.7	107.9 ± 12.7	<0.001
dBP (mmHg)	81.31 ± 3.3	69.5 ± 9.4	<0.001
Total cholesterol (mg/dL)	178.5 ± 48.0	168.7 ± 39.6	<0.001
Triglycerides (mg/dL)	110.5 ± 85.4	89.0 ± 63.6	<0.001
HDL cholesterol (mg/dL)	53.4 ± 16.9	52.5 ± 14.0	<0.001
LDL cholesterol (mg/dL)	104.3 ± 39.7	99.0 ± 31.5	0.001
Current smoker, y (%)	31 (4.5)	110 (4.2)	0.9
Insulin dose (U/Kg/day)	0.83 ± 0.36	0.93 ± 0.39	<0.001
Number of clinical visits (previous year)	4.07 ± 1.7	4.10 ± 1.6	0.6

*Missing cases 268 (7.5%).
**Missing cases 130 (3.6%).
BMI: body mass index; sBP: systolic blood pressure; dBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein.
Overweight/obesity were considered together.
The data are presented as counts (percentage) or means ± SD; *African-Brazilians, Mulattos, Asians, and Native Indians.

older, exhibited longer duration of diabetes and had higher BMI, total cholesterol, triglycerides, LDL-C, and HDL-C values than patients without hypertension ($P < 0.001$ for all comparisons). These data are listed in Table 1.

A greater number of children and adolescents had missing blood pressure data than did adults (258 (96.3%) versus 10 (3.7%), respectively, $P < 0.001$), and these data are indicated in Table 2.

The mean age at the time of hypertension diagnosis was 20 ± 10.3 years, and the self-reported duration of hypertension was 3 years (range < 1 to 44 years). Patients who were aware of their hypertension were older ($P < 0.001$) and exhibited higher sBP ($P = 0.001$) and fewer borderline sBP of 140 mmHg ($P = 0.01$) and borderline dBP of 90 mmHg ($P = 0.02$) compared to patients who were unaware. A total of 370 (53.7%) of the hypertensive patients received treatment. More patients aware of their hypertensive status received treatment than did patients who were unaware of their condition ($P < 0.001$). These data are presented in Table 3.

Higher SBP and dBP values were also observed in treated patients compared to untreated patients (sBP: 132.99 ± 19.4 versus 123.1 ± 21.2 mmHg, respectively, $P < 0.001$, and dBP: 83.00 ± 12.32 versus 79.12 ± 14.20 mmHg, respectively, $P < 0.001$). In total, 207 (55.9%) of the 370 treated patients were administered only one antihypertensive agent; of these, 161 (43.5%) patients used ACE inhibitors and 46 (12.4%) patients received monotherapy with calcium channel blockers ($n = 5$, 1.3%), beta blockers ($n = 10$, 2.7%), angiotensin receptor blockers (ARBs) ($n = 15$, 4.1%), or diuretics ($n = 16$, 4.3%). A total of 122 (33%) patients received two drugs in the following combinations: ACE inhibitors plus diuretics, ARBs, beta blockers or calcium channel blockers, and ARBs

Table 3: Hypertension awareness.

Variables	Hypertension awareness		P value
	Yes (%)	No (%)	
n (%)	453 (65.7)	236 (34.3)	—
Children and adolescents, n (%)	28 (22)	99 (78)	<0.001*
Adults, n (%)	425 (75.6)	137 (24.4)	
sBP (mmHg)	129.8 ± 20.1	124.1 ± 21.9	0.001
dBP (mmHg)	81.5 ± 12.4	80.1 ± 15.2	0.1
Borderline sBP (140 mmHg) (%)	11	17.4	0.01
Borderline dBP (90 mmHg) (%)	17	28.4	0.01
Antihypertensive treatment (%)	67.3	11.8	<0.001

*$P < 0.001$ (children and adolescents versus adults).
sBP: systolic blood pressure; dBP: diastolic blood pressure.
The data are presented as counts (percentage) or means ± SD.

plus diuretics or calcium channel blockers. Forty-one (11.1%) patients received triple therapy with ACE inhibitors and diuretics plus ARBs, beta blockers or calcium channel blockers.

A total of 76 (22.9%) treated hypertensive patients achieved the targeted blood pressure range. The patients' sBP values correlated with age ($r = 0.47$, $P = 0.001$), diabetes duration ($r = 0.41$, $P < 0.001$), total insulin dose ($r = -0.17$, $P < 0.001$), AER ($r = 0.16$, $P < 0.001$), BMI ($r = 0.44$, $P < 0.001$), total cholesterol ($r = 0.11$, $P = 0.001$), triglycerides ($r = 0.10$, $P = 0.001$), HDL cholesterol ($r = 0.05$, $P = 0.01$) and LDL cholesterol ($r = 0.07$, $P = 0.001$). The dBP values correlated with age ($r = 0.40$, $P = 0.001$), diabetes duration ($r = 0.32$, $P < 0.001$), total insulin dose ($r = -0.12$, $P < 0.001$), AER ($r = 0.16$, $P < 0.001$), BMI ($r = 0.38$, $P < 0.001$), total cholesterol ($r = 0.15$, $P = 0.001$), triglycerides ($r = 0.14$, $P < 0.001$), HDL cholesterol ($r = 0.05$, $P = 0.01$), and LDL cholesterol ($r = 0.07$, $P = 0.001$).

Patients with proliferative retinopathy or nonproliferative retinopathy had higher sBP and dBP values than patients without retinopathy (sBP: 124.5 ± 20.6 versus 121.2 ± 19.2 versus 113.1 ± 15.6 mmHg, respectively, $P < 0.001$, and dBP: 78.5 ± 11.9 versus 76.9 ± 11.5 versus 72.5 ± 10.8 mmHg, respectively, $P < 0.001$). Additionally, patients with clinical nephropathy or microalbuminuria had higher sBP and dBP values than patients without nephropathy (sBP: 123.3 ± 21.1 versus 120.9 ± 17.9 versus 113.3 ± 15.8 mmHg, respectively, $P < 0.001$, and dBP: 78.7 ± 12.6 versus 76.6 ± 10.7 versus 72.5 ± 10.7 mmHg, respectively, $P < 0.001$).

Multivariate logistic analysis revealed that hypertension was directly associated with age (OR = 1.06; 95% CI (1.05–1.076; $P < 0.001$)), BMI (OR = 1.13; 95% CI (OR = 1.09–1.17; $P < 0.001$)), AER level [OR = 1.02; 95% CI (1.01–1.03; $P < 0.001$)] and male gender [OR = 1.35; 95% CI (1.02–1.80; $P < 0.001$)]. Caucasian race was also associated with a lower odds ratio of hypertension (OR = 0.68; 95% CI (0.51–0.91; $P = 0.01$)). This model described 25.3% of the probability of hypertension for a given patient.

4. Discussion

This study demonstrated that, while nearly 20% of the patients examined exhibited hypertension, only 53.7% of these patients received treatment. Moreover, only 22.3% of the treated hypertensive patients achieved the targeted sBP and dBP values. Hypertension was more common in non-Caucasian adults and was associated with microvascular complications and other cardiovascular risk factors, such as being overweight or obese and exhibiting dyslipidemia.

The ADA provides recommended blood pressure levels for all diabetic patients, but approximately 7.5% of the patients participating in the current study received no such evaluation in the year prior to the study. This was commonly observed primarily in children and adolescents, as well as individuals from the north/northeast and midwest regions of Brazil. Some diabetes clinical care centers in Brazil may not include blood pressure evaluations in their routine care of children and adolescents. Although hypertension was more frequent among adults (31.3%), in our diabetic study population, 8.3% of diabetic children and adolescents also were hypertensive. Few studies of hypertension in T1D patients have been conducted; the majority of these studies analyzed hypertension in adult diabetic patients and reported a prevalence of 24 to 43% [12–14, 25–29], which is similar to those observed in the current study. In addition, an observational study in Rio de Janeiro, Brazil, identified a hypertension prevalence of 6.8% in nondiabetic children and adolescents [30]. Our data on children and adolescents are similar to those published by the Search Study (5.9%) [26], although our prevalence figures were higher than those published (4%) in a recent Norwegian study [29]. Studies of elevated sBP or dBP in children and adolescents (greater than the 90th percentile for age, gender, and height) have reported a prevalence of hypertension between 6% and 23%, depending on the presence of other cardiovascular risk factors [26–29]. Additionally, the prevalence of hypertension has been shown to increase fourfold in overweight or obese children and adolescents [29]. Age, diabetes duration, the presence of chronic complications, race, and the number of medical visits with available blood pressure evaluations may account for the differences between our study and those conducted previously.

More than one-third of our patients who were unaware of their hypertensive condition were children and adolescents. Importantly, all of these patients were treated by an endocrinologist in secondary and tertiary care settings.

Diabetes treatment in public clinics is financed by the NBHCS, and our data reveal that factors other than medical recommendations might likely interfere with diabetes care in Brazil [18, 31].

The guidelines recommend aggressive hypertension treatment in T1D patients, but only 53.7% of our patients received such treatment; similar results were described in the EURODIAB study [13]. In the current study, the majority of treated patients (55.9%) received only one antihypertensive

drug, whereas 44.1% received two or more drugs. These results are in contrast with the results of previous studies reporting that up to 19% of T1D patients received two antihypertensive agents [12, 13]. In addition, only 11.1% of the patients in the current study received triple therapy, which is higher than the percentage described in the CACT1 (7%) and EURODIAB (1.9%) studies. The abovementioned studies were conducted 5 to 10 years before our study, suggesting that an increase in the intensity of hypertension treatment has occurred in recent years, as previously observed in a temporal analysis of EURODIAB [32]. However, less than one-third of our patients and T1D patients in the EURODIAB study [13] exhibited controlled sBP and dBP levels, which suggests that factors beyond pharmacological treatment might influence blood pressure control. Additionally, compared to our study, a larger percentage of the CACT1 patients (up to 64%) exhibited controlled blood pressure levels [12]. This difference may be attributed to study design, as the patients in the EURODIAB and our corresponding studies were not volunteers.

The Pittsburgh Epidemiology of Diabetes Complications Study utilized different targets for blood pressure and demonstrated small improvements in hypertension control, primarily in younger-aged groups of T1D patients, over a 10-year follow-up period [33]. One study that was performed at academic medical centers observed a low rate of medication management when T1D patients remained above their blood pressure goal [34]. As factors such as hypertension, obesity, and being overweight are indicators of CVD risk, we concluded that the young patients that were evaluated represent a high-risk group for the development of microvascular and macrovascular complications associated with diabetes, as described previously [4, 5, 26–28]. Furthermore, our study demonstrated a clear association between the different stages of retinopathy and nephropathy and increasing levels of blood pressure.

The BrazDiab1SG is the only national registry on the prevalence, awareness, and treatment of hypertension in T1D; the principal strength of our study is our large sample size, which included a representative sample of T1D distribution in the young Brazilian population. Importantly, our study included patients from a wide range of racial backgrounds from all geographic regions of the country, and it maintained a uniform, standard recruitment protocol at all of the participating centers.

However, several limitations of the current study must be addressed. We used a clinical definition of T1D that was assigned by physicians and was applicable to all patients, which is similar to previous studies [15, 16]. However, autoantibody and C-peptide levels were not measured. Therefore, some patients with other types of diabetes may have been included. Nevertheless, it is important to emphasize that 93.1% of our patients were diagnosed before the age of 30, which supports the high probability that these patients had T1D. Also, as all of the patients in this study lived in large cities and were seen in a public center by a specialist, patients who relied on primary care facilities and lived in rural areas may have been overlooked. Although 14% of the Brazilian population lives in rural areas, the prevalence of T1D in this group is very low [34], and consequently rural T1D patients represent the minority of patients who receive treatment in Brazil. Additionally, patients recruitment within each center may have produced a selection bias for age because the majority of our patients were younger than 30 years of age. Moreover, there were missing data for blood pressure measurements, which were primarily observed in the youngest patients. Additionally, the prevalence of hypertension may have been overestimated because diagnosis was based on the measurement of a blood pressure in one day rather than two separate measurements on two separate days. Although we used a standard clinical sphygmomanometer, the possibility for misclassification remains, especially, at borderline diagnosis levels for sBP and dBP. Misclassification was noted in our sample in the analysis of the lack of hypertension awareness, which was more frequent in the borderline group of patients. The use of self-reported hypertension as a criterion for awareness may have also produced a bias in the diagnosis of this condition.

Therefore, to our knowledge, this research constitutes the first national report on the prevalence of hypertension in T1D in Brazil, a disease with increasing incidence in our country [35]. Our results demonstrate that many T1D patients with hypertension do not receive antihypertensive treatment; moreover, few treated T1D patients receive combined therapy, and few of these patients achieve their targeted sBP and dBP values. The evaluation of blood pressure in children and adolescents is likely not included in all routine diabetic clinical care centers. Thus, greater attention should be paid to blood pressure evaluation and hypertension diagnosis and treatment for T1D patients in Brazil.

Appendix

*Brazilian Type 1 Diabetes Study Group (BrazDiab1SG)

Executive Steering Committee: Marilia Brito Gomes (Chair), Roberta Cobas, Sergio Atala Dib and Carlos Negrato. Universidade Estado Rio de Janeiro: Roberta Cobas*, Alessandra Matheus, Lucianne Tannus; Universidade Federal Rio de Janeiro: Lenita Zajdenverg*, Melanie Rodacki; Hospital Geral de Bonsucesso: Neuza Braga Campos de Araújo*, Marilena de Menezes Cordeiro; Hospital Universitário Clementino Fraga Filho—IPPMG: Dr. Jorge Luiz Luescher*; Renata Szundy Berardo; Serviço de Diabetes da Disciplina de Endocrinologia e Metabologia do Hospital das Clínicas da Universidade de São Paulo: Marcia Nery*; Catarina Cani; Maria do Carmo Arruda Marques; Unidade de Endocrinologia Pediátrica da Santa Casa de Misericórdia de São Paulo: Luiz Eduardo Calliari*, Renata Maria de Noronha; Instituto da Criança do Hospital das Clínicas da Universidade de São Paulo: Thais Della Manna*, Roberta Salvodelli, Fernanda Garcia Penha; Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto—USP: Milton Cesar Foss*, Maria Cristina Foss-Freitas; Ambulatório da Faculdade Estadual de Medicina de São José do Rio Preto: Antonio Carlos Pires*, Fernando Cesar Robles; Associação de Diabéticos de

Bauru: Carlos Antonio Negrato*, Maria de Fatima Guedes; Centro de Diabetes da Escola Paulista de Medicina: Sergio Atala Dib*, Patricia Dualib; Clínica de Endocrinologia da Santa Casa de Belo Horizonte Setor Diabetes Tipo 1: Saulo Cavalcanti da Silva*, Janice Sepulveda; Ambulatório Multiprofissional de Atendimento à Diabetes do Hospital de Clínicas da Universidade Estadual de Londrina: Henriqueta Guidio de Almeida*, Emerson Sampaio; Hospital de Clínicas da Universidade Federal do Paraná: Rosangela Roginski Rea*, Ana Cristina Ravazzani de Almeida Faria; Instituto da Criança com Diabete Rio Grande Sul: Balduino Tschiedel*, Suzana Lavigne, Gustavo Adolfo Cardozo; Hospital de Clínicas de Porto Alegre: Mirela Azevedo*, Luis Henrique Canani, Alessandra Teixeira Zucatti; Hospital Universitário de Santa Catarina: Marisa Helena Cesar Coral*, Daniela Aline Pereira; Instituto de Diabetes-Endocrinologia de Joinville: Luiz Antonio de Araujo*; Hospital Regional de Taguatinga, Brasília: Hermelinda Cordeiro Pedrosa*, Monica Tolentino; Flaviene Alves Prado; Hospital Geral de Goiânia: Dr Alberto Rassi: Nelson Rassi*, Leticia Bretones de Araujo; Centro de Diabetes e Endocrinologia do Estado da Bahia: Reine Marie Chaves Fonseca*; Alexis Dourado Guedes, Odelisa Silva de Mattos; Universidade Federal do Maranhão: Manuel Faria*, Rossana Azulay; Centro Integrado de Diabetes e Hipertensão do Ceará: Adriana Costa e Forti*, Maria Cristina Façanha; Universidade Federal do Ceará: Renan Montenegro Junior*, Ana Paula Montenegro; Universidade Federal de Sergipe: Naira Horta Melo*, Karla Freire Rezende; Hospital Universitário Alcides Carneiro: Alberto Ramos*; Hospital Universitário João de Barros Barreto, Pará: João Soares Felicio*, Flavia Marques Santos; Hospital Universitário Getúlio Vargas, Hospital Adriano Jorge: Deborah Laredo Jezini*.

Abbreviations

ADA:	American Diabetes Association
CV:	Cardiovascular
T1D:	Type 1 diabetes
BDS:	Brazilian Diabetes Society
sBP:	Systolic blood pressure
dBP:	Diastolic blood pressure
BMI:	Body mass index
HbA1C:	Glycated hemoglobin
T2D:	Type 2 diabetes
FBG:	Fasting blood glucose
NBHCS:	National Brazilian Health Care System
BrazDiab1SG:	Brazilian Type 1 Diabetes Study Group,
SBGM:	Self-blood glucose monitoring
HDL:	High-density lipoprotein
LDL:	Low-density lipoprotein
NGSP:	National Glycohemoglobin Standardization Program
ACE:	Angiotensin-converting enzyme
ARBs:	Angiotensin receptor blockers
AER:	Albumin excretion rate.

Consent

Written informed consent for the study was obtained from all patients or their parents when necessary.

Acknowledgments

The authors thank Mrs. Karianne Aroeira Davidson, Mrs. Anna Maria Ferreira, and Mrs. Elisangela Santos for their technical assistance. This work was supported by grants from the Farmanguinhos/Fundação Oswaldo Cruz/National Health Ministry, Brazilian Diabetes Society, Fundação do Amparo à Pesquisa do Estado do Rio de Janeiro, and Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 653789, 15 pages
http://dx.doi.org/10.1155/2013/653789

Review Article

Impact of Diabetes on Cardiovascular Disease: An Update

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Received 6 December 2012; Revised 31 January 2013; Accepted 31 January 2013

Academic Editor: Mario Fritsch Neves

Cardiovascular diseases are the most prevalent cause of morbidity and mortality among patients with type 1 or type 2 diabetes. The proposed mechanisms that can link accelerated atherosclerosis and increased cardiovascular risk in this population are poorly understood. It has been suggested that an association between hyperglycemia and intracellular metabolic changes can result in oxidative stress, low-grade inflammation, and endothelial dysfunction. Recently, epigenetic factors by different types of reactions are known to be responsible for the interaction between genes and environment and for this reason can also account for the association between diabetes and cardiovascular disease. The impact of clinical factors that may coexist with diabetes such as obesity, dyslipidemia, and hypertension are also discussed. Furthermore, evidence that justify screening for subclinical atherosclerosis in asymptomatic patients is controversial and is also matter of this review. The purpose of this paper is to describe the association between poor glycemic control, oxidative stress, markers of insulin resistance, and of low-grade inflammation that have been suggested as putative factors linking diabetes and cardiovascular disease.

1. Introduction

Diabetes is an important chronic disease which incidence is globally increasing and though considered as an epidemic [1]. The World Health Organization (WHO) estimated there were 30 million people who had diabetes worldwide in 1985. This number increased to 135 million by 1995 and reached 217 million in 2005. By the year 2030 WHO predicts this number will increase to at least 366 million [1]. This growth in diabetes prevalence, driven principally by an increased prevalence of type 2 diabetes (T2D), is occurring in both developing and developed countries [1]. The incidence of type 1 diabetes (T1D) is also increasing in parallel to that of T2D worldwide [2–4].

Individuals with diabetes and with chronically poor metabolic control can experience microvascular and macrovascular complications leading to a significant burden for the individual and for the society. This burden includes direct costs of medical care and indirect costs, such as loss of productivity, which result from diabetes-related morbidity and premature mortality [5, 6]. Health care expenses for people with diabetes is more than double of that for people without diabetes; the direct and indirect expenditures attributable to diabetes in 2007 in the USA were conservatively estimated at $174 billion, with slightly more spent on chronic complications attributable to diabetes than on diabetes care itself [6]. The International Diabetes Federation (IDF) estimated that diabetes accounts for 5–10% of the total healthcare budget in many countries [3]. The outpatient costs of T2D in Brazil were estimated by the ESCUDI study in 2011 [7]. The total costs were US$ 2,108 per patient/year, which consisted mostly of direct costs (63.3%) [7].

Cardiovascular diseases (CVD) are the most prevalent cause of mortality and morbidity among people with T2D and T1D [8–10]. In 2004, in the USA the presence of CVD

and stroke was found in 68% and 16% of deaths related to diabetes among people older than 65 years, respectively [11]. Adult people with diabetes present rates of mortality due to heart disease and stroke from two to four times higher than those without diabetes [11]. It has been stated that patients with T2D without a previous history of myocardial infarction have the same risk of coronary artery disease (CADs) as nondiabetic subjects with a history of myocardial infarction [12]; this has led the National Cholesterol Education Program to consider diabetes as a coronary heart disease risk equivalent [13]. However, there is still some uncertainty as to whether the cardiovascular risk conferred by diabetes is truly equivalent to that of a previous myocardial infarction [14]. In general, patients with diabetes aggregate other comorbidities such as obesity, hypertension, and dyslipidemia which also contribute to increase the risk for CVD [15]. In the period of 2005 to 2008, the American Diabetes Association (ADA) estimated that 67% of people with diabetes older than 20 years presented blood pressure levels ≥140/90 mmHg or were using antihypertensive drugs [16]. Although there is strong evidence that supports both the efficacy and cost effectiveness of programs directed towards an improvement of glycemic control and other cardiovascular risk factors in patients with T2D [17] and T1D [18], the majority of these patients [19, 20] never achieve the goals established by guidelines issued by diabetes societies [16, 21].

The underlying mechanisms that cause accelerated atherosclerosis in patients with diabetes and consequently an increased prevalence of CVD are poorly understood. The purpose of this paper is to describe the association between poor glycemic control, oxidative stress, markers of insulin resistance, and of low-grade inflammation that have been suggested as putative factors linking these two conditions.

2. The Role of Glycemic Control

In recent decades, several clinical trials have investigated the effect of intensive treatment of hyperglycemia on cardiovascular risk reduction, in both T2D [22–25] and T1D [26] presenting conflicting results. The clinical characteristics of the studied populations regarding the presence of CVD and the duration of diabetes as well as the type of intensive intervention performed and the goals to be achieved partly explain the differences in the results.

In the *United Kingdom Prospective Diabetes Study* (UKPDS) [22], in newly diagnosed T2D patients, the early intensive treatment of hyperglycemia within the first five years of disease resulted in a long-term cardiovascular benefit, compared with patients in the conventional treatment group. This benefit was observed even after the loss of difference in glycemic control between the groups that occurred during the further five years of observational followup. However, the same was not observed in the three other large clinical trials conducted in patients with T2D. In the *Veterans Affairs Diabetes Trial* (VADT) [23], older patients with a 10 years mean duration of diabetes had no cardiovascular benefit when submitted to an intensive glycemic control regimen. This population comprised 40% of patients with a previous history of cardiovascular disease. Similar results were obtained from the *Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation* (ADVANCE) trial [24] which aimed to achieve an A1c of 6.5% through intensive treatment with gliclazide plus other drugs. This strategy did not reduce the rate of major macrovascular events or death, despite a reduction in the incidence of diabetic nephropathy. As in VADT, patients were older (mean age of 66 years) and had a longer duration of diabetes (8 years) than UKPDS patients when the intensive treatment was started. In contrast, more strict intensive treatment aiming to reduce HbA1c below 6% in T2D patients, as occurred in the *Action to Control Cardiovascular Risk in Diabetes* (ACCORD) trial [25], in addition to showing no benefit in reducing macrovascular events resulted in increased mortality, weight gain, and risk of hypoglycemia. Patients in this study presented a high cardiovascular risk profile when first initiated intensive glycemic treatment. Table 1 presents the main differences between these trials.

In T1D patients, *the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications* (DCCT/EDIC) study [26] showed the cardiovascular benefits of an intensive glycemic control after a followup of 17 years. The patients in this study were treated intensively for about 6.5 years and followed for 10 years observationally. Even after losing the strict glycemic control, represented by a glycated hemoglobin level below 7%, during the observational period, the group previously intensively treated presented a reduction of any cardiovascular event by 42%.

The main lesson learned from these results is that intensive treatment of hyperglycemia, targeting glycated hemoglobin levels below 7%, when initiated early in patients with short duration of diabetes and low cardiovascular risk, results in cardiovascular benefits. The same is not true when looking up tighter glycemic targets in older patients exposed to hyperglycemia for years before and with a higher cardiovascular risk profile.

This early protection is postulated to result from a mechanism known as "metabolic memory," which means that the effect of the early glycemic exposure environment is remembered later in target organs [27] resulting in long-term deleterious or protective effects. The mechanisms involved in this process appear to comprehend epigenetic changes and intracellular metabolic changes that result in oxidative stress, low-grade inflammation, and endothelial dysfunction (Figure 1). These topics will be discussed below.

3. Obesity

Obesity, which prevalence is also increasing worldwide [28] is becoming a major public health issue due to its association with chronic diseases such as diabetes mellitus, hypertension, dyslipidemia, sleep apnea, osteoarticular disease, and cardio and cerebrovascular diseases [28]. According to data from the WHO in 2008, the global prevalence of obesity (body mass index (BMI) $\geq 30\ kg/m^2$) was 10% in men and 14% in women. Data from the National Health and Nutrition Examination

Table 1: Differences of the effects of glycemic control in cardiovascular risk reduction in type 2 diabetes.

	UKPDS-10 years	VADT	ADVANCE	ACCORD
Sample size	5,102*	1,791	11,140	10,251
Followup (years)	10	5.6	5	3.4
Baseline characteristics				
Age (years)	54	60.4	58	62.2
Duration of diabetes (years)	Recently diagnosed	11.5	8	10
Presence of cardiovascular disease	9%	40%	32%	35%
Presence of microvascular complications	18%	62%	10%	Albuminuria 14.0 (6.9–45.8)#
A1c levels	6.2%	8.3%	7.5%	8.3%
Effects of intensive treatment				
Difference in A1c levels (intensive/conventional)	7.0/7.9%	6.9/8.4%	6.5/7.3%	6.4/7.5%
Reduction in macrovascular events	Sulfa/insulin group ↓15% MI, ↓13% death Metformin group ↓33% MI, ↓27% death	NS	NS	↓Nonfatal MI ↑Death
Reduction in microvascular events (diabetic retinopathy, nephropathy, or neuropathy)	↓24% (combined)	NS	↓incident nephropathy	—

NS: nonstatistically significant.
A1c: glycated hemoglobin; MI: myocardial infarction.
*3,277 posttrial monitoring.
#The percentage of subjects with microvascular complications is not available. Ratio of urinary albumin (mg) to creatinine (g); median (Interquartile range).

Survey (NHANES) showed that the prevalence of overweight and obesity in adults increased from 55.9% to 64.5% and from 22.9% to 30.5%, from 1988–1994 to 1999-2000, respectively [28].

Obesity, especially with visceral fat deposition, is associated with low-grade inflammation, which plays a role in the pathogenesis of diabetes, and both diseases are associated with significant increase in morbidity and mortality due to CVD [28–30].

The main determinants for the onset of diabetes are beyond genetic factors, obesity and sedentary lifestyle [31]. Several studies have shown decreased incidence of diabetes by nonpharmacologic treatments, lifestyle changes, and body weight reduction. The Finish Diabetes Prevention Study Group showed that the incidence of diabetes was reduced in 58% in the group with only intensive lifestyle changes [31]. The Diabetes Prevention Program (DPP), diabetes incidence was reduced by 58% with intensive lifestyle intervention when compared to placebo and remained reduced by 34% after 10 years of followup [32]. Therefore, efforts should be made to encourage the adoption of healthy lifestyle and thus to combat the obesity epidemic.

4. Dyslipidemia

Dyslipidemia in T2D worsens cardiovascular risk due to the peculiar atherogenic profile composed by increased very low-density lipoprotein (VLDL) cholesterol, triglycerides and small and dense LDL cholesterol levels and decreased high-density lipoprotein (HDL) cholesterol levels. With such lipoproteins modified by oxidation and glycosylation there is a reduction on vascular compliance predisposing to early and aggressive atherosclerosis [33]. This may also occur in T1D, even though they are young patients and seldom present lipid abnormalities, but in this case, the atherogenic profile is not caused exclusively by increased lipid levels, and hyperglycemia *per se* is also pivotal in this process [34]. This was evidenced in an experimental study which concluded that either diabetic hyperlipidemia or hyperglycemia accelerates distinct phases of atherogenesis in diabetes [35]. In this study, it was shown that the dyslipidemia associated with diabetes is not sufficient to initiate the atherosclerotic lesion, because the progression of atherosclerosis process could be normalized after intensive glycemic control with insulin in mice [35].

In many interventional studies, the reduction of LDL cholesterol and triglycerides and increase of HDL cholesterol have been proved to be effective in reducing macrovascular disease and mortality in patients with T2D, especially in those with previous CAD.

The *Collaborative Atorvastatin Diabetes Study* (CARDS) was the first trial that studied T2D patients without previous CVD. Intervention with atorvastatin 10 mg showed 37% reduction in Cardiovascular events and 48% reduction in stroke when compared to placebo [36]. In the *HDL Atherosclerosis Treatment Study* (HATS), the combined use of low doses of simvastatin (10 to 20 mg/day) with high doses

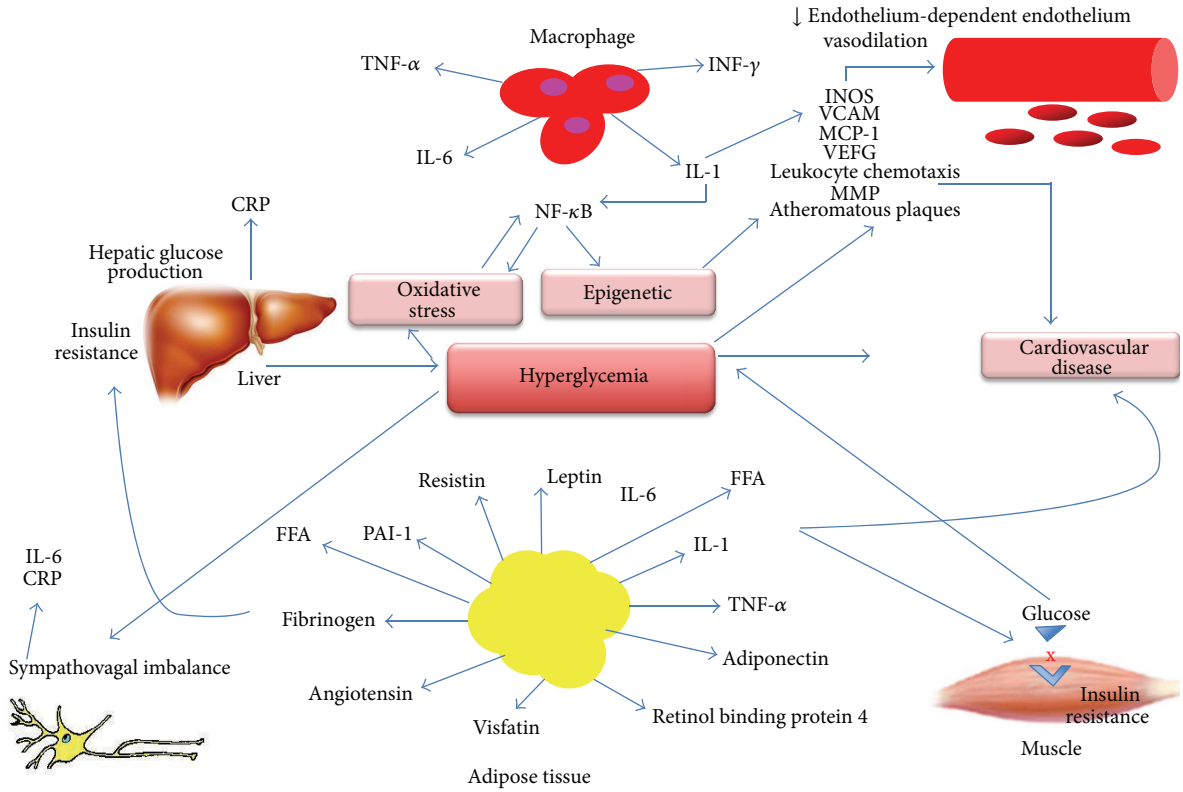

Figure 1: Pathogenesis of cardiovascular disease in diabetes. The mechanisms involved in the pathogenesis of cardiovascular disease in diabetes comprehend epigenetic changes and intracellular metabolic changes that result in oxidative stress, low-grade inflammation, and endothelial dysfunction. CRP: C-reactive protein; FFA: free fatty acids; INOS: inducible nitric oxide synthase; IL-1: interleukin 1; IL-6: interleukin 6; MCP-1: monocyte chemoattractant molecule 1; MMP: matrix metalloproitenase; NF-κB: nuclear factor kappa-β; PAI-1: plasminogen activator inhibitor-1; VCAM-1; vascular cell adhesion molecule-1; VEFG: vascular endothelial growth factor; TNF-α: Tumor necrosis factor-α; INF-γ: Interferon-γ.

of niacin (2 to 4 g/day) showed a reduction in absolute risk of 13% for cardiovascular outcomes when HDL reached the target [37]. The study TNT (treatment to new targets) studied patients with T2D with previous CVD and compared the use of Atorvastatin 10 mg (conventional group) with Atorvastatin 80 mg (intensive group), and the goals were 100 mg and 80 mg for LDL cholesterol, respectively. The aggressive target achieved in this study (1.9 mmol/L) showed the most reduced rates of mortality due to cardiovascular events among all studies with statins [38].

Although decreasing LDL cholesterol has brought enough and established evidence on reducing cardiovascular mortality in T2D, if the treatment of dyslipidemia starts too late it may not be effective in avoiding atherosclerosis progression. According to the Deutsche Diabetes Dialyze Study (4D) that studied 1,255 T2D patients with end-stage renal disease which were randomized to Atorvastatin 20 mg/day or matching placebo during four years, there was no significant reduction in cardiovascular events with the intervention when compared to placebo [39]. Concerning cholesterol goals for diabetics, as far as we know, we should get as lower cholesterol levels as possible as stated by National Cholesterol Education Program Adult Treatment Panel III Guidelines (NCEP ATP III) [40]. It is well established that diabetic subjects are considered to belong to a high-risk category, thus their benefit from LDL-lowering therapy appears when LDL-C goal of 1.8 mmol/L is achieved [40].

In a recent meta-analysis which reviewed 22 trials with statins versus control, it was showed that statin use could be associated with an increased incidence of diabetes [40]. Despite the fact that an immediate doubling in cardiovascular risk in individuals with 5-year risk of major vascular events lower than 10%, such an effect is more than 50-times smaller than the absolute benefit observed with statin therapy in such individuals (about 11 fewer major vascular events per 1,000 treated over 5 years per 1.0 mmol/L reduction in LDL cholesterol) [41].

Considering hypertriglyceridemia, there is little evidence to support the benefits the goals to be achieved can bring. Fibrates are recommended to reduce pancreatitis risk in patients with triglycerides levels above 4.5 mmol/L when lifestyle modification does not succeed [42]. Until recently, there were no data that support that the combined use of statins and fibrates could reduce cardiovascular mortality. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study was a multinational randomized trial

conducted with 9,795 patients with T2D which showed that fenofibrate did not significantly reduce the risk of the primary outcomes of coronary events. Instead, it reduced the number of total cardiovascular events (fewer nonfatal myocardial infarctions and revascularizations) [43]. Another message from this study was that fibrate confers microvascular protection, because it reduced the need for laser treatment for diabetic retinopathy [44].

5. Hypertension

Hypertension is a highly prevalent disease worldwide and very common among patients with diabetes. Approximately from 10 to 30% of T1D and 60% of T2D patients have hypertension [45, 46].

The coexistence of these two conditions increase the risk of developing macrovascular complications (myocardial infarction, stroke) and also microvascular complications (nephropathy and retinopathy) [47]. The vigorous treatment of hypertension may reduce the progression of these complications.

The time hypertension starts differs in different types of diabetes. In patients with T1D, hypertension develops years after diagnosis usually already reflecting the development of diabetic nephropathy [46]. Blood pressure (BP) tends to increase three years after the onset of microalbuminuria [48]. In patients with T2D, hypertension may be present at diagnosis or even before the elevation of blood glucose levels [49]. The association between hypertension and obesity is well established leading to a higher rate of cardiovascular morbidity and mortality in patients with these two conditions [49].

The recommended target blood pressure for patients with diabetes, according to the ADA [50] is characterized by BP < 130/80 mmHg [51]. Although, the European Society of Hypertension Task Force [52] states that BP goals traditionally recommended in diabetes are not supported by outcomes evidence from trials. They also reinforce only to pursue a reasonable BP reduction without indicating a goal which is unproven, since it has also been very difficult to achieve blood pressure goals in the majority of the patients [53].

According to the ADVANCE study in diabetic patients at high cardiovascular risk lower BP levels should be reached [53]. One should always take into consideration the individualization of treatment and its correlation with response to therapy, drug tolerance, and individual characteristics. However, randomized clinical trials have demonstrated that the established therapeutic target (BP < 130/80 mmHg) own benefits in reducing CHD, stroke, and kidney disease [52, 54]. In patients with renal insufficiency and proteinuria above 1 to 2 g per day, the target BP should approach 120/75 mmHg [53].

The treatment of hypertension in diabetic patients aims at the prevention of CVD, minimizing the progression of renal disease and diabetic retinopathy. According to the UKPDS, patients with T2D may benefit more from tight control of BP than with strict control of blood glucose levels [55]. Initial treatment should include nonpharmacological measures such as weight reduction (in overweight and obesity), regular exercising, reducing salt intake (<1500 mg per day), avoiding excessive alcohol consumption (no more than two servings per day in men and no more than one serving per day in women), and smoking cessation. Pharmacological therapy should be initiated in all diabetics who persist with BP > 130/80 mmHg, when a change in lifestyle has already been implemented for 3 months or when the maximum BP levels are already higher than 140/90 mmHg at diagnosis [50, 56].

Pharmacological therapy can be accomplished with various classes of antihypertensive agents. Diuretics, angiotensin converting enzyme inhibitors, angiotensin II antagonists, beta blockers, calcium channel blockers, alpha blockers, and combination of blockers of the renin-angiotensin have shown to be effective in reducing cardiovascular events. In most cases, the association of two or three drugs may be necessary in order to achieve the goals of the treatment.

The ACCORD-BP study, evaluating more intensive treatment of blood pressure (systolic blood pressure reduction aiming at levels lower than 120 mmHg) in patients with T2D and CVD or at least two cardiovascular risk factors, showed no reduction in cardiovascular events rates (myocardial infarction, CHF, and cardiovascular death), although it was observed a reduction in the number of strokes [57].

6. Oxidative Stress

Increased intracellular glucose concentrations result in the activation of alternative pathways of metabolism such as the hexosamine and the aldose reductase pathways, both involved in the pathophysiology of chronic complications of diabetes. These pathways trigger an increased production of reactive oxygen species (ROS) and depletes substrates for important antioxidant enzymes. Additionally, increased intracellular glucose leads to the formation of advanced glycation end products (AGES) and the activation of protein kinase C (PKC). All these mechanisms lead to a common effect, an increased oxidative stress state.

Oxidative stress results from an imbalance between the production of ROS and the antioxidant defense. The ROSs are chemically instable and highly reactive molecules [58] continuously produced by aerobic organisms that function as second messengers regulating the expression of redox signal sensitive genes (e.g., nuclear factor kappa-β (NFκ-B) gene) and in the production of inflammatory mediators. They are generated from enzymes that use oxygen as electron acceptor including the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, nitric oxide synthase (NOS), xanthine oxidase, the mitochondrial chain electron transport, lipoxygenase, cyclooxygenase, and cytochrome P450. The first three are the main sources of ROS in the vascular wall [59].

The active form of NADPH oxidase is responsible for the reduction of the molecular oxygen resulting in the formation of superoxide anion [$NAD(P)H + 2O_2 \rightarrow NAD(P)^+ + H^+ + 2O_2^-$] [58]. This enzyme could act as a sensor of the concentration of oxygen in the vasculature modulating the vascular tone [60]. Components of the NADPH oxidase

were demonstrated in vascular and renal cells in animals and humans [61–67].

The ROSs produced in the vascular wall are involved in various cellular events such as mitosis, apoptosis, migration, hypertrophy and extracellular matrix modification, and changes in gene transcription and protein synthesis [68]. They may also function as mediators of the metabolic memory to hyperglycemia. Human retinal endothelial cells exposed to hyperglycemia in vitro, maintained high levels of oxidative stress markers such as PKC and β subunits of NADPH oxidase p47phox, even after normalization of blood glucose levels [69].

Another important source of ROS in diabetes is the mitochondria. It is postulated that the mitochondrial O_2^- anion acts as a factor initiating a cascade of events that result in increased production of ROS and reactive nitrogen species (RNS) through activation of NFκ-B. This results in the production of inflammatory cytokines, activation of PKC and NADPH oxidase. In addition, NOS can divert the production of nitric oxide (NO) to generate O_2^- in conditions of deficiency of L-arginine or tetrahydropterin in the endothelium of diabetic patients [70]. When both are produced the formation of peroxynitrite (NOO^-) occurs, causing damage to cellular structures such as DNA, lipids, and proteins [71].

Under normal conditions, the presence of ROS induces the expression of antioxidant enzymes as a defense mechanism. This is not a rule under diabetes condition. For instance, in fibroblasts from T1D patients with overt nephropathy, the exposure to hyperglycemia led to an increase in lipid peroxidation without a compensatory increase in the level of the antioxidant enzyme Cu-Zn superoxide dismutase, catalase, and glutathione peroxidase [72]. Even patients with a short diabetes duration and without chronic complications present less antioxidant plasma capacity and uric acid levels suggesting that the oxidative stress occurs early in the disease [73].

Nonenzymatic extracellular antioxidants include α-tocopherol, vitamin A, β-carotene, ascorbic acid, albumin, and uric acid. The lipid solubility properties of α-tocopherol, vitamin A, and β-carotene are particularly important to protect against lipid peroxidation. The role of uric acid in the pathogenesis of CVD and endothelial dysfunction is still conflicting [74–76]. Another important component of the antioxidant defense in diabetes is haptoglobin [77–79]. This plasma protein binds free hemoglobin resulting in the inhibition of iron-induced oxidative damage, since hemoglobin released in the blood after hemolysis of senescent erythrocytes is a potent oxidant.

7. Epigenetics

Nowadays, there is compelling evidence linking epigenetic factors to many human diseases including diabetes and CVD [80]. Epigenetic factors, by different types of reactions, could mediate the interplay between genes and environment resulting in activation or repression of genetic transcription, or even silencing the genetic transcription. The most important epigenetic reactions affecting genetic transcription are acetylation and methylation. These reactions occur mainly in the tail of histones that are proteins where DNA is wrapped. Brownlee et al. [81] have demonstrated in human aortic endothelial cells that excess ROS resulting from hyperglycemia can induce monomethylation of lysine from histone 3 increasing the expression of the subunit p65 of NFκ-B. This reaction is responsible for the increased transcription of vascular cell adhesion molecule 1 (VCAM-1), monocyte chemoattractant molecule 1 (MCP-1), and some inflammatory proteins like interleukin 6 (IL-6), intercellular adhesion molecule 1 (ICAM-1), and NOS that are related to hyperglycemia-induced arterial pathology. Moreover, this reaction persisted after a six-day period of subsequent normoglycemia, supporting the concept of metabolic memory. Epigenetic reactions could be an important mediator between diabetes, CVD, and chronic inflammatory response. Besides, some comorbidities associated with diabetes have also been associated with epigenetics like hypertension [82] and obesity [83]. The epigenetic modifications associated with hypertension are related to intrauterine environmental factors which can limit the development of the nephrons and to other factors that are related to autonomic responsiveness, vessel remodeling, salt sensitivity, and to the renin-angiotensin system. The mechanisms involved in these associations are mainly methylation of histones and of DNA. The relationship between epigenetics and obesity is more complex and is related to genomic imprinting, epigenetic mosaicism, and nonimprinted gene which through different pathways can influence energy balance, body weight, and fat mass.

8. Inflammatory Cascade, Diabetes, and Atherosclerosis

Diabetes, obesity, and insulin resistance are associated with subclinical inflammation characterized by overexpression of cytokines produced by adipose tissue, activated macrophages, and other cells [84, 85]. Inflammatory mediators, such as TNF-α, interleukin-1 (IL-1), IL-6, leptin, resistin, MCP-1, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), fibrinogen, angiotensin, visfatin, retinol binding protein-4, and adiponectin are involved in signaling pathways, in insulin action, and perpetuation of inflammatory response [85]. These cytokines are involved in the chronic inflammatory process of the vessels wall, promoting lipid accumulation with consequent development of atherosclerosis and CVD [86].

Atherosclerosis is a complex multifactorial disease, and the acceleration of atherosclerosis in diabetes may be explained by several conditions including hyperglycemia, increased oxidative stress, advanced glycation end products (AGE), dyslipidemia, autonomic imbalance, hyperinsulinemia, inflammatory markers excess, and genetic variables [35, 87, 88].

It is assumed that the adipose tissue initiates obesity-induced inflammation and leads to the recruitment of immune cells which contributes to the maintenance of inflammatory response [84], besides leading to endothelial

dysfunction with increased expression of adhesion molecules (ICAM-1, VCAM-1, P-selectin, and E-selectin), migration of monocytes, neutrophils, and T lymphocytes [89].

Insulin resistance induces chronic elevation in FFA plasma concentrations leading to increased storage of triglycerides in muscle, promoting reduction of muscle glucose uptake and liver, and increased hepatic glucose production, that have been shown to impair insulin action and promote hyperinsulinemia [90]. Hyperinsulinemia can, per se, induce cardiomyocyte hypertrophy through myocyte growth induced by an activation of PI3 K/Akt-1 pathway and also by enhancing FFA levels. FFA are also implicated in the development of myocardial contractile dysfunction [91].

Several cytokines described to be related with insulin resistance are also involved with the development of atherosclerosis and CVD. TNF-α and other cytokines, FFA and ROS, activate inflammatory pathways and promote the expression of numerous genes involved in insulin resistance [84, 85, 89].

IL-1 is another cytokine produced as a consequence of stress or cell injury mainly by macrophages that modulate key events in the process of atherosclerosis such as vessels wall inflammation, leukocyte chemotaxis and adhesion by increasing expression of VCAM-1 and MCP-1, angiogenesis (through vascular endothelial growth factor—(VEFG) induction), upregulation of matrix metalloproteinases (MMP), and destabilization of atheromatous plaques, that can lead to plaque rupture and thrombosis [86].

CRP is an acute phase protein and is primarily derived from IL-6 hepatic biosynthesis [92]. Atherogenic mechanisms of CRP include impaired production of endothelial NO and prostacyclin; increased production of endothelin-1 and other cell adhesion molecules, monocyte chemoattractant protein-1, IL-8, and PAI-1; ROS and proinflammatory macrophage production; monocyte adhesion and chemotaxis; uptake of oxidized low-density lipoprotein (LDL); CRP also stimulates the expression of metalloproteinases, activates NF-κB, and promotes cell proliferation in vascular smooth muscle cells due to upregulation of the angiotensin type 1 receptor [93].

Adiponectin has many protective actions in the atherosclerosis process due to its inhibition of LDL oxidation, activation of macrophages (via TNF-α), reduction of adhesion molecule (VCAM and ICAM), inhibition of proliferation and migration, of smooth cells, and an increased production of NO in endothelial cells [87]. Adiponectin is markedly reduced with increased obesity, and in diabetes [85] and hypoadiponectinemia is associated with an increase in CVD rates [94].

Leptin is a hormone secreted by adipose tissue and primarily involved in the regulation of energy expenditure and food intake. Plasma leptin concentrations are increased in obese and diabetic patients [95]. Leptin has been shown to participate in the development of atherosclerosis in several ways: inducing oxidative stress; increasing the production of MCP-1, endotelin-1 (ET-1) which leads to cardiomyocyte hypertrophy; promoting migration, proliferation, hypertrophy of vascular smooth muscle cells (VSMC), and vascular cell wall calcification; stimulating platelet aggregation; attenuating cardiomyocyte contractility through increased nitric oxide production, reduction of intracellular calcium, and decreased β-adrenergic response [95].

Therefore, evidences suggest that the hypothesis that is low-grade inflammation would be the causal common factor between diabetes, insulin resistance, obesity, and CVD [32].

9. Endothelial Dysfunction

Endothelial vasodilation and vascular reactivity in diabetes are known to be impaired since its early phases [96, 97]. This is explained by the hypothesis that there are changes in endothelial cells function present in the early atherosclerosis lesion [98]. Thus, oxidative stress, inflammation, and endothelial dysfunction are closely correlated in diabetes, because the formers increase vascular endothelial permeability, generating leukocyte adhesion, which is coupled with impairment in endothelial signal transduction and redox-regulated transcription factors [99, 100]. Another possible mechanism to link these conditions is that the impaired endothelium-dependent vasodilation in diabetes is associated with reduced action of NO secondary to its inactivation, and this is a consequence of oxidative stress, rather than decreased NO production from endothelial cells. Moreover, the abnormal metabolism of NO is related to advanced diabetes microvascular complications [99]. Many factors can explain endothelial dysfunction in diabetes such as hyperlipidemia [96, 98], insulin resistance [86, 98, 101], hyperglycemia [98], hyperamylinemia [101], hypertension [101], and hyperhomocysteinemia [101].

10. Endothelial Dysfunction in T1D

Endothelial function of the macro- and microcirculation, which is usually evaluated through the vasodilator response to endothelium-dependent vasodilators or physiological stimuli, is characteristically impaired in patients with T1D [76, 102–104]. The endothelial response to acetylcholine is correlated with diabetes duration, glycemic control, triglycerides, and age [76, 105, 106].

Endothelial dysfunction in T1D is an important determinant of inflammatory activity regardless of the presence or absence of complications showing that it can be considered an early marker for CVD [107]. The disturbances in vascular responses can be seen even in children with T1D, as evidenced in studies that showed impaired flow-mediated dilation (FMD) responses and also association with increased carotid artery intima-media thickness in this group [97, 108]. And, as evidenced by Davi et al. [97], this alteration represents an early and, in some cases, a reversible event in the natural history of T1D in children and adolescents because it was noted that in approximately 45% of this population the tissue plasminogen activator (tPA) levels were reversed after 1 year.

Several markers of endothelial function in T1D have been described such as Von Willebrand factor, thrombomodulin, selectin, PAI-1, Type IV collagen, and tPA, that are so forth indicators of endothelial cell dysfunction when increased.

VCAM-1 levels are more markedly increased in patients with T1D with retinopathy when compared with those with micro- or macroalbuminuria only [98]. It has been shown that the cellular adhesion molecule E-selectin may enhance CAD prediction beyond traditional risk factors in T1D [98, 107]. Other markers of low-grade inflammation levels are described to be elevated in this group such as of oxidized LDL [109], monocyte IL-6, superoxide anion, plasma CRP, sCD40L, and nitrotyrosine levels [110].

So, endothelial dysfunction in T1D represents a high risk for micro- and macroangiopathy and hyperglycemia, appears to be one of the main causes, that alone seems not to be sufficient to cause it, because other agents such as genes and environmental factors are likely to play a role [76, 98].

11. Endothelial Dysfunction in T2D

T2D is independently associated with impaired FMD, and endothelial dysfunction is considered to be the determinant factor for the vascular complications that is aggravated, rather than caused by hyperglycemia, because of the presence of many other risk factors such as obesity, hypertension, dislypidemia, and ageing as well [96, 101, 111]. One possible explanation for this is the increased calpain (calcium-dependent protease) activity in response to hyperglycemia. Hyperglycemic states can induce loss of NO via a calpain-dependent decrease in the association with endothelial NOS. Moreover, inhibition of calpain activity decreases endothelial cell surface expression of the proinflammatory adhesion molecules ICAM-1 and VCAM-1 during hyperglycemia [112]. Markers of endothelial dysfunction are early signs for the development of microangiopathy.

The hallmark of T2D is insulin resistance, therefore there is sufficient evidence pointing to the coexistence of endothelial dysfunction with this condition [101]. Elevated circulating levels of PAI-1 and ET-1 can be seen in obesity as well as the correlation between endothelial activation and acute-phase reaction with insulin resistance and obesity in T2D. Abnormalities in vascular reactivity and insulin resistance can also be seen in young first-degree relatives of T2D patients independent of the presence of classic cardiovascular risk factors [113].

12. Cardiovascular Autonomic Neuropathy (CAN)

CAN is one of the most common chronic complications of diabetes mellitus and has shown negative impact on survival and quality of life in patients with diabetes [114]. The prevalence of CAN ranges from 2.6% to 90% among subjects with diabetes, and the incidence of CAN increases with age, diabetes duration, and inadequate glycemic control [115].

Recent studies [116, 117] have shown that dysregulation of the Autonomic Nervous System (ANS) with increased sympathetic activity is associated with elevated inflammatory markers such as IL-6 and CRP, demonstrating a link between autonomic imbalance, inflammation, and CVD. The ANS is responsible for modulating the activity of the sinus node (heart rate), ventricular (end systolic and diastolic volume) and blood vessels (systemic vascular resistance), and the dysfunction of the ANS may contribute to the development of arterial stiffness, left ventricular hypertrophy, and ventricular diastolic dysfunction [118].

The clinical manifestations of CAN are described as resting tachycardia, postural hypotension, exercise intolerance, abnormal coronary vasomotor regulation (risk of silent myocardial ischemia and infarction), increased QT interval, perioperative instability, increased risk of renal disease, stroke, and sudden death [114].

CAN represents a strong indicator of cardiovascular risk in both T1D and T2D [119]. In the Detection of Silent Myocardial Ischemia in Asymptomatic Diabetic Subjects (DIAD) study, the strongest predictors for abnormal perfusion tests were abnormal Valsalva maneuver, male sex, and diabetes duration, demonstrating that CAN may have an important role in the screening of CVD [120]. Patients with diabetes and CAN have 5-year mortality rates ranging from 16 to 53%, depending on its severity [119]. The mortality rates from CVD in T1D and T2D are 4.2 and 10 times higher, respectively, than in healthy individuals without diabetes [120, 121].

13. Screening for Subclinical Atherosclerosis

The screening for the detection of subclinical atherosclerosis in asymptomatic diabetic patients is the subject of considerable controversy. There are no prospective studies that support its usefulness and that can modify the natural history of those patients [122, 123]. Even today, there is no consensus on which tests should be performed. Intensive medical therapy seems to provide equal outcomes to invasive revascularization [124]. Which raises questions on how screening results would change management? The clinical risk factors that indicate increased risk of CVD in diabetic patients are CAD, cerebrovascular or peripheral vascular disease, female sex, age greater than 40 years in men and greater than 50 years in women, long duration of diabetes (for every 10 years the risk increases 86% according to the Framingham study), presence of renal disease, autonomic neuropathy and classic risk factors such as hypertension, dyslipidemia, smoking, sedentary lifestyle, family early atherosclerotic disease, metabolic syndrome, and presence of atrial fibrillation [124, 125].

One of the major limitations of the routine screening for subclinical atherosclerosis is the different rates of coronary events in previous studies. The prevalence of silent myocardial ischemia (SMI) in diabetic population varies in different studies, ranging from 12% to almost 57% [126, 127]. This variability underlines the difficulty to have a cost-effective screening and the necessity to define the cardiovascular risk in the asymptomatic diabetic population who could benefit from this screening. The ADA does not recommend the detection of CVD in asymptomatic diabetic patients as a routine. Their recommendations for investigating SMI are very conservative, being the exercise testing in diabetic patients with typical (chest pain, dyspnea) or atypical cardiac symptoms and changes in baseline electrocardiogram. Asymptomatic patients with carotid or peripheral vascular

disease or sedentary patients who want to start high-intensity exercise can also be investigated [50]. The DIAD study [120] accessed 1,123 asymptomatic diabetic patients in a randomized controlled trial. The patients were randomly assigned to be screened with adenosine-stress radionuclide myocardial perfusion imaging (MPI) or not to be screened. The cumulative cardiac event rate was 2.9% over a mean (SD) followup of 4.8 years for an average rate of 0.6% per year. A comparison of the cardiac event rates (0.6% per year) with those reported in ACCORD trial for the subgroup of patients with T2D without previous cardiac events (1.4% per year) which included a selection of older patients with specific additional risk factors for CVD would appear favorable and compatible in these two studies [25]. The data from these two studies show that there is no evidence that the complete survey of subclinical arterial disease may modify the natural history of CAD in asymptomatic diabetic patients with risk factors controlled by recommended goals.

Despite the controversy regarding the screening, several studies using various invasive and noninvasive cardiovascular examinations are being conducted. The presence of calcium in coronary arteries is a specific marker of atherosclerosis, independent of its etiology [128]. The presence of calcified plaques correlates with increasing age, especially after age 50 [129, 130]. Though the calcium score represents an estimate of the total amount of plaque present in an individual, it does not correspond directly to the degree of luminal narrowing of a given vessel [128]. The calcium score was higher than the scores of Framingham and UKPDS for the prediction of events [129]. According to the Patients with Renal Impairment and Diabetes undergoing Computed Tomography (PREDICT) study, the coronary artery calcium (CAC) score was taken as independent risk marker for incremental coronary events and stroke [130].

The US National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) recommends the use of calcium score in a selection of patients with intermediate risk by traditional methods (between 10 and 20% risk in 10 years), and when added to conventional methods, these patients may become high risk, and benefit of a therapy aimed to more restrictive treatment targets [13]. A recently meta-analysis showed that diabetic patients without a history of myocardial infarction had 43% less risk of developing coronary events when compared with patients without diabetes but with prior infarction [14]. Both coronary calcifications as average intimal thickness are increased in this population; however, the classification of these individuals to a higher category of risk is still controversial when using these methods [131]. Although being very promising the use of the calcium score for CVD in asymptomatic diabetic patients still needs further prospective studies and cost effectiveness to demonstrate its benefits.

Revascularization of asymptomatic T2D subjects is still polemic. The Bypass Angioplasty Revascularization Investigation 2 Diabetes Trial (BARI 2D) was a randomized study with 2,368 patients with T2D and SMI comparing revascularization versus intensive medical therapy, showed no differences on reducing rates of death and cardiovascular events among patients undergoing prompt revascularization and those undergoing medical therapy or between strategies insulin sensitizers or insulin provision [123].

According to the 2010 American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults [132], in asymptomatic adults with diabetes, 40 years and older, measurement of CAC score is reasonable for cardiovascular risk assessment (Class IIa, evidence B). Stress MPI may be considered for advanced cardiovascular risk assessment in asymptomatic adults with diabetes or when previous risk assessment testing suggests a high risk of CHD, such as CAC score of 400 or greater (Class IIb, evidence C).

Carotid intima-media thickness (C-IMT) is considered one of the independent predictors of coronary artery disease, a marker of early atherosclerosis and vascular remodeling [133]. According to Irie et al. in an evaluation of 251 asymptomatic T2D patients, the addition of max-IMT (the greatest IMT in the observation-possible areas) to conventional risk factors improves the risk stratification for CAD [133]. In T1D patients, the DCCT/EDIC research group demonstrated that in 12 years after the DCCT intervention the C-IMT progression in the group that received intensive diabetes therapy was slower than the group that received conventional therapy from years 1 to 6. It could be assigned to a durable "metabolic memory" that exists for atherosclerosis. But, the similar C-IMT progression in the original treatment groups over EDIC from years 6 to 12 indicates a "metabolic memory amnesia" over time [134].

The ankle brachial pressure index (ABPI) is a simple method to evaluate the presence of peripheral vascular diseases [135]. A low ABPI (<0.9) was considered to be a marker of cardiovascular diseases risk. The AHA recommends the evaluation of ABPI as a diagnostic criterion for the prevalence of peripheral arterial diseases [136]. In the study of Doza et al. with 1,121 T2D patients in north India, the prevalence of low ABPI was 4.5% in men and 4.7% in woman. The results were similar to those found in studies with Chinese Korean and Brazilian populations [137].

Changing the natural history of silent coronary artery disease, without considering the control of classical risk factors, represents a major challenge facing the global epidemic of diabetes mellitus. More research is needed to identify appropriate screening strategies for diabetic asymptomatic patients with CHD.

14. Perspectives and Conclusions

The incidence of diabetes is sharply increasing worldwide which represents an important burden for patients and for the society as well due to micro- and macrovascular complications that people with this condition may experience and consequently cardiovascular diseases that are the most prevalent causes of morbidity and mortality among patients with diabetes.

The classical risk factors for the development of CVD in subjects with diabetes are the presence of poor glycemic control, obesity, dyslipidemia, and hypertension. In recent

decades, several clinical trials have investigated the effect of intensive treatment of hyperglycemia on cardiovascular risk reduction, in both T1D and T2D, like the DCCT and UKPDS, and the main lesson learned from these trials is that intensive treatment of hyperglycemia initiated early in patients with short duration of diabetes and low cardiovascular risk, result in cardiovascular benefits. The same is not true for older patients exposed to hyperglycemia for a long time and with a high cardiovascular risk profile. This protection might result from a mechanism known as "metabolic memory," which means that the effect of the early glycemic exposure environment is imprinted in target organs resulting in long-term deleterious or protective effects. Obesity, especially with visceral fat deposition, is associated with low-grade inflammation, which plays a role in the pathogenesis of diabetes, and both diseases are associated with significant increase in morbidity and mortality due to CVD. Dyslipidemia mainly that represented by high levels of LDL-cholesterol is also a risk factor for CVD because small increases in LDL-cholesterol levels increase the risk for CVD. The coexistence of hypertension and diabetes increase the risk of developing macrovascular complications (myocardial infarction, stroke) and also microvascular complications (nephropathy and retinopathy).

These clinical conditions might be associated with intracellular and mitochondrial metabolic changes that can result in oxidative stress, a state of low-grade inflammation characterized by overexpression of cytokines produced by adipose tissue, activated macrophages and other cells, and the presence of many inflammatory mediators that will finally cause a generalized endothelial dysfunction or even a cardiovascular autonomic neuropathy, an important cause of sudden death among subjects with diabetes.

The proposed mechanisms that can link accelerated atherosclerosis and increased cardiovascular risk in subjects with diabetes are still poorly understood. It has been suggested that an association between hyperglycemia and epigenetic factors by different types of reactions could be responsible for the interaction between genes and environment and for this reason account for the association between diabetes and cardiovascular disease. Many trials have shown that an early intervention in patients with short duration of diabetes could result in cardiovascular benefits, but there is no robust evidence that justify screening for subclinical atherosclerosis in asymptomatic patients with diabetes.

The purpose of this paper was to describe the association between poor glycemic control, oxidative stress, markers of insulin resistance and of low-grade inflammation that have been suggested as putative factors linking diabetes, and cardiovascular disease and to elucidate the mechanisms involved in the pathogenesis of CVD in this population.

Abbreviations

ABPI: Ankle brachial pressure index
ACCORD: Action to Control Cardiovascular Risk in Diabetes
ACCF: American College of Cardiology Foundation
ADA: American Diabetes Association
ADVANCE: Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation
AGE: Advanced glycation end products
AHA: American Heart Association
ANS: Autonomic Nervous System
BARI 2D: Bypass Angioplasty Revascularization Investigation 2 Diabetes Trial
BMI: Body mass index
BP: Blood pressure
CAC: Coronary artery calcium
CAD: Coronary artery disease
CAN: Cardiac Autonomic Neuropathy
CARDS Collaborative Atorvastatin Diabetes Study
CHF: Congestive heart failure
CIMT: Carotid intima-media thickness
CRP: C-reactive protein
CVD: Cardiovascular disease
DCCT: Diabetes Control and Complications Trial
DIAD: Detection of Silent Myocardial Ischemia in Asymptomatic Diabetic Subjects
DPP: The Diabetes Prevention Program
EDIC: Epidemiology of Diabetes Interventions and Complications Trial
ERK: Extracellular signal regulated kinase
ET-1: Endotelin-1
FFA: Free fatty acids
FIELD: Fenofibrate Intervention and Event Lowering in Diabetes
FMD: flow-mediated dilation
GSK-3: Glycogen synthases kinase-3
HATS: HDL Atherosclerosis Treatment Study
HbA1c; Glycated hemoglobin
HDL: High-density lipoprotein
ICAM-1 Intercellular adhesion molecule 1
IDF: International Diabetes Federation;
IL-1: Interleukin 1
IL-6: Interleukin 6
IKKβ: Inhibitor of nuclear factor kappa-B kinase subunit beta
IRS-1: Insulin receptor substrate-1
LDL: Low-density lipoprotein
MCP-1: Monocyte chemoattractant molecule 1
MMP: Matrix metalloproteinase
MPI: Myocardial perfusion imaging
mTOR: Mammalian target of rapamycin
NADPH: Nicotinamide adenine dinucleotide phosphate
NCEP ATP III: National Cholesterol Education Programme Adult Treatment Panel III
NFAT-3: Nuclear factor in activated lymphocytes
NF-κB: Nuclear factor kappa-β
NCEP ATP III: National Cholesterol Education Program Adult Treatment Panel III Guidelines
NHANES: National Health and Nutrition Examination Survey
NO: Nitric oxide
NOO^-: Peroxynitrite

NOS: Nitric oxide synthase
PAI-1: Plasminogen activator inhibitor-1
PDGF: Platelet derived growth factor
PKC: Protein kinase C
PPAR-α: Peroxisome proliferator-activated receptor α
PREDICT: Patients with Renal Impairment and Diabetes undergoing Computed Tomography
RNS Reactive nitrogen species
ROS: Reactive oxygen species
sBP: Systolic blood pressure
SMI: Silent myocardial ischemia
T1D: Type 1 diabetes
T2D: Type 2 diabetes
TGF-β: Transforming growth factor-β
TNT: Treatment to new targets
tPA: Tissue plasminogen activator
UKPDS: United Kingdom Prospective Diabetes Study
WHO: World Health Organization
VADT: Veterans Affairs Diabetes Trial
VCAM-1; Vascular cell adhesion molecule-1
VEFG: Vascular endothelial growth factor
VLDL: Very low-density lipoprotein
VSMC: Vascular smooth muscle cells.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 541689, 9 pages
http://dx.doi.org/10.1155/2013/541689

Clinical Study

Improved Hypertension Control with the Imidazoline Agonist Moxonidine in a Multinational Metabolic Syndrome Population: Principal Results of the MERSY Study

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Received 19 November 2012; Accepted 12 January 2013

Academic Editor: Eduardo Vera Tibiriçá

This study was designed to assess the effects of moxonidine on blood pressure and aspects of the metabolic syndrome in racially diverse population of patients encountered in routine medical practice. Physicians collected data on a minimum of three consecutive patients with uncontrolled essential hypertension and criteria for metabolic syndrome, eligible to receive moxonidine (0.2–0.4 mg once daily) for 6 months, either as monotherapy or as adjunct therapy to current antihypertensive treatment. Systolic and diastolic blood pressure (BP) declined by an average of 24.5 + 14.3 mmHg and 12.6 + 9.1 mmHg, respectively. BP responder rates defined as attaining BP < 140/90 mmHg were significantly ($P < 0.001$) and substantially higher among younger patients, nonpostmenopausal women, and patients receiving monotherapy. While potentially relevant improvements in the entire cohort were observed in regard to body weight (−2.1 ± 5.4 kg), fasting plasma glucose (from 6.8 to 6.2 mmol/L), and triglycerides (2.4 to 2.0 mmol/L), statistically significant changes in metabolic parameters could only be detected in subgroup analyses. Moxonidine therapy reduced blood pressure and improved rates of blood pressure control in this group of patients. While the observed trend towards improvement in various metabolic parameters merits further investigation, the overall effect of moxonidine treatment is consistent with a reduction of total cardiovascular risk in this hypertensive metabolic syndrome cohort.

1. Introduction

Hypertension is a major contributor to cardiovascular disease (CVD) risk, but a patient's global CVD risk is determined by the interplay of multiple risk factors. In particular, the grouping of risk factors—elevated blood pressure, abdominal obesity, dyslipidaemia, and abnormalities of glucose and insulin metabolism, commonly referred to as metabolic syndrome has been associated with a substantial worsening of cardiovascular prognosis and all-cause mortality [1]. Postmenopausal women may be particularly susceptible to the development of metabolic syndrome and to its CVD consequences [2–5], including resistance to antihypertensive therapy [6].

Abdominal obesity is not only a cardinal feature of the metabolic syndrome but also an important contributor to the development and progression of cardiovascular and metabolic disturbances linked to the syndrome. Overactivity of the sympathetic nervous system (SNS) is of particular importance in this context. The interplay between obesity, elevated SNS activity, and hypertensive target organ damage is already demonstrable in very young overweight or obese adults [7, 8]. Elevated blood pressure may be initiated and sustained by increased SNS activation, as are metabolic alterations, inflammatory pathways, and target organ damage [8].

Targeting the SNS directly, therefore, provides a logical and attractive therapeutic target [7–12] in that it simultaneously addresses several relevant elements of the metabolic syndrome and therefore would be expected to reduce overall CVD risk to a greater extent than its isolated effect on blood pressure (BP) might predict.

Moxonidine is a widely approved antihypertensive drug that lowers BP primarily by reducing central SNS activity via activation of imidazoline type-1 receptors in the rostral ventrolateral medulla [13]. In addition to its efficacy as an antihypertensive, moxonidine has been shown to improve indices of glycaemic control, aspects of the plasma lipid profile, and inflammatory markers [14–16]. Furthermore, its use has been associated with reduction in body weight [16–21]. The unique profile of this agent may provide an opportunity to simultaneously address a large number of factors crucially involved in the pathophysiology of the metabolic syndrome associated with elevated blood pressure and may offer several benefits in the management of hypertensive patients with metabolic syndrome.

To this end, we conducted a large, multinational study to appraise the effects of moxonidine on blood pressure, anthropometric, lipid, and metabolic parameters of the metabolic syndrome in patients encountered in routine (real world) medical practice.

2. Methods

2.1. Study Design. MERSY was a multinational, open-label, observational study with a planned duration of 6 months. Participating physicians were asked to collect data on a minimum of three consecutive patients with uncontrolled essential hypertension and metabolic syndrome, for whom moxonidine might be prescribed.

The primary objective was to evaluate the long-term safety and efficacy of moxonidine in hypertensive patients with metabolic syndrome. The secondary objective was to assess the effect of long-term treatment of moxonidine on laboratory parameters associated with the metabolic syndrome.

Moxonidine was prescribed at a dose of 0.2–0.4 mg once daily, either as monotherapy or as adjunctive therapy when the current antihypertensive treatment (which was to the discretion of the treating physician) was insufficient to achieve individual blood pressure targets or if it was not tolerated. The preferred maintenance dose of moxonidine was 0.4 mg/day, but physicians were permitted to initiate therapy at 0.2 mg and titrate to 0.4 mg/day after 2 weeks. After the baseline visit, a first follow-up visit was scheduled for between 1 and 3 months, according to the treating physician's usual practice for such consultations. A final visit was planned 6 months after starting moxonidine therapy. The study had no formal mechanisms to monitor compliance.

Adult patients (age ≥ 18 years) of either sex were eligible for enrolment if they had essential hypertension of any grade, as defined by the 2003 guidelines of the European Society of Hypertension. Patients were either newly diagnosed as hypertensive or had BP levels that were above target despite the use of other antihypertensive drugs measured according to the 2003 guidelines of the European Society of Hypertension or had failed to tolerate current antihypertensive treatment. For Australia only, supplementary inclusion criteria specified age not greater than 75 years and the persistence of hypertension despite concurrent antihypertensive therapy.

Criteria for a diagnosis of metabolic syndrome were based on the 2005 definition proposed by the International Diabetes Federation and comprised central obesity (defined as waist circumference ≥94 cm for Europid men and ≥80 cm for Europid women, with ethnicity-specific values for other groups) plus any two of the following: triglyceride (TG) levels ≥150 mg/dL (≥1.7 mmol/L) or specific treatment for this lipid abnormality; high-density lipoprotein (HDL) cholesterol <40 mg/dL (<1.03 mmol/L) in men or <50 mg/dL (<1.29 mmol/L) in women, or specific treatment for this lipid abnormality; systolic BP (SBP) ≥ 130 mmHg or diastolic BP (DBP) ≥ 85 mmHg, or treatment of previously diagnosed hypertension; fasting plasma glucose (FPG) ≥ 100 mg/dL (≥5.6 mmol/L) or previously diagnosed type 2 diabetes. If FPG was >5.6 mmol/L (>100 mg/dL), an oral glucose tolerance test was recommended, but this was not compulsory. Specific advice on lifestyle modification was not mandated by the protocol.

The only criterion prohibiting patients from taking part in this study was the presence of contraindications to moxonidine, as identified in the relevant National Summary of Product Characteristics (SPC).

2.2. Statistics and Data Analysis. From observations and experience in a previous postmarketing surveillance study [21], it was estimated that a study population of 2488 patients would be required for satisfactory statistical power. In order to allow for dropout and loss of data, a recruitment target of 3600 patients, recruited via 1200 physicians, was specified.

Nominal qualitative variables were compared using the χ^2 test or Fisher's exact test. Ordinal qualitative variables were compared using the Wilcoxon test or the Kruskal-Wallis test. Quantitative variables were compared using variance analysis.

BP, laboratory and weight parameters were compared between visits using covariance analysis, with the baseline value as the adjusted variable.

The absolute changes in heart rate (HR) between the baseline and postbaseline visits were summarized and analyzed through a one-sample t-test. All tests were two sided, with significance declared at the 5% level.

In addition to pooled results, subgroup analyses were undertaken according to menopause status (as determined by questioning), type of antihypertensive regimen (monotherapy or combination therapy), and age (<65 years and ≥65 years).

Data management and statistical analysis were conducted by the FOVEA Group, Rueil-Malmaison, France. Data entry was performed using Access version 9.0. Double data entry was used. Entered data were verified against case record form data when a discrepancy was found during double data entry. Quality control was performed using SAS version 8.2.

2.3. Efficacy Endpoints. The primary efficacy variable was the percentage of patients responding to antihypertensive therapy during the study. A response was defined as attainment of systemic arterial BP < 140/90 mmHg from baseline to

Table 1: Summary demographic details of the intent-to-treat (ITT) population.

Total patients	$N = 5603$
Sex ($n = 5554$)	
Male	2793 (50.2%)
Female	2772 (49.8%)
Age (yrs) ($n = 5554$)	
<40	397 (7.1%)
40–49	1045 (18.8%)
50–59	1854 (33.4%)
60–69	1458 (26.2%)
>69	804 (14.5%)
<65	4102 (73.9%)
≥65	1452 (26.1%)
Menopause status ($n = 2615$)	
Postmenopausal	1856 (71.0%)
Non postmenopausal	759 (29.0%)
Height (mean ± SD, cm) ($n = 5464$)	168.1 ± 8.9
Weight (mean ± SD, kg) ($n = 5464$)	91.9 ± 15.6
BMI (mean ± SD, kg/m^2) ($n = 5464$)	32.5 ± 5.0
Waist circumference (mean ± SD, cm) ($n = 5195$)	104.6 ± 13.3
Hip circumference (mean ± SD, cm) ($n = 4722$)	107.7 ± 13.7
Race/ethnicity ($n = 4815$)	
White	2312 (48.0%)
American Indian or Alaska native	1496 (31.1%)
Asian	835 (17.3%)
Black of African heritage or African American	149 (3.1%)
Native Hawaiian or other Pacific Islander	17 (0.4%)
Aboriginal/Torres Strait Islander	6 (0.1%)
Smoker status ($n = 5453$)	
Yes	1292 (23.7%)
No	4161 (76.3%)

Table 2: Baseline metabolic indices in the intent-to-treat (ITT) population. The sample sizes for variables are less than the full ITT population ($n = 5603$), due to lack of data.

	Means ± SD (mmol/L)
Fasting plasma glucose ($n = 2551$)	6.8 ± 2.1
Triglycerides ($n = 2288$)	2.4 ± 1.1
Cholesterol ($n = 2305$)	5.8 ± 1.1
HDL-cholesterol ($n = 1893$)	1.2 ± 0.5
LDL-cholesterol ($n = 1421$)	3.5 ± 1.1
Creatinine ($n = 1952$)	0.09 ± 0.06
Urinary albumin ($n = 272$)	92.7 ± 191.6

each follow-up visit. BP limits of <130/80 mmHg were set for patients with a diagnosis of diabetes at baseline.

Secondary efficacy variables comprised the absolute change in BP from baseline to each follow-up visit, the absolute change in laboratory parameters for metabolic syndrome (FPG, TG, total cholesterol, HDL-cholesterol, low-density lipoprotein (LDL)-cholesterol, and creatinine, urinary albumin) from baseline to final visit at 6 months, and the absolute change in weight parameters (body mass index (BMI), waist-hip circumferences) from baseline to each follow-up visit.

2.4. Safety Endpoints. Suspect adverse drug reactions (SADRs)—defined as a response to a drug that was noxious and unintended and that occurred at doses normally used in humans for prophylaxis or treatment of a disease or to modify physical function—were screened for via active enquiry during follow-up and final visits. Each SADR was evaluated for duration, severity (mild, moderate, or severe), and seriousness. Physicians' assessment of the causal relationship to the investigational drug was documented, as was the action taken to address all SADRs and the outcome of each event. Special provisions were made for the reporting of all SADRs regarded as serious. Any pregnancies identified during the study were recorded and monitored as discrete events.

2.5. Administration and Ethical Considerations. The study was conducted in accordance with the ICH GCP (1997) and the Therapeutic Goods Administration (TGA) "Note for Guidance on Good Clinical Practice" (CPMP/ICH/135/95) annotated with TGA comments (July 2000). Patients were free to withdraw from the study at any time, for any reason, specified or unspecified, without prejudice to their medical care. Physicians were free to exclude any patient at any time if this was judged to be in the interests of the patient.

3. Results

3.1. Patient Profile and Treatments. The MERSY study was conducted in 13 countries between December 2006 and March 2008. Figure 1 illustrates the derivation of the four patient populations. The present analysis reports primarily data from the intent-to-treat (ITT) ($N = 5603$) and safety ($n = 5879$) populations. Two countries (Bahrain and Switzerland) did not recruit any patients.

Principal demographic details of the ITT population are depicted in Tables 1 and 2. Mean BP at baseline was (158.3 ± 13.8)/(94.1 ± 8.7) mmHg. Mean SBP and DBP were ~4 mmHg lower in younger patients (<65 years) than in older ones ((157.6 ± 13.5)/(95.1 ± 8.4) mmHg versus (160.3 ± 14.4)/(91.6 ± 9.1) mmHg; $P < 0.001$), ~1 mmHg lower in nonpostmenopausal women (SBP 157.8 ± 13.7 mmHg versus 158.9 ± 14.1 mmHg; $P < 0.001$), and ~5 mmHg higher in patients receiving multiple antihypertensive medications than in those prescribed monotherapy ((159.2 ± 14.1)/(65.0 ± 13.4) mmHg versus (154.7 ± 11.7)/(60.9 ± 12.1) mmHg; $P < 0.001$ versus monotherapy). Differing national legislations precluded a complete audit of ethnicity data.

There was statistical evidence of variations in various metabolic syndrome-related metabolic parameters according

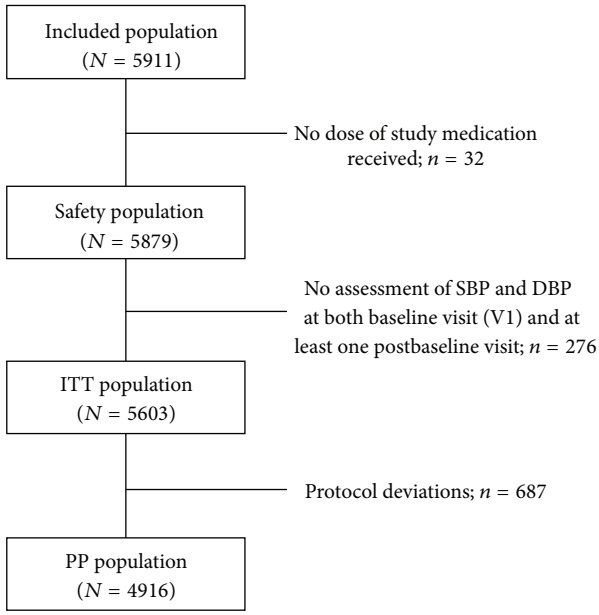

Figure 1: CONSORT summary of population recruitment.

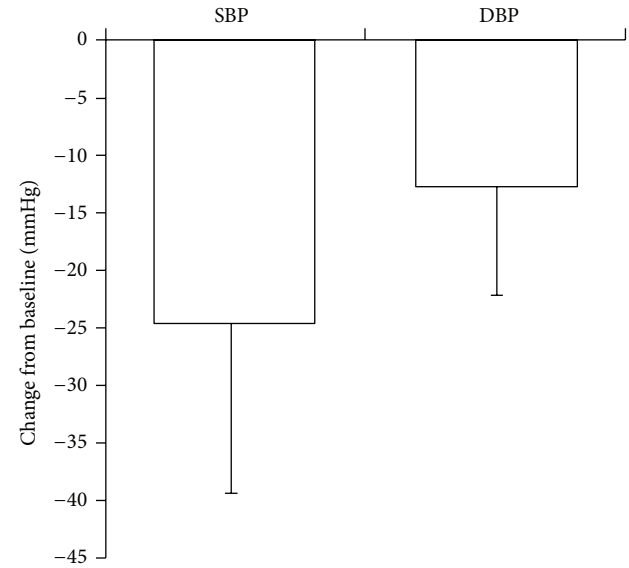

Figure 2: SBP and DBP responses during moxonidine therapy. Mean ± SD.

to age, therapeutic regimen, and menopause status, but with the exception of FPG according to menopause status (6.9 ± 2.1 mmol/L in postmenopausal women versus 6.6 ± 2.1 mmol/L in nonpostmenopausal women) ($P = 0.001$), these differences were numerically small (data not shown).

In the month preceding the baseline visit, most patients ($n = 3506$) had received multiagent combination therapy for BP control. Diuretics were the single most widely prescribed class of drugs recorded in this subset of patients ($n = 2277$). A further 1200 patients had received monotherapy, of which 605 had received either an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB), 199 had received a calcium-channel blocker, 160 had been prescribed a beta-blocker, and 142 had received a diuretic. A further 719 patients had received no antihypertensive medication during that month.

A baseline diagnosis of diabetes was present for 47.1% (2623) of the ITT population for whom data were available ($n = 5567$), with diabetes proportionately more often recorded in older patients (54.2% at age ≥ 65 years versus 44.8% at <65 years), those taking multiple antihypertensive drugs (51.5% versus 28.7% versus monotherapy), and postmenopausal women (50.5% versus 35.2% versus nonpostmenopausal) ($P < 0.001$ for all comparisons).

Documented reasons for initiating moxonidine therapy comprised lack of efficacy of current antihypertensive medication ($n = 3885$), intolerance to current antihypertensive medication ($n = 286$), or a new diagnosis of hypertension ($n = 886$); 138 cases were classified as "other reasons." Lack of efficacy of current therapies was more likely to be the reason in older patients, postmenopausal patients, and patients taking multiple antihypertensive drugs, whereas a new diagnosis of hypertension was proportionately more common in younger nonpostmenopausal patients.

Moxonidine 0.2 mg/day was prescribed to 1731 patients at the baseline visit. Doses up to 0.4 mg/day were prescribed to a further 3635 patients. Among the 4118 patients of the ITT cohort for whom medication data were available from the last study visit, 20.0% ($n = 823$) were being prescribed moxonidine 0.2 mg/day at that time and 76.3% were prescribed doses up to 0.4 mg/day ($n = 3143$). The percentage of patients who received moxonidine as monotherapy (19-20%) or as part of multiple combination therapy (80-81%) remained constant throughout the study.

3.2. Primary Efficacy Endpoint. The proportion of patients classified as responding to hypertension therapy increased progressively during the study, from 24.2% ($n = 1345$) at first in-study clinical visit (between 1 and 3 months) to 41.3% ($n = 2314$) at final assessment at 6 months. (This total comprised nondiabetic patients achieving SBP < 140 mmHg and diabetes patients achieving SBP < 130 mmHg.) Responder rates were significantly ($P < 0.001$) and substantially higher among younger patients (44.3% versus 33.4% in older patients), nonpostmenopausal women (52.8% versus 38.5% in postmenopausal women), and patients receiving monotherapy (55.7% versus 37.8% in those receiving multidrug therapy).

SBP and DBP declined by an average of 24.5 ± 14.3 mmHg and 12.6 ± 9.1 mmHg, respectively, across the study, as illustrated in Figure 2. The mean change in pulse pressure was −11.8 ± 12.8 mmHg. As illustrated in Figures 3(a)–3(d), a range of mostly moderate but statistically significant variations in blood pressure changes were recorded in patient subgroups.

3.3. Secondary Efficacy Endpoints. On-treatment changes were recorded for mean values of every nominated laboratory

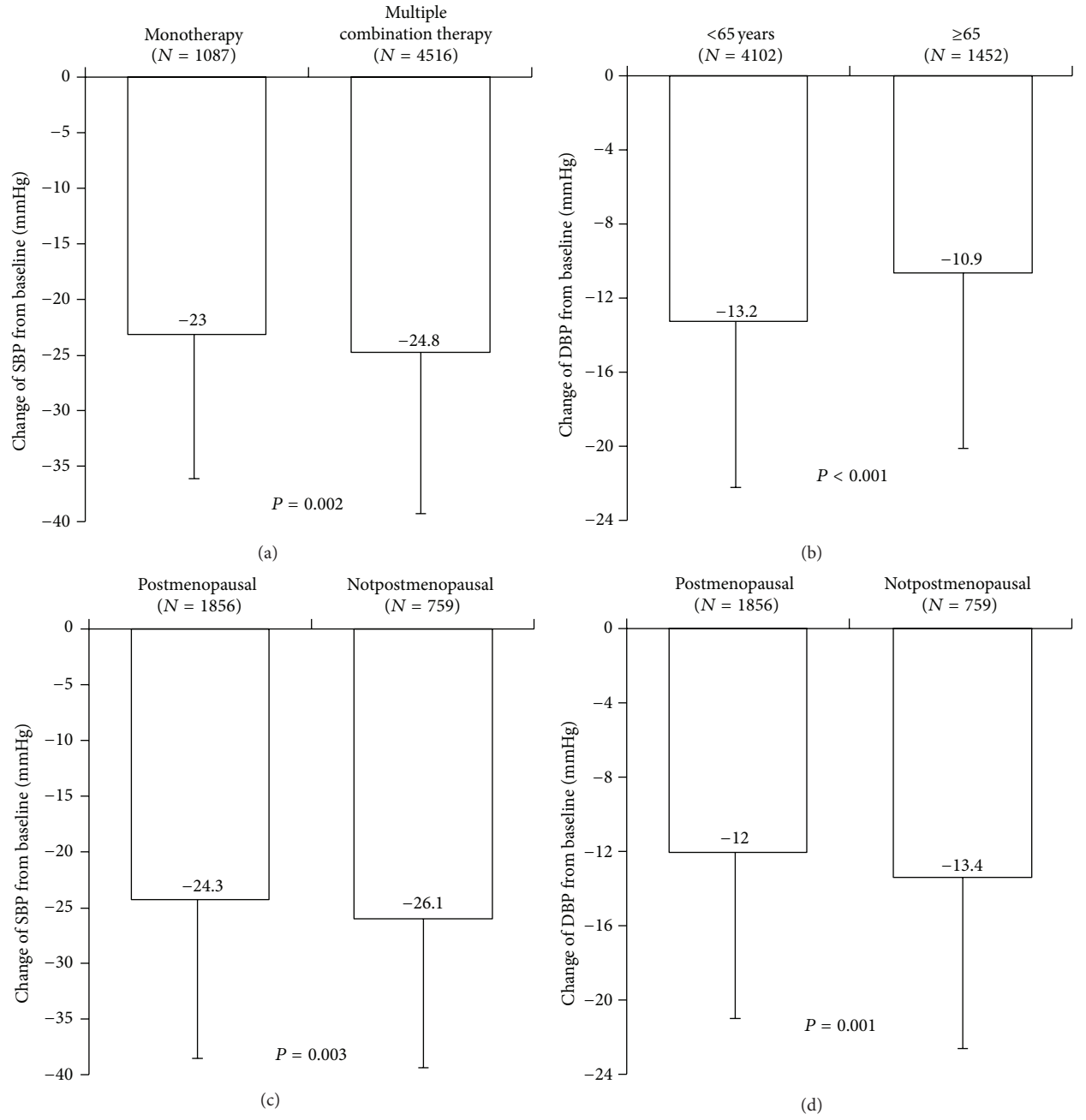

Figure 3: (a) Evolution of SBP by type of antihypertensive treatment (ITT), (b) evolution of DBP by age cohort between V1 and V3 (ITT population, $N = 5603$), (c) evolution of SBP by menopausal status (ITT population; $n = 2772$), and (d) evolution of DBP by menopausal status (ITT population; $n = 2772$).

parameter except creatinine (Table 3). The proportional changes in FPG did not differ significantly between prespecified subgroups ($P > 0.2$), notwithstanding differences in absolute values. There were likewise no subgroup-specific variations in the trend for total cholesterol, creatinine, or urinary albumin.

By contrast, the reduction in TG and the increase in HDL-C levels was more marked in younger (versus older) patients, and the reduction in TGs was significantly larger in nonpostmenopausal women (versus postmenopausal) ($P < 0.001$) for all comparisons, except HDL-C ($P = 0.004$). The reduction in LDL-C during treatment was more marked in younger patients than older ones ($P = 0.007$).

Average weight declined by –2.1 ± 5.4 kg during the study and BMI declined by –0.7 ± 2.0 kg/m^2. Mean HR, assessed in the safety population ($n = 5879$), fell from 79.6 ± 9.1 beats/min to 74.1 ± 7.0 beats/min, an average reduction of –5.7 ± 8.2.

Table 3: In-study trends in laboratory parameters associated with the metabolic syndrome (secondary efficacy endpoints). All results expressed as mmol/L unless indicated otherwise. Data are expressed as mean ± SD.

Total patients	$N = 5603$
Fasting plasma glucose	
At study start	6.8 ± 2.1
At study end	6.2 ± 1.6
In-study change	−0.8 ± 1.6
Triglycerides	
At study start	2.4 ± 1.1
At study end	2.0 ± 0.9
In-study change	−0.6 ± 1.0
Cholesterol	
At study start	5.8 ± 1.1
At study end	5.2 ± 0.9
In-study change	−0.7 ± 1.0
HDL-cholesterol	
At study start	1.2 ± 0.5
At study end	1.3 ± 0.5
In-study change	0.1 ± 0.5
LDL-cholesterol	
At study start	3.5 ± 1.1
At study end	3.0 ± 0.9
In-study change	−0.5 ± 0.9
Creatinine	
At study start	0.09 ± 0.06
At study end	0.10 ± 0.07
In-study change	0.01 ± 0.04
Urinary albumin	
At study start	92.7 ± 191.6
At study end	83.3 ± 205.5
In-study change	−7.6 ± 153.1
Body weight (kg)	
At study start	92.0 ± 15.6
At study end	90.0 ± 15.3
In-study change	−2.1 ± 5.4

Table 4: Summary of suspected adverse drug reactions (SADRs) recorded during the study.

	No. of events	No. of patients (%)
All SADRs	195	132 (2.2%)
SADRs considered related to study treatment	151	97 (1.6%)
SADRs leading to study termination	93	62 (1.1%)
Severe SADRs	15	10 (0.2%)
Serious SADRs	12	6 (0.1%)

Patients' assessments of treatment were "excellent," "good," "tolerable," or "bad" in 44.4%, 48.3%, 6.4%, and 0.9% ($n = 46$) of cases, respectively. The distribution of investigators' impressions of treatment was similar.

3.4. Safety Findings. During the course of the study, 195 SADRs were recorded in 132 patients (2.2% of the study population) (Table 4). Of these events, 12 (in 6 patients) were classified as serious. Just under half led to study termination; the number of severe SADRs was small ($n = 15$). Events contributing at least 5% of the total SADR count comprised gastrointestinal disorders (55 events; 28.2%); nervous system disorders (53 events; 27.2%); general disorders and administration site conditions (29 events; 14.9%); and skin and subcutaneous tissue disorders (10 events; 5.1%). The most frequent SADRs linked with the gastrointestinal system were dry mouth (48 events in 47 subjects) while most of the SADRs linked with the nervous system included dizziness (16 events in 16 subjects) or headache (13 events in 13 subjects). Nervous system disorders were the single largest category of events associated with study termination and the single largest source of events rated as severe ($n = 9$).

Of the 12 serious SADRs, 2 each were classified as nervous system disorders; vascular disorders; infections and infestations; or respiratory, thoracic, and mediastinal disorders. The remaining four serious SADRs comprised one case each in the categories of psychiatric disorder; pregnancy, puerperium, and perinatal conditions; renal and urinary disorders; and cardiac disorders.

No deaths were reported during the study.

4. Discussion

This open-label phase IV trial was designed to assess the effect of moxonidine on BP and laboratory parameters associated with the metabolic syndrome after 6 months of treatment in a general practice setting. We sought to enlarge on previous experience in a single country [21] by recruiting our patients from 11 countries with varying ethnic and racial profiles.

The results of the MERSY study are consistent with the previous experience with moxonidine in the management of hypertension [21–24]. The ~16% increment in responder rates seen in our patient sample was smaller than was reported in a placebo-controlled assessment [25]. Such a difference might have been predicted given the more complex clinical circumstances of our patients, the high degree of treatment resistance at baseline, and the extensive use of moxonidine initially at doses <0.4 mg. Nevertheless, the earlier study [25] provides a useful placebo-controlled benchmark for assessing the scale of the response seen in our patients and persuades us that the improvement in responder rates was a true effect plausibly attributable to the use of moxonidine. We regard the close similarity in the absolute magnitude of SBP and DBP reductions in our patients and in the CAMUS study [21] as also noteworthy in this context. A blood pressure reduction of this magnitude compares favorably with that achieved by other antihypertensive drug classes considered as first choice such as diuretics and others, particularly as add-on treatment at doses commonly used. Recent reports demonstrating the failure to achieve blood pressure goals despite the widespread use of antihypertensive treatment in a multinational European survey of patients with metabolic

syndrome highlights the need for more assertive and effective treatment of blood pressure in this growing segment of the adult population [26]. Data from the MERSY trial support the view that targeting the SNS centrally could be seen as part of the response to this need.

While blood pressure reduction is the primary goal of antihypertensive therapy, potential effects on metabolic parameters also need to be taken into account. Such considerations have led to widespread recommendations in national and international guidelines to avoid beta-blockers and diuretics in patients with metabolic disturbances or diabetes mellitus if not indicated for additional comorbidities. The reasoning for these recommendations relates to the well-described weight gain with beta-blockers and the adverse metabolic effects (such as insulin resistance and hyperuricemia) encountered with both beta-blockers and diuretics. In contrast, antihypertensive agents that exert no or even beneficial metabolic effects, such as calcium channel blockers (considered neutral in this regard) and inhibitors of the renin-angiotensin-system (ACE inhibitors, angiotensin receptor blockers, and direct renin inhibitors), which have been shown to reduce new onset of diabetes [27], are considered preferred choices in this scenario.

Moxonidine clearly falls into the second category, with proven efficacy in regard to blood pressure lowering and beneficial effects in regard to diverse metabolic parameters. While the effects of moxonidine on individual metabolic indices in our study could be considered as modest, the trends of all the changes seen were towards a profile of lower overall CVD risk, which was particularly evident in the subgroup analyses.

In this context, the average reduction in body weight of 2.1 kg in our patients is noteworthy and replicates earlier studies of moxonidine in populations with metabolic syndrome [16, 21]. Weight loss has clearly been associated with improved CV and other outcomes, suggesting that moxonidine may have additional beneficial effects beyond blood pressure reduction, particularly in overweight or obese hypertensive subjects or those with the metabolic syndrome. The recent withdrawal of sibutramine [28] and other primary weight-loss drugs emphasizes the desirability of having an agent with such an effect.

Several lines of evidence suggest that sympathetic activation is of particular relevance in the earlier stages of hypertension [7, 8, 11]. Our data may provide additional support for these observations in that the BP responder rates were significantly ($P < 0.001$) and substantially higher among younger compared to older patients (44.3% versus 33.4%). Interestingly, the reduction in LDL-C and TG ($P < 0.001$) and the increase in HDL-C levels ($P = 0.004$) was also more marked in younger than in older patients. These findings may indicate that younger subjects derive specific benefit from centrally sympatholytic agents.

Our observation of reductions in TG and body weight in conjunction with improvement in blood pressure control are compatible with the suggestion [29] that visceral obesity and dyslipidaemia are central contributors to the resistance of hypertension in metabolic syndrome, but are not an a priori proof of this idea. Indeed, given that we observed advantageous trends in most of the metabolic indices measured, the precise elements of metabolic syndrome involved in blood pressure resistance may be immaterial if inhibition of central sympathetic outflow becomes part of the antihypertensive strategy. It, therefore, appears plausible to suggest that centrally acting sympatholytic agents such as moxonidine may be considered an equally effective and beneficial choice as inhibitors of the renin-angiotensin system in patients with hypertension and metabolic syndrome, perhaps a preferred choice when compared to calcium-channel blockers, and most likely preferable to beta-blockers and diuretics, if no other conditions warrant their use. The validity of this concept has recently been demonstrated by the results of a renal denervation technique to inhibit sympathetic nervous system activation in obese patients with resistant hypertension. Despite being applied on a background of multiple antihypertensive medications, this method achieved an average blood pressure reduction of 32/12 mmHg and was associated with significant improvements in glycemic control and insulin sensitivity [30].

Additional, albeit indirect evidence for this concept comes from the ALMAZ study [15], in which the beneficial effects of moxonidine on indices of glucose homoeostasis were most marked in patients with a heart rate >80 beats/min, considered as an indicator of increased sympathetic drive. We did not stratify responses to therapy by heart rate in MERSY but if heart rate is a valid proxy for SNS status, a 5 beats/min decline in heart rate in our cohort may have been relevant to the overall effects of moxonidine in our study. Since high heart rate may contribute to cardiovascular risk [31, 32], the demonstrated ability of moxonidine to lower heart rate may well be relevant to its therapeutic profile. Given the increasing number of studies indicating potential beneficial effects of moxonidine beyond those on BP and expanding to metabolic parameters, additional studies and meta-analyses of existing studies may be useful to confirm the validity of this concept.

Moxonidine was well tolerated when used in combinations with the range of first-line antihypertensives in MERSY, as was the case in other studies. More generally, the safety profile of moxonidine in the MERSY study was fully in accordance with the known effects of the drug. The overall incidence of SADRs was very low (<2.5%) and the nature of the SADRs observed was consistent with previous experience. No previously unreported terms of SADR were encountered during our study. The incidence of SADRs with moxonidine is usually highest during the first weeks of treatment and thereafter declines to very low levels. The independent decision of many investigators to start therapy at doses <0.4 mg/day may have contributed to the excellent tolerability profile of moxonidine in the MERSY study and may be regarded as an example of skill in clinical practice.

5. Conclusions

In summary, in this large sample of patients with hypertension and concomitant metabolic syndrome, moxonidine enhanced blood pressure control when used alone or in

combination and was associated with improvement in several aspects of metabolic syndrome. Moxonidine was well-tolerated during 6 months of continuous use. Given current estimations suggesting that approximately 70% of all incident hypertension is associated with overweight or obesity, it appears justified to recommend antihypertensive treatment with an agent that targets the underlying pathophysiology including sympathetic activation, particularly so if additional benefits can be achieved in regard to control of body weight and other metabolic markers characterizing the metabolic syndrome.

Summary Table

What Is Known about the Topic.

(i) Hypertension is often associated with relevant comorbidities, particularly metabolic disturbances.

(ii) Antihypertensive drug therapy should be safe, well tolerated, effective in lowering blood pressure and ideally have a beneficial effect on comorbidities.

(iii) Sympathetic activation plays a role in blood pressure elevation and metabolic disturbances and can be targeted therapeutically.

What This Study Adds.

(a) Use of the centrally acting imidazoline receptor agonist moxonidine (0.2–0.4 mg once daily) for 6 months, either as monotherapy or as adjunct therapy to current antihypertensive treatment in patients with uncontrolled essential hypertension and criteria for metabolic syndrome, was associated with (i) improvement in control of blood pressure, (ii) neutral or beneficial trends in a range of metabolic indices including lipid fractions and fasting plasma glucose, and (iii) an average reduction in body weight of ~2 kg.

(b) Antihypertensive treatment with a centrally acting sympatholytic agent that targets common underlying pathophysiologic pathways is a safe and effective treatment strategy in a general practice setting with potential additional benefits in regard to metabolic disturbances frequently encountered in hypertensive populations.

Acknowledgments

The MERSY study was supported administratively and financially by Abbott Products Operations AG, Allschwil, Switzerland. I. Chazova also reports receiving travel support/speaker fees from Abbott, Astra Zeneca, Boehringer Ingelheim, Novartis, Pfizer, Servier, and Solvay. M. Schlaich has received research grant support from Deutsche Forschungsgemeinschaft, National Health and Medical Research Council of Australia, Kidney Health Australia, National Heart Foundation of Australia, Diabetes Australia, Heart Kids Australia, Abbott, Ardian Inc./Medtronic, and Servier and has received travel support/speaker fees/consulting fees from Abbott, Ardian, Astra Zeneca, Boehringer Ingelheim, MMI, Novartis, Omron, Pfizer, Servier, and Solvay.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2013, Article ID 420979, 9 pages
http://dx.doi.org/10.1155/2013/420979

Research Article

Role of Renin-Angiotensin System and Oxidative Stress on Vascular Inflammation in Insulin Resistence Model

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Received 22 October 2012; Accepted 5 December 2012

Academic Editor: Agostino Virdis

(1) This study aims to demonstrate the causal involvement of renin angiotensin system (RAS) and oxidative stress (OS) on vascular inflammation in an experimental model of metabolic syndrome (MS) achieved by fructose administration to spontaneously hypertensive rats (FFHR) during 12 weeks. (2) Chronic treatment with candesartan (C) (10 mg/kg per day for the last 6 weeks) or 4OH-Tempol (T) (10^{-3} mmol/L in drinking water for the last 6 weeks) reversed the increment in metabolic variables and systolic blood pressure. In addition, chronic C treatment reverted cardiovascular remodeling but not T. (3) Furthermore, chronic treatment with C was able to completely reverse the expression of NF-κB and VCAM-1, but T only reduced the expression. C reduced the expression of proatherogenic cytokines as CINC2, CINC3, VEGF, Leptin, TNF-alpha, and MCP-1 and also significantly reduced MIP-3, beta-NGF, and INF-gamma in vascular tissue in this experimental model. T was not able to substantially modify the expression of these cytokines. (4) The data suggest the involvement of RAS in the expression of inflammatory proteins at different vascular levels, allowing the creation of a microenvironment suitable for the creation, perpetuation, growth, and destabilization of vascular injury.

1. Introduction

Inflammation is a ubiquitous pathological process which is central to the development of multiple cardiovascular diseases. Many vascular diseases such as atherosclerosis, restenosis, and transplant vasculopathy are chronic, progressive processes initiated and propagated by local inflammation of large- and medium-sized arteries [1]. This inflammation is mediated by a variety of cell types including macrophages, lymphocytes, endothelial cells (EC), and vascular smooth muscle cells (VSMC). The multiple cell types which participate in vascular inflammation have evolved to produce common cytokines and specific membrane receptors allowing them to transmit their effects into the cell, permitting these diverse cell types to communicate by expression and recognition of multiple pro- and anti-inflammatory cytokines. As such, cytokines and their receptors are the currency of inflammation, and represent attractive targets for therapeutic modalities in numerous vascular inflammatory disorders.

Synthesis and recognition of cytokines and receptors by both vascular and inflammatory cells allows bidirectional communication between these two systems and demonstrates that, under particular conditions, we can consider vascular cells as an extended participant in the adaptive immune response. Cytokines often act in synergy with other cytokines and frequently share receptor subunits which combine into homodimers or heterodimers with receptors of other cytokines. Cytokines can drive multiple, often simultaneous, cellular processes including mitogenesis, development, gene expression, fibrosis, and chemotaxis [2]. Proinflammatory cytokines most often lead to activation of nuclear factor-(NF-κB) which acts as a "master switch" for transcription of numerous genes, the expression of which may be appropriate, as in host defense, or maladaptive, as in chronic vascular disease [3–5].

The inflammatory nature of atherosclerosis has prompted broad investigation into vascular inflammatory processes, and consequently, proinflammatory signaling mechanisms in

the vascular wall have been well characterized [6–9]. Interest has been placed on understanding the potentially protective role of blocking renin-angiotensin system (RAS) and antioxidative systems on vascular wall [10]. Such studies that do exist place a strong emphasis on the role of angiotensin and oxidative stress in the metabolic syndrome vascular remodeling pathophysiology.

Spontaneously hypertensive rats (SHR) provide a model of genetic hypertension that allows the study of essential hypertension. The administration of carbohydrate-rich diets to rats can induce insulin resistance, hyperinsulinemia, dyslipidemia, and moderate hypertension. Chronic fructose-fed rats (FFR) provide a useful experimental model for studying the interaction of the factors that shape the metabolic syndrome. This combined model (FFHR) is representative of hypertensive individuals who eat a modern Western diet rich in refined sugars [11]. We postulate that this dual experimental model could be appropriate for extrapolating results to human pathology.

The hypothesis suggests that RAS and oxygen-free radicals are actively involved in the activation of different molecular inflammatory as cytokines, NF-κB, and VCAM-1 generating a microenvironment that allows cardiovascular remodeling.

2. Methods

2.1. Animals and Experimental Design. All procedures were performed according to institutional guidelines for animal experimentation; protocol was submitted and approved by the Institutional Committee for Laboratory Animal Use and Care (CICUAL) of the School of Medicine-UNCuyo. Thirty-day-old male Wistar Kyoto rats (WKY) and SHR were fed a standard commercial chow diet ad libitum and housed in a room under conditions of controlled temperature (20°C) and humidity, with a 12-hour light/dark cycle during a 12-week experimental period. Candesartan (C) and 4OH-Tempol (T) were administrated to respective groups during the last six weeks.

(I) Control (W): WKY receiving food and drinking water (DW) ad libitum.

(II) SHR: receiving food and DW ad libitum.

(III) Fructose-Fed Rats (FFR): WKY receiving 10% (w/v) fructose (Parafarm, Buenos Aires, Argentina) solution in DW during all 12 weeks.

(IV) Fructose-fed Hypertensive Rats (FFHR): SHR receiving 10% (w/v) fructose solution in DW during all 12 weeks.

(V) FFHR + C: FFHR receiving 10 mg/kg C by intraesophageal administration.

(VI) FFHR + T: receiving 10^{-3} M T in DW ad libitum.

At the end of the experimental period, rats were anesthetized with sodium pentobarbital (50 mg/Kg ip), blood samples were taken and arteries and organs were aseptically excised for measurements.

2.2. Systolic Blood Pressure Measurement. Systolic blood pressure (SBP) was monitored indirectly in conscious prewarmed slightly restrained rats by the tail-cuff method and recorded on a Grass Model 7 polygraph (Grass Instruments Co., Quincy, MA, USA). The rats were trained in the apparatus several times before measurement.

2.3. Biochemical Determinations

2.3.1. HOMA Index and Intraperitoneal Glucose Tolerance Test. Fasting plasma insulin was assayed by ACS:180SE automated chemiluminescence system (Bayer, Germany). Plasma glucose levels were assayed using a commercial colorimetric method (Wiener Lab., Argentina). Homeostasis model assessment (HOMA) was used as an index to measure the degree of insulin resistance; it was calculated using the following formula: [insulin (μU/mL) × glucose (mmol/L)/22.5] [12].

Three days before the end of the experimental period, a glucose tolerance test (GTT) was performed. Rats fasted overnight were slightly anesthetized with pentobarbital, and glucose was administered (2 g/Kg ip). Blood samples were taken by tail-bleeding at 0, 30, 60, and 90 minutes after injection to determine plasma glucose concentration. The total area under the curve was calculated as mmol/L/90 min.

2.3.2. Assessment of the Lipid Profile. At the end of the experimental period blood samples were drawn from the animals, after fasting for 12 hours. Total plasma cholesterol, HDL cholesterol and triglycerides were assessed using photocolorimetric enzymatic methods (Wiener Lab., Rosario, Argentina). Data are expressed in mmol/L.

2.4. Oxidative Stress Determinations

2.4.1. Measurement of Plasma Thiobarbituric Acid-Reactive Substances (TBARS). In order to demonstrate the effect of increased oxidative stress at the vascular level, plasma lipid peroxidation was assessed by TBARS concentration. This method was based on the reaction between plasma malondialdehyde, a product of lipid peroxidation, and thiobarbituric acid, as has been previously described [13]. No correction for sample protein content was necessary because of the nature of sample [14].

2.5. Measurement of Vascular NAD(P)H-Oxidase Activity. The lucigenin-derived chemiluminescence assay was used to determine NAD(P)H-oxidase activity in a segment of thoracic aorta, as previously described [13]. To assess NAD(P)H-oxidase activity, NADPH (500 μmol/L) was added and chemiluminescence was immediately measured in a liquid scintillation counter (LKB Wallac Model 1219 Rack-Beta Scintillation Counter, Finland) set in the out-of-coincidence mode. Time-adjusted and normalized-to-tissue-weight scintillation counters were used for calculations. Measurements were repeated in the absence and presence of diphenylene iodinium (DPI) (10–6 mol/L), which inhibits flavin-containing enzymes, including NAD(P)H-oxidase [15, 16].

2.6. eNOS Activity in Homogenates of Cardiac and Arterial Tissue. The activity of Ca^{2+}/calmodulin-dependent endothelial nitric oxide synthase, (eNOS) was measured in mesenteric arteries homogenates and in left ventricle cardiac tissue, by conversion of L-[3H]arginine into L-[3H]citruline. Values were corrected according to protein contents in the homogenates (Bradford method) and to incubation time and are expressed as dpm/mg protein/min. The material obtained from each animal was processed independently [16].

2.7. Relative Heart Weight. In order to evaluate cardiac hypertrophy, we measured relative heart weight (RHW). Briefly, heart was separated from the great vessels, dropped into a buffered saline solution (PBS), blotted with tissue paper to remove blood, and weighed. Total heart weight was corrected according to the ratio between heart weight (milligrams) and 100 grams of the total body weight before killing.

2.8. Tissue Preservation. Tissue samples for histopathology were processed as has been previously reported [17]. Samples from all rats were used for these observations. Anesthetized animals were briefly perfused with PBS (298 mOsmol/Kg H_2O, pH 7.40, and 4°C) to clear out the blood. Mesenteric arteries were perfused in vivo with the same solution through the mesenteric artery during 5 min. For histological studies, arteries were also perfused with 4% paraformaldehyde solution for 10 min and fixed by paraffin. Five μm-thick tissue slices were transversely cut across the mesenteric tissue on a microstate (Microm HM, Germany) and processed for histological studies. Similar procedure was applied for heart tissue preservation, by aortic retrograde perfusion.

2.9. Quantitative Histomorphometry to Determine Cardiac Hypertrophy. Histomorphological analyses were conducted on slices from the outer (free) wall of the left ventricle (LV) of the heart. Estimations of cardiomyocyte area were made from sections stained with Masson trichrome solution. Areas with transverse sections of myofibers were selected. The contour of the fibers was then drawn manually. Total myocardiocyte area was expressed as square micrometer (μm^2).

2.10. Arterial Structure. Changes in the structure of arterial walls were assessed by measuring the media layer in mesenteric arteries. Dissected mesenteric vascular beds were fixed in 10% formaldehyde, dehydrated, embedded in paraffin, and later cut in microtome. The slices were dyed and examined as has been previously described [17]. Nontransverse sectioned arteries were excluded from investigation. The lumen to media ratio (i.e., internal diameter to medial thickness) (M/L) was then calculated. Fifty slices from each animal were processed were analyzed to obtain an average value for each rat. Average values were then used for final analysis.

2.11. SDS-PAGE and Immunoblot Analysis. Mesenteric tissue was washed in PBS and proteins extracted in cold 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X-100, and a protease inhibitor mixture (P2714, Sigma). After sonication for 15 s (3 times with 10-s intervals) and extraction for 30 min at 4°C, sample extracts were clarified by centrifugation at 14,000 ×g for 20 min and used immediately or stored at −20°C. Proteins were separated on 10% polyacrylamide slab gels and transferred to 0.22-μm nitrocellulose membranes (GE, Germany). Nonspecific reactivity was blocked by incubation for 1 h at room temperature in 5% nonfat dry milk dissolved in washing buffer (PBS, pH 7.6, and 0.2% Tween 20). Blots were incubated with anti-p65 and anti-VCAM-1 antibodies (0.2 μg/mL in blocking solution) for 60 min at room temperature. Horseradish peroxidase-conjugated goat anti-rabbit-IgG and swine anti-goat-IgG dissolved in blocking buffer were used as secondary antibodies (0.25 μg/mL, 45 min at room temperature). Excess first and second antibodies were removed by washing 5 times for 5 min in blocking solution. Detection was accomplished with enhanced chemiluminescence system (ABC, Dako System) and subsequent exposure to Kodak X-AR film (Eastman Kodak) for 5–30 s.

2.12. Immunohistochemistry and Digital Confocal Microscopy (IHC)

Determination of Transcription Factors (WB). Rabbit anti-rat NF-κB p65 subunit [Rel A], C-terminus antibody was obtained from Millipore International Inc. (Amsterdam, Netherlands) (AB1604b), and goat anti-rat VCAM-1 (C-19) antibody was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz USA) (sc-1504). Tissue sections were cut at 3 μm thickness from paraffin-embedded blocks. Deparaffinized sections were used to determine inflammatory response. Tissue was permeabilized in 1% Triton X-100 for 15 min, rinsed well with PBS and blocked with sterile filtered 10% normal rabbit serum for 20 min. All antibody solutions were microfuged for 20 min before use. The antibodies were 1 : 1000 diluted. Primary incubations lasted 1 hour at 21-22∘C, followed by extensive washes in PBS with Triton X-100, six times for 5 min each. Secondary antibodies, anti-rabbit IgG TR, and anti-goat IgG FITC (Sigma-Aldrich) were diluted in PBS alone in compliance with the manufacturer's instructions.

Images were collected with Nikon EZ-C1 3.00 software on a Nikon Diaphot TMD microscope equipped for fluorescence with a xenon lamp and filter wheels (Sutter Instruments, Novato, CA, USA), fluorescent filters (Chroma, Brattleboro, VT, USA), cooled charge-coupled device camera (Cooke, Tonawanda, NY, USA), and stepper motor (Intelligent Imaging Innovations, Inc., Denver, CO, USA). Multifluor images were merged, deconvolved, and renormalized using EZ-C1 3.00 Thumbnailler software.

2.13. Measurement of High-Sensitive C Reactive Protein (hs-CRP) Concentration. Plasma HS-CRP concentrations were measured using a turbidimetric assay (Bayer Advia 1650, AG Leverkiusen). Data are expressed in mg/L.

2.14. Cytokine Determination by "ChemiArray". Cytokine expression was assessed by ChemiArray system (rat antibody arrays) (Chemicon International, USA): neutrophil chemotactic cytokine 2 and 3 (CINC-2 and CINC-3), CX3CL1, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-3 alpha (MIP-3 alpha), nerve growth factor beta (beta-NGF), tissue inhibitor of metalloproteinase-1 (TIMP-1), vascular endothelial growth factor (VEGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (INF-γ), interleukin 1 alpha and beta (IL-1α, IL-1β), interleukin 4, 6, and 10 (IL-4, IL-6, IL-10) LIX, leptin, and tumor necrosis factor alpha (TNF-α). We proceeded, according to the manufacturer's instructions, to block nonspecific reactivity by incubation at room temperature for 1 h with a solution according to the instructive. The membranes were incubated in solutions A and B for 60 min at room temperature. Horseradish peroxidase-conjugated antibodies provided by the manufacturer were used. Excess primary or secondary antibody was removed or after 5 washes of 5 min with washing solution. Detection was performed with chemiluminescence system and subsequent exposure to Kodak X-AR film (Eastman Kodak) for 5–30 s. Citokines were distributed in membranes according the map (Table 1).

2.15. Reagents. Unless otherwise noted, reagents were purchased from Sigma Chemical Co., MO, USA.

2.16. Statistical and Data Analysis. Data are expressed as mean ± SEM. The statistical significance of data comparison between all groups was assessed by one-way ANOVA followed by Bonferroni post-test. A two-sided P value of less than 0.05 was considered significant.

3. Results

3.1. Biochemical Determinations. To categorize experimental models we assessed metabolic profile of the different groups. Chronic administration of fructose induced several alterations included in the cluster of risk factors that characterizes MS. The comparison between HOMA index and areas under the GTT curve evidenced that FFR and FFHR developed glucose intolerance, as proven by the significantly increased HOMA index and area values compared to control rats (Table 2).

On the other hand, the animals in FFR and FFHR groups also showed significant differences in the levels of triglycerides and HDL-cholesterol when compared to their controls (Table 2). SHR, FFR, and FFHR groups also showed significant differences in the levels of hs-CRP when compared to WKY. FFHR group showed higher hs-CRP levels than other groups (Table 2).

Chronic treatment with C significantly reduced the HOMA index and areas under the GTT curve; it also reduced triglyceride levels and HDL-cholesterol, reversing the parameters that comprise the MS. Furthermore T partially but significantly reduced these variables. C significantly reduced the values of hsCRP, while T reduced them only partially. See Table 2.

3.2. Systolic Blood Pressure Measurement. Table 2 also shows the time-course of SBP changes along the experimental period. By the sixth week, SBP of FFHR and SHR were significantly increased compared to the control group, and there was also an increase in pressure in the FFR group, lower but still significant.

C normalized SBP to control values and T partially reduced SBP values. C was more effective and powerful in lowering the SBP. Probably the hypertensive state in this experimental model, although it has a component of endothelial dysfunction, should involve angiotensin 1 receptor (AT1R) in the underlying mechanism.

3.3. Oxidative Stress Determinations. Vascular oxidative status was assessed by measurement of the superoxide producing enzymatic activity and its effects on plasma lipid peroxidation. Table 3 shows that NAD(P)H-oxidase activity was significantly higher in aortas from FFHR when compared to those from other groups. Plasma TBARS values are shown in Table 3. Plasma TBARS concentration was significantly greater in FFR, SHR, and FFHR than in controls.

In addition, the arterial eNOS activity in the proposed models was analyzed as shown in Table 3: FFHR significantly reduced their enzyme activity, contributing to decrease the production and consequent bioavailability of nitric oxide (NO).

These results confirm that these experimental models, essentially FFHR, have a significant superoxide production and decreased NO bioavailability. T was effective to reduce superoxide production by reducing the activity of NAD(P)H-oxidase and TBARS, and was also able to normalize eNOS activity (Table 3). Furthermore C, probably by inhibiting the activity of NAD(P)H-oxidase, was also able to achieve these effects, normalizing endothelial oxidative status (Table 3).

3.4. Quantitative Histomorphometry for Determining Cardiac and Vascular Hypertrophy. The RHW and myocardiocyte area were significantly higher in FFR, SHR, and FFHR than in control rats, demonstrating myocardial hypertrophy in these experimental models (Table 3). The results of *M/L* ratio calculated in mesenteric arteries are shown in Table 2. FFHR always displayed a significantly reduced *M/L* ratio when compared to the corresponding arteries from WKY; this result was also registered for FFR and SHR groups.

Chronic treatment with T significantly reduced myocardial hypertrophy and vascular remodeling, demonstrating that oxidative stress, by activating different mechanisms, actively participates in the process of cardiovascular remodeling.

However, AT_1R blockade by C was more effective in reducing these variables. The results demonstrate that intracellular cascade post-AT_1R activation is fundamental to both cardiac and vascular remodeling processes, not only caused by oxidative stress mechanisms, but also by grown factors.

3.5. Determination of Transcription Factors. As shown in Table 4, some inflammatory markers were evaluated in mesenteric arteries, as well as the expression of NF-kappa B

Table 1: Metabolic and cardiovascular variables.

Variable	WKY	FFR	SHR	FFHR	FFHR + C	FFHR + T
Fasting glucose (mmol/L)	4.88 ± 0.1	6.44 ± 0.2*	5.0 ± 0.2	6.5 ± 0.2$^{*\wedge}$	5.6 ± 0.1**	5.6 ± 0.1**
Fasting triglycerides (mmol/L)	0.8 ± 0.0	1.8 ± 0.0$^{*\#}$	0.9 ± 0.0	1.9 ± 0.1$^{*\#}$	1.1 ± 0.2**	1.7 ± 0.1
HOMA index (μU/mL insulin × mmol/L glucose)/22.5	4.32 ± 0.1	10.93 ± 0.1$^{*\#}$	7.2 ± 0.1*	14.1 ± 0.4$^{*\#\wedge}$	5.7 ± 0.5**	7.2 ± 0.1
Area under glucose tolerance test curve (mmol/L/90 min)	881 ± 64	1392 ± 21$^{*\#}$	1292 ± 31*	1839 ± 51$^{*\#\wedge}$	971 ± 54**	1200 ± 2.4*
HDL-Cholesterol (mg/dL)	22.5 ± 0.7	12.2 ± 0.8$^{*\#}$	19.3 ± 0.9*	13.6 ± 1.2$^{*\#\wedge}$	19.2 ± 1.4**	15.6 ± 2*
High-sensitivity C reactive Protein (mg/dL)	2.55 ± 0.1	3.5 ± 0.0	3.1 ± 0.1	4.5 ± 0.1$^{*\#\wedge}$	2.01 ± 0.0**	4.0 ± 0.0*
Systolic blood pressure (mmHg)						
Baseline	105 ± 3	102 ± 1.0	103 ± 1	105 ± 3	105 ± 2	103 ± 1
6 weeks	113 ± 2.0	131 ± 3.0*	161 ± 3*	162 ± 2$^{*\#}$	165 ± 2*	165 ± 2$^{*\#}$
12 weeks	115 ± 1.3	136 ± 3.0*	177 ± 1$^{*\#}$	181 ± 1$^{*\#\wedge}$	100 ± 2.5**	168 ± 1.1$^{\wedge **}$

The above values correspond to metabolic and cardiovascular variables.
Symbols indicate: $^{*}P < 0.001$ versus WKY; $^{\wedge}P < 0.001$ versus SHR; $^{\#}P < 0.01$ versus FFR; **versus FFHR.

Table 2: Oxidative stress and morphometric variables.

Variable	W	FFR	SHR	FFHR	FFHR + C	FFHR + T
NAD(P)H oxidase activity (counts/min/mg tissue)	40.5 ± 6	133 ± 5*	160 ± 9.1$^{*\#}$	297 ± 9.1$^{*\#\wedge}$	142 ± 9.1$^{*\wedge **}$	97 ± 2.1$^{*\wedge **}$
Arterial eNOS activity (dpm/mg/prot/min)	85.0 ± 2	60.1 ± 2.6*	80.0 ± 2.1	56.4 ± 5.7$^{*\#\wedge}$	86.4 ± 1.1**	84.6 ± 1.1**
TBARS (μmol/L)	1 ± 0.1	2.2 ± 0.1*	1.69 ± 0.1*	2.8 ± 0.1$^{*\#\wedge}$	1.03 ± 0.6$^{\#\wedge **}$	0.73 ± 0.4$^{\#\wedge **}$
Relative heart weight (mg/100 g body weight)	225 ± 4	290 ± 4*	330 ± 1.8$^{*\#}$	400 ± 4$^{*\#\wedge}$	262 ± 4**	289 ± 4**
Myocardiocyte area (μm^2)	1682 ± 69	2066 ± 57*	2222 ± 78$^{*\#}$	3242 ± 55$^{*\#\wedge}$	1588 ± 55**	2188 ± 35**
Media/Lumen ratio mesenteric arteries	13.9 ± 0.3	10.2 ± 0.5*	8.9 ± 0.6$^{*\#}$	8.45 ± 0.2$^{*\#}$	14.5 ± 5**	9.5 ± 5**

The above values correspond to stress oxidative and morphometrics variables.
Symbols indicate: $^{*}P < 0.001$ versus WKY; $^{\wedge}P < 0.001$ versus SHR; $^{\#}P < 0.01$ versus FFR; **versus FFHR.

and VCAM-1, one of the posttranscriptional products that actively participate in vascular inflammation. Both molecules were determined by IHC. The expression of these molecules in the FFHR group increased significantly compared to control groups. In Table 4, right panel shows a representative image of WB of these antibodies. Average optical density significantly increased in mesenteric artery homogenates from FFHR and FFR groups compared to their controls. It can be observed that the distribution of the β-actin marker is similar for all groups.

Moreover, it can be seen in images obtained by IHC in vascular wall of these experimental groups. FFHR shows a large increase in NF-κB expression at the level of EC and VSMC, and also expressed VCAM-1 at subendothelial level.

C completely reduced activation and nuclear translocation of NF-κB (p65 fraction) and VCAM-1 expression. On the other hand, T was not able to significantly reduce the activation of NF-κB and VCAM-1 expression, even though the initial description of the activation of this molecule was by ROS. This could be explained because in FFHR, nuclear factor NF-κB production was more important via superoxide generated by AT1R activation. This finding is very important because blocking AT_1R at vascular level significantly reduced vascular inflammation. This may also be a determining factor in the reduction of vascular remodeling, as previously shown.

3.6. Cytokine Determination by "ChemiArray System". After evaluation and analysis of vascular remodeling and inflammation and increased hsCRP associated to the FFHR model development, we decided to study the local expression of cytokines (Figure 2). By using the previously described kit, we were able to observe a significant increase in several cytokines in FFHR, including the followings: CINC2, CINC3, VEGF, MIP-3, beta-NGF, VEGF, Leptin, TNF-alpha, INF-gamma, and MCP-1 (Table 3). This finding is the first evidence of the presence of local inflammation at vascular level in an experimental model of insulin resistance

Table 3: Cytokine release profiles on different experimental models.

Cytokine[a]	Relative levels[b]	Array location[c]	Fold increase for control group (WKY)[d]				
			SHR	FFR	FFHR	FFHR + T	FFHR + C
CINC-2	H	E1-2	1.18	1.10	2.42	2.07	NC
CINC-3	H	F1-2	1.34	1.30	2.74	2.00	NC
CNTF	—	G1-2	~	~	~	~	~
Fractalkine	—	H1-2	~	~	~	~	~
GM-CSF	—	A4-5	~	~	~	~	~
INF-γ	H	B3-4	5.00	4.50	5.90	4.00	1.50
IL-1α	—	C3-4	~	~	~	~	~
IL-1β	—	D3-4	~	~	~	~	~
IL-4	—	E3-4	~	~	~	~	~
IL-6	—	F3-4	~	~	~	~	~
IL-10	—	G3-4	~	~	~	~	~
LIX	—	H3-4	~	~	~	~	~
Leptin	H	A5-6	1.16	1.33	2.10	1.67	NC
MCP-1	H	B5-6	1.25	NC	4.00	2.62	NC
MIP-3α	H	C5-6	1.16	1.33	3.91	2.50	1.25
β-NGF	H	D5-6	2.40	2.40	3.50	2.93	NC
TIMP-1	H	E5-6	2.60	1.52	2.50	1.90	NC
TNF-α	H	F5-6	3.19	1.10	3.35	2.80	1.30
VEGF	H	G5-6	2.50	NC	3.05	2.60	NC

[a] Name of cytokine.

[b] Relative levels: —: undetectable; H: high; L: low.

[c] See Figure 2 for the location of the duplicate spots in the matrix.

[d] When the control "~" symbol was used to indicate an approximation of zero, the values indicate the fold increase versus Wistar Kyoto (WKY: control group). NC: no change (less than or equal to fold difference from the level in WKY group).

Table 4: ChemiArray Rat Lysate Cytokine Antibody Array I Map.

	A	B	C	D	E	F	G	H
1	Positive	Positive	Negative	Negative	CINC-2	CINC-3	CNTF	Fractalkine
2	Positive	Positive	Negative	Negative	CINC-2	CINC-3	CNTF	Fractalkine
3	GM-CSF	INF-γ	IL-lα	IL-lβ	IL-4	IL-6	IL-10	LIX
4	GM-CSF	INF-γ	IL-lα	IL-1β	IL-4	IL-6	IL-10	LIX
5	Leptin	MCP-1	MIP-3α	β-NGF	TIMP-1	TNF-α	VEGF	Blank
6	Leptin	MCP-1	MIP-3α	β-NGF	TIMP-1	TNF-α	VEGF	Blank
7	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Positive
8	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Positive

Cytokines Names: Neutrophil chemotactic cytokine 2 and 3 (CINC-2 and CINC-3), ciliary neurotrophic factor (CNFT), monocyte chemotactic protein-1 (MCP-1), inflammatory protein macrophage-3 alpha (MIP-3 alpha), nerve growth factor beta (beta-NGF), tissue inhibitor of metalloproteinase-1 (TIMP-1) and vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor, macrophage (GM-CSF), interferon gamma (INF-γ), interleukin 1 alpha and beta (IL-1α, IL-1β), interleukin 4, 6, and 10 (IL-4, IL-6, IL-10), lipopolysaccharide-induced CXC chemokine (LIX or CXCL5), leptin, and tumor necrosis factor alpha (TNF-α).

such as FFHR. Others cytokines measurable by this kit, especially interleukins, could not be evaluated because the study was focused on the mesenteric tissue homogenate but not on peripheral blood, where most of the interleukins are found. This result shows the significant presence of pro-atherogenic cytokines as CINC2, CINC3, VEGF, Leptin, TNF-alpha, MCP-1, and TIMP-1, other with undetermined significance to vascular level even, as MIP-3 and beta-NGF and other as INF-gamma with antiatherogenic effect.

Chronic treatment with T was ineffective in reversing the expression of cytokines in the mesenteric vascular tree (Table 3).

Chronic treatment with C was able to reverse to control values the expression of proatherogenic cytokines as CINC2, CINC3, VEGF, Leptin, beta-NGF, TIMP-1, and MCP-1 and also significantly reduce MIP-3alpha, TNF-alpha, and INF-gamma expression, demonstrating the important role played by the RAS in vascular inflammation in this experimental model (Table 3).

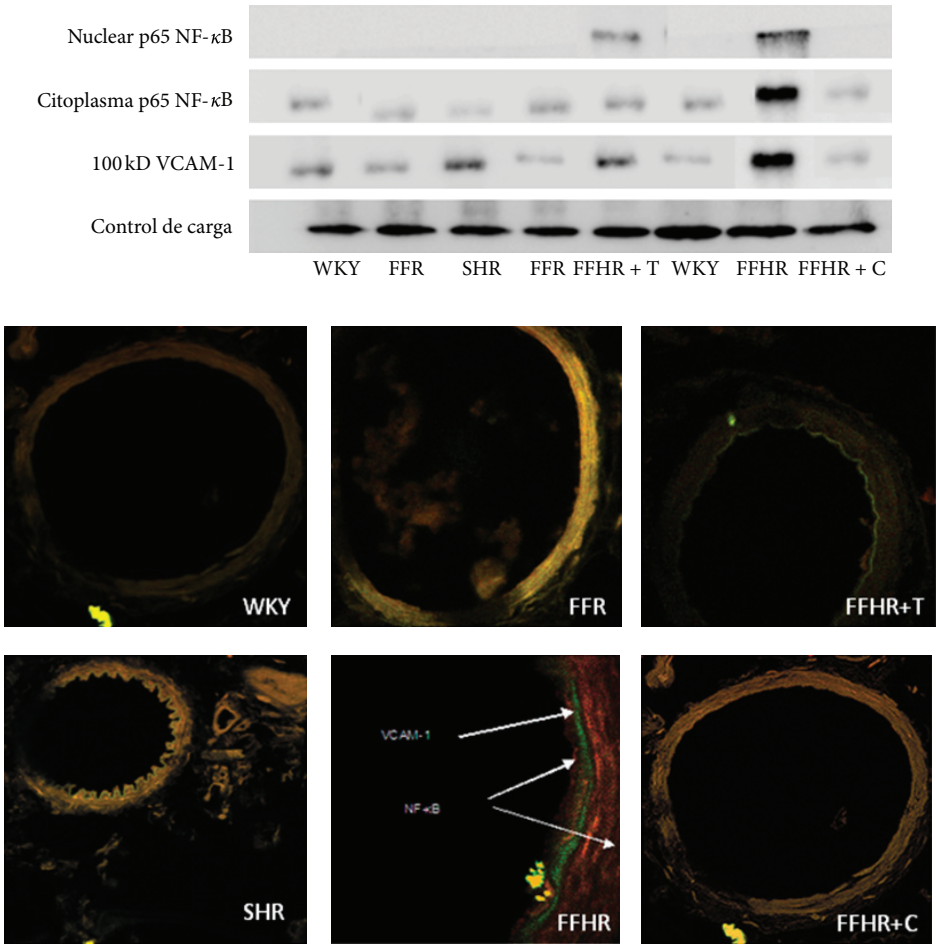

Figure 1: Cytoplasmatic and nuclear p-65 fraction of Nuclear Factor-κB (NF-κB) and Vascular cell adhesion protein 1 (VCAM-1) expression in mesenteric arteries by western blot and Inmunohistochemistry. In up panel shows the western blot representative membrane in which analyzed anti-VCAM-1-FITC and anti-p65-TRITC, the results were obtained by optic density of the bands revealed for each group. In top panel shows microphotographs obtained by laser ICM 600x of mesenteric tissue.

These results allow us to infer that the activation of cytokines is mediated by a different to redox-sensitive way and AT_1R receptor was actively involved in expression of cytokines.

4. Discussion

This study was designed in order to test the hypothesis that suggests the active involvement of RAS and oxidative stress in the activation of different inflammatory molecules as cytokines, NF-κB, and VCAM-1, generating a microenvironment that allows cardiovascular remodeling (Figure 1). We demonstrate the presence of humoral inflammatory markers at vascular level and transcription factors activation, also allows establishing a cause-effect relationship with the AT1R activation.

To our knowledge, this is the first study showing anti-inflammatory effect and inhibition of remodeling progression with a AT1R, candesartan, in an experimental model of MS. AT1-R blocking are associated with reversion of activation of vascular proinflammatory mechanisms found in metabolic syndrome models such as NF-κB expression or cytokine activation. Another important finding is that treatment with a mimetic of superoxide dismutase has similar anti-inflammatory effects to those found with C, although weaker and was not effective in reverting vascular remodeling. Moreover, superoxide blocking production did not overcome protective effects exerted by C.

The experimental model FFHR showed hypertension, dyslipidemia, insulin resistance, vascular and cardiac remodeling, inflammation demonstrated by increased hsCRP and vascular inflammation by increased the NF-κB expression, VCAM-1, and proatherogenic cytokines. The increased expression of VCAM-1, as discussed in the literature, is a marker of vascular inflammation, vascular permeability, and endothelial dysfunction [18].

The inflammatory process found in this experimental model is not only circumscribed at vascular level but it is also systemic, as demonstrated trough the increase in the expression of hsCRP, which is synthesized in the liver in response to increased IL-6. The experimental model showed a very significant increase of this protein [19, 20].

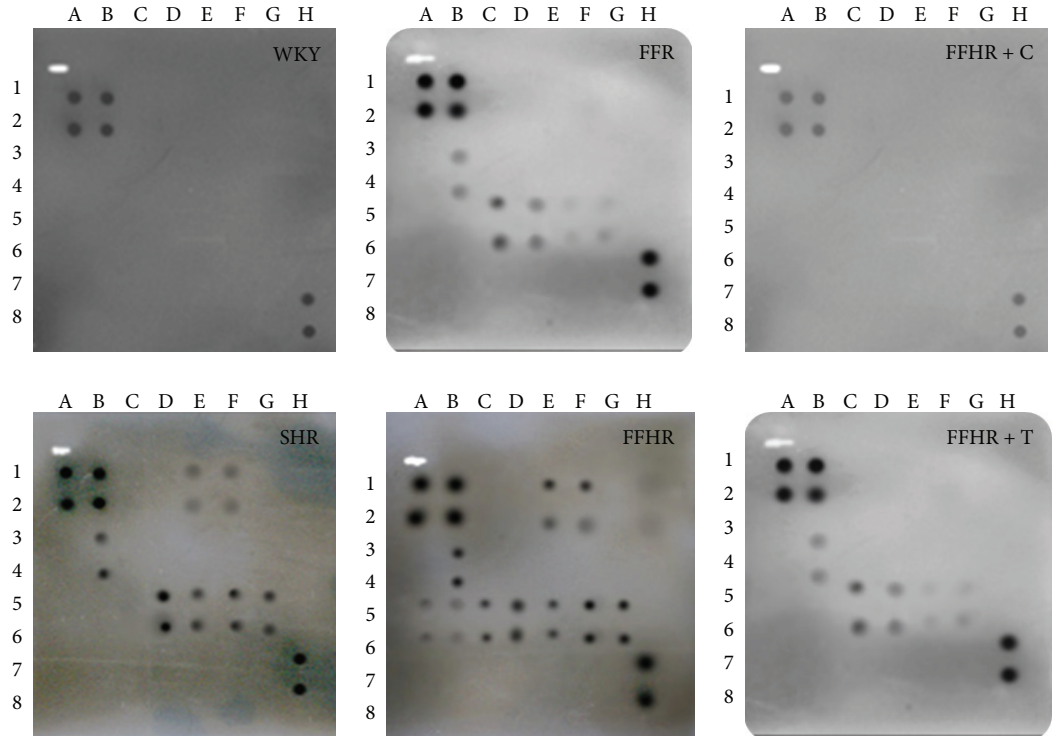

Figure 2: Detection of cytokines on membrane antibody arrays by chemiluminiscence. Each cytokine is represented by duplicate spots in the following locations. See Table 4. Average net light intensity for each pair of cytokine spots detected on the basis of gray-scale levels using US NIH Image software ver. 1.66. Cytokines names: Neutrophil chemotactic cytokine 2 and 3 (CINC-2 and CINC-3), ciliary neurotrophic factor (CNFT), monocyte chemotactic protein-1 (MCP-1), inflammatory protein macrophage-3 alpha (MIP-3 alpha), nerve growth factor beta (beta-NGF), tissue inhibitor of metalloproteinase-1 (TIMP-1) and vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor, macrophage (GM-CSF), interferon gamma (INF-γ), interleukin 1 alpha and beta (IL-1α, IL-1β), interleukin 4, 6, and 10 (IL-4, IL-6, IL-10), lipopolysaccharide induced CXC chemokine (LIX or CXCL5), leptin, and tumor necrosis factor alpha (TNF-α).

The data suggest the involvement of RAS in the expression of inflammatory proteins at different vascular levels, allowing the creation of a microenvironment suitable for the creation, perpetuation, progression, and unstabilization of vascular injury, be it a simple eutrophic vascular remodeling, or an atherosclerotic lesion.

A likely explanation is the central role played by the type 1 angiotensin II receptor. Through its intracellular p40 subunit, it is increasing NAD(P)H-oxidase activity, generating an increment of free radicals at both, extracellular and intracellular levels, and activating different cascades of mediators such as PKC, JAK2, PI3 K, FAK, and PLC. These intracellular signal are able to induce translocation of the p65 subunit of NF-κB and AP-1 activation [21] with increased synthesis of cytokines, demonstrated in our work. This activation can also be mediated by oxygen free radicals without depending on the activation of the AT1R but this answer seems to be inferior, with less recruitment of cytokines. Another important point is close relationship with insulin grown factor (IGF) by Src. This signal favoring growth and remodeling of the extracellular matrix, stimulates production of intracellular inflammatory mediators such as NF-κB or AP-1, amplifying the vascular inflammatory response [22, 23].

This study demonstrates the central role of RAS in the pathophysiology of metabolic syndrome. Moreover, it could help to explain some of the findings obtained in large clinical trials.

Our question on these results analysis was whether the superoxide scavenger can restore the bioavailability of nitric oxide or is there another mechanism? This question was answered. Oxidative state is not the unique pathological mechanism involved in the activation of proinflammatory molecules or vascular remodeling, it is also needed an intricate messengers network, able to activate different cascades to enhance and extend endothelial responses to different cells involved in vascular injury process. RAS activates cascades of growth, cell differentiation, and proinflammatory mechanisms, acting as a key in the process of remodeling and vascular injury.

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Hindawi Publishing Corporation
International Journal of Hypertension
Volume 2012, Article ID 147321, 9 pages
doi:10.1155/2012/147321

Clinical Study

Consumption of High-Polyphenol Dark Chocolate Improves Endothelial Function in Individuals with Stage 1 Hypertension and Excess Body Weight

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Received 3 August 2012; Accepted 28 September 2012

Academic Editor: Mario Fritsch Neves

Background. Hypertension and excess body weight are important risk factors for endothelial dysfunction. Recent evidence suggests that high-polyphenol dark chocolate improves endothelial function and lowers blood pressure. This study aimed to evaluate the association of chocolate 70% cocoa intake with metabolic profile, oxidative stress, inflammation, blood pressure, and endothelial function in stage 1 hypertensives with excess body weight. *Methods*. Intervention clinical trial includes 22 stage 1 hypertensives without previous antihypertensive treatment, aged 18 to 60 years and presents a body mass index between 25.0 and 34.9 kg/m^2. All participants were instructed to consume 50 g of chocolate 70% cocoa/day (2135 mg polyphenols) for 4 weeks. Endothelial function was evaluated by peripheral artery tonometry using Endo-PAT 2000 (Itamar Medical). *Results*. Twenty participants (10 men) completed the study. Comparison of pre-post intervention revealed that (1) there were no significant changes in anthropometric parameters, percentage body fat, glucose metabolism, lipid profile, biomarkers of inflammation, adhesion molecules, oxidized LDL, and blood pressure; (2) the assessment of endothelial function through the reactive hyperemia index showed a significant increase: 1.94 ± 0.18 to 2.22 ± 0.08, $P = 0.01$. *Conclusion*.In individuals with stage 1 hypertension and excess body weight, high-polyphenol dark chocolate improves endothelial function.

1. Introduction

The endothelium has emerged as a key regulator of vascular homeostasis, acting as an active signal transducer for metabolic, hemodynamic, and inflammatory factors that modify the function and morphology of the vessel wall. Alterations in endothelial-cell function can precede the development of atherosclerotic changes and the progression of cardiovascular diseases [1]. Hypertension and excess body weight (body mass index (BMI) $\geq$25 kg/m^2) are conditions with high prevalence, being important risk factors for endothelial dysfunction [2–5]. According to World Health Organization (2009) [3] high blood pressure is responsible for 13% of deaths globally and overweight and obesity are responsible for 5%. Hypertension and obesity are frequently associated, and the causal association between obesity and elevated blood pressure has since long been demonstrated [6].

Lifestyle interventions, including diet, may affect endothelial function: high-fat diets impair endothelial function, and diets such as the Mediterranean diet are associated with improved endothelial function [7]. Recently, cocoa and cocoa-derived products such as chocolate with 70% or more cocoa (dark chocolate) have gained attention

because of evidences that they lower blood pressure and improve endothelial function [8–14]. These beneficial effects have been frequently ascribed to flavonoids, a subgroup of the polyphenolic family of antioxidant chemicals, abundantly present in fruits, vegetables, red wine, teas and cocoa. Catechin and its isomer epicatechin are types of flavonoids with strong antioxidant properties. Cocoa contains high concentrations of epicatechin and has been noted to have antioxidant content that is two times higher than that of red wine and almost three times higher than that of green tea [15, 16].

There are several plausible mechanisms by which polyphenols may improve endothelial function and lower blood pressure. In addition to their antioxidant effects which are assumed to increase the biodisponibility of nitric oxide (NO), polyphenols have been shown to increase the formation of NO by endothelial NO synthase via increased calcium level and redox-sensitive activation of the phosphoinositide 3 (PI3)-kinase/Akt pathway. Polyphenols also (1) enhance the production of endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin and (2) inhibit the synthesis of vasoconstrictors such as endothelin-1 and the angiotensin-converting enzyme [14, 17].

Recent studies have also demonstrated beneficial effects of dark chocolate or cocoa on insulin resistance [9, 18–20], lipid profile [21, 22], and inflammatory status [23]. However, the number of studies evaluating the effect of dark chocolate or cocoa on these cardiovascular risk factors is relatively low, and some authors did not find all these benefits [24, 25].

The effects of dark chocolate or cocoa have already being evaluated in hypertensive individuals [9, 10, 19]. However, there is a lack of studies evaluating its effect in hypertensive individual presenting overweight and obesity. Therefore, the aim of the present study was to association of chocolate 70% cocoa intake with metabolic profile, oxidative stress, biomarkers of inflammation, blood pressure and endothelial function in stage 1 hypertensive subjects with excess body weight.

2. Materials and Methods

This pre-post trial was conducted at the Laboratory of Clinical and Experimental Pathophysiology, CLINEX, located at Pedro Ernesto University Hospital, of Rio de Janeiro State University. Written informed consent was obtained from all the enrolled patients. The study protocol was approved by the Human Ethics Committee of Pedro Ernesto University Hospital. The procedures followed in this study were in accordance with institutional guidelines.

Potential participants were recruited at the Department of Plastic Surgery, among the candidates for lipoplasty. Candidates for the study, underwent eligibility screening by registered dietitians. Subjects were screened according to the following criteria: age between 18 and 60 years, BMI from 25.0 to 34.9 kg/m^2 and diagnosis of stage 1 hypertension (without previous antihypertensive treatment) [26]. The exclusion criteria were current use of antioxidant and dietary supplements; use of any medication known to interfere in body weight, blood pressure, and metabolic profile; recent changes (within previous 6 months) in dietary intake, body weight (>3 kg), and intensity or frequency of physical exercise. Individuals with eating disorders, major depression, or a medical history of drug addiction were excluded. Those with any metabolic disease, such as diabetes mellitus or hypothyroidism or chronic diseases severely affecting the cardiovascular, gastrointestinal, and renal systems were also excluded. Pregnant or lactating women were not allowed into the study.

Of the 550 individuals initially screened, 28 entered the run-in period. During the 2-week run-in period all cocoa foods were excluded, and potential participants were submitted to clinical, dietary, anthropometric, and biochemical evaluation. Six subjects failed to complete the run-in period, four individuals because their levels of blood pressure were not in the range of stage 1 hypertension and two because of lost of interest. So after completing the run-in period, 22 participants were included in the study and 20 completed the follow-up period (4 weeks), being included in the final analysis (Figure 1). The noncompleters left the study because of scheduling conflicts.

During the intervention phase (4 weeks), participants were submitted to clinical and nutritional assessment at baseline (week 0) and weeks 1, 2, 3, and 4. All participants were instructed to consume 50 g of chocolate 70% cocoa/day (containing 2135 mg polyphenols) for 4 weeks (Table 1). They received instructions to consume 25 g in the morning and 25 g in the afternoon. At baseline and at every week participants received the amount of chocolate sufficient for 7 days of the study. The development and reinforcement of strategies for continued success were made at the same time points.

Body weight, waist circumference, hip circumference were measured at baseline and at weeks 1, 2, 3, and 4. At baseline and at week 4 participants were also submitted to ambulatory blood pressure monitoring (ABPM) and evaluation of endothelial function; body composition and fasting plasma levels of circulating insulin, glucose, leptin, lipid profile (triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol), biomarkers of inflammation (C-reactive protein, interleucina-6, and tumor necrosis factor-α), biomarker of oxidative stress (oxidized LDL), and biomarkers of endothelial dysfunction (intracelluar adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin).

The dietary assessments during the run-in period used a 3-day food record, covering 2 weekdays, and 1 weekend day, to estimate current energy and nutrients consumption. To avoid weight gain during the study period, patients were instructed to reduce their habitual energy intake proportionally to the energy supplied by the chocolate (280 Kcal/day; mainly from lipid-rich foods). During the intervention phase, the food records were reviewed and clarified in an interview with a nutritionist every week to assess dietary adherence. Nutrient analysis of the 3-day food record was performed using the software NutWin (São Paulo Federal University, UNIFESP, São Paulo, Brazil).

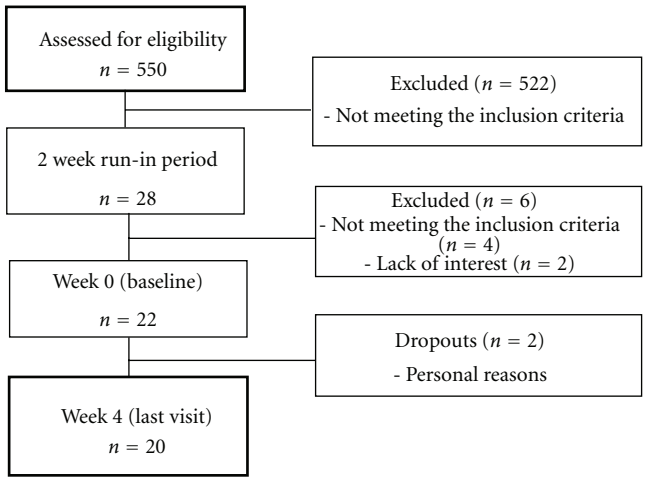

Figure 1: Flow diagram of the study.

Table 1: Nutrient composition of the dark chocolate used in the study (50 g).

Nutrient	Dark chocolate (70% cocoa)
Energy (kcal)	228
Protein (g)	4.8
Carbohydrate (g)	20
Total fat (g)	20
Saturated fat (g)	13.4
Sodium (mg)	60

Reported total energy, percent of energy from protein, fat and carbohydrate, and fiber content at the run-in phase were similar to that of the last week of the study. Subjects were carefully instructed to refrain from flavonoid-rich foods and beverages, including tea and wine; a list of these foods and beverages was given to each participant. All participants were encouraged to continue their usual physical activity throughout the study period.

Height, weight, and waist and hip circumferences were measured from 08:00 to 10:00 h after a 12 h fast. Height was measured using a stadiometer accurate to ±0.5 cm, and weight was obtained with a calibrated scale, accurate to ±0.1 kg (Filizola S.A., São Paulo, SP, Brazil), with participants wearing light clothing and no shoes. BMI was calculated using the standard equation (kilograms per meters squared). Waist circumference was measured in the standing position, midway between the lower margin of the last rib and the iliac crest. The measurements were taken at midexhalation. Hip circumference was measured at the widest point of the hip/buttocks area with the measuring tape parallel to the floor. Waist-to-hip ratio was determined by dividing waist circumference by hip circumference. Anthropometric measurements were taken twice, and mean values were used in all analyses. Percentage of body fat was estimated by electrical bioimpedance using a Biodynamics BIA-450 body fat analyzer (Biodynamics Corp., Seattle, WA, USA).

Blood samples were collected after a 12 h fasting period and were stored at −80°C. Total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed by using an automated analyzer. LDL cholesterol was calculated using the Friedewald formula [27] when triglycerides did not exceed 400 mg/dL. Radioimmunoassay was used to determine plasma leptin and insulin levels (Linco Research, St Charles, MO, USA, double antibody solid-phase enzyme immunoassay). Fasting plasma glucose was determined by the use of glucose oxidase method. The insulin resistance status was assessed by the use of homeostasis model assessment of insulin resistance (HOMA-IR) index, that is, serum insulin (μU/mL) × plasma glucose (mmol/L)/22.5 [28]. The values of VCAM-1, ICAM-1 and E-Selectin were determined by immunonephelometry enzymatic immunoassay using a commercial kit of multiple dosing (LINCO Research, St Charles, MO, USA). Plasma levels of TNF-α and IL-6 were determined by enzymatic immunometric method (TiterZyme EIA) using commercial kits (Assay Designs, Ann Arbor, MI, USA). Highly sensitive CRP (hs-CRP) was determined by turbidimetry, using commercial kit (Biosystems, Barcelona, Spain).

2.1. Endothelial Function. Endothelial function was evaluated by peripheral artery tonometry (PAT) using Endo-PAT 2000 (Itamar Medical, Caesarea, Israel), a finger plethysmographic device that allows the isolated detection of pulsatile arterial volume changes [29]. Endo-PAT 2000 is a noninvasive technology and was approved by the Food and Drug Administration for use as a diagnostic aid in patients with signs and symptoms of ischemic heart disease [30]. There is evidence of a significant relationship between hyperemia-induced finger pulse wave amplitude changes, defined as the PAT hyperemia ratio, and brachial artery flow-mediated dilation [31].

Endo-PAT consists of two finger-mounted probes, which include a system of inflatable latex air cushions within a rigid external case. The probe design allows the application of a constant and evenly distributed near-diastolic counterpressure within the entire probe, which increases sensitivity by unloading arterial wall tension and prevents venous blood pooling to avoid venoarteriolar reflex vasoconstriction. Pulsatile volume changes of the fingertip are sensed by a pressure transducer and transferred to a personal computer where the signal is band pass filtered (0.3 to 30 Hz), amplified, displayed, and stored [29]. The Endo-PAT studies were performed with the patient in the supine position and both hands on the same level in a comfortable, thermoneutral environment. A blood pressure cuff was placed on one upper arm (study arm), while the contralateral arm served as a control (control arm); Endo-PAT probes were placed on one finger of each hand (same finger on both hands). A continuous recording of pulsatile blood volume responses from both hands was initiated. After a 10 min equilibration period, the blood pressure cuff on the study arm was inflated to 60 mmHg above systolic pressure for 5 min. The cuff was then deflated to induce reactive hyperemia, whereas PAT recording was continued. The reactive hyperemia index

(RHI) obtained with Endo-PAT is analyzed by a computer in an operator-independent manner.

2.2. Blood Pressure. During the run-in phase blood pressure was recorded by using a calibrated Dinamap 1846 Critikon automated sphygmomanometer (Critikon, Tampa, FL, USA) after a resting period of at least 10 min in the sitting position. An appropriate arm cuff was used. Arm position was adjusted so that the cuff was at the level of the right atrium. Blood pressure was measured on the nondominant arm every 3 min for 15 minutes. The first value was discarded, and the mean of the last five readings was used in the analysis. Patients were considered to have stage 1 hypertension if their systolic blood pressure levels were between 140–159 mmHg and/or diastolic blood pressure between 90–99 mmHg [26].

At baseline (week 0) and at the end of the study (week 4) blood pressure was evaluated by Ambulatory Blood Pressure Monitoring (ABPM) to improve the estimate of "true" blood pressure. Ambulatory blood pressure was recorded using the SpaceLabs 90207 oscillometric blood pressure monitors (SpaceLabs, Redmond, WA, USA) calibrated against a mercury sphygmomanometer before use on each patient. Monitors were programmed to read blood pressure and heart rate every 20 min from 6:00 to 18:00 h. and every 30 min from 18:00 to 6:00 h. Mean daytime (6:00 to 18:00 h), and nighttime (18:00 to 6:00 h) blood pressure and heart rate were calculated.

2.3. Statistical Analysis. Means ± standard errors were used to summarize continuous variables. To test the possible association of chocolate 70% cocoa intake with nutritional parameters, biochemical variables, endothelial function, and blood pressure, we compared data obtained at baseline (week 0) with data obtained at the end of the study (week 4). Paired Student's t-test was used when variables had normal distribution and Wilcoxon test was used for variables without normal distribution.

Correlation tests were conducted to determine the relationship between RHI and variables of interest. Partial correlations controlled for different confounders were also used.

On the basis of a previous study [9], this trial was designed to have 80% power to detect a significant difference in systolic blood pressure evaluated by ABPM before and after the intake of dark chocolate. Assuming 20% dropout rate, we needed at least 12 participants in the study. GraphPad PRISM 5.0 (GraphPad Software Inc, San Diego, CA, USA) and Stata 10.0 (STATA Corp., College Station, TX, USA) were used for statistical analysis. $P < 0.05$ was considered statistically significant.

3. Results

Twenty participants (10 men and 10 women) completed the study and were included in the final analysis. The average age of these patients was 44.00 ± 2.87 years, and their BMI was 31.29 ± 1.16 kg/m^2. During the run-in phase, systolic and diastolic blood pressure levels were 146.50 ± 1.28 and 93.20 ± 0.74 mmHg, respectively.

As expected from the experimental design, all anthropometric parameters and the percentage of body fat remained almost unchanged after the 4 weeks of chocolate supplementation (Table 2). As presented in Table 3, at the end of the study, there were no significant changes in glucose metabolism, lipid profile, biomarkers of inflammation, and oxidized LDL.

The comparative analysis of the values obtained with ABPM between week 0 and week 4 revealed no significant modifications. However, there was a small decrease in both systolic and diastolic blood pressure at 24 h, daytime and nighttime (Figure 2).

After 4 weeks of chocolate supplementation there was a significant increase in RHI (Figure 3). Serum levels of adhesion molecules, which are biomarkers of endothelial dysfunction, also showed a decrease, however, without reaching statistical significance (Figure 4).

An inverse and a significant association was observed between the RHI at baseline and the modification in this index during the study period ($r = -0.60$; $P = 0.02$). Even after adjusting for confounders, this association remained significant ($r = -0.70$; $P = 0.04$). The confounding factors included in this analysis were age and changes during the study period in BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-CRP, and HOMA.

The changes in RHI during the study presented a negative and significant association with the modifications in diurnal systolic and diastolic blood pressure ($r = -0.69$; $P = 0.04$ and $r = -0.83$; $P = 0.006$) after adjusting for age and changes in BMI, HOMA, and hs-CRP. Modifications in nocturnal and 24 h blood pressure did not present significant associations with changes in RHI.

4. Discussion

In the present study, based on a sample of subjects with stage 1 hypertension and excess body weight, the main finding was that the consumption of high-polyphenol dark chocolate 70% cocoa (50 g/day, during four weeks) improved endothelial function.

An improvement in endothelial function after high-polyphenol cocoa and/or dark chocolate intake was observed in several studies [9, 11, 12, 18, 19, 32–37]. These studies have different designs, varying principally in relation to the time and dose of supplementation and in relation to the criteria of eligibility of the participants. Even with different designs the studies found significant improvement in endothelial function that was evaluated in the great majority of the trials by flow-mediated vasodilatation of the brachial artery [9, 11, 12, 19, 34–36]. To our knowledge only one study [12] evaluated endothelial function using the same method that we used (pulse-wave amplitude on the finger assessed by PAT), although the device that was used in our study is a different one.

The duration of the studies varies widely: there are trials that observe acute effects (in general 2 h after the intake of

Table 2: Anthropometric parameters and percentage of body fat at baseline (week 0) and at the end of the study (week 4).

Characteristic	Week 0 ($n = 20$)	Week 4 ($n = 20$)	P
Body weight (kg)	84.80 ± 3.88	84.63 ± 3.91	0.55
Body mass index (kg/m^2)	31.29 ± 1.16	31.26 ± 1.19	0.83
Waist circumference (cm)	94.30 ± 2.73	94.07 ± 2.83	0.59
Hip circumference (cm)	110.70 ± 2.50	110.65 ± 2.49	0.85
Waist-to-hip ratio	0.85 ± 0.14	0.85 ± 0.15	0.79
Body fat (%)	36.59 ± 1.45	36.46 ± 1.41	0.69

Values are expressed as mean ± standard error.

Table 3: Metabolic variables and biomarkers of inflammation and oxidative stress at baseline (week 0) and at the end of the study (week 4).

Variable	Week 0 ($n = 20$)	Week 4 ($n = 20$)	P
Glucose (mg/dL)	90.60 ± 2.60	88.65 ± 2.75	0.55
Insulin (μU/mL)	21.86 ± 3.11	23.49 ± 2.94	0.19
HOMA-IR	4.94 ± 0.79	5.08 ± 0.63	0.20
Total cholesterol (mg/dL)	199.00 ± 7.41	195.15 ± 9.25	0.55
HDL cholesterol (mg/dL)	50.85 ± 2.31	48.75 ± 2.64	0.43
LDL cholesterol (mg/dL)	122.15 ± 6.71	122.00 ± 9.24	0.98
Triglycerides (mg/dL)	132.80 ± 11.18	122.55 ± 11.77	0.29
High sensitive CRP (mg/dL)	0.93 ± 0.27	0.61 ± 0.12	0.24
Tumor necrosis factor-α (pg/mL)	17.51 ± 8.03	18.96 ± 9.02	0.18
Interleucine-6 (pg/mL)	87.87 ± 20.6	69.40 ± 14.7	0.17
Oxidized LDL (μg/mL)	0.11 ± 0.01	0.12 ± 0.01	0.60

Values are expressed as mean ± standard error.
HDL: high-density lipoprotein, LDL: low density lipoprotein, CRP: C-reactive protein.

cocoa products) [18, 33–36], or short-term effects: 5 days [12], 2 weeks [9, 11, 19, 37], 4 weeks [38], 6 weeks [32] and 12 weeks [18]. The duration of the present study, although was not sufficient to evaluate the long-term effect of dark chocolate intake on endothelial function, it is greater than the duration of several studies.

The participants of the clinical trials that observed improvements in endothelial function with cocoa supplementation included health individuals [11, 12, 37], hypercholesterolemic postmenopausal women [32], heart transplant recipients [33], smokers [35, 36], hypertensives [9], hypertensives with impaired glucose intolerance, [19], individuals with diabetes [38], and overweight and obese individuals [18]. As stated before, only individuals with hypertension and excess body weight were included in the present study, creating a difference from other studies and showing that in this population of high risk to endothelial dysfunction the supplementation of high-polyphenol dark chocolate alone (without specific treatment for hypertension and excess body weight) can improve endothelial function.

The participants of this study presented a decrease in blood pressure levels, although without reaching statistical significance. This finding contrasts with some studies that also evaluated the effects of cocoa and/or cocoa rich chocolate in hypertensive individuals [8–10, 19].

Desch et al. (2010) [8] performed a meta-analysis of randomized controlled trials assessing the antihypertensive effects of flavanol-rich cocoa products. In total 10 trials comprising 297 individuals were included. The populations studied were either healthy normotensive adults or patients with prehypertension/stage 1 hypertension. This meta-analysis confirmed the blood pressure lowering capacity of flavanol-rich cocoa [8]. However, in a recently published meta-analysis [39] newer studies were included, totalizing 15 trials and were performed a subgroup analysis by baseline blood pressure (hypertensive/normotensive). Pooled meta-analysis of all trials revealed a significant blood pressure-reducing effect of cocoa-chocolate compared with control. However, subgroup meta-analysis was significant only for the hypertensive or pre-hypertensive subgroups, while blood pressure was not significantly reduced in the normotensive subgroups [39].

Possible explanations for the lack of significant reduction in blood pressure in the present study are (1) baseline levels of blood pressure, evaluated by ABPM, in our patients were lower than the levels found in others studies and (2) dose of dark chocolate was also lower [9, 19]. Ried et al. (2009) [40] did not find a blood pressure reducing effect of 50 g dark chocolate daily over a period of 8 weeks in a prehypertensive population.

In the present study, the lipid profile had no significant modifications. However, some studies observed significant reductions on total and/or LDL cholesterol after the intake of dark chocolate or cocoa [19, 21, 22]. Mursu et al. (2004) [41] found that the ingestion of 75 g/day of dark chocolate for 15 days increased HDL cholesterol. The changes seen in lipid profile in the studies cited above were highly dependent on the dose of cocoa consumption and health status of

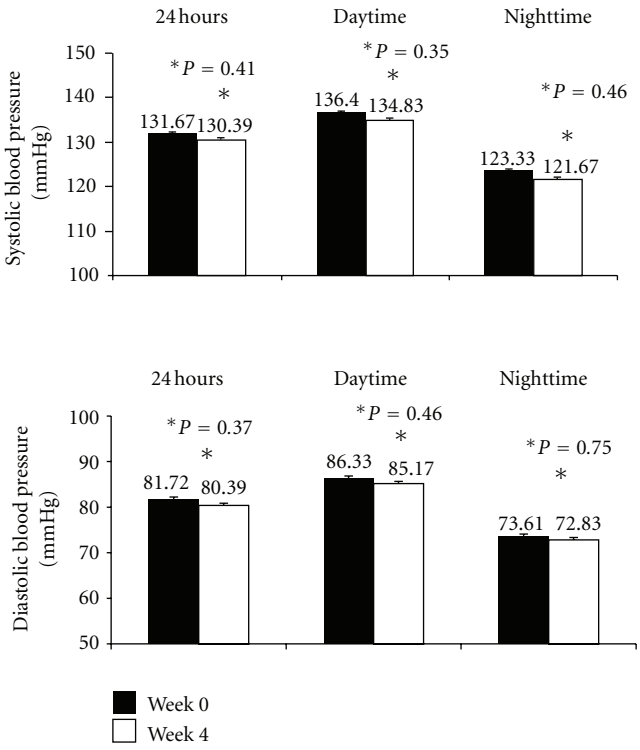

Figure 2: Mean values of systolic and diastolic blood pressure evaluate by ambulatory blood pressure monitoring at baseline (week 0) and at the end of the study (week 4) ($n = 20$).

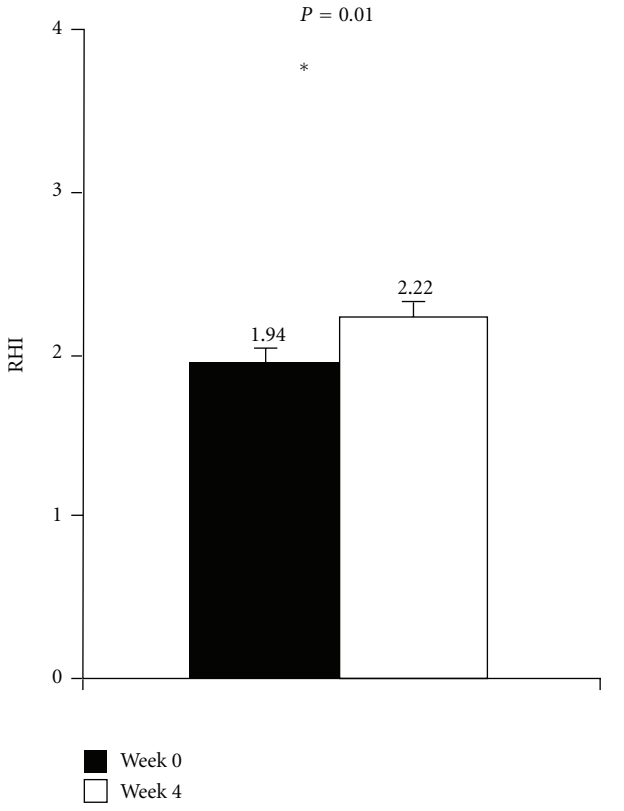

Figure 3: Mean reactive hyperemia index (RHI) evaluated by Endo-PAT2000 at baseline (week 0) and at the end of the study (week 4) ($n = 20$).

patients [16]. The dose of dark chocolate used in the study of Mursu et al. (2004) [41] and Grassi et al. (2008) [19] was 75 g/day and 100 g/day, respectively. Therefore, one possible explanation for the significant changes in serum lipids on our study may be the dose of chocolate. Another possible explanation is that at baseline our participants had mean levels of lipid profile within the normal range according to NCEP (2001) [42].

The content of lipids in dark chocolate is high. As seen in Table 1, in the present study, the ingestion of 50 g/day of chocolate resulted in an intake of 20 g of total fat/day. Despite its high fat content, cocoa itself does not seen to exert untoward effects on serum lipids (and in some studies has beneficial effects), because cocoa butter is composed on average of 33% oleic acid, 25% palmitic acid, and 33% of stearic acid [43]. Oleic acid is a monounsaturated fat that lowers LDL cholesterol [42] and although palmitic and stearic acids are saturated fats, stearic acid in comparison with other saturated fatty acids lowers LDL cholesterol [44, 45].

All participants included in the present study had excess body weight (BMI from 25.0 to 34.9 kg/m^2). As weight loss improves endothelial function and decreases blood pressure, while weight gain has the opposite effect [46–48]; an important issue of our study was to instruct the participants to maintain their body weight. It is important to notice that all the patients included in our study did not present recent changes in body weight before the study and were not planning to begin an energy restricted diet or to increase physical activity. After the end of the study all participants were scheduled to a consultation with a nutritionist and with a physician to begin the treatment for hypertension and weight loss.

There are several limitations in our study. One of them is the lack of a control group, which was not planned in the initial design of the study. However, the eligibility criteria of the present study were to rigorously to try to avoid confounding factors, so as seen in Figure 1 we assessed for eligibility 550 individuals and only 22 could be included in the study.

5. Conclusion

The findings of the present study suggest that, in individuals with stage 1 hypertension and excess body weight, the consumption of high-polyphenol dark chocolate is associated with improvements in endothelial function.

Authors' Contribution

Lívia de Paula Nogueira: study conception and design; data collection, assembly, analysis, and interpretation; paper drafting; approval of the manuscript final version. Marcela Paranhos Knibel: data collection, assembly, analysis, and interpretation; manuscript drafting; approval of the manuscript final version. Márcia Regina Simas Gonçalves Torres: data analysis and interpretation; manuscript drafting; approval of the manuscript final version. José Firmino

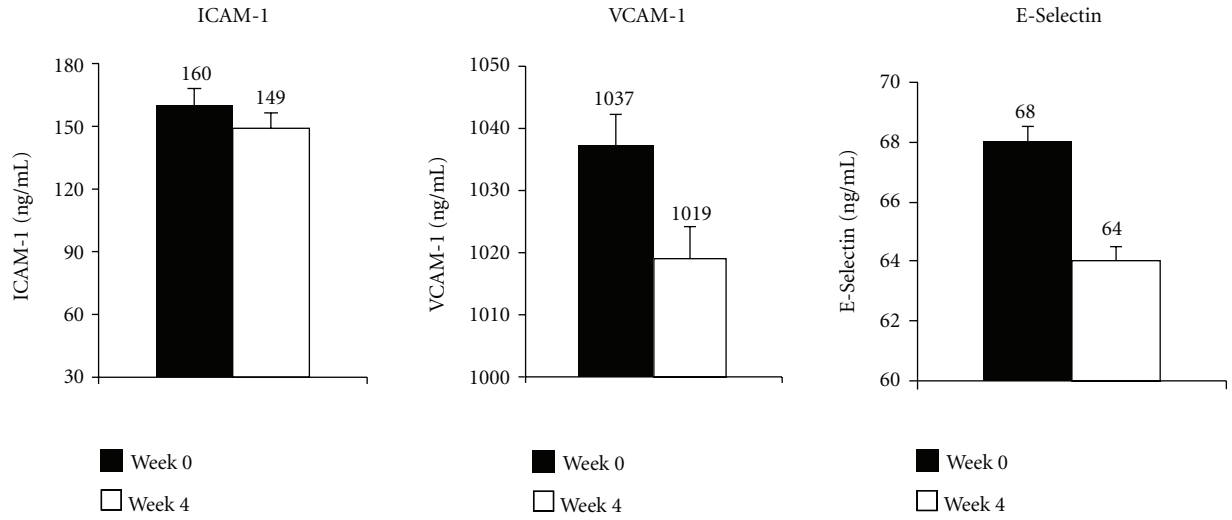

Figure 4: Mean values of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin at baseline (week 0) and at the end of the study (week 4) ($n = 20$).

Nogueira Neto: laboratory analysis, manuscript drafting and approval of the manuscript final version. Antonio Felipe Sanjuliani: study conception and design; data analysis and interpretation; manuscript drafting and revision; approval of the manuscript final version.

Acknowledgment

This study was supported by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

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