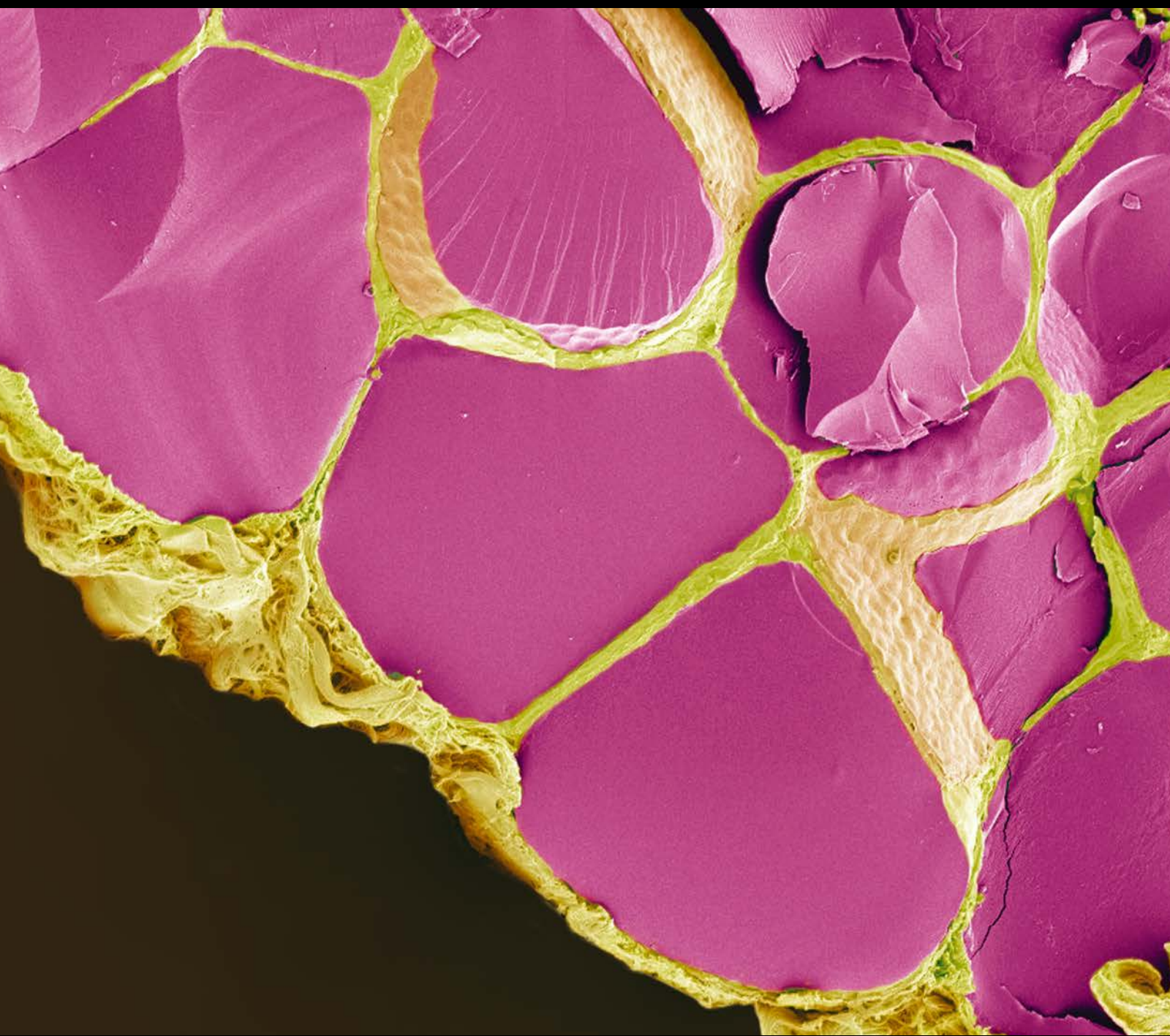
International Journal of Endocrinology

Regulation of Lipid Metabolism and Beyond

Guest Editors: Youngah Jo, Hiroaki Okazaki, Young-Ah Moon, and TongJin Zhao



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Editorial Board

Contents

Regulation of Lipid Metabolism and Beyond
Youngah Jo, Hiroaki Okazaki, Young-Ah Moon, and TongJin Zhao
Volume 2016, Article ID 5415767, 2 pages

Obesity and Insulin Resistance Are the Main Determinants of Postprandial Lipoprotein Dysmetabolism in Polycystic Ovary Syndrome
Tommy Kyaw Tun, Anne McGowan, Niamh Phelan, Neuman Correia, Gerard Boran, Anna-Louise O'Connor, Helen M. Roche, and James Gibney
Volume 2016, Article ID 9545239, 11 pages

The Role of miR-378a in Metabolism, Angiogenesis, and Muscle Biology
Bart Krist, Urszula Florczyk, Katarzyna Pietraszek-Gremplewicz, Alicja Józkowicz, and Jozef Dulak
Volume 2015, Article ID 281756, 13 pages

Betatrophin Acts as a Diagnostic Biomarker in Type 2 Diabetes Mellitus and Is Negatively Associated with HDL-Cholesterol
Min Yi, Rong-ping Chen, Rui Yang, Xian-feng Guo, Jia-chun Zhang, and Hong Chen
Volume 2015, Article ID 479157, 7 pages

Role of FTO in Adipocyte Development and Function: Recent Insights
Myrte Merkestein and Dyan Sellayah
Volume 2015, Article ID 521381, 7 pages

The Intricate Network of Adipokines and Stroke
Ema Kantorová, Ľubica Jesenská, Daniel Čierny, Kamil Zeleňák, Štefan Sivák, Matej Stančík, Peter Galajda, Vladimír Nosáľ, and Egon Kurča
Volume 2015, Article ID 967698, 8 pages

Paraoxonase 2 Induces a Phenotypic Switch in Macrophage Polarization Favoring an M2 Anti-Inflammatory State
Marie Koren-Gluzer, Mira Rosenblat, and Tony Hayek
Volume 2015, Article ID 915243, 9 pages

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International Journal of Endocrinology
Volume 2016, Article ID 5415767, 2 pages
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Editorial

Regulation of Lipid Metabolism and Beyond

Youngah Jo,[1] Hiroaki Okazaki,[2] Young-Ah Moon,[3] and TongJin Zhao[4]

[1]*Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX, USA*
[2]*Department of Diabetes and Metabolic Diseases, University of Tokyo, Tokyo, Japan*
[3]*Department of Molecular Medicine, Inha University School of Medicine, Incheon, Republic of Korea*
[4]*School of Life Sciences, Xiamen University, Xiamen, Fujian, China*

Correspondence should be addressed to Youngah Jo; youngah.jo@utsouthwestern.edu

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One of the worldwide health issues is the increasing number of obese population. In 1975 it was less than 100 million but it reached 600 million in 2014 according to WHO report in 2015. More than one-third of adults and 17% of youth are obese in America [1, 2]. Obesity is one of the major risk factors for diverse human diseases and more and more research papers are coming to dissect the mechanism and find new therapeutic targets. This special issue has been opened to collect recent studies on lipid metabolism including its regulation-dysregulation and new possible biomarkers that can be used for diagnosis of obesity and other related diseases. Also it includes reviews to focus on current studies about regulation mechanism of lipid metabolism.

Lipids play important roles in the body to store energy and as the components of biological membranes, steroid hormones, bile acids, vitamins, and so forth. They are supplied from diets or from the de novo synthesis in the liver. Fatty acids mainly stored as triglycerides are the major energy source for muscle and heart. However, the overproduction and accumulation of triglycerides in adipose tissue and other tissues are closely related to the human metabolic disorders. Disturbance of cholesterol homeostasis is also closely related to atherosclerosis.

The lipids taken from diets are wrapped in chylomicrons to be mobilized to liver or peripheral tissues. Many lines of studies have demonstrated the postprandial hypertriglyceridemia as one of the risk factors for cardiovascular diseases in obese individuals [3]. However it has been unclear why obese patients with polycystic ovarian syndrome (PCOS) have higher incidence of cardiovascular diseases. T. K. Tun et al. have an attention to, in particular, the settings of the postprandial changes in the lipid profile by comparison of two obese groups of PCOS or non-PCOS. The study demonstrates that obese PCOS individuals have higher association with insulin resistance and obesity when compared to the obese control non-PCOS group. Therefore the obesity in polycystic ovary syndrome should be considered as a risk factor and managed to reduce their cardiovascular disease burden.

Another research article demonstrates the one possible biomarker in newly diagnosed type 2 diabetic patients. The role of betatrophin is controversial [4, 5]. M. Yi et al. measured the levels of betatrophin and observed that higher betatrophin level is related to the type 2 diabetes and reversely correlated with the HDL levels. It suggests betatrophin as a new marker for diagnosis of type 2 diabetes in early stage.

Cardiovascular disease (CVD) is a significant health and financial burden to our society that warrants new and more effective therapies. Recent research has discovered novel functions of HDL in the trafficking of microRNAs (miRNAs) as a part of intercellular gene-regulation networks. It has become clear in recent years that miRNAs are one of the many classes of noncoding regulatory small RNAs [6]. Here in this special issue the role of miR-378a has been described. It explains the biological basics and target genes of miR-378a and the roles of miR-378a in lipid metabolism, muscle biology, and proangiogenic effect on blood vessel formation.

Another review article is describing the role of FTO gene, which is associated with obesity in humans and has been identified as the first gene related to obesity in human from the genome-wide association study. The review is explaining

how the SNP of FTO gene in intron 1 regulates the expression of various genes to increase adiposity in various tissues during developmental stages.

As the role of adipose tissue has shed light on the studies of obesity-related diseases, one review article is demonstrating the role of various adipokines in many metabolic diseases. E. Kantorová et al. have summarized the role of adipokines in neurological diseases.

The complication of dyslipidemia is also related to the inflammatory processes involved in the pathogenesis of atherosclerosis. M. Koren-Gluzer et al. are demonstrating the anti-inflammatory role of paraoxonase 2 in induction of polarization of macrophage to M2 state using a knockout mice model of paraoxonase 2.

There are many factors that regulate lipid metabolism. This special issue is representing basic research and clinical works using a small number of samples and reviews of current studies. However it will provide a line of data to make many people understand the mechanism of lipid regulation and to develop new possible biomarkers or drugs to advance our human life.

Youngah Jo
Hiroaki Okazaki
Young-Ah Moon
TongJin Zhao

References

[1] NCD Risk Factor Collaboration, "Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19•2 million participants," *The Lancet*, vol. 387, no. 10026, pp. 1377–1396, 2016.

[2] C. L. Ogden, M. D. Carroll, B. K. Kit, and K. M. Flegal, "Prevalence of childhood and adult obesity in the United States, 2011-2012," *The Journal of the American Medical Association*, vol. 311, no. 8, pp. 806–814, 2014.

[3] A. Pirillo, G. D. Norata, and A. L. Catapano, "Postprandial lipemia as a cardiometabolic risk factor," *Current Medical Research and Opinion*, vol. 30, no. 8, pp. 1489–1503, 2014.

[4] K. H. Kaestner, "Betatrophin—romises fading and lessons learned," *Cell Metabolism*, vol. 20, no. 6, pp. 932–933, 2014.

[5] M. Abu-Farha, D. Sriraman, P. Cherian et al., "Circulating ANGPTL8/betatrophin is increased in obesity and reduced after exercise training," *PLoS ONE*, vol. 11, no. 1, Article ID e0147367, 2016.

[6] S. Vienberg, J. Geiger, S. Madsen, and L. T. Dalgaard, "MicroRNAs in metabolism," *Acta Physiologica*, 2016.

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Research Article

Obesity and Insulin Resistance Are the Main Determinants of Postprandial Lipoprotein Dysmetabolism in Polycystic Ovary Syndrome

Tommy Kyaw Tun,[1] Anne McGowan,[1] Niamh Phelan,[1] Neuman Correia,[1] Gerard Boran,[2] Anna-Louise O'Connor,[3] Helen M. Roche,[3] and James Gibney[1]

[1]*Department of Endocrinology and Diabetes, Tallaght Hospital, Tallaght, Dublin 24, Ireland*
[2]*Department of Chemical Pathology, Tallaght Hospital, Tallaght, Dublin 24, Ireland*
[3]*Nutrigenomics Research Group, UCD Conway Institute of Biomolecular and Biomedical Research, School of Public Health and Population Science, University College Dublin, Belfield, Dublin 4, Ireland*

Correspondence should be addressed to Tommy Kyaw Tun; tommykyawtun@rcsi.ie

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Postprandial dyslipidaemia may be a plausible mechanism by which polycystic ovary syndrome (PCOS) increases cardiovascular risk. We sought to investigate whether the postprandial glucose and insulin and lipid and lipoprotein responses, including that of apolipoprotein B-48 (apoB-48) containing chylomicrons, to a mixed meal are different in obese PCOS women when compared to obese control subjects and whether differences, if any, are related to obesity, insulin resistance (IR), hyperandrogenaemia, or PCOS status. 26 women with PCOS (age 30.4 ± 1.2 years (mean ± SEM), body mass index (BMI) 36.8 ± 1.5 kg/m^2) and 26 non-PCOS subjects (age 34.1 ± 0.9 years, BMI 31.5 ± 1.0 kg/m^2) were studied before and up to 8 hours following a standard mixed meal. AUC-triglyceride (AUC-TG) was higher and AUC-high-density lipoprotein (AUC-HDL) lower in PCOS women. These differences were not apparent when BMI was accounted for. Insulin sensitivity (S_I), AUC-apoB-48, and AUC-apolipoprotein B (AUC-apoB) were found to be independent predictors of AUC-TG, accounting for 55% of the variance. Only AUC-insulin remained significantly elevated following adjustment for BMI. Obesity related IR explains postprandial hypertriglyceridaemia and hyperinsulinaemic responses. Management of obesity in premenopausal women with PCOS is likely to reduce their cardiovascular risk burden.

1. Introduction

There is considerable evidence from epidemiological research, meta-analysis [1, 2], and prospective clinical trials [3, 4] to support an independent role for fasting and postprandial plasma triglycerides (TG) as a risk factor for cardiovascular disease (CVD). Increased plasma remnants of triglyceride-rich lipoproteins (TRLs), caused by delayed elimination of the same, has been shown to be prospectively associated with angiographic evidence of atherosclerosis and cardiac events [5]. Postprandial hypertriglyceridaemia is associated with two other well established cardiovascular risks—IR and obesity—both of which are prevalent in PCOS. An increase in postprandial lipoproteins is often found in IR states [6] and hyperinsulinaemia itself appears to delay and exacerbate postprandial accumulation of intestinally derived chylomicrons [7]. Obesity, especially visceral adiposity, also contributes to a magnified postprandial TG response [8, 9].

Postprandial hypertriglyceridaemia may be a plausible mechanism by which PCOS increases cardiovascular risk. Whilst numerous studies have investigated plasma lipid profile under fasting conditions in women with PCOS, few have investigated changes in the postprandial setting. In a previous study by Velázquez M et al. a strong positive correlation between postprandial TG and increasing waist-to-hip ratio (WHR) was demonstrated [10]. That study

evaluated overweight (BMI 27.41±0.50 kg/m^2) PCOS women and compared them to lean PCOS women and lean control subjects. There were no obese controls and there was no analysis of the influence of androgens on postprandial hypertriglyceridaemia [10]. A separate study by Bahceci et al. evaluated postprandial responses to an oral fat tolerance test, comparing lean PCOS women with lean controls (BMI 23.5 ± 2.6 kg/m^2 versus 23.1 ± 4.0 kg/m^2; $p > 0.05$) [11]. There were no obese or overweight controls in this study. They showed that PCOS women had higher baseline insulin levels, IR as assessed by homeostatic model assessment-IR ($HOMA_{IR}$), AUC-TG, AUC-total-cholesterol, AUC-very-low-density lipoprotein cholesterol (AUC-VLDL-chol), and AUC-apoB. Surprisingly, though, AUC-insulin did not differ between the two groups. As with the study by Velázquez M et al., no analyses were reported between androgens and postprandial lipids [11].

It is also unclear whether postprandial hypertriglyceridaemia is mainly due to the contribution of intestinally derived apoB-48 containing chylomicrons or hepatically derived apoB-100 containing VLDL particles. Both VLDL and chylomicrons share a common lipolytic pathway and are hydrolysed by lipoprotein lipase (LPL), an enzyme predominantly found on the endothelial surfaces of the capillaries of adipose tissue, heart, and skeletal muscle. Hydrolysis results in the formation of a spectrum of smaller, denser particles. There are a number of described receptor mediated pathways through which these particles may eventually be cleared from the circulation [12]. Traditionally it has been difficult to assess what proportions of TRLs are chylomicrons and VLDL. However, using ELISA, it is now possible to directly measure the quantity of apoB-48 and hence chylomicron particles, in whole plasma [13, 14].

We sought to investigate whether the postprandial glucose and insulin and lipid and lipoprotein responses, including that of apolipoprotein B-48 (apoB-48) containing chylomicrons, to a mixed meal are different in obese PCOS women when compared to obese control subjects and whether differences, if any, are related to obesity, insulin resistance (IR), hyperandrogenaemia, or PCOS status.

2. Methods

2.1. Subjects. Fifty-two obese premenopausal women with ($n = 26$) and without ($n = 26$) PCOS were recruited. Women with PCOS were recruited from the endocrinology outpatient clinics (Tallaght Hospital, Dublin, Ireland). Normal women were recruited by local advertisement. PCOS was defined according to the National Institute of Health (NIH) criteria as chronic oligomenorrhea (fewer than nine menstrual cycles per year) and clinical and/or biochemical evidence of hyperandrogenism, in the absence of other disorders causing the same phenotype [15]. Clinical criteria included hirsutism with a Ferriman-Gallwey score greater than 9, acne, or male pattern alopecia; biochemical criteria included total testosterone, androstenedione, or dehydroepiandrosterone sulphate (DHEAS) greater than the laboratory reference range. All normal subjects were eumenorrheic with testosterone levels within the normal range and were studied in the follicular phase of the menstrual cycle. Subjects were excluded if they were younger than 18 years old or older than 45 years old; were non-Caucasian, pregnant, or lactating; had a recent or chronic illness or medication likely to influence results; or were taking any medications likely to influence the results including hormonal contraception, antihypertensives, lipid-lowering medications, and antiplatelet or anti-inflammatory agents. Fourteen women in the PCOS group and 3 women without PCOS had a first-degree relative with type 2 diabetes mellitus. All study subjects gave their written signed consent to the study, which was approved by the Research Ethics Committee of Tallaght Hospital and St. James's Hospital (Dublin, Ireland).

2.2. Clinical Protocol. This was a cross-sectional study. All subjects had their height measured with a Harpenden stadiometer and weight measured in light clothing. BMI was calculated as weight (kg)/height squared (m^2). Waist circumference (WC) and hip circumference (HC) were measured with a nondistensible flexible tape, and WHR was calculated accordingly. All subjects also underwent estimation of body composition including percentage body fat (%BF) and percentage lean mass (%LM) using bioimpedance analysis using the Bodystat 1500 system (Bodystat Ltd., UK).

2.3. Frequently Sampled Intravenous Glucose Tolerance Test (fsIVGTT). Subjects attended the Clinical Investigation Unit after a 12-hour overnight fast and were requested to refrain from vigorous exercise and alcohol on the day prior to their fsIVGTT. On the morning of the fsIVGTT, two cannulae were inserted into the antecubital veins of both forearms. A fasting blood sample was taken from cannula 1. Then a bolus of 50% glucose solution (0.3 g/kg body weight (BW)) was infused into cannula 2 over a 1-minute period followed by 20 mL of 0.9% saline. Twenty minutes later, a dose of insulin (0.03 U/kg BW) was infused into the same cannula (cannula 2), which was then removed at 30 min after initiation of glucose administration. Meanwhile, blood was sampled through cannula 1 at frequent intervals over a 3-hour period (−5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min after the start of the glucose injection) for determination of glucose and insulin at each time point. Blood samples were spun at 3000 rpm for 10 min after which the plasma was aliquoted and stored at −80°C until required for future analysis. Insulin sensitivity (S_I), glucose effectiveness (S_G), acute insulin response (AIR_G), and disposition index (DI) were estimated using the MINMOD computer program (version 3.0, copyright R. N. Bergman).

2.4. Mixed Meal and Sampling. Following a 12-hour overnight fast, subjects came to the Clinical Investigation Unit in Tallaght Hospital for blood sampling and a high calorific meal. The mixed meal was designed and analyzed by a qualified dietician. It consisted of 948 kcal. 48% of the total calories were derived from fat (20% saturated, 16% monounsaturated, and 8% polyunsaturated). 36% and 15% of the calories were derived from carbohydrate and protein, respectively. The meal was ingested within 20 minutes after baseline bloods. Bloods were taken at 2, 4, 6, and 8 hours

after ingestion of the meal. Subjects were advised to restrict their physical activity to the minimum until the end of the sampling period.

2.5. Laboratory Methods

2.5.1. Hormones, Glucose, HbA1c, Lipids, and Apolipoproteins A-I, A-II, and B. Glucose was measured by an enzymatic (hexokinase) method on the Roche P Module (Roche, Stockholm, Sweden) and insulin was measured by electrochemiluminescence immunoassay on the Roche E Module (coefficients of variation (CVs) <5% for both). Glucose and insulin levels were used to calculate $HOMA_{IR}$. Nonesterified fatty acids (NEFA) were measured by a kit using the Randox Colorimetric Method (Randox, Antrim, UK) and analyzed on a Hitachi modular analyzer (Tokyo, Japan) (CV < 5%). Luteinising hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), DHEAS, oestradiol, thyroid stimulating hormone (TSH), free thyroxine (fT4), prolactin, and cortisol were measured by standard chemiluminescence immunoassays (CVs < 5% for all). Total testosterone was measured by electrochemiluminescence immunoassay on the Roche E Module. Free androgen index (FAI) was calculated by the following formula: FAI = 100 × total testosterone/SHBG. Androstenedione was measured by radioimmunoassay (CV < 5%). Total-cholesterol, TG, and HDL-C were measured using standard laboratory techniques (CV < 5%). LDL-C was calculated using the Friedewald equation. Apolipoproteins A-I (apoA-I), A-II (apoA-II), and B (apoB) were measured by standard nephelometry on a BNII nephelometer (Dade Behring, Deerfield, IL) (CV < 5%).

2.5.2. ApoB-48 ELISA. ApoB-48 was measured using a commercially available ELISA kit (AKHB48, Gentaur BVBA corporation) and using a modified version of the method described by Lorec et al. [13]. The anti-apoB-48 coated plate is initially washed to remove buffer. The appropriately diluted samples are added to the plate and left to react for one hour. Samples were diluted 1 : 250 to ensure that the concentration was not above the assay range. The plate was subsequently washed 4 times, and biotin-conjugated anti-apoB-48 antibody is added. This binds to human apoB-48 bound to the anti-apoB-48 antibody immobilised on the coated plate. This reaction is stopped after 1 hour and the plate washed 4 times. Finally a peroxidase-conjugated avidin is then added to the plate. The avidin and biotin readily bind to ensure that the horseradish peroxidase enzyme is immobilised on the plate. After washing, the chromogenic substrate tetramethyl benzidine (TMB) is added. This forms a blue colour on reaction with horseradish peroxidase enzyme. The reaction stopper (1 M H_2SO_4) is added, resulting in a yellow colour formation which is proportional to substrate concentration. The plate was read at an absorbance of 450 nm. Interassay and intra-assay CVs were 14% and 9%, respectively.

2.6. Statistical Analysis.

Data are presented as mean ± SEM. Skewed variables were logarithmically transformed to normalise data prior to analysis. Initial comparisons between groups were performed using independent t-test. Analysis of covariance (ANCOVA) was used to compare differences between the two groups adjusting for 3 separate covariates: BMI, $HOMA_{IR}$, and S_I. Correlations were made using Pearson's correlation coefficient. Multiple linear regression analysis was used to identify independent contributors to postprandial TG, apoB-48, and HDL-chol. Independent variables that correlated significantly in univariate analysis were entered into the multiple regression models in forward stepwise fashion. Statistical significance was defined as $p < 0.05$.

Table 1: Baseline characteristics of all subjects ($n = 52$).

	PCOS ($n = 26$)	Controls ($n = 26$)	p
Age (yrs)	30.4 ± 1.2	34.1 ± 1.5	0.063
Systolic BP (mmHg)	126.3 ± 1.8	121.6 ± 2.5	0.131
Diastolic BP (mmHg)	79.0 ± 1.1	77.8 ± 2.3	0.652
Waist (cm)	112.0 ± 2.2	99.8 ± 2.4	<0.001
Hip (cm)	120.5 ± 1.7	110.9 ± 1.9	<0.001
Waist : Hip	0.930 ± 0.014	0.900 ± 0.014	0.139
Weight (kg)	95.4 ± 2.6	83.2 ± 2.8	0.003
BMI (kg/m^2)	36.8 ± 0.9	31.5 ± 1.0	<0.001
Smoking (%)	26	31	0.696
% Body fat mass (%)	40.4 ± 1.3	33.6 ± 2.0	0.006
T (nmol/L)*	3.22 ± 0.30	1.57 ± 0.13	<0.001
SHBG (nmol/L)*	29.8 ± 2.00	47.4 ± 3.48	<0.001
FAI*	12.2 ± 1.59	3.67 ± 0.39	<0.001
Androstenedione (nmol/L)	15.58 ± 0.84	11.82 ± 0.84	0.003
DHEAS (μmol/L)	7.62 ± 0.71	4.98 ± 0.49	0.003
FSH (IU/L)*	5.54 ± 0.35	8.93 ± 0.90	<0.001
LH (IU/L)*	6.37 ± 0.74	7.49 ± 1.41	0.654
$HOMA_{IR}$ ($\mu mol^2/L^2$)*	4.40 ± 0.41	2.56 ± 0.30	<0.001
S_I ($\times 10^{-4}$ min^{-1}/mU/L)*	1.99 ± 0.18	4.05 ± 0.46	<0.001
S_G (min^{-1})	0.02 ± 0.00	0.02 ± 0.00	0.352
AIR_G ($mU \cdot L^{-1} \cdot min$)*	985 ± 171	613 ± 85	0.207
DI	1756 ± 281	2308 ± 358	0.226

*t-tests analysis after log transformation.

3. Results

3.1. Baseline Demographic Data and Hormonal Profile. Table 1 shows the baseline data of both groups of subjects. Women with PCOS had greater BMI, WC, HC, and %BF. All androgens were higher whilst SHBG and FSH were lower in women with PCOS. Women with PCOS were more insulin resistant with a higher $HOMA_{IR}$ (4.40 ± 0.41 $\mu mol^2/L^2$ in PCOS versus 2.56 ± 0.30 $\mu mol^2/L^2$ in controls) and lower S_I ($1.99 \pm 0.18 \times 10^{-4}$ min^{-1}/mU/L in PCOS versus $4.05 \pm 0.46 \times 10^{-4}$ min^{-1}/mU/L in controls).

3.2. Postprandial Lipids, NEFA, and Apolipoprotein. Table 2 shows the pre- and postprandial results of TG, HDL, and NEFA. Postprandial TG at 2 hours was significantly greater in

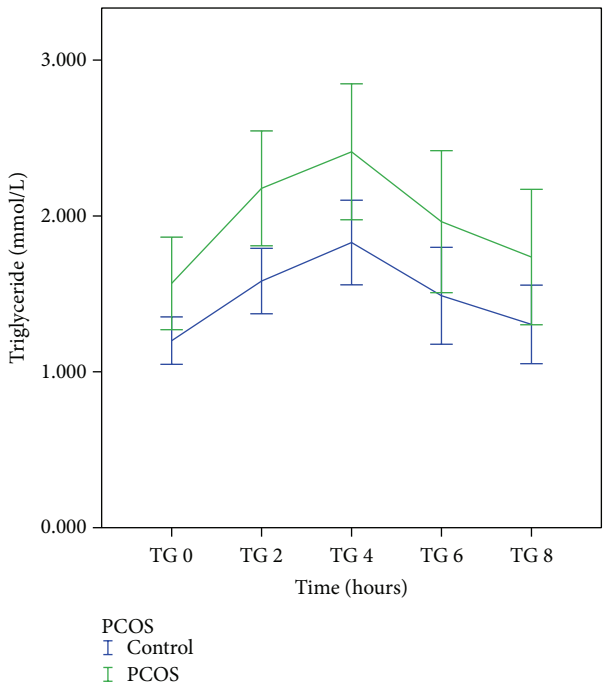

Figure 1: Postprandial triglyceride response in PCOS and control subjects.

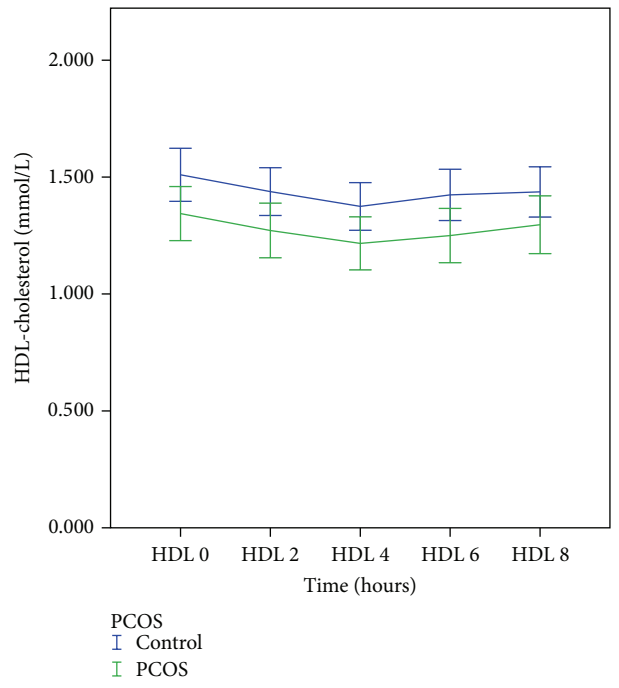

Figure 2: Postprandial HDL-cholesterol response in PCOS and control subjects.

PCOS women (2.18±0.18 mmol/L versus 1.58±0.10 mmol/L, $p = 0.019$) and nonsignificantly greater at the other time points compared to controls (Figure 1). AUC-TG, but not the iAUC-TG, was also significantly higher in women with PCOS. Conversely, HDL-chol was significantly lower in PCOS women compared to controls at $T = 0$, 2, 4, and 6 hours (Figure 2). AUC-HDL was also significantly lower in PCOS compared with controls. NEFA levels did not differ significantly between the two groups. There were no significant differences in apoA-I, apoA-II, apoB, and apoB-48 at any of the time points and the AUC or iAUC of these apolipoproteins between the two groups.

3.3. Postprandial Glucose and Insulin. Table 3 shows the pre- and postprandial results for glucose and insulin. Glucose levels were nonsignificantly greater at 2 hours and significantly greater at 4 hours in women with PCOS compared to controls (Figure 3). AUC-glucose was also greater in PCOS. With the exception of insulin at 8 hours, insulin level at all other time points, AUC-insulin, and iAUC-insulin were all greater in women with PCOS (Figure 4).

3.4. Comparisons following Adjustment for BMI, $HOMA_{IR}$, and S_I. Tables 4 and 5 show results following adjustment for BMI, $HOMA_{IR}$, and S_I. After adjusting for BMI, androgens remained higher and SHBG lower in PCOS women compared to control subjects (Table 4). There were no differences in TG, HDL, and apoB-48 at any time points nor in AUC-TG, AUC-HDL, AUC-apoB-48. Insulin remained significantly higher at all time points except at 8 hours. AUC-insulin and iAUC-insulin were also significantly higher (Table 5). There were no differences in the other postprandial apolipoproteins.

Following adjustment for $HOMA_{IR}$, androgens and SHBG were significantly different between the two groups (Table 4). ApoB-48 at 4 hours was significantly ($p = 0.034$) higher in the PCOS women (19.8 ± 1.68 μg/mL) compared to controls (15.2 ± 1.64 μg/mL) and insulin at 4 and 6 hours as well as AUC-insulin and iAUC-insulin also remained significantly higher (Table 5).

Finally, following adjustment for S_I, androgens remained significantly greater in PCOS women, but SHBG was no longer significantly different (Table 4). Only insulin at 4 hours was higher in the PCOS women, but AUC-insulin and iAUC-insulin were not different between the two groups (Table 5). ApoB-48 was nonsignificantly ($p = 0.058$) greater at 4 hours in PCOS women (19.2 ± 1.90 μg/mL) compared to controls (16.8 ± 2.00 μg/mL) but not at other time points (Table 5). There were no significant differences in apoB, apoA-I, or apoA-II at any of the time points or in the AUC or iAUC between the two groups.

3.5. Correlations and Multiple Regression Analyses. Table 6 shows the correlations between AUC-TG, AUC-HDL, AUC-apoB-48, AUC-apoB, AUC-glucose, AUC-insulin, and all other variables. Taking the entire cohort of 52 subjects, AUC-TG correlated positively ($p < 0.05$ for all variables unless otherwise specified) with WC ($r = 0.314$), weight ($r = 0.357$), BMI ($r = 0.316$), and body fat content ($r = 0.378$) and negatively with body water content ($r = -0.342$) and SHBG ($r = -0.378$, $p < 0.01$). AUC-TG did not correlate with androgens except for FAI ($r = 0.294$) and this is likely to be a

Table 2: Postprandial triglycerides, HDL-chol, NEFA, and apolipoproteins.

	PCOS ($n = 26$)	Controls ($n = 26$)	p
TG 0 hrs (mmol/L)*	1.57 ± 0.15	1.20 ± 0.08	0.075
TG 2 hrs (mmol/L)*	2.18 ± 0.18	1.58 ± 0.10	0.019
TG 4 hrs (mmol/L)*	2.41 ± 0.22	1.83 ± 0.14	0.057
TG 6 hrs (mmol/L)*	1.96 ± 0.23	1.49 ± 0.16	0.074
TG 8 hrs (mmol/L)*	1.74 ± 0.22	1.30 ± 0.13	0.129
AUC-TG (mmol/L·8 hrs)*	8.20 ± 0.77	6.15 ± 0.45	0.041
iAUC-TG (mmol/L·8 hrs)	1.93 ± 0.28	1.35 ± 0.24	0.127
HDL-chol 0 hrs (mmol/L)	1.34 ± 0.06	1.51 ± 0.06	0.046
HDL-chol 2 hrs (mmol/L)	1.27 ± 0.06	1.44 ± 0.05	0.037
HDL-chol 4 hrs (mmol/L)	1.22 ± 0.06	1.37 ± 0.05	0.043
HDL-chol 6 hrs (mmol/L)	1.25 ± 0.06	1.42 ± 0.05	0.034
HDL-chol 8 hrs (mmol/L)	1.30 ± 0.06	1.44 ± 0.05	0.092
AUC-HDL-chol (mmol/L·8 hrs)	5.06 ± 0.23	5.71 ± 0.21	0.041
iAUC-HDL-chol (mmol/L·8 hrs)	−0.32 ± 0.03	−0.33 ± 0.07	0.883
NEFA 0 hrs (mmol/L)	0.65 ± 0.04	0.61 ± 0.05	0.644
NEFA 2 hrs (mmol/L)	0.19 ± 0.02	0.18 ± 0.05	0.658
NEFA 4 hrs (mmol/L)	0.33 ± 0.03	0.36 ± 0.05	0.524
NEFA 6 hrs (mmol/L)	0.62 ± 0.05	0.67 ± 0.05	0.544
NEFA 8 hrs (mmol/L)	0.81 ± 0.05	0.84 ± 0.05	0.698
AUC NEFA (mmol/L·8 hrs)	1.87 ± 0.10	1.94 ± 0.05	0.681
iAUC NEFA (mmol/L·8 hrs)	−0.72 ± 0.14	−0.52 ± 0.05	0.400
ApoB-48 0 hrs (μg/mL)*	11.9 ± 1.45	9.89 ± 1.29	0.252
ApoB-48 2 hrs (μg/mL)*	17.0 ± 1.43	15.7 ± 1.08	0.468
ApoB-48 4 hrs (μg/mL)*	19.2 ± 1.56	15.8 ± 1.55	0.058
ApoB-48 6 hrs (μg/mL)*	18.3 ± 1.70	15.8 ± 1.93	0.184
ApoB-48 8 hrs (μg/mL)*	12.9 ± 1.34	11.3 ± 1.48	0.239
AUC-apoB-48 (μg/mL·8 hrs)*	66.4 ± 5.32	57.6 ± 4.41	0.157
iAUC-apoB-48 (μg/mL·8 hrs)	19.35 ± 3.38	18.27 ± 2.45	0.796
ApoB 0 hrs (g/dL)	0.73 ± 0.07	0.82 ± 0.06	0.328
ApoB 2 hrs (g/dL)	0.76 ± 0.07	0.73 ± 0.06	0.816
ApoB 4 hrs (g/dL)	0.83 ± 0.06	0.76 ± 0.06	0.364
ApoB 6 hrs (g/dL)	0.79 ± 0.05	0.77 ± 0.05	0.779
ApoB 8 hrs (g/dL)	0.83 ± 0.05	0.79 ± 0.05	0.603
AUC-apoB (g/dL·8 hrs)	3.16 ± 0.17	3.07 ± 0.14	0.688
iAUC-apoB (g/dL·8 hrs)	0.22 ± 0.22	−0.22 ± 0.19	0.143
ApoA-I 0 hrs (g/dL)	1.15 ± 0.07	1.10 ± 0.10	0.679
ApoA-I 2 hrs (g/dL)	1.04 ± 0.09	1.14 ± 0.09	0.437
ApoA-I 4 hrs (g/dL)	1.19 ± 0.08	1.28 ± 0.08	0.465
ApoA-I 6 hrs (g/dL)	1.18 ± 0.08	1.22 ± 0.08	0.715
ApoA-I 8 hrs (g/dL)	1.08 ± 0.08	1.16 ± 0.09	0.481
AUC-apoA-I (g/dL·8 hrs)	4.52 ± 0.19	4.75 ± 0.18	0.391
iAUC-apoA-I (g/dL·8 hrs)	−0.08 ± 0.30	0.35 ± 0.34	0.342
ApoA-II 0 hrs (g/dL)	0.32 ± 0.02	0.30 ± 0.03	0.544
ApoA-II 2 hrs (g/dL)	0.29 ± 0.03	0.30 ± 0.02	0.796
ApoA-II 4 hrs (g/dL)	0.26 ± 0.03	0.25 ± 0.03	0.764
ApoA-II 6 hrs (g/dL)	0.26 ± 0.02	0.30 ± 0.02	0.195
ApoA-II 8 hrs (g/dL)	0.25 ± 0.02	0.26 ± 0.02	0.761
AUC-apoA-II (g/dL·8 hrs)	1.10 ± 0.04	1.13 ± 0.04	0.657
iAUC-apoA-II (g/dL·8 hrs)	−0.19 ± 0.09	−0.08 ± 0.09	0.410

* t-tests analysis after log transformation.

Table 3: Postprandial glucose and insulin.

	PCOS ($n = 26$)	Controls ($n = 26$)	p
Glucose 0 hrs (mmol/L)	5.18 ± 0.11	4.99 ± 0.10	0.191
Glucose 2 hrs (mmol/L)	5.67 ± 0.30	4.90 ± 0.25	0.053
Glucose 4 hrs (mmol/L)	5.18 ± 0.18	4.73 ± 0.12	0.044
Glucose 6 hrs (mmol/L)	4.66 ± 0.08	4.66 ± 0.06	0.959
Glucose 8 hrs (mmol/L)	4.66 ± 0.06	4.62 ± 0.09	0.713
AUC-glucose (mmol/L·8 hrs)	20.50 ± 0.48	19.10 ± 0.38	0.024
iAUC-glucose (mmol/L·8 hrs)	−0.33 ± 0.32	−0.86 ± 0.40	0.306
Insulin 0 hrs (mU/L)*	19.3 ± 1.80	11.7 ± 1.28	<0.001
Insulin 2 hrs (mU/L)*	105 ± 13.8	47.6 ± 5.41	<0.001
Insulin 4 hrs (mU/L)*	49.9 ± 7.02	21.4 ± 2.21	<0.001
Insulin 6 hrs (mU/L)*	21.7 ± 2.35	10.4 ± 0.85	<0.001
Insulin 8 hrs (mU/L)*	15.3 ± 1.59	12.6 ± 2.05	0.084
AUC-insulin (mU/L·8 hrs)*	194 ± 21.6	91.5 ± 8.03	<0.001
iAUC-insulin (mU/L·8 hrs)*	117 ± 17.5	44.8 ± 5.70	<0.001

*t-tests analysis after log transformation.

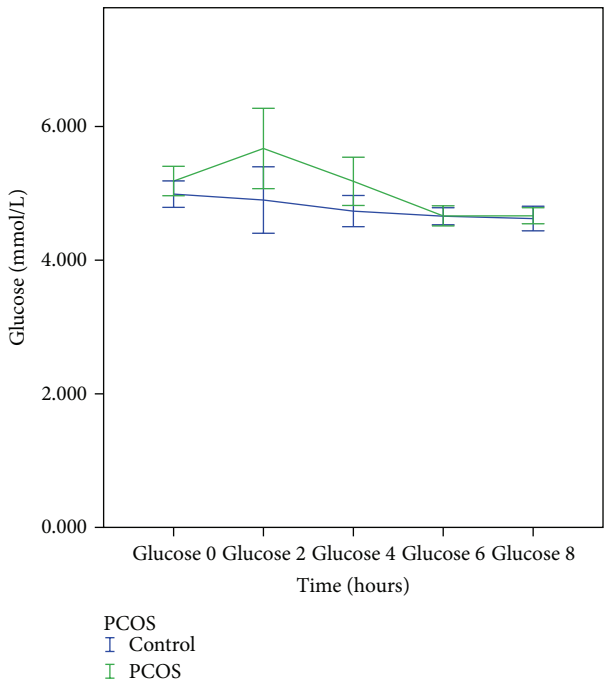

Figure 3: Postprandial glucose response in PCOS and control subjects.

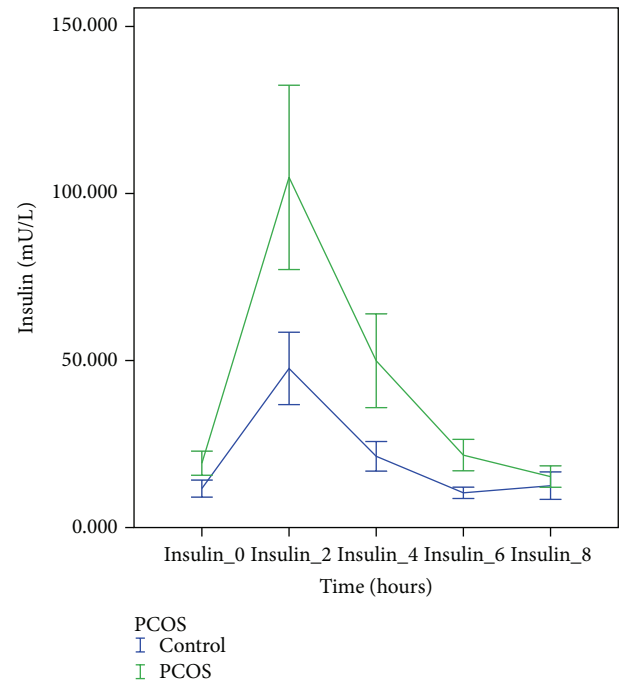

Figure 4: Postprandial insulin response in PCOS and control subjects.

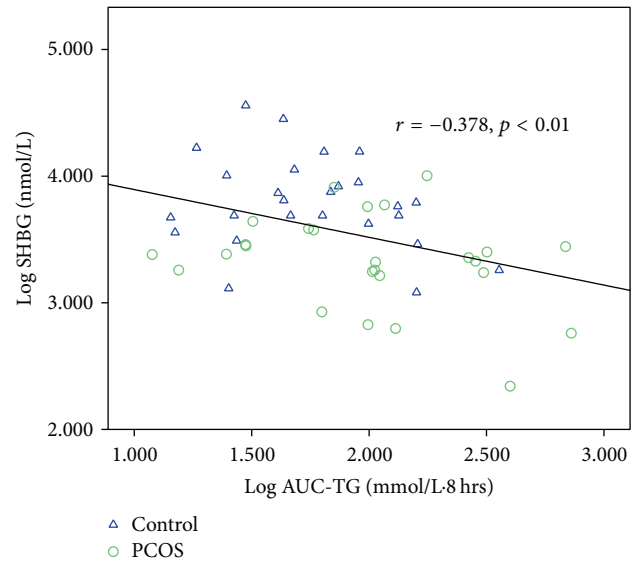

Figure 5: Correlation between AUC-TG and SHBG.

result of the inverse correlation with SHBG, which causes FAI to be higher. Figures 5–8 show the correlations graphically between AUC-TG and SHBG (Figure 5), FAI (Figure 6), androstenedione (Figure 7), and DHEAS (Figure 8), respectively. As expected, AUC-TG also correlated strongly with $HOMA_{IR}$, S_I ($r = -0.601$, $p < 0.01$), and AUC-HDL ($r = -0.540$). Insulin at 0, 2, 4, and 6 hours, AUC-insulin, and iAUC-insulin also correlated significantly with AUC-TG (Figure 9). There were also positive correlations between AUC-TG and apoB-48 at 0, 2, 6, and 8 hours, AUC-apoB-48 (Figure 10), apoB at 2, 4, 6, and 8 hours, and AUC-apoB suggesting that both chylomicrons and VLDL contribute to postprandial hypertriglyceridaemia. Glucose at 0 and 2 hours, AUC-glucose, S_G, and DI also correlated with AUC-TG. Stepwise multiple regression analysis revealed that S_I, AUC-apoB-48, and AUC-apoB were independent predictors of AUC-TG, accounting for 55% of the overall variance. This suggests that postprandial chylomicron particles probably play an important role in contributing towards postprandial

Table 4: Baseline characteristics adjusted for BMI, $HOMA_{IR}$, and log S_I.

	Covariate: BMI		Covariate: $HOMA_{IR}$		Covariate: S_I	
	PCOS	Controls	PCOS	Controls	PCOS	Controls
Systolic BP (mmHg)	124.6 ± 2.2	123.3 ± 2.2	124.4 ± 2.2	123.5 ± 2.2	125.0 ± 2.4	123.0 ± 2.6
Diastolic BP (mmHg)	78.0 ± 1.9	78.9 ± 1.9	78.6 ± 2.0	78.2 ± 2.0	78.6 ± 2.1	78.9 ± 2.3
Waist (cm)	107.3 ± 1.5	104.5 ± 1.5	109.0 ± 2.2	102.8 ± 2.2	108.8 ± 2.4	104.3 ± 2.6
Hip (cm)	116.6 ± 1.1	114.8 ± 1.1	118.7 ± 1.9[a]	112.6 ± 1.9	118.4 ± 2.0	113.3 ± 2.2
Waist : Hip	0.920 ± 0.015	0.910 ± 0.015	0.917 ± 0.015	0.913 ± 0.015	0.919 ± 0.016	0.921 ± 0.017
Weight (kg)	89.2 ± 1.4	89.5 ± 1.4	91.2 ± 2.5	87.4 ± 2.5	91.0 ± 2.8	88.9 ± 3.0
BMI (kg/m^2)	—		35.3 ± 1.0	32.9 ± 0.9	35.4 ± 1.1	33.7 ± 1.2
% Body fat mass (%)	37.6 ± 1.2	36.2 ± 1.2	38.2 ± 1.7	35.7 ± 1.7	38.5 ± 1.8	36.2 ± 1.9
T (nmol/L)*	3.37 ± 0.25[b]	1.52 ± 0.24	3.24 ± 0.25[b]	1.56 ± 0.25	3.49 ± 0.27[b]	1.30 ± 0.279
SHBG (nmol/L)*	32.2 ± 3.04[b]	45.2 ± 2.98	31.4 ± 2.98[b]	45.7 ± 2.98	34.2 ± 3.19	43.4 ± 3.45
FAI*	12.2 ± 1.25[b]	4.08 ± 1.22	12.2 ± 1.24[b]	3.70 ± 1.24	12.5 ± 1.38[b]	3.30 ± 1.50
Androstenedione (nmol/L)	15.67 ± 0.9[b]	11.99 ± 0.89	15.07 ± 0.92[a]	12.30 ± 0.90	15.98 ± 0.99[b]	11.37 ± 1.05
DHEAS (μmol/L)	8.00 ± 0.68[b]	4.80 ± 0.66	7.88 ± 0.66	4.72 ± 0.66	8.55 ± 0.66[b]	3.80 ± 0.72
$HOMA_{IR}$ ($\mu mol^2/L^2$)*	3.99 ± 0.35[a]	3.02 ± 0.34	—		3.91 ± 0.38	3.39 ± 0.41
S_I ($\times 10^{-4}$ min^{-1}/mU/L)*	2.23 ± 0.31[b]	3.78 ± 0.34	2.33 ± 0.30[a]	3.66 ± 0.32	—	
S_G (min^{-1})	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
AIR_G ($mU \cdot L^{-1} \cdot min$)*	986 ± 144	612 ± 155	867 ± 133	747 ± 143	909 ± 149	700 ± 161
DI	1846 ± 321	2206 ± 345	1893 ± 326	2151 ± 351	2174 ± 312	1831 ± 338

*ANCOVA analysis after log transformation. [a] $p < 0.05$ versus controls. [b] $p < 0.01$ versus controls.

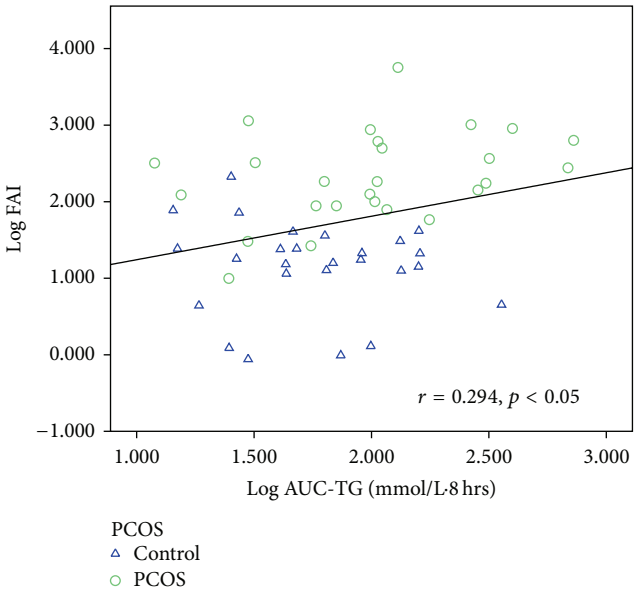

Figure 6: Correlation between AUC-TG and FAI.

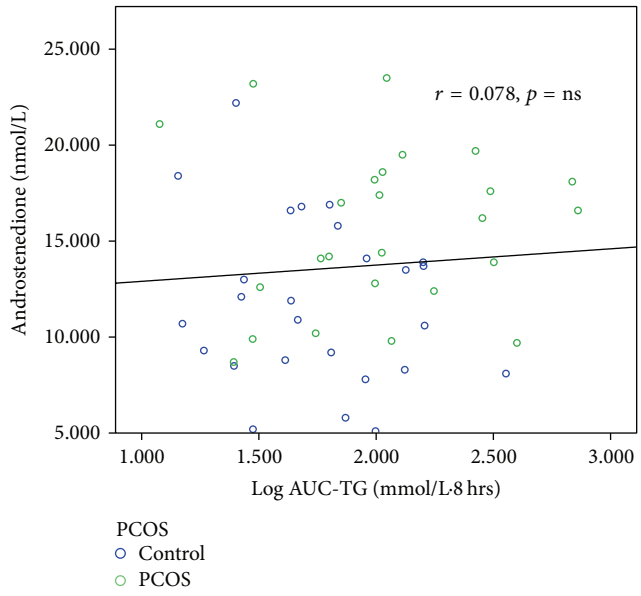

Figure 7: Correlation between AUC-TG and androstenedione.

hypertriglyceridaemia, together with apoB and insulin sensitivity. AUC-TG was the only independent predictor of AUC-apoB-48, explaining 25.8% of the variance.

AUC-HDL correlated significantly negatively with WC ($r = -0.483$, $p < 0.01$), HC ($r = -0.285$), weight ($r = -0.439$, $p < 0.01$), BMI ($r = -0.456$, $p < 0.01$), body fat ($r = -0.444$), FAI ($r = -0.350$), $HOMA_{IR}$ ($r = -0.499$, $p < 0.01$), AUC-TG ($r = -0.540$, $p < 0.01$), AUC-glucose ($r = -0.309$), and AUC-insulin ($r = -0.488$, $p < 0.01$). AUC-HDL correlated significantly positively with body water ($r = 0.389$), SHBG ($r = 0.443$, $p < 0.01$), and S_I ($r = 0.454$). AUC-HDL also correlated negatively with TG at all time points, iAUC-TG, iAUC-B48, apoA-I at 2, 4, and 6 hours and AUC-apoA-I, glucose at 0 and 2 hours, AUC-glucose, insulin at 0, 2, 4, and 6 hours, AUC, and iAUC-insulin (results not shown). There was a nonsignificant negative correlation with androstenedione ($r = -0.27$, $p = 0.055$). AUC-TG and WC were the only independent predictors of AUC-HDL,

Table 5: Postprandial TG, HDL, apoB-48, glucose, and insulin levels adjusted for BMI, $HOMA_{IR}$, and S_I.

	Covariate: BMI		Covariate: $HOMA_{IR}$		Covariate: S_I	
	PCOS	Controls	PCOS	Controls	PCOS	Controls
TG 0 hrs (mmol/L)*	1.54 ± 0.13	1.25 ± 0.12	1.40 ± 0.11	1.36 ± 0.11	1.47 ± 0.13	1.33 ± 0.14
TG 2 hrs (mmol/L)*	2.14 ± 0.16	1.66 ± 0.16	1.97 ± 0.14	1.79 ± 0.14	2.04 ± 0.17	1.73 ± 0.18
TG 4 hrs (mmol/L)*	2.37 ± 0.20	1.90 ± 0.19	2.14 ± 0.16	2.10 ± 0.16	2.24 ± 0.20	2.10 ± 0.22
TG 6 hrs (mmol/L)*	1.92 ± 0.21	1.57 ± 0.21	1.70 ± 0.18	1.75 ± 0.18	1.80 ± 0.22	1.77 ± 0.24
TG 8 hrs (mmol/L)*	1.71 ± 0.19	1.37 ± 0.19	1.48 ± 0.16	1.56 ± 0.16	1.60 ± 0.20	1.56 ± 0.22
AUC-TG (mmol/L·8 hrs)*	8.06 ± 0.68	6.45 ± 0.66	7.25 ± 0.54	7.10 ± 0.54	7.61 ± 0.70	7.04 ± 0.76
iAUC-TG (mmol/L·8 hrs)	1.87 ± 0.29	1.45 ± 0.29	1.63 ± 0.27	1.65 ± 0.27	1.64 ± 0.31	1.82 ± 0.33
HDL-chol 0 hrs (mmol/L)	1.40 ± 0.06	1.45 ± 0.06	1.41 ± 0.06	1.44 ± 0.06	1.40 ± 0.06	1.38 ± 0.07
HDL-chol 2 hrs (mmol/L)	1.33 ± 0.06	1.38 ± 0.06	1.34 ± 0.05	1.37 ± 0.05	1.34 ± 0.06	1.33 ± 0.07
HDL-chol 4 hrs (mmol/L)	1.27 ± 0.06	1.32 ± 0.05	1.28 ± 0.05	1.31 ± 0.05	1.28 ± 0.06	1.27 ± 0.06
HDL-chol 6 hrs (mmol/L)	1.30 ± 0.06	1.37 ± 0.06	1.32 ± 0.06	1.36 ± 0.06	1.31 ± 0.06	1.31 ± 0.07
HDL-chol 8 hrs (mmol/L)	1.35 ± 0.06	1.38 ± 0.06	1.36 ± 0.06	1.37 ± 0.06	1.36 ± 0.06	1.32 ± 0.07
AUC-HDL-chol (mmol/L·8 hrs)	5.28 ± 0.23	5.49 ± 0.22	5.33 ± 0.22	5.44 ± 0.22	5.31 ± 0.24	5.26 ± 0.26
iAUC-HDL-chol (mmol/L·8 hrs)	−0.33 ± 0.06	−0.31 ± 0.06	−0.33 ± 0.06	−0.32 ± 0.06	−0.30 ± 0.06	−0.27 ± 0.07
ApoB-48 0 hrs (μg/mL)*	12.4 ± 1.54	9.70 ± 1.47	11.9 ± 1.49	9.86 ± 1.46	11.9 ± 1.70	10.4 ± 1.80
ApoB-48 2 hrs (μg/mL)*	17.3 ± 1.43	15.6 ± 1.36	17.0 ± 1.37	15.7 ± 1.34	16.5 ± 1.53	16.9 ± 1.62
ApoB-48 4 hrs (μg/mL)*	19.6 ± 1.77	15.5 ± 1.69	19.8 ± 1.68[a]	15.2 ± 1.64	19.2 ± 1.90	16.8 ± 2.00
ApoB-48 6 hrs (μg/mL)*	18.9 ± 2.06	15.6 ± 1.97	17.2 ± 1.94	16.9 ± 1.90	17.9 ± 2.22	17.5 ± 2.34
ApoB-48 8 hrs (μg/mL)*	13.2 ± 1.60	11.2 ± 1.53	11.7 ± 1.47	12.4 ± 1.44	12.3 ± 1.70	12.5 ± 1.79
Log ApoB-48 (μg/mL·8 hrs)*	67.8 ± 5.51	57.1 ± 5.26	65.6 ± 5.30	58.4 ± 5.18	65.0 ± 5.97	62.5 ± 6.30
iAUC apoB-48 (μg/mL·8 hrs)	19.02 ± 3.33	18.31 ± 3.18	17.57 ± 3.19	19.98 ± 3.12	17.92 ± 3.70	21.06 ± 3.91
Insulin 0 hrs (pmol/L)*	17.9 ± 1.56[a]	13.4 ± 1.2	15.4 ± 0.30	15.6 ± 0.30	17.5 ± 1.66	15.1 ± 1.79
Insulin 2 hrs (pmol/L)*	95.7 ± 10.3[a]	59.5 ± 10.1	88.7 ± 8.91	63.8 ± 8.91	91.6 ± 11.3	64.1 ± 12.2
Insulin 4 hrs (pmol/L)*	47.6 ± 5.59[b]	24.6 ± 5.47	43.7 ± 4.92[a]	27.6 ± 4.92	45.7 ± 5.94[b]	27.7 ± 6.42
Insulin 6 hrs (pmol/L)*	20.9 ± 1.90[b]	11.6 ± 1.86	19.2 ± 1.57[b]	12.9 ± 1.57	19.9 ± 1.94	13.2 ± 2.11
Insulin 8 hrs (pmol/L)*	13.6 ± 1.89	14.4 ± 1.84	13.4 ± 1.81	14.3 ± 1.81	14.1 ± 2.17	14.7 ± 2.35
AUC-insulin (pmol/L·8 hrs)*	180 ± 16.2[b]	110 ± 15.8	166 ± 13.0[a]	119 ± 13.0	173 ± 17.5	120 ± 18.9
iAUC-insulin (pmol/L·8 hrs)*	108 ± 13.5[a]	55.9 ± 13.2	104 ± 13.0[a]	57.0 ± 13.0	103 ± 14.7	59.6 ± 15.9

* ANCOVA analysis after log transformation. [a] $p < 0.05$ versus controls. [b] $p < 0.01$ versus controls.

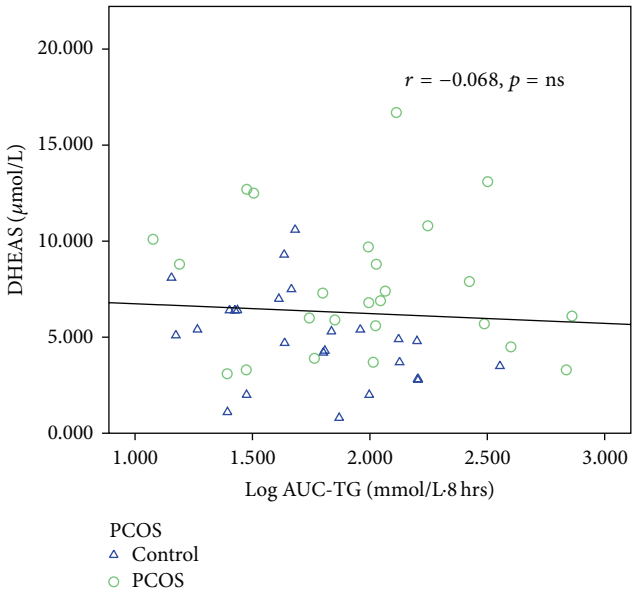

Figure 8: Correlation between AUC-TG and DHEAS.

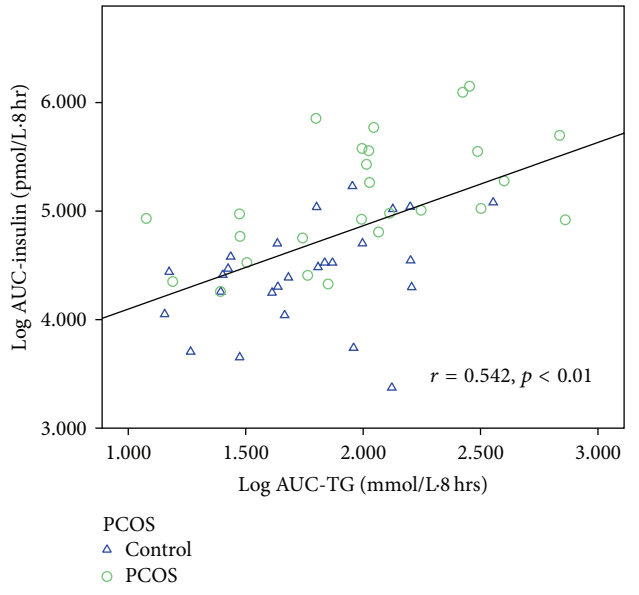

Figure 9: Correlation between AUC-TG and AUC-insulin.

Table 6: Correlations between AUC-TG, AUC-HDL-chol, AUC-apoB-48, AUC-apoB, AUC-glucose, AUC-insulin, and other variables.

	AUC-TG* (mmol/L·8 hrs)	AUC-HDL (mmol/L·8 hrs)	AUC-apoB-48* (μg/mL·8 hrs)	AUC-B (g/dL·8 hrs)	AUC-glucose (mmol/L·8 hrs)	AUC-insulin* (pmol/L·8 hrs)
AUC-TG* (mmol/L·8 hrs)	1.000	−0.540[b]	0.524[b]	0.484[b]	0.304[a]	0.542[b]
AUC-HDL-chol (mmol/L·8 hrs)	−0.540[b]	1.000	−0.229	−0.225	−0.309[a]	−0.488[b]
AUC-apoB-48* (μg/mL·8 hrs)	0.524[b]	−0.229	1.000	0.298[a]	0.171	0.165
AUC-apoB (g/dL·8 hrs)	0.484[b]	−0.225	0.298[a]	1.000	0.038	0.206
AUC-glucose (mmol/L·8 hrs)	0.304[a]	−0.309[a]	0.171	0.038	1.000	0.634[b]
AUC-insulin* (pmol/L·8 hrs)	0.542[b]	−0.488[b]	0.165	0.206	0.634[b]	1.000
Age, PCOS status						
Age (years)	0.125	−0.010	−0.025	0.134	0.342[a]	−0.014
PCOS	0.284[a]	−0.284[a]	0.201	0.057	0.315[a]	0.579[b]
Androgens, SHBG						
T* (nmol/L)	0.133	−0.163	0.115	−0.023	0.092	0.331
SHBG* (nmol/L)	−0.378[b]	0.443[b]	−0.277[a]	−0.277[a]	−0.305[a]	−0.550[b]
FAI*	0.294[a]	−0.350[a]	0.227	0.125	0.226	0.530[b]
Androstenedione (nmol/L)	0.078	−0.271	−0.014	−0.056	0.146	0.416
DHEAS (μmol/L)	−0.068	−0.048	0.108	−0.289[a]	0.125	0.158
Anthropometrics						
Waist (cm)	0.314[a]	−0.483[b]	0.063	0.080	0.372[b]	0.661[b]
Hip (cm)	0.216	−0.285[a]	−0.064	0.154	0.356[a]	0.609[b]
Waist : Hip	0.237	−0.439[b]	0.163	−0.047	0.193	0.360[a]
Weight (kg)	0.357[a]	−0.378[b]	0.059	0.276	0.271	0.639[b]
BMI (kg/m^2)	0.316[a]	−0.456[b]	0.033	0.184	0.414[b]	0.686[b]
% Body fat mass (%)	0.378[a]	−0.444[b]	0.009	0.164	0.368[a]	0.539[b]
% Body lean mass (%)	−0.128	0.240	0.085	−0.152	−0.348[a]	−0.381[a]
% Body water (%)	−0.342[a]	0.389[a]	−0.031	−0.178	−0.314	−0.487[b]
IR, insulin sensitivity, and variables derived from the fsIVGTT						
$HOMA_{IR}$* (μmol^2/L^2)	0.568[b]	−0.499[b]	0.130	0.229	0.366[b]	0.800[b]
S_I ($\times 10^{-4}$ min^{-1}/mU/L)*	−0.601[b]	0.454[b]	−0.223	−0.262	−0.453[b]	−0.810[b]
S_G (min^{-1})	−0.364[a]	0.159	−0.266	−0.232	−0.083	−0.171
AIR_G (mU·L^{-1}·min)*	0.029	−0.090	−0.282	0.100	−0.410[b]	0.153
DI	−0.364[a]	0.226	−0.328[a]	−0.139	−0.558[b]	−0.391[b]

*Correlations after log transformation.
[a]$p < 0.05$. [b]$p < 0.01$.

explaining 37.2% of the variance using multiple regression analysis.

As expected, significant correlations were found between AUC-insulin and SHBG. AUC-insulin also correlated strongly with FAI, AUC-TG, AUC-HDL, AUC-glucose, PCOS status, and anthropometrics. Finally AUC-insulin was negatively correlated with DI, suggesting a correlation with possible subsequent β-cell function failure and development of diabetes.

4. Discussion

Dyslipidaemia, including both increased TG and low HDL-cholesterol, is common in PCOS [16, 17] and is a well-recognized feature of the metabolic syndrome which confers increased risk of cardiovascular disease [18, 19]. Hypertriglyceridaemia signifies the presence of excess TRL. TRL consists of hepatically derived VLDL, characterised by the presence of apoB-100, and intestinally derived chylomicrons, which contain apoB-48. Both VLDL and chylomicrons share a common lipolytic pathway and are hydrolysed by lipoprotein lipase (LPL) and there are a number of described receptor mediated pathways through which these particles may eventually be cleared from the circulation [12]. It is postulated that an excess production and/or a delay in clearance of postprandial lipoproteins of both TRL particles may be implicated in atherosclerosis [20, 21].

To the best of the author's knowledge, this is the first study to compare the postprandial responses in obese PCOS women with that of obese control subjects. Although some

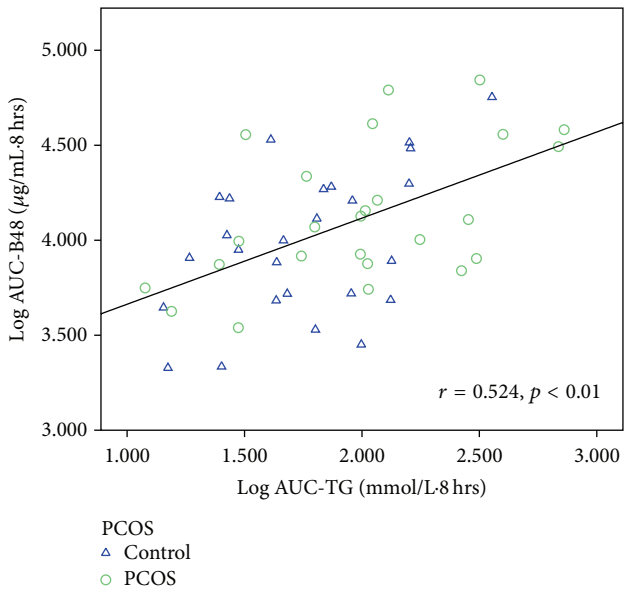

Figure 10: Correlation between AUC-TG and AUC-apoB-48.

postprandial studies in PCOS women have been done previously, this is the first one to evaluate the contribution of the two main metabolic/endocrine disturbances in PCOS women—IR and hyperandrogenaemia—to postprandial dyslipidaemia in obese women with PCOS. Both are implicated in accelerated atherosclerosis but the relative contribution of either is a subject of controversy [22–24]. Obesity, in particular central obesity, is strongly associated with insulin resistance, whilst androgens are also associated with an android distribution of fat. In a previous study by Velázquez M et al. a strong correlation between postprandial triglycerides and waist-to-hip ratio was demonstrated. That study evaluated overweight (BMI 27.41±0.50 kg/m^2) PCOS women and compared them to lean PCOS women and lean controls. Only free testosterone was measured, and there was no analysis between this and postprandial hypertriglyceridaemia [10]. A separate study by Bahceci et al. evaluated postprandial responses to an oral fat tolerance test, in lean PCOS women with lean controls (BMI 23.5 ± 2.6 kg/m^2 versus 23.1 ± 4.0 kg/m^2; $p > 0.05$). They showed that PCOS women had higher baseline insulin, $HOMA_{IR}$, AUC-TG, AUC-total-cholesterol, AUC-VLDL-cholesterol, and AUC-apoB. Surprisingly, though, AUC-insulin did not differ between the two groups. Again, no analyses were reported between androgens and postprandial lipids [11].

In this study, comparing obese women with PCOS with obese control subjects, AUC-TG, AUC-HDL, AUC-glucose, and AUC-insulin were all significantly different. HDL-chol and insulin were different at most of the time points, but TG was only different at 2 hours. Independent predictors of AUC-TG included S_I, AUC-apoB-48, and AUC-apoB, accounting for 55% of the variance. This suggests that postprandial chylomicron particles have an important role to play in postprandial hypertriglyceridaemia, together with insulin sensitivity and apoB. On the other hand, AUC-TG was the only independent predictor of AUC-apoB-48, explaining 25.8% of the variance.

Following adjustment for BMI, lipid differences were no longer noted but insulin levels at all time points and AUC-insulin and iAUC-insulin were still much greater in PCOS women consistent with the fact that PCOS women are more insulin resistant than their BMI matched counterparts [25, 26]. In this study, IR was determined by the more convenient $HOMA_{IR}$ and insulin sensitivity was determined by the more sophisticated fsIVGTT derived S_I. When comparisons were made adjusting for $HOMA_{IR}$, postprandial insulins were still significantly higher in the PCOS group. However, these differences were largely no longer present when adjustments were made for S_I, which may be a better marker for insulin sensitivity than $HOMA_{IR}$, since the latter is calculated from fasting values of insulin and glucose.

There was only weak or no correlation between androgens and TG, HDL-chol, apoB-48, apoA-I, apoA-II, and apoB. There were very strong correlations between SHBG and TG, HDL-chol, AUC-apoB-48, AUC-apoB, and AUC-apoA-II. FAI but not testosterone *per se* also strongly correlated with HDL at all time points, TG at 0 and 2 hours, and both AUC-HDL-chol and AUC-TG. This suggests that IR, and perhaps obesity, both of which are strongly negatively correlated with SHBG, rather than androgens, may be the major determinant of postprandial lipids.

This study had certain limitations. PCOS women and the controls were not entirely matched for BMI. However, both groups were obese and results were analyzed with BMI as a covariate. The two groups did not match for IR as assessed by $HOMA_{IR}$ nor S_I as assessed by the fsIVGTT. However this is to be expected based on the well accepted notion that women with PCOS are more insulin resistant than BMI matched counterparts. In addition, after using $HOMA_{IR}$ and S_I as covariates, results still revealed that hyperinsulinaemia and possibly a low SHBG level are the main factors influencing postprandial hypertriglyceridaemia. Postprandial chylomicrons also appear to play a significant role in postprandial hypertriglyceridaemia in premenopausal women with and without PCOS.

In conclusion, this study provides evidence that, in obese women with PCOS, IR plays a more important role than hyperandrogenaemia in postprandial dyslipidaemia and cardiovascular risk. Targeting obesity and thereby improving IR by lifestyle measures and perhaps the use of agents such as metformin should be a priority in the treatment of obese PCOS women.

Conflict of Interests

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

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References

[1] M. A. Austin, "Epidemiology of hypertriglyceridemia and cardiovascular disease," *The American Journal of Cardiology*, vol. 83, no. 9, pp. 13F–16F, 1999.

[2] J. E. Hokanson and M. A. Austin, "Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies," *Journal of Cardiovascular Risk*, vol. 3, no. 2, pp. 213–219, 1996.

[3] A. Fontbonne, E. Eschwege, F. Cambien et al., "Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes. Results from the 11-year follow-up of the Paris Prospective Study," *Diabetologia*, vol. 32, no. 5, pp. 300–304, 1989.

[4] L. E. Eberly, J. Stamler, and J. D. Neaton, "Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease," *Archives of Internal Medicine*, vol. 163, no. 9, pp. 1077–1083, 2003.

[5] N. R. Phillips, D. Waters, and R. J. Havel, "Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events," *Circulation*, vol. 88, no. 6, pp. 2762–2770, 1993.

[6] J. Jeppesen, C. B. Hollenbeck, M.-Y. Zhou et al., "Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity, and postprandial lipemia," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 15, no. 3, pp. 320–324, 1995.

[7] A. Harbis, C. Defoort, H. Narbonne et al., "Acute hyperinsulinism modulates plasma apolipoprotein b-48 triglyceride-rich lipoproteins in healthy subjects during the postprandial period," *Diabetes*, vol. 50, no. 2, pp. 462–469, 2001.

[8] C. Couillard, N. Bergeron, A. Pascot et al., "Evidence for impaired lipolysis in abdominally obese men: postprandial study of apolipoprotein B-48- and B-100-containing lipoproteins," *The American Journal of Clinical Nutrition*, vol. 76, no. 2, pp. 311–318, 2002.

[9] C. Couillard, N. Bergeron, D. Prud'homme et al., "Gender difference in postprandial lipemia: importance of visceral adipose tissue accumulation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 19, no. 10, pp. 2448–2455, 1999.

[10] E. Velázquez M, G. A. Bellabarba, S. Mendoza, and L. Sánchez, "Postprandial triglyceride response in patients with polycystic ovary syndrome: relationship with waist-to-hip ratio and insulin," *Fertility and Sterility*, vol. 74, no. 6, pp. 1159–1163, 2000.

[11] M. Bahceci, M. Aydemir, and A. Tuzcu, "Effects of oral fat and glucose tolerance test on serum lipid profile, apolipoprotein, and CRP concentration, and insulin resistance in patients with polycystic ovary syndrome," *Fertility and Sterility*, vol. 87, no. 6, pp. 1363–1368, 2007.

[12] R. W. Mahley and Z. S. Ji, "Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E," *The Journal of Lipid Research*, vol. 40, no. 1, pp. 1–16, 1999.

[13] A.-M. Lorec, C. Juhel, Y. Pafumi et al., "Determination of apolipoprotein B-48 in plasma by a competitive ELISA," *Clinical Chemistry*, vol. 46, no. 10, pp. 1638–1642, 2000.

[14] N. Sakai, Y. Uchida, K. Ohashi et al., "Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA," *Journal of Lipid Research*, vol. 44, no. 6, pp. 1256–1262, 2003.

[15] J. K. Zawadzki and A. Dunaif, "Diagnostic criteria for polycystic ovary syndrome: towards a rational approach," in *Polycystic Ovary Syndrome*, A. Dunaif, J. R. Givens, F. P. Haseltine, and G. R. Merriam, Eds., pp. 377–384, Blackwell Scientific, Boston, Mass, USA, 1992.

[16] R. S. Legro, A. R. Kunselman, and A. Dunaif, "Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome," *The American Journal of Medicine*, vol. 111, no. 8, pp. 607–613, 2001.

[17] T. Apridonidze, P. A. Essah, M. J. Iuorno, and J. E. Nestler, "Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 4, pp. 1929–1935, 2005.

[18] E. S. Ford, "The metabolic syndrome and mortality from cardiovascular disease and all-causes: findings from the National Health and Nutrition Examination Survey II Mortality study," *Atherosclerosis*, vol. 173, no. 2, pp. 309–314, 2004.

[19] B. Isomaa, P. Almgren, T. Tuomi et al., "Cardiovascular morbidity and mortality associated with the metabolic syndrome," *Diabetes Care*, vol. 24, no. 4, pp. 683–689, 2001.

[20] D. B. Zilversmit, "Atherogenesis: a postprandial phenomenon," *Circulation*, vol. 60, no. 3, pp. 473–485, 1979.

[21] P. H. E. Groot, W. A. H. J. Van Stiphout, X. H. Krauss et al., "Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 11, no. 3, pp. 653–662, 1991.

[22] A. Dunaif, "Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis," *Endocrine Reviews*, vol. 18, no. 6, pp. 774–800, 1997.

[23] L. C. Morin-Papunen, I. Vauhkonen, R. M. Koivunen, A. Ruokonen, and J. S. Tapanainen, "Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome," *Human Reproduction*, vol. 15, no. 6, pp. 1266–1274, 2000.

[24] A. D. Coviello, R. S. Legro, and A. Dunaif, "Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance," *The Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 2, pp. 492–497, 2006.

[25] A. Dunaif, K. R. Segal, W. Futterweit, and A. Dobrjansky, "Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome," *Diabetes*, vol. 38, no. 9, pp. 1165–1174, 1989.

[26] E. Carmina and R. A. Lobo, "Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome," *Fertility and Sterility*, vol. 82, no. 3, pp. 661–665, 2004.

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Review Article

The Role of miR-378a in Metabolism, Angiogenesis, and Muscle Biology

Bart Krist, Urszula Florczyk, Katarzyna Pietraszek-Gremplewicz, Alicja Józkowicz, and Jozef Dulak

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30–387 Krakow, Poland

Correspondence should be addressed to Jozef Dulak; jozef.dulak@uj.edu.pl

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MicroRNA-378a (miR-378a, previously known as miR-378) is one of the small noncoding RNA molecules able to regulate gene expression at posttranscriptional level. Its two mature strands, miR-378a-3p and miR-378a-5p, originate from the first intron of the peroxisome proliferator-activated receptor gamma, coactivator 1 beta (*ppargc1b*) gene encoding PGC-1β. Embedding in the sequence of this transcriptional regulator of oxidative energy metabolism implies involvement of miR-378a in metabolic pathways, mitochondrial energy homeostasis, and related biological processes such as muscle development, differentiation, and regeneration. On the other hand, modulating the expression of proangiogenic factors such as vascular endothelial growth factor, angiopoietin-1, or interleukin-8, influencing inflammatory reaction, and affecting tumor suppressors, such as SuFu and Fus-1, miR-378a is considered as a part of an angiogenic network in tumors. In the latter, miR-378a can evoke broader actions by enhancing cell survival, reducing apoptosis, and promoting cell migration and invasion. This review describes the current knowledge on miR-378a linking oxidative/lipid metabolism, muscle biology, and blood vessel formation.

1. Introduction

Cell metabolism governing the growth and functioning of each cell and a whole organism refers to chemical transformations and enzyme-catalyzed energy producing and energy utilizing reactions of carbohydrates, proteins, and lipids. Amongst the most metabolically active organs are liver, brain, gut, kidneys, and heart [1–3]. Although the rate of metabolic reactions is lower in skeletal muscles, they account for around 20% of the total energy expenditure due to a 50–60% contribution to a total body mass [3]. Several microRNAs were reported to control processes related to metabolism such as insulin secretion (miR-9, miR-375), adipocyte differentiation (miR-143), fatty acid metabolism (miR-122), and myogenesis (miR-1, miR-133a, miR-133b, and miR-206) (reviewed in [4]). Of potential meaning is also miR-378a, located in the gene encoding master metabolic regulator, peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PGC-1β) [5]. miR-378a was found to affect lipid and xenobiotic metabolism, lipid storage, mitochondrial function, and shift towards a glycolytic pathway (Warburg effect) [5, 6]. Moreover, it affects muscle differentiation via regulation of myogenic repressor, MyoR [7]. Because nutrients supply for metabolic processes is a matter of circulation, metabolically active tissues require high vascular density. Recently, miR-378a was reported to regulate tumor angiogenesis mainly via inhibition of tumor suppressors SuFu and Fus-1 [8, 9]. Thus, a growing body of evidence suggests a role of miR-378a as a mediator controlling reciprocally dependent processes such as metabolism, muscle differentiation/regeneration, and angiogenesis.

2. MicroRNAs

MicroRNAs (miRNAs; miRs) are small noncoding RNA molecules with an average length of 21-22 nucleotides which can regulate gene expression posttranscriptionally by targeting mostly the 3′untranslated region (3′UTR) of mRNAs. However, miRNA target sites were also found on the 5′UTR regions of human mRNA [10]. Since their discovery in *C. elegans* in 1993 [11], miRNAs currently can be recognized as potent players in wide spectrum of biological processes like

Table 1: Classification of miR-378 variants. Source: miRBase, version 21, September 2015 [28]. The seed sequence (defined as nucleotides 2–8 from the miRNA 5′-end of the mature miRNA) is in bold font.

Name	Mature strand	Previous ID	Sequence	Location	Host gene
			Human		
hsa-miR-378a	hsa-miR-378a-5p	miR-378*	5′-c**uccugac**uccagguccugugu-3′	chr5: 149732825–149732890	*PPARGC1B*
	hsa-miR-378a-3p	miR-422b miR-378	5′-a**cuggacu**uggagucagaaggc-3′	chr5: 149732825–149732890	*PPARGC1B*
hsa-miR-378b	hsa-miR-378b		5′-a**cuggacu**uggaggcagaa-3′	chr3: 10330229–10330285	*ATP2B2*
hsa-miR-378c	hsa-miR-378c		5′-a**cuggacu**uggagucagaagagugg-3′	chr10: 130962588–130962668	—
hsa-miR-378d-1	hsa-miR-378d		5′-a**cuggacu**uggagucagaaa-3′	chr4: 5923275–5923328	—
hsa-miR-378d-2	hsa-miR-378d		5′-a**cuggacu**uggagucagaaa-3′	chr8: 93916022–93916119	*PDP1*
hsa-miR-378e	hsa-miR-378e		5′-a**cuggacu**uggagucagga-3′	chr5: 170028488–170028566	*DOCK2*
hsa-miR-378f	hsa-miR-378f		5′-a**cuggacu**uggagccagaag -3′	chr1: 23929070–23929147	—
hsa-miR-378g	hsa-miR-378g		5′-a**cugggcu**uggagucagaag-3′	chr1: 94745860–94745900	*LINC01057*
hsa-miR-378h	hsa-miR-378h		5′-a**cuggacu**uggugucagaugg-3′	chr5: 154829458–154829540	*FAXDC2*
hsa-miR-378i	hsa-miR-378i		5′-a**cuggacu**aggagucagaagg-3′	chr22: 41923222–41923297	*TNFRSF13C*
hsa-miR-378j	hsa-miR-378j		5′-a**cuggauu**uggagccagaa-3′	chr17: 37614931–37615039	*DDX52*
			Murine		
mmu-miR-378a	mmu-miR-378a-5p	miR-378*	5′-c**uccugac**uccagguccugugu-3′	chr18: 61397835–61397900	*PPARGC1B*
	mmu-miR-378a-3p	miR-378	5′-a**cuggacu**uggagucagaagg-3′	chr18: 61397835–61397900	*PPARGC1B*
mmu-miR-378b	mmu-miR-378b		5′-c**uggacuu**ggagucagaaga-3′	chr11: 88352773–88352864	*MSI2*
mmu-miR-378c	mmu-miR-378c		5′-a**cuggacu**uggagucagaagc-3′	chr14: 46954830–46954928	*SAMD4*
mmu-miR-378d	mmu-miR-378d		5′-a**cuggccu**uggagucagaaggu-3′	chr10: 126710282–126710391	—

The "∗" sign refers to a nucleotide position not present in the murine and rat miR-378a-3p mature sequence, which is present in the mature human sequence.

development, differentiation, cellular defense mechanisms, and others. Conservative estimates state that over 30% of mRNA expression is regulated by miRNAs [12, 13]. However, others suggest that even up to 60% of the mRNA expression is targeted by miRNAs [14]. miRNAs are often located in the introns of coding genes or noncoding sequences but can also be located in exons. Intronic miRNAs can be expressed together with their host gene mRNA being derived from a common RNA transcript [15, 16]. Other miRNAs can also have their own promoters, which enable independent expression, or can be organized in clusters sharing a common transcriptional regulation [17, 18].

miRNAs transcription is RNA polymerase II-dependent [17]. In the case of miRNAs that are encoded in their own genes, the primary miRNA transcript (pri-miRNA) is several kilobases long, while miRNAs encoded in intronic regions of other genes (miRtrons) have shorter transcripts. The miRNA stem loop is excised from pri-miRNA by endoribonuclease drosha/DGCR8 (microprocessor complex) and a hairpin called pre-miRNA is exported from the nucleus by exportin-5 in a Ran-GTP dependent manner [19]. An endoribonuclease dicer removes the hairpin loop sequence from pre-miRNA, creating a double stranded miRNA duplex. Depending on the relative stability of the miRNA duplex, one or, more rarely, both strands can be incorporated in a multiprotein RNA-induced silencing complex (RISC). When there is perfect pairing between the miRNA sequence and its target site, mRNA is cleaved by a protein part of the RISC called argonaute (AGO). If the pairing is partial, deadenylation of the mRNA via recruitment of the CCR4-NOT complex by the GW182 proteins inside the RISC takes place and the poly-A tail is lost, leaving the mRNA vulnerable to RNase activity, ubiquitination, and mRNA degradation. Alternatively, miRNA-induced RISC can also cause repression of translation by mechanisms such as, for example, the promotion of ribosome drop-off from the mRNA transcript or destabilization of the mRNA binding cap protein (Figure 1) (reviewed in [20, 21]).

3. miR-378a: Basics

miR-378a is embedded in the first intron of the *ppargc1b* gene encoding PGC-1β [5]. The pre-miR gives rise to a leading strand (miR-378a-3p, previous IDs for murine sequence: mmu-miR-422b, mmu-miR-378, and mmu-miR-378-3p; for human: hsa-miR-422b and hsa-miR-378) and a passenger strand (miR-378a-5p, previous IDs for murine sequence: mmu-miR-378, mmu-miR-378*, and mmu-miR-378-5p; for human: hsa-miR-378 and hsa-miR-378*). miRNA-378a-3p mature strand was first identified in 2004 in humans (originally named miR-422b) [22]. Recently, other miRs with similar sequences but other localizations in the genome have been discovered and named: mmu-miR-378b,c,d in mouse and hsa-miR-378-b,c,d1,d2,e,f,g,h,i,j in human [23–27] (Table 1). In humans, miR-378a is by far the most expressed of the miR-378 sequences, with 7030 reads per million, in 78 experiments during deep sequencing, compared with 101–3220 reads per million, in 42–72 experiments for the other forms, respectively. In mice, miR-378a and miR-378b have similar expression levels, at 11700 and 11000 reads per million

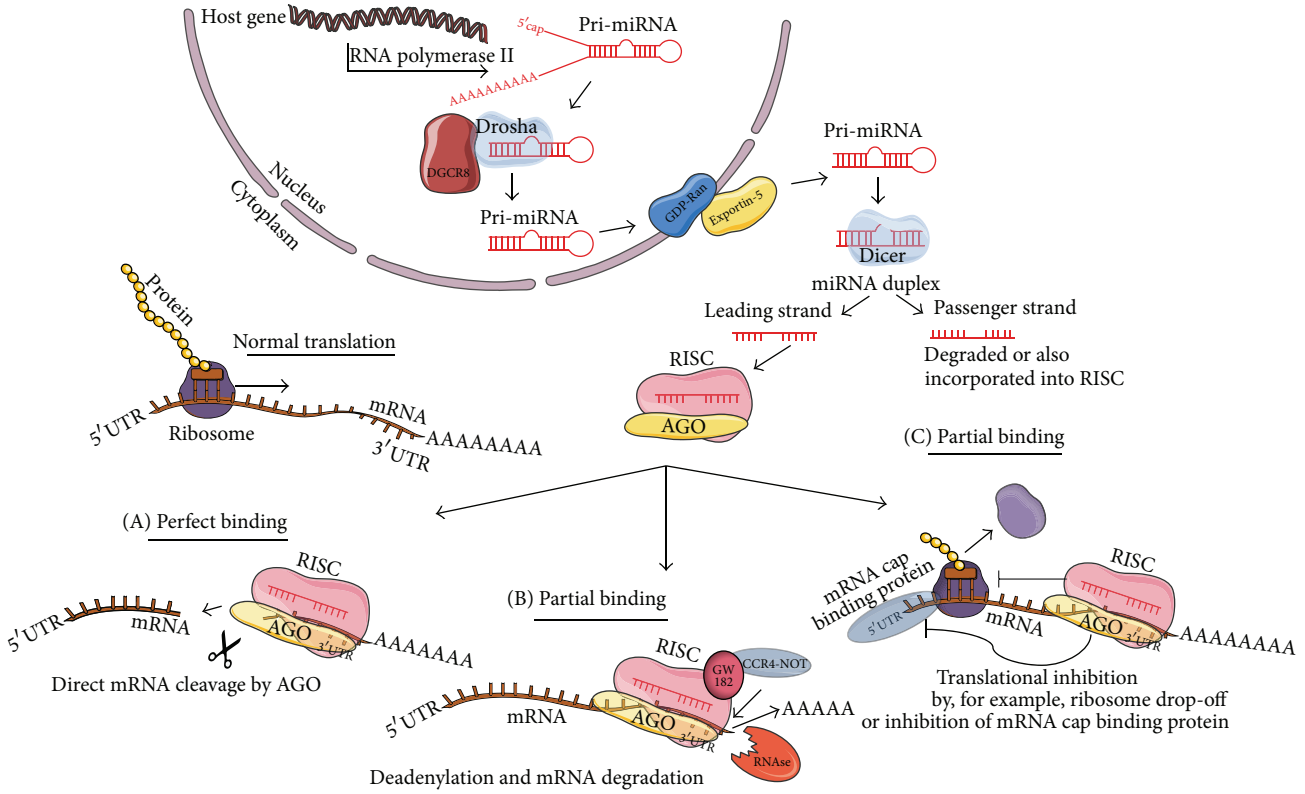

Figure 1: miRNA biogenesis. miRNAs are transcribed as mRNA transcripts from the genome by polymerase II as pre-miRs. Endoribonuclease drosha and DGCR8 excise pre-miRs from the primary transcripts. Pri-miRs are exported from the nucleus by exportin-5. An endoribonuclease dicer processes the pre-miRNA and removes the hair loop sequence, creating a double stranded miRNA duplex. One or both strands can be incorporated in RNA-induced silencing complex RISC, which allows the miRNA to suppress translation of their target mRNA or cleave the mRNA and lead to the degradation of it. miRNA-induced RISC can act on their targets by three ways. When there is perfect pairing between the miRNA sequence and its target site, the mRNA is cleaved (A). If the pairing is partial, deadenylation of the mRNA via recruitment of the CCR4-NOT complex takes place and the poly-A tail is lost, leaving the mRNA vulnerable to RNAse activity and mRNA degradation (B). As a second manner of action when pairing is not perfect, the miRNA-induced RISC can also induce repression of translation by blocking initiation or further steps of translation, by mechanisms such as, for example, the promotion of ribosome drop-off from the mRNA transcript or destabilization of the mRNA cap binding protein (C).

(miRBase, version 21, September 2015) [28]. The sequence of miR-378a mature strands is highly conserved between species, with the miR-378a-5p strand being identical in both human and mice and the miR-378a-3p strand only differing in one nucleotide (Table 2) [6, 27].

PGC-1β may regulate several facets of energy metabolism such as mitochondrial biogenesis, thermogenesis, and glucose and fatty acid metabolism [6]. Both strands of miR-378a are coexpressed with PGC-1β as shown, for example, in the liver and during adipocyte differentiation [6, 29]. The coexpression of miR-378a with its host gene implies they may share the same transcriptional activators, and miR-378a might be involved in similar processes as PGC-1β. Accordingly, high levels of (porcine) miR-378-1 (Table 2) expression are found in developing muscle, postnatal muscle, and myocardium and in brown adipose tissue [29, 30].

To date, only a limited number of miR-378a targets, which can be predicted based on *in silico* analysis, have been experimentally validated. The latter, however, imply a role of miR-378a in mitochondrial energy homeostasis, glycolysis, and skeletal muscle development and in tumor angiogenesis and other processes (Table 3).

Table 2: miR-378a is highly conserved between species. Source: miRBase, version 21, September 2015 [28]. The seed sequence (defined as nucleotides 2–8 from the miRNA 5′-end of the mature miRNA) is in bold font.

Species	Name	Sequence
Human	hsa-miR-378a-5p	5′-c**uccugac**uccagguccugugu-3′
	hsa-miR-378a-3p	5′-a**cuggacu**uggagucagaaggc-3′
Mouse	mmu-miR-378a-5p	5′-c**uccugac**uccagguccugugu-3′
	mmu-miR-378a-3p	5′-a**cuggacu**uggagucagaagg•-3′
Rat	rno-miR-378a-5p	5′-c**uccugac**uccagguccugugu-3′
	rno-miR-378a-3p	5′-a**cuggacu**uggagucagaagg•-3′
Pig	ssc-miR-378-1	5′-a**cuggacu**uggagucagaaggc-3′
Cow	bta-miR-378-1	5′-a**cuggacu**uggagucagaaggc-3′
Thirteen-lined ground squirrel	itr-miR-378a	5′-a**cuggacu**uggagucagaaggc-3′

4. miR-378a in Metabolism

A major source of energy production comprises oxidation of glucose in glycolysis followed by oxidation of pyruvate in well-oxygenated cells (or followed by lactic acid fermentation in cancer, the Warburg effect) and from β-oxidation of lipids, which yields even more ATP per gram then carbohydrates metabolism. A complicated net of metabolic pathways requires advanced regulation by signaling molecules and hormones.

A location of miR-378a in the gene encoding PGC-1β [5] implies an involvement of miR-378a in metabolic pathways. Unlike its homologue, PGC-1α, the expression of PGC-1β is not elevated in response to cold exposure [31] but occurs in response to hypoxia, exercise, caloric restriction, or aging (reviewed in [32]). PGC-1β is preferentially expressed in tissues with relatively high mitochondrial content, such as heart, skeletal muscle, and brown adipose tissue [6]. In 2002, PGC-1β was first cloned and shown to be upregulated in the liver during fasting [31]. PGC-1β strongly activates hepatic nuclear factor 4 (HNF4) and PPARα, both of these nuclear receptors being important for the adaptation of hepatocytes to the effects of fasting. These findings could hint to a possible role of PGC-1β in the regulation of gluconeogenesis and fatty acid oxidation in the liver [31]. PGC-1β is also involved in the regulation of energy expenditure or in the pathway of estrogen receptor-related receptors (ERRs) [33–37]. Since miRNAs originating in the introns of host genes may modulate the protein encoded by their parental genes and may be involved in the same mechanisms [38–40], miR-378a is proposed to be involved in the metabolic pathways affected by PGC-1β [6].

It was reported that mice lacking the first intron of the *ppargc1b* gene (and thus miR-378a) have a significantly higher oxygen capacity and mitochondrial function [6]. Such mice also exhibit a resistance to high fat induced obesity. They identified a mediator complex subunit 13 (MED13), involved in nuclear receptor signaling, and carnitine acetyltransferase (CRAT), a mitochondrial enzyme involved in fatty acid metabolism, as targets of miR-378a-5p and miR-378a-3p, respectively [6]. It implies that miR-378a plays a regulatory role in lipid metabolism. miR-378a-5p regulated also cytochrome P450 2E1 (CYP2E1) being involved in the metabolism of, for example, drugs and toxins [41].

In addition, it has been discovered that transcription factor nuclear respiratory factor-1 (NRF-1), a critical regulator of the expression of some important metabolic genes in mitochondria regulating cellular growth, is inhibited by miR-378a-3p [42]. Thus, miR-378a can be considered as a regulator of mitochondrial function in cells overexpressing miR-378a.

Moreover, miR-378a-5p inhibits the mRNAs of ERRγ and GA-binding protein-α in breast cancer, which both interact with PGC-1β and together control oxidative metabolism [5]. This leads to a reduction of tricarboxylic acid gene expression and oxygen consumption and an increase in lactate production, which shifts cells from an oxidative towards a glycolytic pathway. In this way, miR-378a-5p is believed to be a switch regulating the Warburg effect in breast cancer [5]. Moreover, in situ hybridization experiments in this study showed that miR-378a-5p expression correlates with progression of breast cancer [5]. The proposed regulating role of miR-378a-5p on the Warburg effect is in parallel with the effects of PGC-1β, which mediates gluconeogenesis and fatty acid metabolism after periods of fasting or intense exercise [31]. Coactivation by PGC-1β of ERRα and PPARα makes muscle fibers in PGC-1β transgenic mice more rich in mitochondria and highly oxidative [43]. Accordingly, such animals were able to run for longer times and at higher workloads [43].

Increased glycolytic rates and increased cell proliferation can be related to lactate production by lactate dehydrogenase (LDH). LDHA was found to be a direct target of miR-378a in the study of Mallat et al. [44]. In this way, hsa-miR-378a-3p represses cell growth and increases cell death by targeting LDHA. Of note, hsa-miR-378a-3p and hsa-miR-378a-5p had opposite effects on LDHA expression. LDHA was significantly downregulated by miR-378a-3p overexpression and upregulated by miR-378a-5p overexpression [44].

In addition, miR-378a is also considered as an important factor in adipogenesis and lipid storage. There is a complex family of factors regulating those processes such as insulin [45], insulin-like growth factors (IGFs), glucagon, and thyroid hormones T3 and T4 (reviewed in [46–49]). As mentioned before, it was demonstrated that miR-378a-knockout mice do not get fat after 8 weeks of high fat diet [6]. Such animals show an enhanced mitochondrial fatty acid metabolism and have elevated oxidative capacity of tissues targeted by insulin (e.g., liver, muscles, and adipose tissues) [6]. In accordance with that, it was shown that mature strands of bta-miR-378-1 (Table 1) are expressed at higher level in cows with high (versus low) amount of back fat [50]. Similarly, an inhibition of both mmu-miR-378a-3p and its host gene, PGC-1β, by the flavonoid fisetin lowered the accumulation of fat in the liver [42]. Interestingly, mmu-miR-378a-5p was downregulated in mice that were fed a high fat diet for five months [51]. In addition, miR-378a is highly induced during adipogenesis [29]. Overexpression of miR-378a-3p/-5p during adipogenesis increased the transcriptional activity of CCAAT/enhancer-binding proteins (cEBP) alpha and beta, which can stimulate the expression of leptin, a hormone produced mainly by adipocytes which controls the homeostasis of body weight [29] (reviewed in [52, 53]). On the other hand, TNF-α, IL-6, and leptin are reported to increase the expression of miR-378a-3p in mature human adipocytes *in vitro* [54]. These cytokines are mainly secreted in the adipose tissue and are suggested to be involved in development of insulin resistance [55, 56]. In addition, miR-378a-3p was shown to target insulin growth factor 1 receptor (IGF1R) and reduce the Akt signaling cascade in cardiomyocytes during cardiac development [57]. Moreover, in tissues where IGF1 levels were high (e.g., fibroblasts and fetal hearts), miR-378-3p levels were very low, showing an inverse relation and suggesting a negative feedback loop between miR-378a-3p, IGF1R, and IGF1 [57].

As already mentioned, PGC-1β is a coactivator of PPARγ [5]. The latter functions as a master regulator of adipogenesis and is involved in the formation of peroxisomes and the catabolism of very long chain fatty acids [58, 59]. PPARγ facilitates also the storage of fat in part by inhibiting leptin

Table 3: The known interactions of miR-378a.

miR-378a-3p		miR-378a-5p		Both/unspecified	
Target	Function	Target	Function	Target	Function
			Metabolism		
NRF1 [42]*	Critical regulator of the mitochondrial function	*CYP2E1* [41]*	Involved in conversion of acetyl-CoA to glucose	*cEBPα* [27]#	Transcription factor, promotes expression of leptin
TPM2 [44]*	Involved in the regulation of ATPase activity	*ERRγ* [5]*	Involved in control of oxidative metabolism	*cEBPβ* [27]#	Transcription factor, regulation of genes involved in immune and inflammatory responses
IGF1R [57]#	Tyrosine kinase receptor, mediates the effects of IGF-1	*GABPα* [5]*	Involved in control of oxidative metabolism, nuclear control of mitochondrial function		
RORA [69]#	Orphan receptor, possibly involved in circadian rhythm	*GDP* [67]*	Enzyme involved in GDP-mannose production		
LDHA [44]*	Involved in the lactic acid cycle	*DDAH* [44]*	Involved in the synthesis of arginine from citrulline		
CRAT [6]#	Catalyzes exchange of acyl groups between carnitine and coenzyme A	*LDHA* [44]*	Involved in the lactic acid cycle		
		MED13 [6]#	Component of the mediator complex (transcriptional coactivator)		
			Muscle differentiation and regeneration		
MyoR [7]#	Represses MyoD (and thus myogenesis)	*VIM* [44]*	Cytoskeletal protein anchoring position of organelles	*Purβ* [69]#	Controlling transcription of smooth muscle actin
ACTN4 [44]*	Nonmuscle α-actinin isoform, cytoskeletal protein	*Actin* [44]*	Cytoskeletal protein, involved in muscle contraction		
14-3-3-γ [44]*	Regulatory protein highly expressed in muscle	*Hsp70.3* [76]#	Involved in cytoprotective responses against stress induced stimuli		
CASP3 [73]®	Involved in activation of apoptosis				
CTGF [74]#	Connective tissue growth factor				
TGFβ1 [74]#	Involved in cell growth, proliferation, differentiation, and apoptosis				
IGF1R [57]#	Tyrosine kinase receptor, mediates the effects of IGF-1				
MAPK [77, 78]®#	Involved in proliferation, differentiation cell survival, and apoptosis				
			Angiogenesis		
TOB2 [88]*	Suppressing cyclin D1	*VEGF-A* [89]*	Induction of angiogenesis		
		SuFu [8]*	Involved in inhibition of SHH pathway		
		Fus-1 [8]*	Involved in RNA binding and tumor suppression		
		FN1 [112]#	Receptor binding integrin		
		BAX [93]*	Involved in apoptosis, activation of caspases		
		ITGB3 [116]#	Involved in cell adhesion and cell-surface signaling		
		VIM [116]#	Involved in anchoring position of organelles		

Studies were performed in human (∗), mouse (#), or rat (®).

[60]. Accordingly, the amount of adipose tissue does not increase in mice lacking PPARγ when they are fed a high fat diet [61]. It was also reported that in cultured adipocytes mmu-miR-378a and PGC-1β expression is PPARγ, or rosiglitazone (a PPARγ ligand), dependent, finding two peroxisome proliferator response elements in the miR-378a loci [62]. On the other hand, overexpression of miR-378a elevated the expression of PPARγ isoform 2 [29], suggesting positive feedback loop and confirming the involvement of miR-378a in the storage of fat.

There are several activators known to induce expression of PPARγ such as the members of the E2F transcription factor family and prostaglandin J-2 (PGJ-2) [63–65]. The latter may act through RAR-related orphan receptor alpha (RORA), which is frequently found in myocardium [66]. In addition to PPARγ, RORA regulates also MyoD, a major transcription factor involved in skeletal muscle differentiation [67, 68]. Interestingly, RORA is a possible (but not yet validated) target for miR-378a-3p [69].

A proteomics-based study revealed several other proteins that are potentially targeted by rat miR-378a-3p or miR-378a-5p. miR-378a-3p was shown to regulate mannose-1-phosphate guanylyltransferase (GDP), dimethylarginine dimethylaminohydrolase 1 (DDAH1), and lactate dehydrogenase A (LDHA); all those proteins are participating in metabolic processes [44]. On the other hand, tropomyosin beta chain, which is involved in the regulation of ATPase activity, was found to be a target of miR-378a-5p [44].

5. miR-378a in Muscle Development, Differentiation, and Regeneration

High levels of murine and rat miR-378a-3p, miR-378a-5p, and porcine miR-378-1 are reported in both developing and adult skeletal muscles [7, 30, 44]. miR-378a expression is enhanced during skeletal muscle differentiation [30].

MyoD and MyoG play a role in the processes of myogenesis and muscle regeneration, in which dormant satellite cells are activated upon muscle damage and start proliferating and differentiating into muscle fibers (reviewed in [70, 71]). It has been shown that miR-378a-3p targets the myogenic repressor MyoR during myoblast differentiation, which directly inhibits MyoD [7]. On the other hand, MyoD is upregulated in response to miR-378a-3p overexpression and, conversely, the level of miR-378a-3p may be enhanced by MyoD [7]. Thus, there is evidence for a feedback loop in which miR-378a-3p regulates muscle differentiation via inhibiting MyoR, leading to an increase of MyoD, which in turn enhances miR-378a-3p [7].

It has been suggested by Davidsen et al. that miR-378a may also control the development of skeletal muscle mass after training [72]. In this study, miR-378a (strand not specified) was significantly downregulated in men who obtained low training-induced muscle mass gain compared to men who obtained high training-induced muscle mass gain [72].

A growing body of data shows a role of miR-378a-3p in the myocardium. miR-378a-3p is expressed mostly by cardiomyocytes, but not by nonmuscle cells, whereas the level of miR-378a-5p was reported to be very low in the heart [57]. Fang et al. showed that miR-378-3p is significantly downregulated both *in vitro* in cardiomyocytes cell cultures exposed to hypoxia and *in vivo* during myocardial injury in rats [73]. Overexpression of miR-378a-3p enhanced cell viability and inhibited apoptosis via caspase-3 inhibition [73]. In contrast to this finding, another study found that miR-378a-3p downregulation enhanced the survival of cardiac stem cells via focal adhesion kinase activation and releasing connective tissue growth factor (CTGF), the latter being a target of miR-378a-3p [74]. miR-378a inhibition enhanced cardiomyocytes survival after H_2O_2 treatment [57]. Overexpression of miR-378a-3p in the study of Knezevic et al. increased apoptosis of cardiomyocytes via the direct targeting of IGF1R leading to a decrease of Akt signaling [57]. This is in opposition to the previously mentioned study of Fang et al. which showed apoptosis was decreased during miR-378a-3p overexpression due to targeting of caspase-3 [73]. The converse findings of the studies could be explained by different models used by Knezevic et al. and Fang et al. Because of those discrepancies, the role of miR-378a in apoptosis of cardiomyocytes requires further investigation. The finding that miR-378a-3p affects both IGF1R and the Akt pathway was confirmed [75] in a study which found that overexpression of miR-378a-3p in rhabdomyosarcoma suppressed IGF1R expression and affected phosphorylation of the Akt protein [75]. miR-378a-5p was shown to target heat shock protein 70.3 (Hsp70.3) in mouse hearts in normoxic conditions, but in hypoxic conditions a transcript variant of Hsp70.3 without miR-378a-5p target site in its 3′-UTR is not repressed and can exert its cytoprotective properties [76].

Potential involvement of miR-378a in cardiac remodeling was also proposed. miR-378a-3p prevented cardiac hypertrophy by targeting either Ras signaling or the mitogen-activated protein kinase (MAPK) pathway [77, 78].

More studies on the effect of miR-378a expression in muscle disorders would also be desirable. In both Golden Retriever muscular dystrophy dogs and Duchenne muscular dystrophy patients, miR-378a expression was dysregulated, suggesting some relation between miR-378a expression and muscle dystrophy [79].

All in all, these findings suggest miR-378a-3p can be considered as an important player in cardiac development, remodeling, and hypertrophy.

6. miR-378a in Angiogenesis

Angiogenesis comprises development of new blood vessels from existing ones, regulated by cytokines and growth factors such as, for example, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and angiopoietin-1 (Ang-1). Their expression can be posttranscriptionally controlled by microRNAs such as miR-126, miR-296, miR-210, miR-21, and the miR-17~92 cluster [80] (reviewed in [81]).

Skeletal muscles and heart muscle are tissues which, due to their oxygen and energy consumption, need to be sufficiently vascularized. One of the major regulators of angiogenesis is the hypoxia-inducible factor-1 (HIF-1), which

controls over 100 genes [82] involved mainly in the glycolytic pathway and blood vessel formation, including VEGF-A or interleukin-8 [83–85]. VEGF is generally induced by hypoxia, while IL-8 in at least some cancers and endothelial cells can be diminished by HIF-1 via inhibition of c-Myc and Sp-1 transcription factors [86, 87]. c-Myc, known as a regulator of cell cycle progression, apoptosis, and cellular transformation, is also a potent activator of PGC-1β and, in turn, miR-378a-3p, upregulating their expression [88].

In addition, miR-378a has been shown to affect VEGF-A in two ways. Human hsa-miR-378a-5p (by the study of Hua et al. named as miR-378) can directly affect VEGF-A by competing with hsa-miR-125a for the same seed-region in the VEGF-A 3′UTR causing upregulation of VEGF-A [89]. miR-378a-5p can also indirectly regulate VEGF-A affecting sonic hedgehog (SHH) signaling via Sufu inhibition, which is an inhibitory component of this signaling pathway [8]. The SHH pathway in turn can upregulate VEGF-A and also other regulators of blood vessels formation, Ang-1 and Ang-2 expression [90–92]. Increased expression of VEGF-A, as well as PDGFβ and TGFβ1, was also seen in mesenchymal stromal cells (MSCs) transfected with rno-miR-378a-5p [93].

In skeletal muscles, VEGF-induced angiogenesis appears not to be regulated by the well-known HIF pathway but by PGC-1α, which coactivates estrogen-related receptor alpha (ERR-α) on binding sites in the promoter and the first intron of the VEGF gene, inducing its expression [94]. This angiogenic pathway shows new roles for PGC-1α and ERR-α, which are important regulators of mitochondrial activity in response to stimuli like exercise. If there might be a role for PGC-1β in this pathway, it is yet to be examined. It is noteworthy, however, that miR-378a-5p is known to affect the estrogen receptors by inhibiting ERRγ, another estrogen-related receptor [5].

A role for miR-378a in cell cycle regulation and stimulation of cell growth is also proposed. In human mammary epithelial and breast cancer cell lines, miR-378a-3p can target the antiproliferative protein TOB2, which is a suppressor of cyclin D1, which in turn is required for cell cycle G1-phase to S-phase progression [88]. Enhancing endothelial cell proliferation via cell cycle regulation contributes to the angiogenic process. Whether miR-378 affects endothelial cell proliferation by regulation of cell cycle remains to be established.

The role of miR-378a in the formation of blood vessels nourishing tumor and enabling tumor growth was revealed. miR-378a was found to be differentially regulated in different types of cancers [95] being downregulated in gastric cancer [96, 97], oral [98], and colon carcinoma [99], while being upregulated in renal [100] and lung cancer [9, 101]. Since it is also changed in serum or plasma of patients with prostate cancer [102], renal cancer [100, 103], and gastric cancer [104] and frequently found to be overexpressed in cryopreserved bone marrow mononuclear cells from acute myeloid leukemia patients [105], miR-378a might be considered as a biomarker.

The role of miR-378a in tumorigenesis, tumor growth, and tumor vascularization was revealed for the first time by Lee and coworkers in glioblastoma [8]. They showed that miR-378a-5p enhances cell survival, reduces caspase-3 activity, and promotes tumor growth and angiogenesis, through repression of two tumor suppressors, Sufu and Fus-1 [8]. Strikingly, nude mice injected with miR-378a-5p transfected cancer cells formed tumors of bigger volume and with larger blood vessels compared to GFP-transfected cells. On the other hand, high expression of miR-378a-5p in NSCLC correlated with brain metastases due to higher cell migration, invasion, and tumor angiogenesis [9]. Another study confirmed the downregulation of Fus-1 by miR-378a-5p and showed that in the HepG2 liver cancer cells miR-378a-5p overexpression enhanced proliferation, migration, and, when injected in mice, invasion [106]. Also in rhabdomyosarcoma, enhanced expression of miR-378a, VEGF, and MMP9 correlated with increased vascularization and metastasis [107]. Taken together, these studies suggest that miR-378a may serve as a prognostic marker in cancer due to its effects on angiogenesis.

Our recent data confirmed the proangiogenic effect of miR-378a (both strands) in non-small cell lung carcinoma (NSCLC) and pointed at its correlation with heme-degrading enzyme, heme oxygenase-1 (HO-1). An involvement of HO-1 in angiogenesis and VEGF-A as well as IL-8 signaling was shown by us previously [108]; however, its action in tumors seems to be complex [109]. In NCI-H292 cell line overexpressing HO-1, miR-378a (both strands) levels decreased [101]. Conversely, when HO-1 was silenced using siRNA, miR-378a expression was enhanced. Also overexpression of the miR-378a precursor sequence diminished HO-1 expression. Conditioned medium from NCI-H292 cells overexpressing miR-378a enhanced angiogenic potential of HMEC-1 endothelial cell line. Tumors formed by such cells in subcutaneous xenografts showed enhanced growth, vascularization, oxygenation, and distal metastasis *in vivo* [101]. These interactions between miR-378a and HO-1 were confirmed in our studies on the role of the Nrf-2 transcription factor/HO-1 axis in NSCLC cell lines [110, 111].

On the other hand, enhanced expression of mmu-miR-378a-5p in 4T1 murine breast cancer cells decreased the proliferation, migration, and invasiveness of these cancer cells *in vitro* and *in vivo* by targeting fibronectin, resulting in inhibition of tumor growth [112].

Recent study showed that miR-378a may act as a biomarker for response to antiangiogenic treatment in ovarian cancer [113]. Low expression of miR-378a was associated with longer progressive-free survival in patients with recurrent ovarian cancer treated with the antiangiogenic drug bevacizumab [113]. Overexpression of the miR-378a precursor in ovarian cancer cells altered expression of genes associated with angiogenesis (ALCAM, EHD1, ELK3, and TLN1), apoptosis (RPN2, HIPK3), and cell cycle regulation (SWAP-70, LSM14A, and RDX) [113]. High miR-378a (strand not specified) expression in renal carcinoma correlated with higher levels of endothelial surface marker CD34 in these tumors [114].

Notably, a recent study suggested clinical relevance for miR-378a in metastatic colorectal cancer, in which enhanced miR-378a expression significantly improved the sensitivity to cetuximab treatment in these patients [115].

Interestingly, recent data indicate a role of miR-378a in stem cells. miR-378a-5p transfection of MSCs has been shown to enhance their survival and angiogenic potential under hypoxic conditions *in vitro* [93]. In coculture with human umbilical vein endothelial cells (HUVECs), miR-378a-5p-transfected MSCs formed a larger number of vascular branches on Matrigel. In the MSCs transfected with miR-378a-5p, the expression of Bcl-2-associated X protein (BAX), which is an important proapoptotic regulator, was decreased, leading to a better survival [93].

It still has to be determined if the proangiogenic effect of miR-378a *in vivo* is confined to tumor angiogenesis, or if this effect is also present in physiological angiogenesis and regenerative neovascularization. Interestingly, new findings in wound healing studies found a rather opposite conclusion. Recently, it was reported that anti-miR-378a-5p enhances wound healing process by upregulating integrin beta-3 and vimentin [116].

The role of the host gene of miR-378a on angiogenesis has also been studied. PGC-1β was reported to have opposite effects in ischemia-induced angiogenesis. It was reported that PGC-1β induces angiogenesis in skeletal muscle, enhancing the expression of VEGF both *in vitro* and *in vivo* after (transgenic) overexpression [117]. Accordingly, it was also found that VEGFA is upregulated in C2C12 myoblast cell line with PGC-1β overexpression. However, after a PCR-based gene array of 84 known angiogenic factors and further RT-PCR of individual genes, they concluded that PGC-1β triggered an antiangiogenic program [118]. After inducing hind limb ischemia in PGC-1β overexpressing mice, an impaired reperfusion was noticed when compared to wild type littermates [118].

7. miR-378a in Inflammation

The role of inflammation in angiogenesis is studied the most in the context of cancer (e.g., reviewed in [119, 120]) but is certainly not limited to this pathology. Both lymphoid (reviewed in [121, 122]) and myeloid (reviewed in [123]) derived inflammatory cells affect angiogenesis in a stimulating or inhibitory manner. The role of miR-378a in inflammatory cells was reported and its anti-inflammatory effect could be suggested.

NK cells exert potent cytotoxic effects when activated by type I IFN from the host once infected [124]. miR-378a was found to be downregulated in activated NK cells and further proved to target granzyme B. Thus, IFN-α activation decreases miR-378a expression and in turn augments NK cell cytotoxicity [124]. Accordingly, suppression of miR-378 targeting granzyme B in NK cells resulted in inhibition of Dengue virus replication *in vivo* [125].

Macrophages are known to play either inhibitory or stimulatory roles in angiogenesis (reviewed by [126]). miRNAs have been proposed to regulate activation and polarization of macrophages (reviewed by [127, 128]). In a study of Rückerl et al. miR-378a-3p was identified as a part of the IL-4-driven activation program of anti-inflammatory macrophages (M2) [129]. miR-378a-3p was highly upregulated after stimulation with IL-4 of peritoneal exudate cells of mice injected with the parasite *Brugia malayi* compared to controls and infected IL-4-knockout mice. The study identified several targets for miR-378a-3p within the PI3 K/Akt signaling pathway, which are important for proliferation but only partially responsible for M2 phenotype [129]. Another study found miR-378a (strand not specified) expression upregulated after stimulation with cytokines like, for example, TNF-α and IL-6 [130].

In line with its potential role in macrophages, miR-378a has been suggested as being of importance in the osteoclastogenesis [131]. Mmu-miR-378a (strand not specified) has been found to be upregulated during osteoclastogenesis *in vitro* [131]. Furthermore, serum levels of miR-378a-3p have been shown to correlate with bone metastasis burden in mice injected with mouse mammary tumor cell lines 4T1 and 4T1.2 [132].

8. Conclusions

A growing body of evidence suggests a role for miR-378a as a mediator controlling reciprocally dependent processes in metabolism, muscle differentiation/regeneration, and angiogenesis.

As miR-378a was found to be differentially regulated in different types of cancers and its level is changed in serum of prostate, renal, and gastric cancer patients, it can be considered as a biomarker for those diseases. The correlation between miR-378a expression and disease progression in lung cancer, liver cancer, and rhabdomyosarcoma suggests a further role of this microRNA as a prognostic marker.

Currently, miR-378a is not utilized as a therapeutic molecule. However, if more research will be done to the mechanisms of action, possibilities for therapeutic use of miR-378a could be sought in the field of metabolic disorders, obesity, or tumors. More studies on the effect of miR-378a expression in muscle disorders would also be desirable.

The proangiogenic effect of miR-378a was observed in tumors; however, no studies have been performed on the angiogenic effects of miR-378a in physiological settings or diseases where angiogenesis plays important roles, such as diabetes and cardiovascular diseases. More study has to be done to assess the mechanisms of miR-378a function in blood vessel formation. Of note, in contrast with proangiogenic role of miR-378a, inhibition of miR-378a-5p enhanced wound healing process. This might suggest a role for miR-378a-5p in diseases such as diabetes or in decubitus ulcers, in which wound healing is impaired.

Of note is the confusion that has arises because of a disarray in nomenclature with studies describing the same molecule, miR-378a, as miR-422b, miR-378, or miR-378*. In addition, it is not always clear which of the two mature strands of miR-378a is studied. This could lead to misunderstandings and errors in interpreting the data published so far.

Disclosure

The graphical art (Figure 1) was performed with the use of Servier Medical Art.

Conflict of Interests

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

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References

[1] Z. Wang, Z. Ying, A. Bosy-Westphal et al., "Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure," *The American Journal of Clinical Nutrition*, vol. 92, no. 6, pp. 1369–1377, 2010.

[2] K. L. Blaxter, *Energy Metabolism in Animals and Man*, Cambridge University Press, Cambridge, UK, 1989.

[3] D. Gallagher, D. Belmonte, P. Deurenberg et al., "Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass," *American Journal of Physiology*, vol. 275, no. 2, part 1, pp. E249–E258, 1998.

[4] M. N. Poy, M. Spranger, and M. Stoffel, "microRNAs and the regulation of glucose and lipid metabolism," *Diabetes, Obesity and Metabolism*, vol. 9, supplement 2, pp. 67–73, 2007.

[5] L. J. Eichner, M. C. Perry, C. R. Dufour et al., "miR-378(*) mediates metabolic shift in breast cancer cells via the PGC-1β/ERRγ transcriptional pathway," *Cell Metabolism*, vol. 12, no. 4, pp. 352–361, 2010.

[6] M. Carrer, N. Liu, C. E. Grueter et al., "Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 38, pp. 15330–15335, 2012.

[7] J. Gagan, B. K. Dey, R. Layer, Z. Yan, and A. Dutta, "MicroRNA-378 targets the myogenic repressor MyoR during myoblast differentiation," *The Journal of Biological Chemistry*, vol. 286, no. 22, pp. 19431–19438, 2011.

[8] D. Y. Lee, Z. Deng, C.-H. Wang, and B. B. Yang, "MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 51, pp. 20350–20355, 2007.

[9] L.-T. Chen, S.-D. Xu, H. Xu, J.-F. Zhang, J.-F. Ning, and S.-F. Wang, "MicroRNA-378 is associated with non-small cell lung cancer brain metastasis by promoting cell migration, invasion and tumor angiogenesis," *Medical Oncology*, vol. 29, no. 3, pp. 1673–1680, 2012.

[10] I. Lee, S. S. Ajay, I. Y. Jong et al., "New class of microRNA targets containing simultaneous 5′-UTR and 3′-UTR interaction sites," *Genome Research*, vol. 19, no. 7, pp. 1175–1183, 2009.

[11] R. C. Lee, R. L. Feinbaum, and V. Ambros, "The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14," *Cell*, vol. 75, no. 5, pp. 843–854, 1993.

[12] B. P. Lewis, C. B. Burge, and D. P. Bartel, "Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets," *Cell*, vol. 120, no. 1, pp. 15–20, 2005.

[13] I. Bentwich, "Prediction and validation of microRNAs and their targets," *FEBS Letters*, vol. 579, no. 26, pp. 5904–5910, 2005.

[14] R. C. Friedman, K. K.-H. Farh, C. B. Burge, and D. P. Bartel, "Most mammalian mRNAs are conserved targets of microRNAs," *Genome Research*, vol. 19, no. 1, pp. 92–105, 2009.

[15] S.-Y. Ying and S.-L. Lin, "Intronic microRNAs," *Biochemical and Biophysical Research Communications*, vol. 326, no. 3, pp. 515–520, 2005.

[16] S. Baskerville and D. P. Bartel, "Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes," *RNA*, vol. 11, no. 3, pp. 241–247, 2005.

[17] Y. Lee, M. Kim, J. Han et al., "MicroRNA genes are transcribed by RNA polymerase II," *The EMBO Journal*, vol. 23, no. 20, pp. 4051–4060, 2004.

[18] Y. Altuvia, P. Landgraf, G. Lithwick et al., "Clustering and conservation patterns of human microRNAs," *Nucleic Acids Research*, vol. 33, no. 8, pp. 2697–2706, 2005.

[19] E. P. Murchison and G. J. Hannon, "miRNAs on the move: miRNA biogenesis and the RNAi machinery," *Current Opinion in Cell Biology*, vol. 16, no. 3, pp. 223–229, 2004.

[20] A. E. Pasquinelli, "MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship," *Nature Reviews Genetics*, vol. 13, no. 4, pp. 271–282, 2012.

[21] D. P. Bartel, "MicroRNAs: genomics, biogenesis, mechanism, and function," *Cell*, vol. 116, no. 2, pp. 281–297, 2004.

[22] K. Kasashima, Y. Nakamura, and T. Kozu, "Altered expression profiles of microRNAs during TPA-induced differentiation of HL-60 cells," *Biochemical and Biophysical Research Communications*, vol. 322, no. 2, pp. 403–410, 2004.

[23] M. S. Stark, S. Tyagi, D. J. Nancarrow et al., "Characterization of the melanoma miRNAome by deep sequencing," *PLoS ONE*, vol. 5, no. 3, Article ID e9685, 2010.

[24] D. D. Jima, J. Zhang, C. Jacobs et al., "Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs," *Blood*, vol. 116, no. 23, pp. e118–e127, 2010.

[25] E. Berezikov, G. Van Tetering, M. Verheul et al., "Many novel mammalian microRNA candidates identified by extensive cloning and RAKE analysis," *Genome Research*, vol. 16, no. 10, pp. 1289–1298, 2006.

[26] L. A. Goff, J. Davila, M. R. Swerdel et al., "Ago2 immunoprecipitation identifies predicted MicroRNAs in human embryonic stem cells and neural precursors," *PLoS ONE*, vol. 4, no. 9, Article ID e7192, 2009.

[27] M. Dannemann, K. Prüfer, E. Lizano, B. Nickel, H. A. Burbano, and J. Kelso, "Transcription factors are targeted by differentially expressed miRNAs in primates," *Genome Biology and Evolution*, vol. 4, no. 4, pp. 552–564, 2012.

[28] A. Kozomara and S. Griffiths-Jones, "MiRBase: integrating microRNA annotation and deep-sequencing data," *Nucleic Acids Research*, vol. 39, no. 1, pp. D152–D157, 2011.

[29] I. Gerin, G. T. Bommer, C. S. McCoin, K. M. Sousa, V. Krishnan, and O. A. MacDougald, "Roles for miRNA-378/378* in adipocyte gene expression and lipogenesis," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 299, no. 2, pp. E198–E206, 2010.

[30] X. Hou, Z. Tang, H. Liu, N. Wang, H. Ju, and K. Li, "Discovery of MicroRNAs associated with myogenesis by deep sequencing of serial developmental skeletal muscles in pigs," *PLoS ONE*, vol. 7, no. 12, Article ID e52123, 2012.

[31] J. Lin, P. Puigserver, J. Donovan, P. Tarr, and B. M. Spiegelman, "Peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β), a novel PGC-1-related transcription coactivator associated with host cell factor," *The Journal of Biological Chemistry*, vol. 277, no. 3, pp. 1645–1648, 2002.

[32] I. S. Patten and Z. Arany, "PGC-1 coactivators in the cardiovascular system," *Trends in Endocrinology and Metabolism*, vol. 23, no. 2, pp. 90–97, 2012.

[33] Y. Kamei, H. Ohizumi, Y. Fujitani et al., "PPARγ coactivator 1β/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 21, pp. 12378–12383, 2003.

[34] J. Lin, R. Yang, P. T. Tarr et al., "Hyperlipidemic effects of dietary saturated fats mediated through PGC-1β coactivation of SREBP," *Cell*, vol. 120, no. 2, pp. 261–273, 2005.

[35] J. Lin, P. T. Tarr, R. Yang et al., "PGC-1β in the regulation of hepatic glucose and energy metabolism," *The Journal of Biological Chemistry*, vol. 278, no. 33, pp. 30843–30848, 2003.

[36] C. Wolfrum and M. Stoffel, "Coactivation of Foxa2 through Pgc-1β promotes liver fatty acid oxidation and triglyceride/VLDL secretion," *Cell Metabolism*, vol. 3, no. 2, pp. 99–110, 2006.

[37] J. St-Pierre, J. Lin, S. Krauss et al., "Bioenergetic analysis of peroxisome proliferator-activated receptor γ coactivators 1α and 1β (PGC-1α and PGC-1β) in muscle cells," *The Journal of Biological Chemistry*, vol. 278, no. 29, pp. 26597–26603, 2003.

[38] E. M. Small and E. N. Olson, "Pervasive roles of microRNAs in cardiovascular biology," *Nature*, vol. 469, no. 7330, pp. 336–342, 2011.

[39] E. van Rooij, D. Quiat, B. A. Johnson et al., "A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance," *Developmental Cell*, vol. 17, no. 5, pp. 662–673, 2009.

[40] S. Wang, A. B. Aurora, B. A. Johnson et al., "The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis," *Developmental Cell*, vol. 15, no. 2, pp. 261–271, 2008.

[41] T. Mohri, M. Nakajima, T. Fukami, M. Takamiya, Y. Aoki, and T. Yokoi, "Human CYP2E1 is regulated by miR-378," *Biochemical Pharmacology*, vol. 79, no. 7, pp. 1045–1052, 2010.

[42] T.-I. Jeon, J. W. Park, J. Ahn, C. H. Jung, and T. Y. Ha, "Fisetin protects against hepatosteatosis in mice by inhibiting miR-378," *Molecular Nutrition & Food Research*, vol. 57, no. 11, pp. 1931–1937, 2013.

[43] Z. Arany, N. Lebrasseur, C. Morris et al., "The transcriptional coactivator PGC-1beta drives the formation of oxidative type IIX fibers in skeletal muscle," *Cell Metabolism*, vol. 5, no. 1, pp. 35–46, 2007.

[44] Y. Mallat, E. Tritsch, R. Ladouce et al., "Proteome modulation in H9c2 cardiac cells by microRNAs miR-378 and miR-378," *Molecular and Cellular Proteomics*, vol. 13, no. 1, pp. 18–29, 2014.

[45] G. Ailhaud, P. Grimaldi, and R. Négrel, "Hormonal regulation of adipose differentiation," *Trends in Endocrinology and Metabolism*, vol. 5, no. 3, pp. 132–136, 1994.

[46] C. Villar-Palasi, "The hormonal regulation of glycogen metabolism in muscle," *Vitamins and Hormones*, vol. 26, pp. 65–118, 1968.

[47] R. P. Brun, J. B. Kim, E. Hu, S. Altiok, and B. M. Spiegelman, "Adipocyte differentiation: a transcriptional regulatory cascade," *Current Opinion in Cell Biology*, vol. 8, no. 6, pp. 826–832, 1996.

[48] M. I. Lefterova and M. A. Lazar, "New developments in adipogenesis," *Trends in Endocrinology and Metabolism*, vol. 20, no. 3, pp. 107–114, 2009.

[49] A. T. Ali, W. E. Hochfeld, R. Myburgh, and M. S. Pepper, "Adipocyte and adipogenesis," *European Journal of Cell Biology*, vol. 92, no. 6-7, pp. 229–236, 2013.

[50] W. Jin, M. V. Dodson, S. S. Moore, J. A. Basarab, and L. L. Guan, "Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development," *BMC Molecular Biology*, vol. 11, article 29, 2010.

[51] D. V. Chartoumpekis, A. Zaravinos, P. G. Ziros et al., "Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice," *PLoS ONE*, vol. 7, no. 4, Article ID e34872, 2012.

[52] S. Collins, C. M. Kuhn, A. E. Petro, A. G. Swick, B. A. Chrunyk, and R. S. Surwit, "Role of leptin in fat regulation," *Nature*, vol. 380, no. 6576, p. 677, 1996.

[53] P. Trayhurn and D. V. Rayner, "Hormones and the ob gene product (leptin) in the control of energy balance," *Biochemical Society Transactions*, vol. 24, no. 2, pp. 565–570, 1996.

[54] L.-L. Xu, C.-M. Shi, G.-F. Xu et al., "TNF-α, IL-6, and leptin increase the expression of miR-378, an adipogenesis-related microRNA in human adipocytes," *Cell Biochemistry and Biophysics*, vol. 70, no. 2, pp. 771–776, 2014.

[55] J. M. Stephens, J. Lee, and P. F. Pilch, "Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction," *The Journal of Biological Chemistry*, vol. 272, no. 2, pp. 971–976, 1997.

[56] S. Müller, S. Martin, W. Koenig et al., "Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors," *Diabetologia*, vol. 45, no. 6, pp. 805–812, 2002.

[57] I. Knezevic, A. Patel, N. R. Sundaresan et al., "A novel cardiomyocyte-enriched MicroRNA, miR-378, targets insulin-like growth factor 1 receptor: implications in postnatal cardiac remodeling and cell survival," *The Journal of Biological Chemistry*, vol. 287, no. 16, pp. 12913–12926, 2012.

[58] E. D. Rosen and B. M. Spiegelman, "Peroxisome proliferator–activated receptor γ ligands and atherosclerosis: ending the heartache," *The Journal of Clinical Investigation*, vol. 106, no. 5, pp. 629–631, 2000.

[59] L. Fajas, D. Auboeuf, E. Raspé et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *The Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[60] J. R. Zierath and H. Wallberg-Henriksson, *Muscle Metabolism*, Frontiers in Animal Diabetes Research, Taylor & Francis, London, UK, 2002.

[61] J. R. Jones, C. Barrick, K.-A. Kim et al., "Deletion of PPARγ in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 17, pp. 6207–6212, 2005.

[62] E. John, A. Wienecke-Baldacchino, M. Liivrand, M. Heinäniemi, C. Carlberg, and L. Sinkkonen, "Dataset integration identifies transcriptional regulation of microRNA genes by PPARγ in differentiating mouse 3T3-L1 adipocytes," *Nucleic Acids Research*, vol. 40, no. 10, pp. 4446–4460, 2012.

[63] L. Fajas, R. L. Landsberg, Y. Huss-Garcia, C. Sardet, J. A. Lees, and J. Auwerx, "E2Fs regulate adipocyte differentiation," *Developmental Cell*, vol. 3, no. 1, pp. 39–49, 2002.

[64] S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann, "A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation," *Cell*, vol. 83, no. 5, pp. 813–819, 1995.

[65] A. Jozkowicz, J. Dulak, E. Piatkowska, W. Placha, and A. Dembinska-Kiec, "Ligands of peroxisome proliferator-activated receptor-gamma increase the generation of vascular endothelial growth factor in vascular smooth muscle cells and in macrophages," *Acta Biochimica Polonica*, vol. 47, no. 4, pp. 1147–1157, 2000.

[66] H. Migita and J. Morser, "15-Deoxy-Δ12,14-postaglandin J2 (15d-PGJ2) signals through retinoic acid receptor-related orphan receptor-α but not peroxisome proliferator-activated receptor-γ in human vascular endothelial cells: the effect of 15d-PGJ2 on tumor necrosis factor-α-induced gene expression," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 4, pp. 710–716, 2005.

[67] H. Sundvold and S. Lien, "Identification of a novel peroxisome proliferator-activated receptor (PPAR) gamma promoter in man and transactivation by the nuclear receptor RORalpha1," *Biochemical and Biophysical Research Communications*, vol. 287, no. 2, pp. 383–390, 2001.

[68] P. Lau, P. Bailey, D. H. Dowhan, and G. E. O. Muscat, "Exogenous expression of a dominant negative RORα1 vector in muscle cells impairs differentiation: RORα1 directly interacts with p300 and MyoD," *Nucleic Acids Research*, vol. 27, no. 2, pp. 411–420, 1999.

[69] S. J. Matkovich, Y. Hu, and G. W. Dorn II, "Regulation of cardiac microRNAs by cardiac microRNAs," *Circulation Research*, vol. 113, no. 1, pp. 62–71, 2013.

[70] A. Musarò, "The basis of muscle regeneration," *Advances in Biology*, vol. 2014, Article ID 612471, 16 pages, 2014.

[71] M. H. Parker, P. Seale, and M. A. Rudnicki, "Looking back to the embryo: defining transcriptional networks in adult myogenesis," *Nature Reviews Genetics*, vol. 4, no. 7, pp. 497–507, 2003.

[72] P. K. Davidsen, I. J. Gallagher, J. W. Hartman et al., "High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression," *Journal of Applied Physiology*, vol. 110, no. 2, pp. 309–317, 2011.

[73] J. Fang, X.-W. Song, J. Tian et al., "Overexpression of microRNA-378 attenuates ischemia-induced apoptosis by inhibiting caspase-3 expression in cardiac myocytes," *Apoptosis*, vol. 17, no. 4, pp. 410–423, 2012.

[74] S. W. Kim, H. W. Kim, W. Huang et al., "Cardiac stem cells with electrical stimulation improve ischaemic heart function through regulation of connective tissue growth factor and miR-378," *Cardiovascular Research*, vol. 100, no. 2, pp. 241–251, 2013.

[75] F. Megiorni, S. Cialfi, H. P. McDowell et al., "Deep sequencing the microRNA profile in rhabdomyosarcoma reveals down-regulation of miR-378 family members," *BMC Cancer*, vol. 14, article 880, 2014.

[76] M. Tranter, R. N. Helsley, W. R. Paulding et al., "Coordinated post-transcriptional regulation of Hsp70.3 gene expression by microRNA and alternative polyadenylation," *The Journal of Biological Chemistry*, vol. 286, no. 34, pp. 29828–29837, 2011.

[77] R. S. Nagalingam, N. R. Sundaresan, M. P. Gupta, D. L. Geenen, R. J. Solaro, and M. Gupta, "A cardiac-enriched microRNA, miR-378, blocks cardiac hypertrophy by targeting Ras signaling," *The Journal of Biological Chemistry*, vol. 288, no. 16, pp. 11216–11232, 2013.

[78] J. Ganesan, D. Ramanujam, Y. Sassi et al., "MiR-378 controls cardiac hypertrophy by combined repression of mitogen-activated protein kinase pathway factors," *Circulation*, vol. 127, no. 21, pp. 2097–2106, 2013.

[79] L. Jeanson-Leh, J. Lameth, S. Krimi et al., "Serum profiling identifies novel muscle miRNA and cardiomyopathy-related miRNA biomarkers in golden retriever muscular dystrophy dogs and duchenne muscular dystrophy patients," *American Journal of Pathology*, vol. 184, no. 11, pp. 2885–2898, 2014.

[80] P. Carmeliet and R. K. Jain, "Molecular mechanisms and clinical applications of angiogenesis," *Nature*, vol. 473, no. 7347, pp. 298–307, 2011.

[81] S. Wang and E. N. Olson, "AngiomiRs—Key regulators of angiogenesis," *Current Opinion in Genetics and Development*, vol. 19, no. 3, pp. 205–211, 2009.

[82] D. J. Manalo, A. Rowan, T. Lavoie et al., "Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1," *Blood*, vol. 105, no. 2, pp. 659–669, 2005.

[83] J. A. Forsythe, B.-H. Jiang, N. V. Iyer et al., "Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1," *Molecular and Cellular Biology*, vol. 16, no. 9, pp. 4604–4613, 1996.

[84] A. Loboda, A. Jozkowicz, and J. Dulak, "HIF-1 and HIF-2 transcription factors—similar but not identical," *Molecules and Cells*, vol. 29, no. 5, pp. 435–442, 2010.

[85] A. Loboda, A. Jozkowicz, and J. Dulak, "HIF-1 versus HIF-2—is one more important than the other?" *Vascular Pharmacology*, vol. 56, no. 5-6, pp. 245–251, 2012.

[86] U. Florczyk, A. Jazwa, M. Maleszewska et al., "Nrf2 regulates angiogenesis: effect on endothelial cells, bone marrow-derived proangiogenic cells and hind limb ischemia," *Antioxidants and Redox Signaling*, vol. 20, no. 11, pp. 1693–1708, 2014.

[87] A. Loboda, A. Stachurska, U. Florczyk et al., "HIF-1 induction attenuates Nrf2-dependent IL-8 expression in human endothelial cells," *Antioxidants and Redox Signaling*, vol. 11, no. 7, pp. 1501–1517, 2009.

[88] M. Feng, Z. Li, M. Aau, C. H. Wong, X. Yang, and Q. Yu, "Myc/miR-378/TOB2/cyclin D1 functional module regulates oncogenic transformation," *Oncogene*, vol. 30, no. 19, pp. 2242–2251, 2011.

[89] Z. Hua, Q. Lv, W. Ye et al., "Mirna-directed regulation of VEGF and other angiogenic under hypoxia," *PLoS ONE*, vol. 1, no. 1, article e116, 2006.

[90] T. Nagase, M. Nagase, K. Yoshimura, T. Fujita, and I. Koshima, "Angiogenesis within the developing mouse neural tube is dependent on sonic hedgehog signaling: possible roles of motor neurons," *Genes to Cells*, vol. 10, no. 6, pp. 595–604, 2005.

[91] R. Pola, L. E. Ling, M. Silver et al., "The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors," *Nature Medicine*, vol. 7, no. 6, pp. 706–711, 2001.

[92] K. J. Lavine, A. C. White, C. Park et al., "Fibroblast growth factor signals regulate a wave of Hedgehog activation that is essential for coronary vascular development," *Genes and Development*, vol. 20, no. 12, pp. 1651–1666, 2006.

[93] Y. Xing, J. Hou, T. Guo et al., "microRNA-378 promotes mesenchymal stem cell survival and vascularization under hypoxic-ischemic conditions *in vitro*," *Stem Cell Research & Therapy*, vol. 5, no. 6, article 130, 2014.

[94] Z. Arany, S.-Y. Foo, Y. Ma et al., "HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1α," *Nature*, vol. 451, no. 7181, pp. 1008–1012, 2008.

[95] J. Jiang, E. J. Lee, Y. Gusev, and T. D. Schmittgen, "Real-time expression profiling of microRNA precursors in human cancer cell lines," *Nucleic Acids Research*, vol. 33, no. 17, pp. 5394–5403, 2005.

[96] J. Guo, Y. Miao, B. Xiao et al., "Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 4, pp. 652–657, 2009.

[97] Y. Yao, A. L. Suo, Z. F. Li et al., "MicroRNA profiling of human gastric cancer," *Molecular Medicine Reports*, vol. 2, no. 6, pp. 963–970, 2009.

[98] L. Scapoli, A. Palmieri, L. Lo Muzio et al., "MicroRNA expression profiling of oral carcinoma identifies new markers of tumor progression," *International Journal of Immunopathology and Pharmacology*, vol. 23, no. 4, pp. 1229–1234, 2010.

[99] Y. X. Wang, X. Y. Zhang, B. F. Zhang, C. Q. Yang, X. M. Chen, and H. J. Gao, "Initial study of microRNA expression profiles of colonic cancer without lymph node metastasis," *Journal of Digestive Diseases*, vol. 11, no. 1, pp. 50–54, 2010.

[100] M. Redova, A. Poprach, J. Nekvindova et al., "Circulating miR-378 and miR-451 in serum are potential biomarkers for renal cell carcinoma," *Journal of Translational Medicine*, vol. 10, no. 1, article 55, 2012.

[101] K. Skrzypek, M. Tertil, S. Golda et al., "Interplay between heme oxygenase-1 and miR-378 affects non-small cell lung carcinoma growth, vascularization, and metastasis," *Antioxidants and Redox Signaling*, vol. 19, no. 7, pp. 644–660, 2013.

[102] H. C. N. Nguyen, W. Xie, M. Yang et al., "Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer," *Prostate*, vol. 73, no. 4, pp. 346–354, 2013.

[103] M. Fedorko, M. Stanik, R. Iliev et al., "Combination of MiR-378 and MiR-210 serum levels enables sensitive detection of renal cell carcinoma," *International Journal of Molecular Sciences*, vol. 16, no. 10, pp. 23382–23389, 2015.

[104] H. Liu, L. Zhu, B. Liu et al., "Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer," *Cancer Letters*, vol. 316, no. 2, pp. 196–203, 2012.

[105] J. Qian, J. Lin, W. Qian et al., "Overexpression of miR-378 is frequent and may affect treatment outcomes in patients with acute myeloid leukemia," *Leukemia Research*, vol. 37, no. 7, pp. 765–768, 2013.

[106] J. Ma, J. Lin, J. Qian et al., "MiR-378 promotes the migration of liver cancer cells by down-regulating fus expression," *Cellular Physiology and Biochemistry*, vol. 34, no. 6, pp. 2266–2274, 2014.

[107] K. Skrzypek, A. Kusienicka, B. Szewczyk et al., "Constitutive activation of MET signaling impairs myogenic differentiation of rhabdomyosarcoma and promotes its development and progression," *Oncotarget*, vol. 6, no. 31, pp. 31378–31398, 2015.

[108] A. Jazwa, A. Loboda, S. Golda et al., "Effect of heme and heme oxygenase-1 on vascular endothelial growth factor synthesis and angiogenic potency of human keratinocytes," *Free Radical Biology and Medicine*, vol. 40, no. 7, pp. 1250–1263, 2006.

[109] H. Was, J. Dulak, and A. Jozkowicz, "Heme oxygenase-1 in tumor biology and therapy," *Current Drug Targets*, vol. 11, no. 12, pp. 1551–1570, 2010.

[110] M. Terti, K. Skrzypek, U. Florczyk et al., "Regulation and novel action of thymidine phosphorylase in non-small cell lung cancer: crosstalk with Nrf2 and HO-1," *PLoS ONE*, vol. 9, no. 5, Article ID e97070, 2014.

[111] M. Tertil, S. Golda, K. Skrzypek et al., "Nrf2-heme oxygenase-1 axis in mucoepidermoid carcinoma of the lung: antitumoral effects associated with down-regulation of matrix metalloproteinases," *Free Radical Biology and Medicine*, vol. 89, pp. 147–157, 2015.

[112] F. Liu, Q. Lv, W. W. Du et al., "Specificity of miR-378a-5p targeting rodent fibronectin," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1833, no. 12, pp. 3272–3285, 2013.

[113] J. K. Chan, T. K. Kiet, K. Blansit et al., "*MiR-378* as a biomarker for response to anti-angiogenic treatment in ovarian cancer," *Gynecologic Oncology*, vol. 133, no. 3, pp. 568–574, 2014.

[114] H.-C. Li, J.-P. Li, Z.-M. Wang et al., "Identification of angiogenesis-related miRNAs in a population of patients with renal clear cell carcinoma," *Oncology Reports*, vol. 32, no. 5, pp. 2061–2069, 2014.

[115] W.-H. Weng, W.-H. Leung, Y.-J. Pang, and H.-H. Hsu, "Lauric acid can improve the sensitization of Cetuximab in KRAS/BRAF mutated colorectal cancer cells by retrievable microRNA-378 expression," *Oncology Reports*, 2015.

[116] H. Li, L. Chang, W. W. Du et al., "Anti-microRNA-378a enhances wound healing process by upregulating integrin beta-3 and vimentin," *Molecular Therapy*, vol. 22, no. 10, pp. 1839–1850, 2014.

[117] G. C. Rowe, C. Jang, I. S. Patten, and Z. Arany, "PGC-1β regulates angiogenesis in skeletal muscle," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 301, no. 1, pp. E155–E163, 2011.

[118] V. Yadav, A. Matsakas, S. Lorca, and V. A. Narkar, "PGC1β activates an antiangiogenic program to repress neoangiogenesis in muscle ischemia," *Cell Reports*, vol. 8, no. 3, pp. 783–797, 2014.

[119] C. Stockmann, D. Schadendorf, R. Klose, and I. Helfrich, "The impact of the immune system on tumor: angiogenesis and vascular remodeling," *Frontiers in Oncology*, vol. 4, article 69, 2014.

[120] M. Ono, "Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy," *Cancer Science*, vol. 99, no. 8, pp. 1501–1506, 2008.

[121] B. J. Nickoloff, "Characterization of lymphocyte-dependent angiogenesis using a SCID mouse: human skin model of psoriasis," *Journal of Investigative Dermatology Symposium Proceedings*, vol. 5, no. 1, pp. 67–73, 2000.

[122] A. Naldini, A. Pucci, C. Bernini, and F. Carraro, "Regulation of angiogenesis by Th1- and Th2-type cytokines," *Current Pharmaceutical Design*, vol. 9, no. 7, pp. 511–519, 2003.

[123] S. E. J. Chambers, C. L. O'Neill, T. M. O'Doherty, R. J. Medina, and A. W. Stitt, "The role of immune-related myeloid cells in angiogenesis," *Immunobiology*, vol. 218, no. 11, pp. 1370–1375, 2013.

[124] P. Wang, Y. Gu, Q. Zhang et al., "Identification of resting and type I IFN-activated human NK cell miRNomes reveals MicroRNA-378 and MicroRNA-30e as negative regulators of NK cell cytotoxicity," *The Journal of Immunology*, vol. 189, no. 1, pp. 211–221, 2012.

[125] S. Liu, L. Chen, Y. Zeng et al., "Suppressed expression of miR-378 targeting gzmb in NK cells is required to control dengue virus infection," *Cellular & Molecular Immunology*, 2015.

[126] F. O. Martinez, A. Sica, A. Mantovani, and M. Locati, "Macrophage activation and polarization," *Frontiers in Bioscience*, vol. 13, pp. 453–461, 2008.

[127] G. Liu and E. Abraham, "MicroRNAs in immune response and macrophage polarization," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 33, no. 2, pp. 170–177, 2013.

[128] D. Zhou, C. Huang, Z. Lin et al., "Macrophage polarization and function with emphasis on the evolving roles of coordinated regulation of cellular signaling pathways," *Cellular Signalling*, vol. 26, no. 2, pp. 192–197, 2014.

[129] D. Rückerl, S. J. Jenkins, N. N. Laqtom et al., "Induction of IL-4Ralpha-dependent microRNAs identifies PI3K/Akt signaling as essential for IL-4-driven murine macrophage proliferation in vivo," *Blood*, vol. 120, no. 11, pp. 2307–2316, 2012.

[130] X. Jiang, M. Xue, Z. Fu et al., "Insight into the effects of adipose tissue inflammation factors on miR-378 expression and the underlying mechanism," *Cellular Physiology and Biochemistry*, vol. 33, no. 6, pp. 1778–1788, 2014.

[131] T. Kagiya and S. Nakamura, "Expression profiling of microRNAs in RAW264.7 cells treated with a combination of tumor necrosis factor alpha and RANKL during osteoclast differentiation," *Journal of Periodontal Research*, vol. 48, no. 3, pp. 373–385, 2013.

[132] B. Ell, L. Mercatali, T. Ibrahim et al., "Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis," *Cancer Cell*, vol. 24, no. 4, pp. 542–556, 2013.

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Research Article

Betatrophin Acts as a Diagnostic Biomarker in Type 2 Diabetes Mellitus and Is Negatively Associated with HDL-Cholesterol

Min Yi, Rong-ping Chen, Rui Yang, Xian-feng Guo, Jia-chun Zhang, and Hong Chen

Department of Endocrinology, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, China

Correspondence should be addressed to Hong Chen; rubychq@163.com

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Objective. By assessing its circulating concentrations in type 2 diabetes mellitus (T2DM) patients, we aimed to explore the associations of betatrophin with various metabolic parameters and evaluate its diagnostic value in T2DM. *Methods*. A total of 58 non-diabetes-mellitus (NDM) subjects and 73 age- and sex-matched newly diagnosed T2DM patients were enrolled. Correlation analyses between circulating betatrophin levels and multiple metabolic parameters were performed. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic value of betatrophin concentration in T2DM. *Results*. Circulating betatrophin levels were approximately 1.8 times higher in T2DM patients than in NDM individuals (median 747.12 versus 407.41 pg/mL, $P < 0.001$). Correlation analysis showed that betatrophin was negatively associated with high-density lipoprotein cholesterol (HDL-C) levels in all subjects. ROC curve analysis identified betatrophin as a potent diagnostic biomarker for T2DM. The optimal cut-off point of betatrophin concentration for predicting T2DM was 501.23 pg/mL. *Conclusions*. Serum betatrophin levels were markedly increased in newly diagnosed T2DM patients and further elevated in obese T2DM subjects. Betatrophin was negatively correlated with HDL-C levels. Our findings indicate that betatrophin could be a potent diagnostic biomarker for T2DM.

1. Introduction

Diabetes mellitus prevalence is increasing at alarming rates, and this ailment has become a major public health problem worldwide. According to the International Diabetes Federation (IDF), 360 million individuals suffered from diabetes in 2011, a number expected to rise to 522 million with a prevalence of 7.7% in 2030 [1]. Type 2 diabetes, characterized by insulin resistance and pancreatic β cell function defect, makes up about 90% of all cases [2]. Replenishing insulin-producing pancreatic β cell mass and alleviating insulin resistance are considered the ideal ways for diabetes care. Targeting the pathophysiological defects that characterize the onset of diabetes can achieve a durable glucose control and benefit to essential components in disease pathogenesis.

Betatrophin, a newly characterized circulatory hormone secreted by liver and adipose tissues, is believed to promote β cell proliferation, therefore attracting increasing attention. After injection of the insulin receptor antagonist S961 into mice, Melton and colleagues discovered a secreted protein of 198 amino acids that specifically induces dramatic and specific pancreatic β cell proliferation, improving glucose tolerance [3]. The notion that betatrophin may interfere with the compensatory response to insulin resistance has raised hope for new diabetes therapeutic in humans. However, other studies found that mouse betatrophin has no effect on human beta cell proliferation and differentiation [4, 5]. In addition, overexpression and silencing of the betatrophin gene in mice do not support a role for this hormone in controlling beta cell growth but point to a clear function in regulating plasma lipid profiles [6]. Meanwhile, opinions regarding the associations of betatrophin with T2DM and obesity in humans are also discrepant. Some studies suggested that circulating betatrophin levels are elevated in type 2 diabetes and obesity [7–10], correlating with lipid profiles, while others reported that betatrophin is associated only with lipid metabolism and

has nothing to do with glucose homeostasis [11, 12]. Is it possible that betatrophin is involved in T2DM development and lipid metabolism? Whether betatrophin is a potential target for diabetes and dyslipidemia medications or solely a diagnostic biomarker remains poorly understood. Therefore, we assessed serum betatrophin concentrations in Chinese population. We hypothesized that betatrophin levels might be increased in obese individuals with T2DM and constitute a potential diagnostic biomarker for T2DM.

2. Materials and Methods

2.1. Study Population. From 2013 to 2014, a total of 131 Chinese subjects (69 males and 62 females) were recruited at the Diabetes Clinics and Medical Examination Center of Zhujiang Hospital. Eligible patients were males and females over 18 years, including 58 non-diabetes-mellitus subjects (NDM: 18 lean, 22 overweight, and 18 obese individuals) and 73 age- and sex-matched patients with T2DM (22 lean, 29 overweight, and 22 obese individuals). T2DM was diagnosed according to the World Health Organization (WHO) diagnostic criteria for diabetes [13]. The exclusion criteria are as follows: (1) subjects being treated with oral hypoglycemic agents and those with macrovascular complications; (2) subjects taking any medications known to affect glucose tolerance within one month; (3) individuals with type 1 diabetes or gestational diabetes; (4) subjects with viral hepatitis, cancer, severe psychiatric disturbances, hepatic failure, chronic renal failure on hemodialysis, congestive heart failure, or other known major diseases. All subjects enrolled provided written informed consent. The study protocol was in agreement with the guidelines of the Human Research Ethics Committees of Zhujiang Hospital and performed in accordance with the ethical principles of the Declaration of Helsinki.

2.2. Anthropometric and Biochemical Measurements. All subjects underwent comprehensive anthropometric measurements, including height, weight, and waist and hip circumferences, whereby body mass index (BMI) and waist-to-hip ratio (WHR) were calculated. Weights were measured in light clothing without shoes. Heights were obtained with a portable, rigid measuring rod. BMI was derived as body weight divided by body height squared. Waist circumference was measured at the midpoint between the lowest rib margin and the iliac crest in a standing position. Hip circumference was measured at the widest point.

Blood samples were collected after 8 hours of fasting without taking any medications, for the assessment of fasting plasma glucose, insulin level, C peptide, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and betatrophin concentrations. Serum betatrophin levels were determined with a commercially available human ELISA kit (Wuhan Eiaab Science, Wuhan, China; Catalogue number E11644h) according to the manufacturer's instructions. ELISA was performed in duplicate, and samples with coefficient of variation (CV) values exceeding 5% were excluded. A standard curve was constructed by plotting mean OD_{450} for each standard against its concentration, generating a best fit curve through the graph points. Fasting plasma glucose amounts were measured by the glucose oxidase method; fasting insulin and C peptide levels were measured by enzyme-amplified chemiluminescence assays. Serum TC, triglycerides, LDL-C, and HDL-C were assessed by enzymatic methods; ALT and AST were quantitated by kinetic methods (Beckman Coulter Inc., Brea, CA); serum uric acid levels were evaluated by the uricase method. Obesity was defined as BMI $\geq 30\,kg/m^2$ and overweight was defined as a BMI between 25 and $30\,kg/m^2$. Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) [14]. Pancreatic β cell function was assessed by homeostasis model assessment of β cell function (HOMA-%β) [15]. HOMA-IR and HOMA-%β were derived using the following equations: HOMA-IR = insulin [lU/mL] $*$ glucose [mmol/L]/22.5; HOMA-%β = 20 $*$ insulin [lU/mL]/(glucose [mmol/L] $-$ 3.5).

2.3. Statistical Analysis. All statistical analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). Normally distributed and continuous variables were presented as mean ± standard deviation (SD) and nonnormally distributed variables as median and quartiles (25% and 75%). Comparisons between groups were assessed by independent-samples t-test; alternatively, analysis of variance (ANOVA) followed by LSD tests was conducted as appropriate. Correlations between variables were assessed using Pearson correlation analysis, controlling for covariates. Two-tailed $P < 0.05$ was considered statistically significant. Performance of betatrophin concentration in detecting T2DM was evaluated using receiver operating characteristic (ROC) curve analysis. Based on ROC analysis, the best cut-off value for betatrophin concentration was determined from the highest Youden index, which is defined as sensitivity + specificity $-$ 1.

3. Results

3.1. Circulating Betatrophin Levels Are Significantly Increased in T2DM and Obese Subjects. A total of 58 NDM subjects and 73 age- and sex-matched T2DM patients were assessed in this study. Baseline characteristics of all participants are shown in Table 1. No significant differences were found in age, gender, BMI, WHR, TG, LDL-C, ALT, and AST levels between the two patient groups. Interestingly, serum betatrophin concentrations were approximately 1.8 times higher in T2DM patients than in NDM individuals (median 747.12 versus 407.41 pg/mL, $P < 0.001$). When stratified by BMI, betatrophin levels in obese T2DM subjects were almost 6.5 times higher than values obtained for healthy NDM subjects (1003.28 versus 155.29 pg/mL, $P < 0.001$, Figure 1).

Table 1: General characteristics of normal glucose participants and newly diagnosed T2DM patients.

Variables	NDM	T2DM	*P* value
Gender (male/*N*)	31/58 (53.4%)	38/73 (52.1%)	0.874
Age (years)	39.43 ± 12.08	41.03 ± 9.74	0.404
BMI (kg/m^2)	26.80 ± 4.26	27.66 ± 3.39	0.204
WHR	0.91 ± 0.07	0.93 ± 0.06	0.100
Betatrophin (pg/mL)	407.41 (172.76–528.32)	747.12 (544.26–1074.96)	<0.001*
Fasting glucose (mM)	5.51 ± 0.63	12.11 ± 3.89	<0.001*
Fast insulin (mIU/L)	10.42 ± 5.83	15.10 ± 8.18	<0.001*
C peptide (μg/L)	1.65 ± 0.76	2.58 ± 2.97	0.021*
HOMA-IR	2.88 ± 1.98	7.90 ± 3.89	<0.001*
HOMA-%β	92.45 ± 46.83	44.77 ± 37.37	<0.001*
QUICKI	0.34 ± 0.04	0.29 ± 0.02	<0.001*
TC (mM)	4.89 ± 1.23	5.47 ± 1.15	0.007*
TG (mM)	1.29 (0.71–2.06)	1.66 (1.09–2.35)	0.213
LDL-C (mM)	3.32 ± 0.92	3.42 ± 0.95	0.559
HDL-C (mM)	1.50 ± 0.47	1.01 ± 0.37	<0.001*
Uric acid (μmol/L)	378.32 ± 110.26	335.40 ± 93.81	0.018*
ALT (IU/L)	21 (14–37.8)	21 (14.5–31)	0.308
AST (IU/L)	24.28 ± 9.18	20.99 ± 12.48	0.096

BMI: body mass index; WHR: waist-to-hip ratio; FINS: fasting blood insulin; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-%β: homeostasis model assessment of β cell function; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; QUICKI: Quantitative Insulin Sensitivity Check Index; ALT: alanine aminotransferase; AST: aspartate aminotransferase.
*$P < 0.05$.

3.2. Betatrophin Is Negatively Associated with HDL-C Levels in All Subjects. Next, we assessed the associations of circulating betatrophin levels with various metabolic parameters. In the T2DM group, betatrophin amounts were correlated only with age and HDL-cholesterol levels; meanwhile, serum betatrophin concentrations were associated with multiple metabolic parameters, including fasting glucose, WHR, insulin, C peptide, HOMA-IR, QUICK index, and lipid profiles (TG and HDL-C) in the NDM group (Table 2). When stratified by BMI, betatrophin is negatively associated with HDL-C levels in all subgroups (Figure 2).

3.3. Performance of Betatrophin Concentration in Detecting T2DM. The ROC curve shown in Figure 3 depicts the diagnostic accuracy of betatrophin level for T2DM. The optimal cut-off point (betatrophin concentration) to predict T2DM was 501.23 pg/mL. Using this cut-off value, diagnostic efficiency for T2DM reached the highest value: the area under the ROC curve was 0.824 (95% CI 0.748–0.885, $P < 0.001$), with sensitivity and specificity of 83.56% and 72.41%, respectively.

4. Discussion

Betatrophin is believed to promote pancreatic beta cell proliferation and to improve metabolic control by increasing beta cell division rate [3, 16] in insulin resistant mice. In humans, the associations of serum betatrophin levels with diabetes, obesity, and lipid profiles remain controversial [7, 9–12, 17–19]. We found that circulating betatrophin concentrations were significantly increased in T2DM and obese patients. Interestingly, for the first time, this study demonstrated that betatrophin was negatively correlated with HDL-C levels in both NDM and T2DM groups. Using a ROC curve, we found that circulation betatrophin concentration could be a diagnostic biomarker for T2DM, with optimal cut-off point of 501.23 pg/mL.

As shown above, serum betatrophin levels were approximately 1.8 times higher in T2DM patients than in NDM individuals; in addition, they were almost 6.5 times higher in obese T2DM subjects compared with values obtained for healthy NDM subjects. These findings corroborate several studies [7, 17, 20, 21] but contradict data reported by Gómez-Ambrosi et al. [11] showing significantly decreased circulating betatrophin in obese individuals, with further drop in IGT and T2DM participants. Several possible reasons may explain these conflicting results: firstly, different study populations and ethnicities were assessed. Increasing evidence shows that betatrophin mRNA is originally expressed in liver and white and brown adipose tissues [22, 23]; therefore, adipose distribution differences between the Europeans and Asians may affect betatrophin concentrations. In addition, T2DM patients assessed in the latter study were taking hypoglycemic medications. Since the effects of hypoglycemic agents on serum betatrophin levels are unclear, it would be challenging to distinguish the potential confounding effects. Lastly,

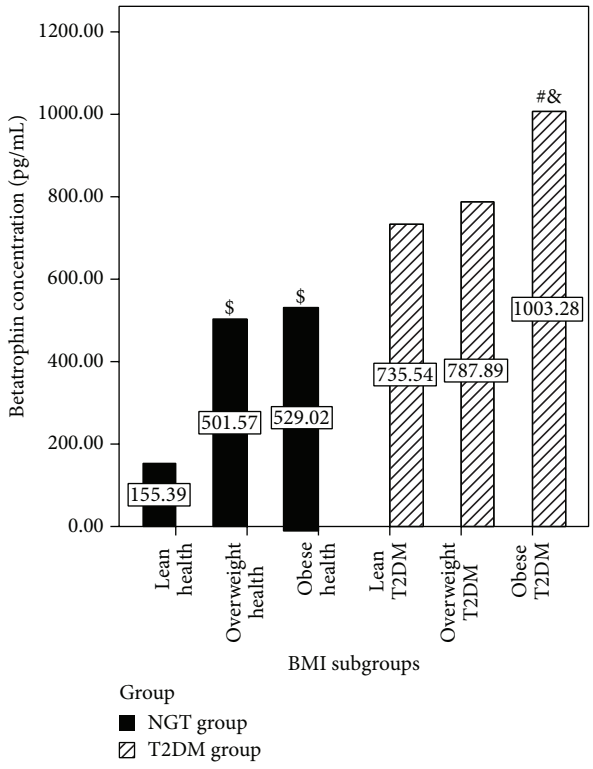

Figure 1: Serum betatrophin concentrations in each subgroup. Serum betatrophin concentration in NDM group: lean health (n = 18), overweight health (n = 22), and obese health (n = 18). Type 2 diabetes group: lean T2DM (n = 22), overweight T2DM (n = 29), and obese T2DM (n = 22). Data are mean ± SEM; $^{\$}P < 0.05$ versus lean health; $^{\#}P < 0.05$ versus lean T2DM; $^{\&}P < 0.05$ versus overweight T2DM.

betatrophin concentrations were determined by ELISA kits manufactured by different companies in both studies. Betatrophin proteolytic regulation leads to different circulating protein levels. Betatrophin ELISA kits may detect either the N- or C-terminus of the protein [24], resulting in differential or even conflicting data.

Our research revealed that betatrophin concentrations were negatively associated with HDL-C levels in both NDM and T2DM groups for the first time. So far, multiple animal-based studies have proposed that betatrophin level is closely related to altered blood lipid metabolism [22, 23, 25, 26]. Mice lacking the betatrophin gene (Gm6484) display lower serum triacylglycerol (TAG) levels, associated with reduced amounts of very low-density lipoprotein (VLDL) as well as elevated lipoprotein lipase (LPL) activity, whereas adenovirus-mediated betatrophin overexpression can elevate circulating triacylglycerol levels [23, 25]. Betatrophin may affect blood lipid profiles by regulating hepatic VLDL secretion and altering LPL activity. Betatrophin induces triglyceride elevation through reduced triglyceride clearance by inhibiting LPL activity. In addition, betatrophin may also act via a functional interaction with ANGPTL3 [23, 27], a known lipid regulator in mice and humans, which regulates HDL-C levels by inhibiting endothelial lipase [28]. Plasma TAG levels are unchanged in mice expressing ANGPTL3 alone, whereas coexpression with betatrophin results in hypertriglyceridemia despite a reduction in circulating ANGPTL3, suggesting betatrophin may regulate lipid metabolism by activating ANGPTL3 [25]. In accordance with animal studies, we found a significant association between serum betatrophin and triglyceride levels in this clinical trial. According to genome-wide association studies, betatrophin sequence variations are related to blood lipid levels in humans [23]. A betatrophin transcript variant and its expression levels are associated with clinical or pathological symptoms. For instance, single-nucleotide polymorphism in the betatrophin gene substituting tryptophan for arginine at residue 59 is reportedly associated with reduced HDL-C and LDL-C in African American and Hispanic participants [5]. Notably, we found that betatrophin was negatively correlated with HDL-C levels in all subjects, including the NDM and T2DM groups. When stratified by BMI, these trends still exist in all subgroups.

Another novel finding of this study is that betatrophin could be used as a diagnostic biomarker for T2DM. By performing ROC curve analysis, we found that circulation betatrophin concentration is a potent diagnostic biomarker for T2DM, with an optimal cut-off point of 501.23 pg/mL (AUROC = 0.824, 95% CI, 0.748–0.885, and $P < 0.001$). The notion that betatrophin could induce pancreatic beta cell proliferation has attracted attention at the very beginning [3, 29, 30]; however, further studies in betatrophin/Angptl8 knockout mice do not support a role for betatrophin in controlling beta cell growth [4, 6, 27, 31]. Meanwhile, new data showing that betatrophin levels are elevated in diabetes but not correlated with glucose homeostasis have greatly questioned the ability of betatrophin to increase beta cell replication in humans [18]. Betatrophin is much more likely to be a diagnostic biomarker rather than a potential therapeutic target for type 2 diabetes [5]. Our research, for the first time, assessed the diagnostic value of betatrophin in type 2 diabetes. Diabetes autoantibodies constitute an important indicator to distinguish type 1 from type 2 diabetes. The current testing method is complicated and more expensive. If betatrophin can be a reliable diagnostic biomarker and classification index for diabetes, it will allow great savings in medical work and expenses. However, our studies did not enroll type 1 diabetes patients; thus, future studies are needed to test this hypothesis.

Other limitations of this study should be taken into account. Firstly, the sample size was relatively small in this single center study, which may affect the statistical power. Secondly, the present research was a cross-sectional study, which could not address the cause-effect relationship between serum betatrophin and T2DM as well as obesity. Lastly, our cross-sectional trial only estimated serum betatrophin levels at a single point, which cannot reflect betatrophin levels over time.

In conclusion, although not definitive our data confirmed previous findings: increased serum betatrophin levels in T2DM and obese subjects. In addition, we observed for the first time a negative correlation between serum betatrophin levels and HDL-cholesterol amounts in all subjects.

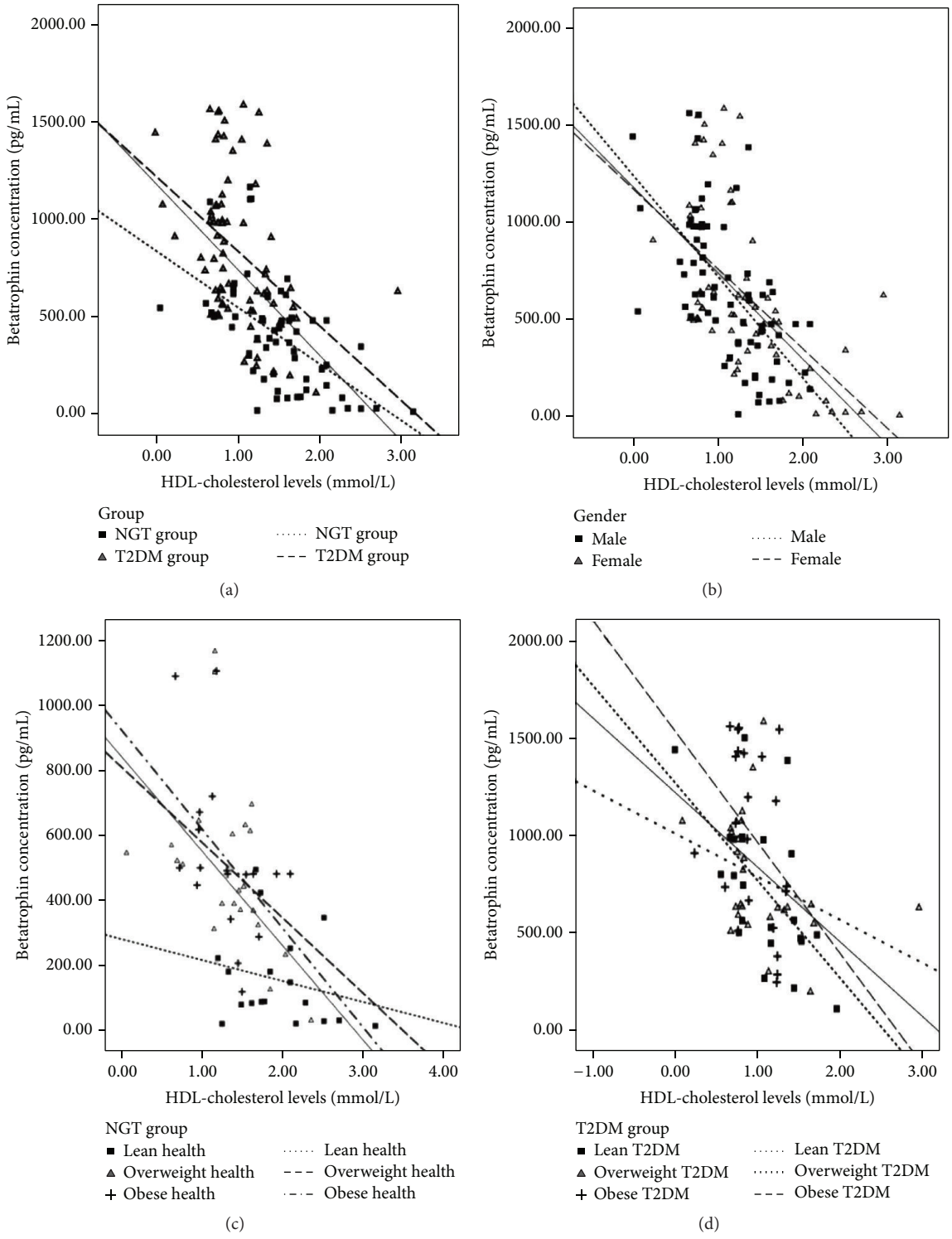

Figure 2: Correlations of serum betatrophin levels with HDL-cholesterol levels in each subgroup. (a) Correlations of serum betatrophin levels with HDL-cholesterol in NDM and T2DM groups. (b) Correlations of serum betatrophin levels with HDL-cholesterol in males and females. (c) Correlations of serum betatrophin levels with HDL-cholesterol in NDM group when stratified by BMI. (d) Correlations of serum betatrophin levels with HDL-cholesterol in T2DM group when stratified by BMI.

Table 2: Correlations between betatrophin levels and various parameters of the study subjects.

Variables	Overall	NDM	T2DM
Age (years)	$r = -0.213$; $P = 0.813$	$r = -0.249$; $P = 0.059$	$r = 0.273$; $P = 0.020^*$
BMI (kg/m^2)	$r = 0.325$; $P < 0.001^*$	$r = 0.240$; $P = 0.070$	$r = 0.162$; $P = 0.170$
WHR	$r = 0.199$; $P = 0.023$	$r = 0.403$; $P = 0.002^*$	$r = -0.003$; $P = 0.977$
Fasting glucose (mM)	$r = -0.465$; $P < 0.001^*$	$r = 0.474$; $P < 0.001^*$	$r = 0.141$; $P = 0.233$
Fast insulin (mIU/L)	$r = 0.351$; $P < 0.001^*$	$r = 0.448$; $P < 0.001^*$	$r = 0.067$; $P = 0.575$
C peptide (μg/L)	$r = 0.184$; $P < 0.001^*$	$r = 0.354$; $P = 0.006^*$	$r = 0.057$; $P = 0.631$
HOMA-IR	$r = 0.461$; $P < 0.001^*$	$r = 0.432$; $P = 0.001^*$	$r = 0.129$; $P = 0.278$
HOMA-%β	$r = -0.249$; $P = 0.004^*$	$r = 0.011$; $P = 0.933$	$r < 0.001$; $P = 0.997$
QUICKI	$r = -0.633$; $P < 0.001^*$	$r = -0.555$; $P < 0.001^*$	$r = -0.056$; $P = 0.638$
TC (mM)	$r = 0.194$; $P = 0.027$	$r = 0.063$; $P = 0.640$	$r = 0.137$; $P = 0.249$
TG (mM)*	$r = 0.337$; $P < 0.001^*$	$r = 0.396$; $P = 0.002^*$	$r = 0.171$; $P = 0.147$
LDL-C (mM)	$r = 0.159$; $P = 0.069$	$r = -0.223$; $P = 0.092$	$r = 0.077$; $P = 0.519$
HDL-C (mM)	$r = -0.596$; $P < 0.001^*$	$r = -0.578$; $P < 0.001^*$	$r = -0.426$; $P < 0.001^*$
Uric acid (μmol/L)	$r = 0.172$; $P = 0.049$	$r = 0.493$; $P = 0.000^*$	$r = 0.067$; $P = 0.572$
ALT (IU/L)*	$r = 0.157$; $P = 0.073$	$r = 0.223$; $P = 0.092$	$r = 0.183$; $P = 0.121$
AST (IU/L)	$r = 0.008$; $P = 0.923$	$r = 0.102$; $P = 0.445$	$r = 0.120$; $P = 0.313$

BMI: body mass index; WHR: waist-to-hip ratio; FINS: fasting blood insulin; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-%β: homeostasis model assessment of β cell function; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; QUICKI: Quantitative Insulin Sensitivity Check Index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; NAFLD: nonalcoholic fatty liver disease.
* $P < 0.05$.

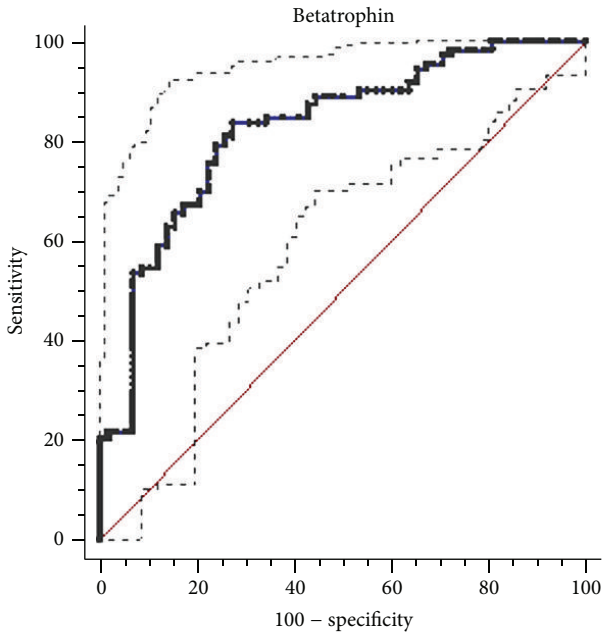

Figure 3: Receiver operating characteristics (ROC) curves showing the performance of betatrophin concentration in detecting type 2 diabetes mellitus. The receiver operating characteristic curve analysis. The optimal cut-off point was 501.23 pg/mL. The area under the ROC curve (area between the solid line and x-axis) was 0.824 (95% CI, area between the dashed lines and x-axis, 0.748–0.885, $P < 0.001$). The sensitivity and specificity are 83.56% and 72.41%, respectively.

Our findings indicate for the first time that circulation betatrophin concentrations could be a diagnostic biomarker for T2DM. These intriguing results warrant further researches; for example, prospective studies should be carried out to clarify the detailed mechanism and association of betatrophin with T2DM and lipid profiles.

Conflict of Interests

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

Authors' Contribution

Min Yi and Rong-ping Chen contributed equally to the work.

References

[1] D. W. Lam and D. LeRoith, "The worldwide diabetes epidemic," *Current Opinion in Endocrinology, Diabetes and Obesity*, vol. 19, no. 2, pp. 93–96, 2012.

[2] Y. Shi and F. B. Hu, "The global implications of diabetes and cancer," *The Lancet*, vol. 383, no. 9933, pp. 1947–1948, 2014.

[3] P. Yi, J.-S. Park, and D. A. Melton, "Betatrophin: a hormone that controls pancreatic β cell proliferation," *Cell*, vol. 153, no. 4, pp. 747–758, 2013.

[4] V. Gusarova, C. A. Alexa, E. Na et al., "ANGPTL8/betatrophin does not control pancreatic beta cell expansion," *Cell*, vol. 159, no. 3, pp. 691–696, 2014.

[5] K. H. Kaestner, "Betatrophin—promises fading and lessons learned," *Cell Metabolism*, vol. 20, no. 6, pp. 932–933, 2014.

[6] Y. Jiao, J. Le Lay, M. Yu, A. Naji, and K. H. Kaestner, "Elevated mouse hepatic betatrophin expression does not increase human β-cell replication in the transplant setting," *Diabetes*, vol. 63, no. 4, pp. 1283–1288, 2014.

[7] D. Espes, M. Martinell, and P.-O. Carlsson, "Increased circulating betatrophin concentrations in patients with type 2 diabetes," *International Journal of Endocrinology*, vol. 2014, Article ID 323407, 6 pages, 2014.

[8] D. Espes, J. Lau, and P.-O. Carlsson, "Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes," *Diabetologia*, vol. 57, no. 1, pp. 50–53, 2014.

[9] Z. Fu, F. Berhane, A. Fite, B. Seyoum, A. B. Abou-Samra, and R. Zhang, "Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity," *Scientific Reports*, vol. 4, article 5013, 2014.

[10] H. Hu, W. Sun, S. Yu et al., "Increased circulating levels of betatrophin in newly diagnosed type 2 diabetic patients," *Diabetes Care*, vol. 37, no. 10, pp. 2718–2722, 2014.

[11] J. Gómez-Ambrosi, E. Pascual, V. Catalán et al., "Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes," *The Journal of Clinical Endocrinology & Metabolism*, vol. 99, no. 10, pp. E2004–E2009, 2014.

[12] K. Guo, J. Lu, H. Yu et al., "Serum betatrophin concentrations are significantly increased in overweight but not in obese or type 2 diabetic individuals," *Obesity*, vol. 23, no. 4, pp. 793–797, 2015.

[13] K. G. M. M. Alberti and P. Z. Zimmet, "Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation," *Diabetic Medicine*, vol. 15, no. 7, pp. 539–553, 1998.

[14] L. U. Monzillo and O. Hamdy, "Evaluation of insulin sensitivity in clinical practice and in research settings," *Nutrition Reviews*, vol. 61, no. 12, pp. 397–412, 2003.

[15] D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner, "Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man," *Diabetologia*, vol. 28, no. 7, pp. 412–419, 1985.

[16] J. Chen, S. Chen, P. Huang et al., "In vivo targeted delivery of ANGPTL8 gene for beta cell regeneration in rats," *Diabetologia*, vol. 58, no. 5, pp. 1036–1044, 2015.

[17] X. Chen, P. Lu, W. He et al., "Circulating betatrophin levels are increased in patients with type 2 diabetes and associated with insulin resistance," *The Journal of Clinical Endocrinology & Metabolism*, vol. 100, no. 1, pp. E96–E100, 2014.

[18] A. Fenzl, B. K. Itariu, L. Kosi et al., "Circulating betatrophin correlates with atherogenic lipid profiles but not with glucose and insulin levels in insulin-resistant individuals," *Diabetologia*, vol. 57, no. 6, pp. 1204–1208, 2014.

[19] S. Wu, H. Gao, Y. Ma, L. Fu, C. Zhang, and X. Luo, "Characterisation of betatrophin concentrations in childhood and adolescent obesity and insulin resistance," *Pediatric Diabetes*, 2014.

[20] H. Yamada, T. Saito, A. Aoki et al., "Circulating betatrophin is elevated in patients with type 1 and type 2 diabetes," *Endocrine Journal*, vol. 62, no. 5, pp. 417–421, 2015.

[21] M. Abu-Farha, J. Abubaker, I. Al-Khairi et al., "Higher plasma betatrophin/ANGPTL8 level in type 2 diabetes subjects does not correlate with blood glucose or insulin resistance," *Scientific Reports*, vol. 5, Article ID 10949, 2015.

[22] G. Ren, J. Y. Kim, and C. M. Smas, "Identification of RIFL, a novel adipocyte-enriched insulin target gene with a role in lipid metabolism," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 303, no. 3, pp. E334–E351, 2012.

[23] R. Zhang, "Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels," *Biochemical and Biophysical Research Communications*, vol. 424, no. 4, pp. 786–792, 2012.

[24] Z. Fu, A. B. Abou-Samra, and R. Zhang, "An explanation for recent discrepancies in levels of human circulating betatrophin," *Diabetologia*, vol. 57, pp. 2232–2234, 2014.

[25] F. Quagliarini, Y. Wang, J. Kozlitina et al., "Atypical angiopoietin-like protein that regulates ANGPTL3," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 48, pp. 19751–19756, 2012.

[26] R. Zhang and A. B. Abou-Samra, "Emerging roles of Lipasin as a critical lipid regulator," *Biochemical and Biophysical Research Communications*, vol. 432, no. 3, pp. 401–405, 2013.

[27] Y. Wang, F. Quagliarini, È. Gusaroèa et al., "Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 40, pp. 16109–16114, 2013.

[28] M. Shimamura, M. Matsuda, H. Yasumo et al., "Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 2, pp. 366–372, 2007.

[29] H. Lickert, "Betatrophin fuels beta cell proliferation: first step toward regenerative therapy," *Cell Metabolism*, vol. 18, no. 1, pp. 5–6, 2013.

[30] R. Raghow, "Betatrophin: a liver-derived hormone for the pancreatic β-cell proliferation," *World Journal of Diabetes*, vol. 4, no. 6, pp. 234–237, 2013.

[31] P. Yi, J.-S. Park, and D. A. Melton, "Perspectives on the activities of ANGPTL8/Betatrophin," *Cell*, vol. 159, no. 3, pp. 467–468, 2014.

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Review Article

Role of FTO in Adipocyte Development and Function: Recent Insights

Myrte Merkestein[1] and Dyan Sellayah[2]

[1]*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, Oxfordshire OX1 3PT, UK*
[2]*School of Biological Sciences, University of Reading, Reading, Berkshire RG6 6AS, UK*

Correspondence should be addressed to Dyan Sellayah; d.sellayah@reading.ac.uk

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In 2007, *FTO* was identified as the first genome-wide association study (GWAS) gene associated with obesity in humans. Since then, various animal models have served to establish the mechanistic basis behind this association. Many earlier studies focussed on FTO's effects on food intake via central mechanisms. Emerging evidence, however, implicates adipose tissue development and function in the causal relationship between perturbations in FTO expression and obesity. The purpose of this mini review is to shed light on these new studies of FTO function in adipose tissue and present a clearer picture of its impact on obesity susceptibility.

1. Introduction

Obesity has risen to become the major health crisis of the current and potentially future generations. The latest figures from the World Health Organization (WHO) reveal a global estimate of 1.4 billion overweight individuals [1]. Most troubling is the devastating socioeconomic impact of obesity and related metabolic disturbances [2]. The WHO has estimated that 2–7% of global spending on health care is driven by a high body mass index (BMI) [3]. One such example is the financial strain being applied to the UK's National Health Service by obesity [4]. It is clear that more effective strategies are required, in terms of public policy, medical treatment, and biomedical research, to provide financially sustainable health care to be able to deal with the obesity crisis head-on. In recent years, genome-wide association studies (GWAS) have offered renewed promise in the quest to understand the genetics of obesity which has for too long eluded modern science.

In 2007, several independent GWAS and population based approaches identified associations between SNPs in intron 1 of *FTO* and human obesity in various European populations [5–8]. Since then, many studies have confirmed the association between SNPs in intron 1 of *FTO* and BMI in non-European populations, notable East Asians [9–11], South Asians [12–16], Africans [17], Hispanics [18, 19], and Native Americans [20], clearly demonstrating that the influence of FTO SNPs on obesity is a global trend. No association has been found between these obesity-associated SNPs and *FTO* expression levels in adipose tissue [21–25]. The fact that the obesity-associated *FTO* SNPs are intronic has led to the notion that the SNPs may affect obesity through influencing the expression of genes proximal to *FTO* in the locus, namely, *RPGRIP1L*, *IRX3*, and *IRX5* [26–29]. The obesity-associated allele of SNP rs8051036 has been suggested to decrease *RPGRIP1L* expression via reduced affinity for a transcriptional activator [26]. *IRX3* expression has recently been documented to account for the obesity association with *FTO* SNPs in human cerebellar tissue [28] and in the pancreas in zebrafish [30]. Furthermore, long-range functional connections were observed between enhancers within the obesity-associated *Fto* interval and *Irx3* expression (and not *Fto*) in adult mouse brain tissues [28]. Another recent study found that an obesity-associated SNP in intron 1 of *FTO* was located in a long enhancer region in preadipocytes specifically. The risk allele disrupted the binding of the gene regulator *ARID5b*, which in turn leads to increased expression of *IRX3* and *IRX5* in preadipocytes [29].

These studies indicate that the obesity-associated region in intron 1 is likely to regulate the expression of various genes, which might be tissue- and developmental stage-specific. Future studies will address how the obesity-associated SNPs contribute to increased adiposity in other tissues and at early developmental stages. Thus, it remains a possibility that the obesity associated with the SNP is mediated by FTO during development or in peripheral tissues. As *FTO* was considered the "obesity-gene" in 2007 when the first GWAS papers were published, many research groups sought out to examine FTO's role in the regulation of body weight. Regardless of whether the obesity-associated SNP affects *FTO* expression levels, these studies have clearly proven an important role of FTO in the regulation of body weight, independent of *IRX3*, *IRX5*, and *RPGRIP1L*.

One important peripheral tissue that has increasingly been shown to be consequential to metabolic regulation and obesity susceptibility is adipose tissue. Interestingly, while it has been long established that *FTO* is highly expressed in adipose tissues [5], its function remained largely obscured. The purpose of this review is to examine the emerging role of FTO in adipose tissue and its relevance to obesity susceptibility.

2. Mouse Models of FTO Function

The first murine model of global germline *Fto* loss was described by Fischer et al. in 2009 [31]. *Fto* deficient mice exhibited high perinatal lethality as well as postnatal growth retardation. Furthermore, *Fto* deficient mice had reduced lean and fat mass with respect to wild type mice. Intriguingly, fat mass was progressively reduced over the time in *Fto* deficient mice, to the extent that by 15 months of age *Fto* deficient mice were almost totally void of gonadal white adipose tissue (gWAT). Fischer and colleagues demonstrated that *Fto* deficiency causes relatively increased food intake as well as energy expenditure, potentially due to enhanced sympathetic tone resulting from elevated serum noradrenalin levels. The effect on energy expenditure in *Fto* deficient mice might have been due to the way in which the data were corrected to lean mass [32]. Others did not find an increase in energy expenditure in *Fto* deficient mice [33]. Interestingly, a model in which a point mutation in *Fto* resulted in reduced FTO protein expression and catalytic activity resulted in reduced lean mass and fat mass without any effects on perinatal lethality [34]. Paradoxically, adult onset *Fto* deficiency in mice (6 weeks of age) resulted in increased adiposity compared to wild type mice, with no effects on lean mass [33]. In line with these findings, a recent study reported increased body weight and adiposity of *Fto* knockout mice compared to wild type mice in response to a high fat diet [35].

To compound the inconsistency in body composition phenotypes with *Fto* deficiency, neural knockout of *Fto* led to a reduction in body weight accompanied by a decrease in lean mass, not fat mass [36], reflecting some of the germline FTO-KO models. However, adult onset knockout of *Fto* in the mediobasal hypothalamus decreased body weight without affecting body composition [33].

Fto overexpression models appear to be more consistent in describing an enhanced adiposity in *Fto* overexpression compared to wild type mice, an effect which is most pronounced on a high fat diet. Church and colleagues documented that *Fto* overexpression is accompanied by increased gWAT mass and increased adipocyte size [37], while Merkestein et al. revealed that adipocyte hypertrophy is preceded by increased gWAT hyperplasia in response to HFD, with respect to wild type mice demonstrating that FTO influences adipogenesis [38]. Conversely, *Fto* deficient mice have been shown to have reduced adipocyte size, both under chow conditions [31] and in response to high fat diet [39]; however, effects on adipose tissue hyperplasia were not studied in these models.

Although many questions remain to be answered and a clear understanding of the effects of FTO on body composition remains to be established, it is beyond doubt that FTO exerts an influence over adiposity and it is plausible that FTO directly regulates adipocyte development and metabolism.

3. Regulation of FTO Expression

The expression of *FTO* in adipose tissue is likely regulated in various ways. There is ample evidence for crosstalk between FTO and the LepRb-STAT3 signalling pathway and the involvement of the p110 isoform of the CUX1 transcription factor.

FTO risk alleles have been associated with changes in circulating levels of the appetite regulating hormones leptin and ghrelin. Several papers have shown that *FTO* risk allele is associated with increased circulating leptin levels [40–47]. However, this association appears to be mediated via increased adiposity, as in several studies the association disappears when correcting for BMI [42, 44–46]. Another study reported an association of an *FTO* risk allele with a decrease in circulating leptin levels which was independent of BMI in older participants [48]. Carriers of *FTO* risk alleles have been shown to have a reduced postprandial suppression of circulating acyl-ghrelin levels [49]. Similarly, another study found increased plasma ghrelin levels after overnight fast in people with *FTO* risk alleles [48].

Leptin increased *FTO* expression in cardiomyocytes, which was dependent on LepRb-STAT3 signalling and a subsequent increase in the p110 CUX1 isoform [50]. Overexpression of *FTO* in the arcuate nucleus of the hypothalamus in rats resulted in an increase in STAT3 mRNA [51]. On the other hand, leptin activated the STAT3 signalling pathway and reduced *FTO* expression in the arcuate nucleus, which was dependent on the LepRb receptor [52]. In hepatocytes, leptin administration, LepRb overexpression, and activation of the STAT3 pathway with IL6 induced FTO protein expression, whereas knockdown of STAT3 inhibited leptin-induced *FTO* mRNA expression. Conversely, overexpression of *FTO* reduced leptin-induced STAT3 phosphorylation and affected downstream events of this signalling pathway. Furthermore, overexpressing of *Fto in vivo* in mouse livers affected STAT3

phosphorylation and its downstream effects in a similar way [53].

Further evidence of the crosstalk between leptin and FTO comes from animal studies. In response to HFD, *Fto* deficient mice did not develop leptin resistance, whereas wild type mice did [35]. Furthermore, knocking out *Fto* improved the features of the metabolic syndrome normally observed in leptin deficient ob/ob mice [54].

Intron 1 of the *FTO* gene contains a binding site for the p110 isoform of the CUX1 transcription factor. The obesity-associated rs8050136 SNP is located in this region. Binding of the p110 CUX1 isoform enhances *FTO* expression [26]. Therefore, this transcription factor is likely to regulate *FTO* expression.

The *FTO* promoter region has also been shown to contain a C/EBPα binding site and C/EBPα promoted *FTO* expression in HEK293 and HeLa cells [55]. Furthermore, miR-33 was shown to regulate *FTO* expression. miR-33 is transcribed from an intronic region within *SREBF2*. SREBF2 is a transcriptional activator of many genes involved in the synthesis and uptake of cholesterol, triglycerides, fatty acids, and phospholipids. miR-33 is expressed in many tissues in the chicken, including adipose tissue. Knocking down miR-33 in primary chicken hepatocytes increased the expression of *FTO* [56].

4. FTO and Adipogenesis

Several studies have examined the expression of *FTO* during adipogenesis, the process by which new adipocytes are formed from preadipocytes, which in turn derive from mesenchymal stem cells. This process which has been well documented *in vitro* occurs in 7–10 days. In cultured preadipocytes and MEFs, *Fto* has been consistently shown to be highly expressed in the early phase of adipogenesis and to decline during the course of adipogenesis *in vitro* [24, 57–59].

These studies indicated a role for FTO in the adipogenic process. Indeed, knockdown of *Fto* decreased adipogenesis in 3T3-L1 preadipocytes [58, 60] and in porcine preadipocytes [61]. MEFs from *Fto*-KO mice exhibited reduced adipogenic potential, whereas overexpression of *Fto* led to an enhanced adipogenic program in primary murine preadipocytes, 3T3L1 preadipocytes, and porcine preadipocytes [38, 60, 61].

In line with these findings, gonadal WAT from *Fto* overexpressing mice fed a high fat diet for 8 weeks from weaning contained an increased number of adipocytes compared with WT mice; however, no differences in adipocyte number were evident between *Fto* overexpression mice and WT mice at weaning, clearly demonstrating that FTO promotes obesogenic adipogenesis in adulthood but plays no role in developmental adipogenesis [38]. This is important as obesogenic adipogenesis, which occurs in response to HFD in adulthood, has recently been shown to operate via distinct signalling mechanisms and requiring different subpopulations of preadipocytes [62]. Notably, the AKT2/PI3K signalling pathway has been evidenced to be essential for obesogenic adipogenesis but not required for developmental adipogenesis, possibly alluding to the insulin signalling aspect of obesogenic adipogenesis which is clearly dependent on dietary factors, in contrast to developmental adipogenesis which responds to developmental cues that predominate during organogenesis [62].

4.1. Catalytic Activity of FTO Necessary for Its Role in Adipogenesis. FTO is 2-oxoglutarate dependent demethylase of single stranded nucleic acids [63]. Its main substrate is likely 6-methyladenosine (m6A) in RNA [64]. In 3T3L1 preadipocytes, overexpression of full length wild type *Fto* enhanced adipogenesis whereas overexpression of catalytically inactive R96Q *Fto* did not affect adipogenesis [60]. Similarly, the effect of *Fto* knockdown on adipogenesis in 3T3-L1 cells could be rescued by reexpressing wild type *Fto*, but not by reexpressing catalytic inactive *Fto* [58]. The reduction in cellular proliferation during the mitotic clonal expansion phase of adipogenesis in *Fto*-KO MEFs could be rescued by expressing WT *Fto*, but not by catalytic inactive R313A *Fto* [38].

In porcine adipocytes, overexpression of *FTO* increased m6A levels, and knockdown of *FTO* reduced these levels. Interestingly, overexpression of the m6A methylase *METTL3* induced similar effects of *FTO* knockdown, whereas knockdown of METTL3 had no effect. Chemically increasing (via methyl donor betaine) m6A levels mimicked *FTO* knockdown, whereas chemical reduction of m6A levels (via methylation inhibitor cycloleucine) reflected the effects seen with *FTO* overexpression [61]. In another study, knockdown of *ALKBH5*, another m6A demethylase, did not affect adipogenesis. Knockdown of *METTL3* on the other hand increased lipid accumulation during adipogenesis [58]. So, m6A is likely to play a role in adipogenesis, but the effect is substrate-specific, as FTO's catalytic activity is essential for adipogenesis; however, that of ALKBH5 is unlikely to be important.

4.2. Mechanism via Which FTO Affects Adipogenesis. FTO was shown to influence adipogenesis at an early stage indeed, during mitotic clonal expansion, which takes place during the first 48 hours after adipogenic stimulation *in vitro* [38]. A potential mechanism via which FTO regulates adipogenesis is RUNX1T1. The SR proteins are important for splice site recognition and intron processing, and FTO was shown to regulate the splicing of SRFS2 target genes via modification of m6A levels. One of these target genes is *RUNX1T1*, which has 2 isoforms. The short isoform is proadipogenic, whereas the long isoform reduces adipogenesis. FTO knockdown decreases the expression of the short isoform [58]. RUNX1T1 has been shown to regulate C/EBPβ activity [65]. Interestingly enough, FTO was shown to act as a transcriptional coactivator for the transcriptional regulators C/EBPα, C/EBPβ, and C/EBPδ [66]. One study found that *FTO* deficiency did not affect the expression levels of C/EBPβ, suggesting FTO acts via a C/EBPβ independent route [39]. But FTO is likely to affect the activity of C/EBPβ, rather than its expression.

5. FTO and Lipogenesis

Besides adipogenesis, FTO might play a role in lipogenesis. In one study, knocking down *FTO* in the human preadipocyte

SGBS cells did not affect adipogenic differentiation, lipolysis, or glucose uptake but did attenuate de novo lipogenesis [67]. And in human myotubes, *FTO* overexpression caused an increase in lipogenesis and upregulation of the expression of genes involved in lipogenesis [57]. Church and colleagues reported a gross increase in adipocyte size after prolonged high fat diet feeding in mice [37], and as adipogenesis also relies upon lipogenesis to assemble lipids into triglycerides during terminal differentiation, these findings suggest that FTO may play two distinct roles in adipogenesis (during MCE at the start and in lipid filling at the culmination of the process). A recent study showed a link between lipogenesis and adipogenesis via the carbohydrate-response element-binding protein (chREBP) which was shown to regulate *PPARγ* [68]. It is conceivable that FTO may influence chREBP and it would be interesting to assess the effects on *chREBP* expression in preadipocytes and MEFs from *Fto*-KO and *Fto* overexpression mice.

6. FTO Deficiency and Browning of White Adipose Tissue

An obesity-associated SNP in intron 1 of *FTO* has been associated with decreased browning of white adipose tissue, which coincided with an increase in expression of *IRX3* and *IRX5* in preadipocytes [29]. Browning of WAT has also been reported in *Irx3*-KO mice [28]. Furthermore, also *Fto*-KO mice showed signs of WAT browning. Expression of *Ucp1* in gonadal and inguinal fat pads was increased in *Fto*-KO mice, and *Fto* deficient adipocytes showed increased expression of *Ucp1* and increased mitochondrial respiration [67]. Furthermore, *FTO* variants were suggested to influence adipose tissue lipolysis and metabolism [24, 46]. Given that *Fto* deficiency is associated with increased circulating noradrenaline levels [31, 34, 67], increased thermogenic capacity and lipolysis of *Fto* deficient adipocytes may result from increased adrenergic receptor activation.

7. FTO and Developmental Programming

Given that *Fto* deficiency results in developmental abnormalities consistent with postnatal growth retardation [31], characterized by reduced lean mass and body length, it would be appropriate to postulate that FTO may act developmentally to influence body composition. Interestingly, Dina and colleagues demonstrated that FTO SNPs are associated not only with adult obesity but also with childhood obesity [8]. In fact, the association between *FTO* SNPs and obesity peaks during early adolescence and tapers off thereafter [45, 69–71], suggesting that *FTO* SNPs may impact obesity by influencing events during the developmental period. Interestingly, one study found that the *FTO* risk allele is actually associated with a reduction in BMI under the age of 2.5 years, and with a shift in the timing of the BMI adiposity rebound, which is a developmental marker. The obesity risk alleles accelerate the developmental age, which subsequently results in an increase in BMI during later time-points [71].

A recent study in rats found that *FTO* mRNA expression in the hypothalamus was increased in offspring following maternal obesity and was associated with a predisposition to high fat diet induced obesity in adulthood, potentially due to increased food intake [72]. It remains to be elucidated whether a similar expression pattern is observed with maternal obesity in offspring adipose tissue, although maternal nutrient restriction had no impact on offspring adipose tissue mRNA levels [73]. Epigenetic processes have been shown to be highly active during the developmental period and may represent a mechanism independent of changes in the DNA sequence by which maternal diet may impact offspring obesity susceptibility. Interestingly, FTO risk allele rs8050136 has been shown to have increased methylation on a per-allele basis [74]. This could represent an exciting new mechanism by which *FTO* SNPs affect obesity and warrants further investigation. Furthermore, a very recent study observed a parent-of-origin effect of *FTO* SNPs, albeit in a very small population in Germany, suggesting that these parent-of-origin effects may modulate the association between *FTO* SNPs and obesity [75].

Recently, it was shown that adipogenesis during development and obesity are regulated by distinct signalling pathways and utilize separate preadipocyte populations (both of which are marked by birth) [62]. Adulthood adipogenesis in mice, which only occurs under HFD conditions, requires Akt2/PI3K signalling and requires smooth muscle actin (SMA) positive preadipocytes, whereas developmental adipogenesis (organogenesis) is not dependent on this pathway [62]. To this end, we have shown that *Fto* overexpression does not affect developmental adipogenesis but has profound effects on obesogenic adipogenesis [38]. Interestingly, in endometrial cancer cells, β-estradiol- (E2-) induced proliferation and invasion were shown to be mediated by FTO. E2 stimulated *FTO* expression via the PI3K/Akt and MAPK signalling pathways. *FTO* knockdown attenuated cancer cell growth and proliferation which was mediated via cyclin D1 regulation [76]. This is particularly intriguing as FTO has been shown to induce adipogenesis through augmenting cyclin D1 expression during the MCE phase [38]. These studies thus indicate that AKT/PI3K signalling may be a crucial part of the as yet unidentified signalling process of FTO-mediated adipogenesis; however, more direct evidence is required to confirm this hypothesis.

8. Conclusion

Whether or not alterations in *FTO* expression are responsible for the obesity-associated SNPs in intron 1 of *FTO* remains to be unequivocally answered. Nevertheless, FTO clearly plays an important role in adipogenesis. Given the similarities between *Fto* and *Irx3* animal models, these genes might act well in concert to regulate adipogenesis and the occurrence of browning of white adipose tissue. The discovery of FTO as a demethylase of single stranded DNA and RNA, and its target m6A, has revealed the importance of m6A in RNA and how it is crucial for important physiological processes, such as adipogenesis. Future studies will address the differences

between FTO's roles in development and in adulthood, not only in adipose tissue but also in other tissues.

Conflict of Interests

The authors declare no conflict of interests.

References

[1] V. S. Malik, W. C. Willett, and F. B. Hu, "Global obesity: trends, risk factors and policy implications," *Nature Reviews Endocrinology*, vol. 9, no. 1, pp. 13–27, 2013.

[2] A. H. Anis, W. Zhang, N. Bansback, D. P. Guh, Z. Amarsi, and C. L. Birmingham, "Obesity and overweight in Canada: an updated cost-of-illness study," *Obesity Reviews*, vol. 11, no. 1, pp. 31–40, 2010.

[3] McKinsey Global Institute, *Overcoming Obesity: An Initial Economic Analysis*, McKinsey Global Institute, 2014.

[4] S. Allender and M. Rayner, "The burden of overweight and obesity-related ill health in the UK," *Obesity Reviews*, vol. 8, no. 5, pp. 467–473, 2007.

[5] T. M. Frayling, N. J. Timpson, M. N. Weedon et al., "A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity," *Science*, vol. 316, no. 5826, pp. 889–894, 2007.

[6] A. Scuteri, S. Sanna, W.-M. Chen et al., "Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits," *PLoS Genetics*, vol. 3, no. 7, article e115, 2007.

[7] A. Hinney, T. T. Nguyen, A. Scherag et al., "Genome Wide Association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants," *PLoS ONE*, vol. 2, no. 12, Article ID e1361, 2007.

[8] C. Dina, D. Meyre, S. Gallina et al., "Variation in FTO contributes to childhood obesity and severe adult obesity," *Nature Genetics*, vol. 39, no. 6, pp. 724–726, 2007.

[9] Y. S. Cho, M. J. Go, Y. J. Kim et al., "A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits," *Nature Genetics*, vol. 41, no. 5, pp. 527–534, 2009.

[10] W. Wen, Y.-S. Cho, W. Zheng et al., "Meta-analysis identifies common variants associated with body mass index in east Asians," *Nature Genetics*, vol. 44, no. 3, pp. 307–311, 2012.

[11] Y. Okada, M. Kubo, H. Ohmiya et al., "Common variants at CDKAL1 and KLF9 are associated with body mass index in east Asian populations," *Nature Genetics*, vol. 44, no. 3, pp. 302–306, 2012.

[12] S. D. Rees, M. Islam, M. Z. I. Hydrie et al., "An FTO variant is associated with Type 2 diabetes in South Asian populations after accounting for body mass index and waist circumference," *Diabetic Medicine*, vol. 28, no. 6, pp. 673–680, 2011.

[13] C. S. Yajnik, C. S. Janipalli, S. Bhaskar et al., "FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians," *Diabetologia*, vol. 52, no. 2, pp. 247–252, 2009.

[14] K. Ramya, V. Radha, S. Ghosh, P. P. Majumder, and V. Mohan, "Genetic variations in the FTO gene are associated with type 2 diabetes and obesity in South Indians (CURES-79)," *Diabetes Technology and Therapeutics*, vol. 13, no. 1, pp. 33–42, 2011.

[15] S. C. Moore, M. J. Gunter, C. R. Daniel et al., "Common genetic variants and central adiposity among Asian-Indians," *Obesity*, vol. 20, no. 9, pp. 1902–1908, 2012.

[16] S. K. Vasan, T. Fall, M. J. Neville et al., "Associations of variants in FTO and near MC4R with obesity traits in south Asian Indians," *Obesity*, vol. 20, no. 11, pp. 2268–2277, 2012.

[17] K. L. Monda, G. K. Chen, K. C. Taylor et al., "A meta-analysis identifies new loci associated with body mass index in individuals of African ancestry," *Nature Genetics*, vol. 45, no. 6, pp. 690–696, 2013.

[18] M. Villalobos-Comparán, M. Teresa Flores-Dorantes, M. Teresa Villarreal-Molina et al., "The FTO gene is associated with adulthood obesity in the Mexican population," *Obesity*, vol. 16, no. 10, pp. 2296–2301, 2008.

[19] C. Dong, A. Beecham, S. Slifer et al., "Genome-wide linkage and peak-wide association study of obesity-related quantitative traits in Caribbean Hispanics," *Human Genetics*, vol. 129, no. 2, pp. 209–219, 2011.

[20] R. Rong, R. L. Hanson, D. Ortiz et al., "Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians," *Diabetes*, vol. 58, no. 2, pp. 478–488, 2009.

[21] C. Zabena, J. L. González-Sánchez, M. T. Martínez-Larrad et al., "The FTO obesity gene. Genotyping and gene expression analysis in morbidly obese patients," *Obesity Surgery*, vol. 19, no. 1, pp. 87–95, 2009.

[22] T. Lappalainen, M. Kolehmainen, U. Schwab et al., "Gene expression of FTO in human subcutaneous adipose tissue, peripheral blood mononuclear cells and adipocyte cell line," *Journal of Nutrigenetics and Nutrigenomics*, vol. 3, no. 1, pp. 37–45, 2010.

[23] L. G. Grunnet, E. Nilsson, C. Ling et al., "Regulation and function of FTO mRNA expression in human skeletal muscle and subcutaneous adipose tissue," *Diabetes*, vol. 58, no. 10, pp. 2402–2408, 2009.

[24] K. Wåhlén, E. Sjölin, and J. Hoffstedt, "The common rs9939609 gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis," *Journal of Lipid Research*, vol. 49, no. 3, pp. 607–611, 2008.

[25] N. Klöting, D. Schleinitz, K. Ruschke et al., "Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans," *Diabetologia*, vol. 51, no. 4, pp. 641–647, 2008.

[26] G. Stratigopoulos, C. A. LeDuc, M. L. Cremona, W. K. Chung, and R. L. Leibel, "Cut-like homeobox 1 (CUX1) regulates expression of the fat mass and obesity-associated and retinitis pigmentosa GTPase regulator-interacting protein-1-like (RPGRIP1L) genes and coordinates leptin receptor signaling," *The Journal of Biological Chemistry*, vol. 286, no. 3, pp. 2155–2170, 2011.

[27] G. Stratigopoulos, J. F. M. Carli, D. R. O'Day et al., "Hypomorphism for RPGRIP1L, a ciliary gene vicinal to the fto locus, causes increased adiposity in mice," *Cell Metabolism*, vol. 19, no. 5, pp. 767–779, 2014.

[28] S. Smemo, J. J. Tena, K.-H. Kim et al., "Obesity-associated variants within FTO form long-range functional connections with IRX3," *Nature*, vol. 507, no. 7492, pp. 371–375, 2014.

[29] M. Claussnitzer, S. N. Dankel, K.-H. Kim et al., "*FTO* obesity variant circuitry and adipocyte browning in humans," *The New England Journal of Medicine*, vol. 373, pp. 895–907, 2015.

[30] A. Ragvin, E. Moro, D. Fredman et al., "Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4, and IRX3," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 2, pp. 775–780, 2010.

[31] J. Fischer, L. Koch, C. Emmerling et al., "Inactivation of the Fto gene protects from obesity," *Nature*, vol. 458, no. 7240, pp. 894–898, 2009.

[32] J. R. Speakman, "FTO effect on energy demand versus food intake," *Nature*, vol. 464, no. 7289, pp. E1–E2, 2010.

[33] F. McMurray, C. D. Church, R. Larder et al., "Adult onset global loss of the Fto gene alters body composition and metabolism in the mouse," *PLoS Genetics*, vol. 9, no. 1, Article ID e1003166, 2013.

[34] C. Church, S. Lee, E. A. L. Bagg et al., "A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene," *PLoS Genetics*, vol. 5, no. 8, Article ID e1000599, 2009.

[35] Y. C. L. Tung, P. Gulati, C.-H. Liu et al., "FTO is necessary for the induction of leptin resistance by high-fat feeding," *Molecular Metabolism*, vol. 4, no. 4, pp. 287–298, 2015.

[36] X. Gao, Y.-H. Shin, M. Li, F. Wang, Q. Tong, and P. Zhang, "The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice," *PLoS ONE*, vol. 5, no. 11, Article ID e14005, 2010.

[37] C. Church, L. Moir, F. McMurray et al., "Overexpression of Fto leads to increased food intake and results in obesity," *Nature Genetics*, vol. 42, no. 12, pp. 1086–1092, 2010.

[38] M. Merkestein, S. Laber, F. McMurray et al., "FTO influences adipogenesis by regulating mitotic clonal expansion," *Nature Communications*, vol. 6, article 6792, 2015.

[39] J. Ronkainen, T. J. Huusko, R. Soininen et al., "Fat mass- and obesity-associated gene Fto affects the dietary response in mouse white adipose tissue," *Scientific Reports*, vol. 5, article 9233, 2015.

[40] M. Arrizabalaga, E. Larrarte, J. Margareto, S. Maldonado-Martín, L. Barrenechea, and I. Labayen, "Preliminary findings on the influence of *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms on resting energy expenditure, leptin and thyrotropin levels in obese non-morbid premenopausal women," *Journal of Physiology and Biochemistry*, vol. 70, no. 1, pp. 255–262, 2014.

[41] A. Shahid, S. Rana, S. Saeed, M. Imran, N. Afzal, and S. Mahmood, "Common variant of FTO Gene, rs9939609, and obesity in Pakistani females," *BioMed Research International*, vol. 2013, Article ID 324093, 7 pages, 2013.

[42] F. Rutters, A. G. Nieuwenhuizen, F. Bouwman, E. Mariman, and M. S. Westerterp-Plantenga, "Associations between a single nucleotide polymorphism of the FTO gene (rs9939609) and obesity-related characteristics over time during puberty in a Dutch children cohort," *The Journal of Clinical Endocrinology & Metabolism*, vol. 96, no. 6, pp. E939–E942, 2011.

[43] I. Labayen, J. R. Ruiz, F. B. Ortega et al., "Association between the FTO rs9939609 polymorphism and leptin in European adolescents: a possible link with energy balance control. the HELENA study," *International Journal of Obesity*, vol. 35, no. 1, pp. 66–71, 2011.

[44] E. Zimmermann, K. Skogstrand, D. M. Hougaard et al., "Influences of the common FTO rs9939609 variant on inflammatory markers throughout a broad range of body mass index," *PLoS ONE*, vol. 6, no. 1, Article ID e15958, 2011.

[45] L. Qi, K. Kang, C. Zhang et al., "Fat mass-and obesity-associated (FTO) gene variant is associated with obesity: longitudinal analyses in two cohort studies and functional test," *Diabetes*, vol. 57, no. 11, pp. 3145–3151, 2008.

[46] R. Do, S. D. Bailey, K. Desbiens et al., "Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study," *Diabetes*, vol. 57, no. 4, pp. 1147–1150, 2008.

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

[48] C. Benedict, T. Axelsson, S. Söderberg et al., "Fat mass and obesity-associated gene (FTO) is linked to higher plasma levels of the hunger hormone ghrelin and lower serum levels of the satiety hormone leptin in older adults," *Diabetes*, vol. 63, no. 11, pp. 3955–3959, 2014.

[49] E. Karra, O. G. O'Daly, A. I. Choudhury et al., "A link between FTO, ghrelin, and impaired brain food-cue responsivity," *The Journal of Clinical Investigation*, vol. 123, no. 8, pp. 3539–3551, 2013.

[50] X. T. Gan, G. Zhao, C. X. Huang, A. C. Rowe, D. M. Purdham, and M. Karmazyn, "Identification of fat mass and obesity associated (FTO) protein expression in cardiomyocytes: regulation by leptin and its contribution to leptin-induced hypertrophy," *PLoS ONE*, vol. 8, no. 9, Article ID e74235, 2013.

[51] Y.-C. L. Tung, E. Ayuso, X. Shan et al., "Hypothalamic-specific manipulation of Fto, the ortholog of the human obesity gene FTO, affects food intake in rats," *PLoS ONE*, vol. 5, no. 1, Article ID e8771, 2010.

[52] P. Wang, F.-J. Yang, H. Du et al., "Involvement of leptin receptor long isoform (LepRb)-STAT3 signaling pathway in brain fat mass- and obesity-associated (FTO) downregulation during energy restriction," *Molecular Medicine*, vol. 17, no. 5-6, pp. 523–532, 2011.

[53] A. Bravard, G. Vial, M.-A. Chauvin et al., "FTO contributes to hepatic metabolism regulation through regulation of leptin action and STAT3 signalling in liver," *Cell Communication and Signaling*, vol. 12, article 4, 2014.

[54] K. Ikels, S. Kuschel, J. Fischer et al., "FTO is a relevant factor for the development of the metabolic syndrome in mice," *PLoS ONE*, vol. 9, no. 8, Article ID e105349, 2014.

[55] W. Ren, J. Guo, F. Jiang et al., "CCAAT/enhancer-binding protein α is a crucial regulator of human fat mass and obesity associated gene transcription and expression," *BioMed Research International*, vol. 2014, Article ID 406909, 7 pages, 2014.

[56] F. Shao, X. Wang, J. Yu, H. Jiang, B. Zhu, and Z. Gu, "Expression of miR-33 from an SREBF2 intron targets the FTO gene in the chicken," *PLoS ONE*, vol. 9, no. 3, Article ID e91236, 2014.

[57] A. Bravard, A. Veilleux, E. Disse et al., "The expression of FTO in human adipose tissue is influenced by fat depot, adiposity, and insulin sensitivity," *Obesity*, vol. 21, no. 6, pp. 1165–1173, 2013.

[58] X. Zhao, Y. Yang, B.-F. Sun et al., "FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis," *Cell Research*, vol. 24, no. 12, pp. 1403–1419, 2014.

[59] D. Tews, P. Fischer-Posovszky, and M. Wabitsch, "Regulation of FTO and FTM expression during human preadipocyte differentiation," *Hormone and Metabolic Research*, vol. 43, no. 1, pp. 17–21, 2011.

[60] M. Zhang, Y. Zhang, J. Ma et al., "The demethylase activity of FTO (fat mass and obesity associated protein) is required for preadipocyte differentiation," *PLoS ONE*, vol. 10, no. 7, Article ID e0133788, 2015.

[61] X. Wang, L. Zhu, J. Chen, and Y. Wang, "mRNA m^6A methylation downregulates adipogenesis in porcine adipocytes," *Biochemical and Biophysical Research Communications*, vol. 459, no. 2, pp. 201–207, 2015.

[62] E. Jeffery, C. D. Church, B. Holtrup, L. Colman, and M. S. Rodeheffer, "Rapid depot-specific activation of adipocyte precursor cells at the onset of obesity," *Nature Cell Biology*, vol. 17, no. 4, pp. 376–385, 2015.

[63] T. Gerken, C. A. Girard, Y.-C. L. Tung et al., "The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase," *Science*, vol. 318, no. 5855, pp. 1469–1472, 2007.

[64] G. Jia, Y. Fu, X. Zhao et al., "*N*6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO," *Nature Chemical Biology*, vol. 7, no. 12, pp. 885–887, 2011.

[65] J. J. Rochford, R. K. Semple, M. Laudes et al., "ETO/MTG8 is an inhibitor of C/EBPβ activity and a regulator of early adipogenesis," *Molecular and Cellular Biology*, vol. 24, no. 22, pp. 9863–9872, 2004.

[66] Q. Wu, R. A. Saunders, M. Szkudlarek-Mikho, I. de la Serna, and K.-V. Chin, "The obesity-associated Fto gene is a transcriptional coactivator," *Biochemical and Biophysical Research Communications*, vol. 401, no. 3, pp. 390–395, 2010.

[67] D. Tews, P. Fischer-Posovszky, T. Fromme et al., "FTO deficiency induces UCP-1 expression and mitochondrial uncoupling in adipocytes," *Endocrinology*, vol. 154, no. 9, pp. 3141–3151, 2013.

[68] N. Witte, M. Muenzner, J. Rietscher et al., "The glucose sensor ChREBP links de novo lipogenesis to PPARγ activity and adipocyte differentiation," *Endocrinology*, vol. 156, no. 11, pp. 4008–4019, 2015.

[69] R. Hardy, A. K. Wills, A. Wong et al., "Life course variations in the associations between FTO and MC4R gene variants and body size," *Human Molecular Genetics*, vol. 19, no. 3, pp. 545–552, 2009.

[70] P. Rzehak, A. Scherag, H. Grallert et al., "Associations between BMI and the FTO gene are age dependent: results from the GINI and LISA birth cohort studies up to age 6 years," *Obesity Facts*, vol. 3, no. 3, pp. 173–180, 2010.

[71] U. Sovio, D. O. Mook-Kanamori, N. M. Warrington et al., "Association between common variation at the FTO locus and changes in body mass index from infancy to late childhood: the complex nature of genetic association through growth and development," *PLoS Genetics*, vol. 7, no. 2, Article ID e1001307, 2011.

[72] V. Caruso, H. Chen, and M. J. Morris, "Early hypothalamic fto overexpression in response to maternal obesity—potential contribution to postweaning hyperphagia," *PLoS ONE*, vol. 6, no. 9, Article ID e25261, 2011.

[73] S. P. Sébert, M. A. Hyatt, L. L. Y. Chan et al., "Influence of prenatal nutrition and obesity on tissue specific fat mass and obesity-associated (FTO) gene expression," *Reproduction*, vol. 139, no. 1, pp. 265–274, 2009.

[74] C. G. Bell, S. Finer, C. M. Lindgren et al., "Integrated genetic and epigenetic analysis identifies haplotype-specific methylation in the FTO type 2 diabetes and obesity susceptibility locus," *PLoS ONE*, vol. 5, no. 11, Article ID e14040, 2010.

[75] X. Liu, A. Hinney, M. Scholz et al., "Indications for potential parent-of-origin effects within the FTO gene," *PLoS ONE*, vol. 10, no. 3, Article ID e0119206, 2015.

[76] Z. Zhang, D. Zhou, Y. Lai et al., "Estrogen induces endometrial cancer cell proliferation and invasion by regulating the fat mass and obesity-associated gene via PI3K/AKT and MAPK signaling pathways," *Cancer Letters*, vol. 319, no. 1, pp. 89–97, 2012.

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Review Article

The Intricate Network of Adipokines and Stroke

Ema Kantorová,[1] Ľubica Jesenská,[2] Daniel Čierny,[2] Kamil Zeleňák,[3] Štefan Sivák,[1] Matej Stančík,[4] Peter Galajda,[4] Vladimír Nosáľ,[1] and Egon Kurča[1]

[1]*Jessenius Faculty of Medicine, Comenius University, Clinic of Neurology, Malá Hora 4A, 03601 Martin, Slovakia*
[2]*Jessenius Faculty of Medicine, Comenius University, Department of Medical Biochemistry, Malá Hora 4A, 03601 Martin, Slovakia*
[3]*Jessenius Faculty of Medicine, Comenius University, Clinic of Radiodiagnostics, Malá Hora 4A, 03601 Martin, Slovakia*
[4]*Jessenius Faculty of Medicine, Comenius University, Clinic of Internal Medicine I, Malá Hora 4A, 036 01 Martin, Slovakia*

Correspondence should be addressed to Ema Kantorová; kantorova@jfmed.uniba.sk

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Cerebrovascular disorders, particularly ischemic stroke, are one of the most common neurological disorders. High rates of overweight and obesity support an interest in the role of adipose tissue and adipose tissue releasing cytokines in inducing associated comorbidities. Adipokines can serve as a key messenger to central energy homeostasis and metabolic homeostasis. They can contribute to the crosstalk between adipose tissue and brain. However recent research has offered ambiguous data on the network of adipose tissue, adipokines, and vascular disorders. In our paper we provide a critical insight into the role of adipokines in evolution of ischemic stroke.

1. Introduction

Adipose tissue is a highly specialized organ that stores excess energy and releases it when needed by other tissues [1]. Obesity develops when the intake of calories exceeds energy expenditure [1]. Depending on the time of onset, obesity is associated with increased adipocyte size and number [1]. Further, an increase in adiposity results in fat accumulation in depots with different metabolic properties. Visceral fat has a higher risk for development of metabolic diseases, whereas subcutaneous fat does not [2].

Adipose tissue is an active secretory organ that elaborates a variety of messenger molecules. Adipocyte-derived hormones have structural homology to cytokines that actively participate in regulating many biological processes [3]. Major target receptors for the messenger molecules are located in adipose tissue [4]. They are also found in the hypothalamus, skeletal muscle, and liver [1]. Adipokines can also exert endocrine effects and participate in an interplay between several tissues [4].

It is still not fully clear whether adipokines are obesity-dependent or obesity-independent risk factors for neurological diseases and how they coordinately regulate physiological functions. However, the link between obesity-associated systemic inflammation and cerebrovascular [3], autoimmune [5], and degenerative neurological disorders has become of major interest [6, 7].

2. Ischemic Stroke and Obesity

Stroke, a leading course of death or disability, shares many risk factors with cardiovascular diseases (CVD), such as age, smoking, hypertension, diabetes mellitus, inactivity, overweight or obesity, and dyslipidemia [1]. Their role in ischemic stroke (IS) is still less elucidated than for CVD [8]. Some studies have suggested that obesity is an independent risk factor for cerebrovascular diseases [8–12]. The measures of obesity include body mass index (BMI), waist-to-hip ratio, and waist circumference (WC). BMI helps to classify rate of excess adipose tissue as overweight when BMI is >25 kg/m^2 and as obesity when BMI > 30 kg/m^2 [8, 9]. Study results have not always been conclusive and have often been contradictive. For example, markers of abdominal adiposity showed a graded and significant association with risk of IS, independent of other vascular risk factors. It was reported that for cerebrovascular events predictive function of WC is better than the one of BMI [12]. On the other hand one of the studies

recorded higher BMI, especially BMI > 30 kg/m^2, in male subjects, to correlate with an increased risk of cerebrovascular accidents [13]. Controversially, Wannamethee and colleagues found BMI and WC not to be associated with risk of IS, showing the lowest risk of IS in obese men (BMI > 30 kg/m^2) [14]. The findings were consistent with previously published studies that showed no association of obesity and IS in older men [15, 16]. The phenomenon called "obesity paradox," described especially in older patients, may reflect changes of body morphology in the elderly [14].

The correlation of excessive body weight and carotid artery disease indicates a role of obesity in development of IS [17]. The diameter and stiffness of carotid arteries appear to increase with higher BMI. Carotid distensibility decreases with higher BMI more at young than at old age. In elastic arteries, the relationship between arterial stiffness and BMI is more complex and varied with gender and age [17].

Excess adipose tissue is responsible for a wide variety of released adipokines such as leptin, adiponectin, resistin, visfatin, and apelin that have been associated with cerebrovascular diseases [18]. Now we are going to discuss those important adipokines that are considered to be associated with ischemic stroke.

3. Adipokines and Ischemic Stroke

3.1. Leptin. Leptin (LEP) is expressed mostly in adipose tissue, although low levels were found in other organs [19]. It circulates as both a bound and a free hormone, the latter representing the bioavailable hormone. LEP concentration is dependent on the quantity of stored energy fat, as well as the status of energy balance. For example, plasma leptin is higher in obese than in lean individuals, falls rapidly during fasting, and increases after feeding. Leptin acts primarily in the brain, bounding specific receptors in the lateral hypothalamus [19].

LEP resistance appears to be a mechanism that is a part of the burden of obesity on health that extends across multiple organ systems. LEP resistance is characterized by decreased availability of LEP to the brain despite normal or even higher plasmatic and cerebrospinal fluid (CSF) levels and peripheral activity. The high levels would be expected to depress appetite but fail to do so [20]. Mechanisms of leptin resistance are complex including genetic mutation of leptin receptor, failure of self-regulation of hypothalamic centers, limited transport of leptin through blood-brain barrier, and intracellular molecular mechanisms [21].

A Finnish twin study established that a substantial percentage of variance in intensity of relationship between hyperleptinemia and vascular complications is attributable to genetic background [22]. According to the study results, genetic effects contributed 72% of total variance in men and 66.4% in nonpregnant women, while 27.8% of variations among men and 33.6% among women were due to environmental effects [22]. In particular important environmental factors are dietary habits, high-fat diet, and overeating with subsequent obesity. In another study both obesity and increased plasma leptin concentration correlated with high blood pressure [23]. Both male and female ischemic stroke patients had higher blood pressure than control subjects [24]. In association with high blood pressure hyperleptinemia in men was found to predict both ischemic and hemorrhagic stroke subtypes [25]. Hyperleptinemia was reported to be a good predictor of uncontrollable high blood pressure [25]. In obese individuals leptin-mediated platelet activation represented a direct link between leptin and the risk of thrombotic complications [26]. A relationship between several coagulation factors (factor VIIa and von Willebrand) and leptin levels supported a role of LEP in procoagulation states frequently associated with vascular complications [27].

Signore and colleagues showed that triglycerides (TG) are capable of reducing leptin transport across the blood brain barrier and modulate function of hypothalamic centers. Therefore the presence of higher plasma triglyceride levels in obese individuals is thought to be directly responsible for the lack of brain LEP concentration and a LEP resistance [20]. Association of higher plasma triglycerides (TG > 2,0 g/L, resp., > 2,8 g/L) with ischemic stroke was also proved by a large Multi-Risk Factor Interventional Trial [28]. The study involving multiethnical patients showed the correlation between hyperlipidemia and ischemic stroke, particularly in women. Considering ethnical differences, White, Afro-American, and Hispanic women had higher levels of TG and both low-density cholesterol (LDL) and high-density cholesterol (HDL) than men with ischemic stroke [28]. The benefits of having higher HDL were seen only in Asian stroke women [28]. A systematic review of epidemiological studies revealed a positive association between elevated TG levels and increased risk of stroke. The study showed a need for new large prospective studies, especially in stroke subtypes [29]. In contrast, in our assessment, LEP correlated with neither TG nor HDL in stroke patients (male and female) [30].

LEP resistance is most often associated with impaired insulin signaling [31] or insulin resistance in patients with IS [32]. Leptin is supposed to promote hyperglycemia-dependent endothelial dysfunction [33–36].

Several population-based studies have reported strong positive association of increased plasma LEP levels with pathogenesis of IS [34, 36–38]. High leptin was significantly and independently associated with IS in both men and women [35, 38]. In our assessment LEP was also reported to be a stroke risk factor for both men and women, with levels found to be three times higher in women than in men with ischemic stroke, but still higher than in controls [30]. Other authors showed that there are sex-differences in leptin activity. Increased LEP was found to be associated with significantly increased risk of ischemic stroke in older men after adjustment for age and BMI [14]. These results originate from a large prospective population study of British men aged 60–79 with no previous diagnosis of myocardial infarction, heart failure, or stroke, in which no women were included [14]. In a case-nested study Söderberg et al. reported that high leptin predicted stroke in men but not in women independently of traditional risks [24]. In a large population-based study, LEP levels were higher in women than men, showing an increased risk of nearly twofold for stroke in women when compared to men [35]. An analysis from another population-based study demonstrated that LEP is a risk factor of stroke in African-American women, independently of age, smoking, obesity

status, and hypertension. The differences in leptin signaling pathways in the central nervous system were considered to be based on a sex-specific relationship between leptin and insulin resistance [32]. In female patients severe obesity coexisted with insulin resistance.

In ischemic stroke patients intracerebral LEP deficiency caused by leptin resistance can trigger atherosclerotic/inflammatory vascular changes and vascular stiffness [39]. The study assessed arterial stiffness by carotid-femoral pulse wave velocity. It suggested that leptin represents the relationship between abdominal adiposity and arterial stiffness [39]. The correlation between leptin and intima-media thickness (IMT) was demonstrated in both men and women, independently of age and other vascular risks [40]. The leptin/adiponectin ratio could be a good predictor of IMT, as noted by Norata et al. [41]. On the other hand Gardener and colleagues have found adiponectin to be a better predictor of arterial stiffness than LEP, being inversely associated with IMT after adjustment for demographics and other vascular risks. Moreover, the relationship between adiponectin and IMT appears to be stronger among patients with diabetes [42].

Knowledge of LEP resistance, absence of its central brain activity, and consequences of LEP resistance stimulated researchers to assess LEP as a potential medicament. Signore and colleagues reported potential neuroprotective role of LEP after its intracerebral application [20]. Experimental treatment by leptin has been evaluated in animal models [43]. In mice intraperitoneal administration of leptin was found to decrease infarction volume following middle cerebral artery occlusion [43]. Leptin protection was dose-dependent and remained effective when leptin administration was delayed up to 90 min after the onset of reperfusion by recombinant tissue plasminogen-activator (rtPA). LEP was demonstrated to exhibit neuroprotective effects against ischemic stroke [43]. It seemed promising to use leptin in tandem with rtPA, administering it following rtPA treatment. In the experiment dual treatment extended the time window of efficacy for rtPA treatment and subsequently reduced reperfusion injury [43]. In another study investigation of potential role of leptin in acute IS contradicted animal studies results. Increased plasma LEP levels correlated with final larger infarction volume and leptin was evaluated as harmful in IS development, potentiated by insulin resistance. LEP efficacy was not confirmed in stroke patients treated by rtPA [33]. Further studies are required to elucidate treatment approaches by leptin in humans.

Beneficial effects of leptin in obesity and neuroendocrine/metabolic dysfunction were reported only in rare cases of human congenital leptin deficiency [44]. In affected children leptin administration resulted in sustained gradual improvement of insulin sensitivity, dyslipidemia, and weight normalization [44].

Doubled effect of LEP resistance and obesity were reported to play an important role in altered sensitivity to preventive medication. The increased levels of isoprostanes observed in visceral obesity and hyperleptinemia could be involved both in the persistent platelet activation *in vivo* and in the resistance to antiplatelet effects of aspirin [45].

Dietary options are essential for normalization of adipoendocrinal status. Restriction of leptin resistance and sensitization of leptin receptors are based on weight reduction, insulin sensitivity reactivation, and lipid profile improvement via dietary and medicament management. Fenofibrate was published to depress leptin levels [46].

3.2. Adiponectin. Adiponectin (ADI) is one of the most abundant adipokines produced by adipocytes. ADI performs a fundamental role in vascular physiology by modulating the crosstalk between endothelial cells, smooth muscle cells, leukocytes, and platelets [47]. Apart from maintaining vascular homeostasis, ADI also seems to protect from vascular injury and atherogenesis [47, 48]. Since 2001, ADI has attracted much attention because of its potential antidiabetic and anti-inflammatory activities and its potential role as a plasma biomarker of metabolic syndrome [49]. In endothelial cells, ADI was shown to increase nitric oxide (NO) production and improve endothelial-dependent vasodilation [4]. ADI was reported to suppress tumor necrosis factor α- (TNFα-) induced production of proinflammatory chemokines and adhesion molecules and inhibit cell proliferation [47]. ADI levels were found to be inversely associated with inflammatory markers [50]. ADI in circulation were decreased in obese subjects, with strongly negative correlation between plasma ADI, BMI, and total fat mass [47].

ADI appears to attenuate secretory cytokine profile of blood-brain barrier cells [51]. ADI receptors were found to be expressed on endothelial vascular cells in brain [48]. Since adiponectin may protect the endothelium from early atherosclerotic events such as the expression of adhesion molecules or the attachment of monocytic cells, hypoadiponectinemia could be linked to endothelial damage [52]. Shimabukuro et al. showed lower adiponectin levels closely related to endothelial dysfunction measured by forearm blood flow, proportionally to the severity of obesity. They considered hypoadiponectinemia to enhance endothelial dysfunction and predict future cerebro- and cardiovascular diseases [53].

Hypoadiponectinemia was associated with stroke in patients with advanced intracranial atherosclerosis, especially in men [30, 54]. Obesity-dependent hypoadiponectinemia was associated with increased common carotid IMT in young and middle-aged women [54]. Lo and colleagues reported traditional risk factors of atherosclerosis such as age, diastolic blood pressure, and triglyceride levels to be significantly associated with carotid IMT. In multivariate modeling adiponectin, age, smoking, and subcutaneous abdominal fat were also significantly related to IMT in healthy women across a range of weights. The study considered ADI associated with IMT to be a novel predictive factor for future stroke [55].

Both lower adiponectin and higher leptin showed significant associations with increased frequency of atherothrombotic (large-artery) stroke [54, 56]. ADI levels were found to be highest in cardioembolic stroke patients and lowest in intracranial atherothrombotic stroke groups [30, 54]. Patients with advanced intracranial atherosclerosis defined by ≥ 1 additional lesion outside the symptomatic arterial territory

had lower ADI levels than those with isolated intracranial atherosclerosis [54]. Chen and colleagues showed plasma adiponectin to be significantly lower in ischemic stroke patients than in healthy subjects. According to the authors ADI level remains an independent stroke risk factor [57]. They did not find differences of plasma ADI levels between patients with small- and large-artery infarction [57]. Similarly both extracranial atherothrombotic stroke and small-artery stroke patients have displayed the same levels of plasma ADI [54]. Both these results are partly in contrast with our finding. Our stroke patients showed evidently lower levels of ADI than controls, and the lowest levels were found in men with atherothrombotic stroke and in women with small-artery stroke [30].

However, several studies found no relationship of ADI levels and IS incidence in both older women [58] and men [14]. Rajpathak and colleagues also found circulating levels of ADI not to be independently associated with an increased risk of IS in postmenopausal women. In these patients ADI levels were dependent on obesity and other cardiovascular disease risk factors [59]. However, in another study, increased levels of plasma ADI correlated with increased risk of incident IS among African-American women. Adiponectin levels were significantly higher among the stroke participants with coronary heart disease compared to those without it. Harmful ADI properties have been suggested to be caused by mechanism of "adiponectin resistance" similar to leptin resistance [37]. Confusion in the literature partly relates to complexities in interpreting benefits of higher levels of adiponectin versus its pathological increase as in heart failure [60]. An alternative explanation sees adiponectin overproduced in response to vascular inflammation to counter the atherosclerotic process in arteries [48, 60].

Hypoadiponectinemia can serve as an independent predictor of mortality after IS. Efstathiou et al. reported low plasma ADI to be related to an increased risk of 5-year mortality after first-ever ischemic stroke, independently of other adverse predictors [50]. Plasma ADI levels were found to be positively associated with age, despite higher frequency of vascular risk factors in older patients [61, 62]. It has been suggested that aging and advanced stages of cardio- and cerebrovascular diseases may trigger a counterregulatory response that raises plasma ADI [60].

Carnevale and colleagues reported an inverse relationship between serum ADI and CHA2DS2-VASc in anticoagulated patients with atrial fibrillation, when CHA2DS2-VASc score determines stroke risk for patients with atrial fibrillation. Atrial fibrillation is burdened by enhanced systemic inflammation and platelet activation. In the study it was documented by increased blood levels of soluble proinflammatory marker, a CD40-receptor ligand (CD40L), and low levels of ADI even after administration of anticoagulants [63]. Low levels of ADI in patients with atrial fibrillation suggested a role of ADI to favor platelet activation *in vivo* [63].

According to other reports, lower baseline ADI concentrations inversely correlated with poor outcomes of IS independently of other adverse predictors [50]. All differences between stroke subgroups, stratified according to adiponectin levels, did not reach significance, suggesting relatively weak association of ADI with the etiology of IS [50, 58]. Correlation of reduced adiponectin levels with the studied inflammatory markers (IL-6, IL-18, TNFα, and CRP) was not very strong either [60].

The potential beneficial effects of ADI support the rationale for administration of ADI as medication. It has been demonstrated experimentally that the decreased secretion of ADI in obesity alters lipid metabolism and insulin sensitivity in the liver. However, administration of recombinant adiponectin to adiponectin-deficient obese mice fed a high-fat diet dramatically alleviated hepatomegaly, steatosis, and inflammation [64]. Exogenous administration of ADI might counteract the consequences of obesity state and activate its antiatherogenic, vasoprotective, and anticancer actions [65]. Direct supplementation of recombinant ADI in human subjects would be extremely expensive. An alternative approach is to use pharmacological or dietary intervention to enhance ADI actions in target tissues. Thiazolidinediones (rosiglitazone, pioglitazone), inhibitors of angiotensin-converted enzyme, and angiotensin II blockers reinforce positive vascular effects [49, 50]. Statins, thought to improve vascular endothelial functions, have shown ambiguous role when atorvastatin was not proved to decrease ADI levels in diabetic or prediabetic patients [66]. Metformin, a commonly used antidiabetic drug, was shown to mimic the action of ADI and may be potentially used in supplementation of ADI [49]. Other possible treatment targets might be proinflammatory cytokines and chemokines or their receptors, through the use of their agonists or monoclonal antibodies [65].

3.3. Resistin. The name resistin (RES) of this adipokine has been derived from the observation that it induced insulin resistance in mice. In contrast, in healthy individuals, comparison of plasma and CSF resistin levels showed a positive correlation of plasma RES with increasing age, but no correlation with BMI or index of insulin resistance (HOMA-IR) [67]. Furthermore, in this study of neurologically intact individuals, CSF resistin levels did not correlate with age or HOMA-IR index, and they remained unaltered by diabetic status [67]. This might be explained by the fact that RES emerges dominantly as a critical mediator of insulin resistance associated with inflammatory settings or sepsis [18]. According to other authors RES could induce similar effects to those of leptin. Increased levels of RES seem to be positively associated with atherosclerosis due to induction of endothelial cells and consequent expression of adhesion molecules, chemokines, and cell proliferation [65, 68]. Resistin can increase the risk of stroke by promoting systemic inflammation and endothelial dysfunction, both playing a significant role in atherosclerosis [68].

Rajpathak et al. found that the association between resistin and IS remained significant after adjustment for obesity as well as markers for inflammation and endothelial dysfunction. The effects of resistin on stroke risk could not be explained by the obesity-associated pathways and might involve additional unidentified biological mechanisms [59]. Resistin is supposed to mediate intensity of ischemic cerebrovascular events. Higher concentrations of resistin and TNFα were observed at the first day after IS in female patients

after comparing with controls. Follow-up revealed sustained elevation of TNFα levels and RES on the 10th day after the onset. Resistin positively correlated with TNFα and stroke severity [69].

The participation of RES in endothelial dysfunction in insulin-resistant patients related to its direct effect on endothelial cells promoting the release of endothelin-1 [70]. The proliferative effect of RES was suggested to underlie the increased incidence of restenosis after artery stenting common among diabetic patients [70].

3.4. Apelin. Apelin (APE) is a bioactive peptide that was originally identified as the endogenous ligand of the orphan G-protein-coupled receptor. In obese mice, increased levels of apelin corresponded with mild inflammation induced by obesity, characterized by an increase in macrocytes count and high TNFα levels [71].

In obesity, increased levels in plasma and adipose tissue were reported [65]. APE was associated with a positive hemodynamic profile, having a positive inotropic effect in normal and failing rat hearts [65]. Reduced apelin levels were found in patients affected by single atrial fibrillation and chronic heart failure [72, 73]. Apelin has been recently identified as an angiotensin II homologue with an impact on vasoreactivity [74]. In contrast, APE treatment was reported to have beneficial effect on aortic wall, causing its relaxation [74]. The function of APE in development of cerebrovascular disorders is not fully clear. A recently reported case-control study did not find any differences in apelin plasma levels between IS patients and healthy controls [75]. In humans apelin levels in acute IS were not elevated, contrary to leptin levels [75].

3.5. Visfatin. Visfatin (VIS) is a recently discovered adipokine, secreted mainly by visceral adipose tissue [71]. According to other authors, VIS plasma levels were shown to correlate with measures of global obesity but not visceral-fat mass or waist-to-hip ratio [18]. VIS is an insulin-mimetic adipokine that was originally discovered in liver, skeletal muscle, and bone marrow as a growth factor for B lymphocyte precursors. It was upregulated in models of acute lung injury and sepsis. Circulating visfatin levels closely correlated with white adipose tissue accumulation. VIS synthesis seems to be regulated by several factors, including glucocorticoids, TNFα, interleukin-6 (IL-6), and growth hormone [71]. Relationship of VIS and type 2 diabetes was also described [18].

Role of VIS in stroke has been studied and reported by several authors. They showed increased plasma VIS levels in acute IS [75, 76]. After adjustment for diabetes, hypertension, dyslipidemia, and age, visfatin was assessed as independent predictor of acute IS [75]. Predictive role of VIS was demonstrated in 6-month follow-up [76].

VIS seems to be a prognostic factor of cardiovascular mortality [75]. Visfatin seems to have a key role in plaque destabilization, associated with its increased expression in macrophages of human unstable carotic and coronary atherosclerosis. In microarray VIS gene was markedly enhanced in carotic plaques in symptomatic individuals compared with plaques in asymptomatic individuals [77]. The relationship between inflammatory markers and VIS levels in patients with symptomatic carotic atherosclerosis supported an inflammatory role of VIS as a mediator in carotic atherosclerosis [77] and future stroke. Higher plasma VIS levels were reported in patients with acute IS associated with symptomatic carotid stenosis (>50%) [75].

4. Conclusion

Increase in rates of overweight and obese people in industrial countries has awakened the interest in the role of adipose tissue in metabolic and hormonal balance in the human body. Adipokines, hormones released by adipose tissue, are divided into two groups. One includes those with anti-inflammatory, antidiabetic, and anabolic functions such as adiponectin. The other includes hormones with proinflammatory, prodiabetic, and catabolic functions, for example, leptin, resistin, visfatin, and probably apelin. Now we know that adipokines regulate metabolism via hypothalamic receptors, and they also function as cytokines, being linked to innate immunity. Moreover, adipokines that appear to regulate endovascular compartment can be crucial for later development of arterial stiffness. Increase in adipose tissue disturbs the balance in adipokine production and activity. Participation of certain adipokines in pathomechanisms of ischemic stroke has been proved by independent studies. However, studies have shown them to be both obesity-dependent and obesity-independent factors. It is obvious that there are differences between populations in prevalence of obesity, as well as in diet, calorie intake, and level of physical activity. Interindividual variability of impact of adipokines on target tissues of subjects in high risk of IS poses the question about the role of genetic background of adipocytes' reactivity to environmental influence. Precise mechanism of functioning of adipokines is probably dependent on other not fully known factors. Despite the volume of current information about adipokines, our knowledge is still incomplete and requires further studies. Even so, it is possible to link excessive cumulation of adipose tissue to ischemic stroke risk.

Abbreviations

ADI:	Adiponectin
APE:	Apelin
BMI:	Body mass index
CD40L:	Ligand of a CD40 receptor, expressed by T cells under inflammatory conditions
CRP:	C-reactive protein
CSF:	Cerebrospinal fluid
CVD:	Cardiovascular diseases
HDL:	High-density cholesterol
HOMA/IR:	Index of insulin resistance
CHA2DS2-VASc:	A stroke risk score for patients with atrial fibrillation
IL-6:	Interleukin-6
IL-18:	Interleukin-18
IMT:	Intima/media thickness
IS:	Ischemic stroke
LDL:	Low-density cholesterol

LEP: Leptin
NO: Nitric oxide
RES: Resistin
TNFα: Tumor necrosis factor α
TG: Triglycerides
rtPA: Recombinant tissue plasminogen-activator
VIS: Visfatin
WC: Waist circumference.

Conflict of Interests

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

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References

[1] T. B. Levine and A. B. Levine, "Adipose tissue and overweight," in *Metabolic Syndrome and Cardiovascular Disease*, S. Pioli, Ed., pp. 264–278, Elsevier, New York, NY, USA, 2nd edition, 2006.

[2] A. F. Tarantal and L. Berglund, "Obesity and lifespan health—importance of the fetal environment," *Nutrients*, vol. 6, no. 4, pp. 1725–1736, 2014.

[3] P. Trayhurn and J. H. Beattie, "Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ," *Proceedings of the Nutrition Society*, vol. 60, no. 3, pp. 329–339, 2001.

[4] G. Frühbeck, "Vasoactive factors and inflammatory mediators produced in adipose tissue," in *Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease*, G. Fantuzi and T. Mazzoneeds, Eds., pp. 63–77, Humana Press, Totowa, NJ, USA, 2007.

[5] G. Matarese, "Leptin in autoimmune diseases," in *Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease*, G. Fantuzzi and T. Mazzone, Eds., pp. 91–100, Humana Press, Totowa, NJ, USA, 2007.

[6] B. N. Rocha, P. L. Scalzo, I. G. Barbosa et al., "Circulating levels of adipokines in Parkinson's disease," *Journal of the Neurological Sciences*, vol. 339, no. 1-2, pp. 64–68, 2014.

[7] D. R. Gustafson, "Adiposity hormones and dementia," *Journal of the Neurological Sciences*, vol. 299, no. 1-2, pp. 30–34, 2014.

[8] B. Boden-Albala, R. L. Sacco, H.-S. Lee et al., "Metabolic syndrome and ischemic stroke risk. Northern Manhattan Study," *Stroke*, vol. 39, no. 1, pp. 30–35, 2008.

[9] M. Bosnar-Puretič, V. Bašič-Kes, M. J. Jurašič, I. Zavoreo, and V. Demarin, "The association of obesity and cerebrovascular disease in young adults—a pilot study," *Acta Clinica Croatica*, vol. 48, no. 3, pp. 295–298, 2009.

[10] H. J. Milionis, E. Rizos, J. Goudevenos, K. Seferiadis, D. P. Mikhailidis, and M. S. Elisaf, "Components of the metabolic syndrome and risk for first-ever acute ischemic nonembolic stroke in elderly subjects," *Stroke*, vol. 36, no. 7, pp. 1372–1376, 2005.

[11] S.-H. Suk, R. L. Sacco, B. Boden-Albala et al., "Abdominal obesity and risk of ischemic stroke: the Northern Manhattan Stroke Study," *Stroke*, vol. 34, no. 7, pp. 1586–1592, 2003.

[12] Y. Winter, S. Rohrmann, J. Linseisen et al., "Contribution of obesity and abdominal fat mass to risk of stroke and transient ischemic attacks," *Stroke*, vol. 39, no. 12, pp. 3145–3151, 2008.

[13] T. Kurth, J. M. Gaziano, K. Berger et al., "Body mass index and the risk of stroke in men," *Archives of Internal Medicine*, vol. 162, no. 22, pp. 2557–2562, 2002.

[14] S. G. Wannamethee, A. G. Shaper, P. H. Whincup, L. Lennon, and N. Sattar, "Adiposity, adipokines, and risk of incident stroke in older men," *Stroke*, vol. 44, no. 1, pp. 3–8, 2013.

[15] J. R. Kizer, M. L. Biggs, J. H. Ix et al., "Measures of adiposity and future risk of ischemic stroke and coronary heart disease in older men and women," *American Journal of Epidemiology*, vol. 173, no. 1, pp. 10–25, 2011.

[16] K. K. Andersen and T. S. Olsen, "The obesity paradox in stroke: lower mortality and lower risk of readmission for recurrent stroke in obese stroke patients," *International Journal of Stroke*, vol. 10, no. 1, pp. 99–104, 2015.

[17] B. Zebekakis, T. Nawrota, L. Thijsa et al., "Obesity is associated with increased arterial stiffness from adolescence until old age," *Journal of Hypertension*, vol. 23, no. 10, pp. 1839–1846, 2005.

[18] G. Frühbeck, J. Gómez-Ambrosi, F. J. Muruzábal, and M. A. Burrell, "The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 280, no. 6, pp. 827–847, 2001.

[19] M. B. Jackson and R. S. Ahima, "Leptin," in *Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease*, G. Fantuzzi and T. Mazzone, Eds., pp. 35–46, Humana Press Inc, Totowa, NJ, USA, 2007.

[20] A. P. Signore, F. Zhang, Z. Weng, Y. Gao, and J. Chen, "Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials," *Journal of Neurochemistry*, vol. 106, no. 5, pp. 1977–1990, 2008.

[21] S. S. Martin, A. Quasim, and M. P. Reilly, "Leptin resistance," *Journal of the American College of Cardiology*, vol. 52, pp. 1201–1210, 2008.

[22] J. Kaprio, J. Eriksson, M. Lehtovirta, M. Koskenvuo, and J. Tuomilehto, "Heritability of leptin levels and the shared genetic effects on body mass index and leptin in adult Finnish twins," *International Journal of Obesity*, vol. 25, no. 1, pp. 132–137, 2001.

[23] J. E. Hall, D. W. Jones, J. J. Kuo, A. da Silva, L. S. Tallam, and J. Liu, "Impact of the obesity epidemic on hypertension and renal disease," *Current Hypertension Reports*, vol. 5, no. 5, pp. 386–392, 2003.

[24] S. Söderberg, B. Stegmayr, H. Stenlund et al., "Leptin, but not adiponectin, predicts stroke in males," *Journal of Internal Medicine*, vol. 256, no. 2, pp. 128–136, 2004.

[25] C. de Haro Moraes, V. N. Figueiredo, A. P. C. de Faria et al., "High-circulating leptin levels are associated with increased blood pressure in uncontrolled resistant hypertension," *Journal of Human Hypertension*, vol. 27, no. 4, pp. 225–230, 2013.

[26] S. Konstantinides, K. Schäfer, S. Koschnick, and D. J. Loskutoff, "Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity," *Journal of Clinical Investigation*, vol. 108, no. 10, pp. 1533–1540, 2001.

[27] M. T. Guagnano, M. Romano, A. Falco et al., "Leptin increase is associated with markers of the hemostatic system in obese

healthy women," *Journal of Thrombosis and Haemostasis*, vol. 1, no. 11, pp. 2330–2334, 2003.

[28] T. Gezmu, D. Schneider, K. Demissie, Y. Lin, C. Giordano, and M. S. Sgizzi, "Lipid profiles and ischemic stroke risk: variations by sex within racial/ethnic groups," *International Journal of Women's Health*, vol. 6, no. 1, pp. 585–594, 2014.

[29] J. Labreuche, P.-J. Touboul, and P. Amarenco, "Plasma triglyceride levels and risk of stroke and carotid atherosclerosis: a systematic review of the epidemiological studies," *Atherosclerosis*, vol. 203, no. 2, pp. 331–345, 2009.

[30] E. Kantorová, M. Chomová, E. Kurča et al., "Leptin, adiponectin and ghrelin, new potential mediators of ischemic stroke," *Neuroendocrinology Letters*, vol. 32, no. 5, pp. 716–721, 2011.

[31] M. J. Mcneely, S. D. Chessler, E. J. Boyko et al., "Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans," *Diabetes Care*, vol. 22, no. 1, pp. 65–70, 1999.

[32] J. Liu, K. R. Butler, S. G. Buxbaum, J. H. Sung, B. W. Campbell, and H. A. Taylor, "Leptinemia and its association with stroke and coronary heart disease in the Jackson Heart Study," *Clinical Endocrinology*, vol. 72, no. 1, pp. 32–37, 2010.

[33] A. I. Calleja, E. Cortijo, P. García-Bermejo et al., "Blood biomarkers of insulin resistance in acute stroke patients treated with intravenous thrombolysis: temporal profile and prognostic value," *Journal of Diabetes Research & Clinical Metabolism*, vol. 2, no. 1, article 2, 2013.

[34] C. Savopoulos, K. Michalakis, M. Apostolopoulou, A. Miras, and A. Hatzitolios, "Adipokines and stroke: a review of the literature," *Maturitas*, vol. 70, no. 4, pp. 322–327, 2011.

[35] J. Sierra-Johnson, A. Romero-Corral, F. Lopez-Jimenez et al., "Relation of increased leptin concentrations to history of myocardial infarction and stroke in the United States population," *The American Journal of Cardiology*, vol. 100, no. 2, pp. 234–239, 2007.

[36] D. E. Flanagan, J. C. Vaile, G. W. Petley et al., "Gender differences in the relationship between leptin, insulin resistance and the autonomic nervous system," *Regulatory Peptides*, vol. 140, no. 1-2, pp. 37–42, 2007.

[37] A. Bidulescu, J. Liu, Z. Chen et al., "Associations of adiponectin and leptin with incident coronary heart disease and ischemic stroke in African Americans: the Jackson Heart Study," *Front in Endocrin*, vol. 1, no. 16, pp. 1–8, 2013.

[38] S. Söderberg, B. Stegmayr, C. Ahlbeck-Glader, L. Slunga-Birgander, B. Ahrén, and T. Olsson, "High leptin levels are associated with stroke," *Cerebrovascular Diseases*, vol. 15, no. 1-2, pp. 63–69, 2003.

[39] B. G. Windham, M. E. Griswold, S. M. Farasat et al., "Influence of leptin, adiponectin, and resistin on the association between abdominal adiposity and arterial stiffness," *American Journal of Hypertension*, vol. 23, no. 5, pp. 501–507, 2010.

[40] M. Ciccone, R. Vettor, N. Pannacciulli et al., "Plasma leptin is independently associated with the intima-media thickness of the common carotid artery," *International Journal of Obesity*, vol. 25, no. 6, pp. 805–810, 2001.

[41] G. D. Norata, S. Raselli, L. Grigore et al., "Leptin:adiponectin ratio is an independent predictor of intima media thickness of the common carotid artery," *Stroke*, vol. 38, no. 10, pp. 2844–2846, 2007.

[42] H. Gardener, C. Sjoberg, M. Crisby et al., "Adiponectin and carotid intima-media thickness in the Northern Manhattan Study," *Stroke*, vol. 43, no. 4, pp. 1123–1125, 2012.

[43] F. Zhang, S. P. Wang, A. P. Signore, and J. Chen, "Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia," *Stroke*, vol. 38, no. 8, pp. 2329–2336, 2007.

[44] I. Sadaf Farooqi, G. Matarese, G. M. Lord et al., "Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency," *Journal of Clinical Investigation*, vol. 110, no. 8, pp. 1093–1103, 2002.

[45] G. Anfossi, I. Russo, G. Doronzo, A. Pomero, and M. Trovati, "Adipocytokines in atherothrombosis: Focus on platelets and vascular smooth muscle cells," *Mediators of Inflammation*, vol. 2010, Article ID 174341, 26 pages, 2010.

[46] T. Damci, S. Tatliagac, Z. Osar, and K. Ilkova, "Fenofibrate treatment is associated with better glycemic control and lower serum leptin and insulin levels in type 2 diabetic patients with hypertriglyceridemia," *European Journal of Internal Medicine*, vol. 14, no. 6, pp. 357–360, 2003.

[47] A. G. Vaiopoulos, K. Marinou, C. Christodoulides, and M. Koutsilieris, "The role of adiponectin in human vascular physiology," *International Journal of Cardiology*, vol. 155, no. 2, pp. 188–193, 2012.

[48] M. Nishimura, Y. Izumiya, A. Higuchi et al., "Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase-dependent mechanisms," *Circulation*, vol. 117, no. 2, pp. 216–223, 2008.

[49] A. Xu, Y. Wang, and K. S. L. Lam, "Adiponectine," in *Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease*, G. Fantuzzi and T. Mazzone, Eds., pp. 47–59, Humana Press, Totowa, NJ, USA, 2007.

[50] S. P. Efstathiou, D. I. Tsioulos, A. G. Tsiakou, Y. E. Gratsias, A. V. Pefanis, and T. D. Mountokalakis, "Plasma adiponectin levels and five-year survival after first-ever ischemic stroke," *Stroke*, vol. 36, no. 9, pp. 1915–1919, 2005.

[51] J. Spranger, S. Verma, I. Göhring et al., "Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells," *Diabetes*, vol. 55, no. 1, pp. 141–147, 2006.

[52] N. Ouchi, J. L. Parker, J. J. Lugus, and K. Walsh, "Adipokines in inflammation and metabolic disease," *Nature Reviews Immunology*, vol. 11, no. 2, pp. 85–97, 2011.

[53] M. Shimabukuro, N. Higa, T. Asahi et al., "Hypoadiponectinemia is closely linked to endothelial dysfunction in man," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 7, pp. 3236–3240, 2003.

[54] O. Y. Bang, J. L. Saver, B. Ovbiagele, Y. J. Choi, S. R. Yoon, and K. H. Lee, "Adiponectin levels in patients with intracranial atherosclerosis," *Neurology*, vol. 68, no. 22, pp. 1931–1937, 2007.

[55] J. Lo, S. E. Dolan, J. R. Kanter et al., "Effects of obesity, body composition, and adiponectin on carotid intima-media thickness in healthy women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 5, pp. 1677–1682, 2006.

[56] B. J. Kim, S.-H. Lee, W.-S. Ryu, C. K. Kim, and B.-W. Yoon, "Adipocytokines and ischemic stroke: differential associations between stroke subtypes," *Journal of the Neurological Sciences*, vol. 312, no. 1-2, pp. 117–122, 2012.

[57] M. P. Chen, J. C. Tsai, F. M. Chung et al., "Hypoadiponectinemia is associated with ischemic cerebrovascular disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, pp. 821–828, 2005.

[58] A. D. Ogorodnikova, S. Wassertheil-Smoller, P. Mancuso et al., "High-molecular-weight adiponectin and incident ischemic

stroke in postmenopausal women: a women's health initiative study," *Stroke*, vol. 41, no. 7, pp. 1376–1381, 2010.

[59] S. N. Rajpathak, R. C. Kaplan, S. Wassertheil-Smoller et al., "Resistin, but not adiponectin and leptin, is associated with the risk of ischemic stroke among postmenopausal women: results from the women's health initiative," *Stroke*, vol. 42, no. 7, pp. 1813–1820, 2011.

[60] D. J. Stott, P. Welsh, A. Rumley et al., "Adipocytokines and risk of stroke in older people: a nested case—control study," *International Journal of Epidemiology*, vol. 38, no. 1, pp. 253–261, 2009.

[61] M. Cnop, P. J. Havel, K. M. Utzschneider et al., "Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex," *Diabetologia*, vol. 46, no. 4, pp. 459–469, 2003.

[62] M. Adamczak, E. Rzepka, J. Chudek et al., "Ageing and plasma adiponectin concentration in apparently healthy males and females," *Clinical Endocrinology*, vol. 62, no. 1, pp. 114–118, 2005.

[63] R. Carnevale, D. Pastori, M. Peruzzi et al., "Total adiponectin is inversely associated with platelet activation and CHA_2DS_2-VASc score in anticoagulated patients with atrial fibrillation," *Mediators of Inflammation*, vol. 2014, Article ID 908901, 6 pages, 2014.

[64] D. Estadella, C. M. da Penha Oller do Nascimento, L. M. Oyama, E. B. Ribeiro, A. R. Dâmaso, and A. de Piano, "Lipotoxicity: effects of dietary saturated and transfatty acids," *Mediators of Inflammation*, vol. 2013, Article ID 137579, 13 pages, 2013.

[65] C. R. Balistreri, C. Caruso, and G. Candore, "The role of adipose tissue and adipokines in obesity-related inflammatory diseases," *Mediators of Inflammation*, vol. 2010, Article ID 802078, 19 pages, 2010.

[66] G. K. Shetty, P. A. Economides, E. S. Horton, C. S. Mantzoros, and A. Veves, "Circulating adiponectin and resistin levels in relation to metabolic factors, inflammatory markers, and vascular reactivity in diabetic patients and subjects at risk for diabetes," *Diabetes Care*, vol. 27, no. 10, pp. 2450–2457, 2004.

[67] K. Kos, A. L. Harte, N. F. da Silva et al., "Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus," *The Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 3, pp. 1129–1136, 2007.

[68] M. P. Reilly, M. Lehrke, M. L. Wolfe, A. Rohatgi, M. A. Lazar, and D. J. Rader, "Resistin is an inflammatory marker of atherosclerosis in humans," *Circulation*, vol. 111, no. 7, pp. 932–939, 2005.

[69] J. Kochanowski, M. Grudniak, A. Baranowska-Bik et al., "Resistin levels in women with ischemic stroke," *Neuroendocrinology Letters*, vol. 33, no. 6, pp. 603–607, 2012.

[70] S. Verma, S.-H. Li, C.-H. Wang et al., "Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction," *Circulation*, vol. 108, no. 6, pp. 736–740, 2003.

[71] F. Lago, C. Dieguez, J. Gómez-Reino, and O. Gualillo, "Adipokines as emerging mediators of immune response and inflammation," *Nature Clinical Practice Rheumatology*, vol. 3, no. 12, pp. 716–724, 2007.

[72] K. S. Chong, R. S. Gardner, J. J. Morton, E. A. Ashley, and T. A. McDonagh, "Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure," *European Journal of Heart Failure*, vol. 8, no. 4, pp. 355–360, 2006.

[73] P. T. Ellinor, A. F. Low, and C. A. MacRae, "Reduced apelin levels in lone atrial fibrillation," *European Heart Journal*, vol. 27, no. 2, pp. 222–226, 2006.

[74] J.-C. Zhong, X.-Y. Yu, Y. Huang, L.-M. Yung, C.-W. Lau, and S.-G. Lin, "Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice," *Cardiovascular Research*, vol. 74, no. 3, pp. 388–395, 2007.

[75] N. P. E. Kadoglou, G. Fotiadis, V. Lambadiari, E. Maratou, G. Dimitriadis, and C. D. Liapis, "Serum levels of novel adipokines in patients with acute ischemic stroke: potential contribution to diagnosis and prognosis," *Peptides*, vol. 57, pp. 12–16, 2014.

[76] C.-G. Yin, L. Jiang, B. Tang, H. Zhang, Q. Qian, and G.-Z. Niu, "Prognostic significance of plasma visfatin levels in patients with ischemic stroke," *Peptides*, vol. 42, pp. 101–104, 2013.

[77] T. B. Dahl, A. Yndestad, M. Skjelland et al., "Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization," *Circulation*, vol. 115, no. 8, pp. 972–980, 2007.

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Research Article

Paraoxonase 2 Induces a Phenotypic Switch in Macrophage Polarization Favoring an M2 Anti-Inflammatory State

Marie Koren-Gluzer,[1] Mira Rosenblat,[1] and Tony Hayek[1,2]

[1] *The Lipid Research Laboratory, Technion Faculty of Medicine, The Rappaport Family Institute for Research in the Medical Sciences, and Rambam Health Care Campus, 31096 Haifa, Israel*
[2] *Internal Medicine E Department, Rambam Health Care Campus, 31096 Haifa, Israel*

Correspondence should be addressed to Tony Hayek; t_hayek@rambam.health.gov.il

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Inflammatory processes are involved in atherosclerosis development. Macrophages play a major role in the early atherogenesis, and they are present in the atherosclerotic lesion in two phenotypes: proinflammatory (M1) or anti-inflammatory (M2). Paraoxonase 2 (PON2) is expressed in macrophages, and it was shown to protect against atherosclerosis. Thus, the aim of our study was to analyze the direct effect of PON2 on macrophage inflammatory phenotypes. Ex vivo studies were performed with murine peritoneal macrophages (MPM) harvested from control C57BL/6 and PON2-deficient (PON2KO) mice. PON2KO MPM showed an enhanced proinflammatory phenotype compared to the control, both in the basal state and following M1 activation by IFNγ and lipopolysaccharide (LPS). In parallel, PON2KO MPM also showed reduced anti-inflammatory responses in the basal state and also following M2 activation by IL-4. Moreover, the PON2-null MPM demonstrated enhanced phagocytosis and reactive oxygen species (ROS) production in the basal state and following M1 activation. The direct effect of PON2 was shown by transfecting human PON2 (hPON2) into PON2KO MPM. PON2 transfection attenuated the macrophages' response to M1 activation and enhanced M2 response. These PON2 effects were associated with attenuation of macrophages' abilities to phagocyte and to generate ROS. We conclude that PON2 promotes an M1 to M2 switch in macrophage phenotypes.

1. Introduction

Inflammatory processes are involved in atherosclerosis development [1]. Macrophages play a major role in the early atherogenesis [2, 3], and they are present in the atherosclerotic lesion in two phenotypes: proinflammatory (M1) or anti-inflammatory (M2) [4–7]. In the plaque, serum lipids, serum lipoproteins, and various pro- or anti-inflammatory stimuli such as cytokines, chemokines, and small bioactive molecules could greatly influence the macrophage phenotype inducing switch towards more proinflammatory or anti-inflammatory properties. The M1/M2 balance in plaques is dynamic, with M1 predominating in disease progression and M2 in regression [8–11]. In vitro, the classic macrophage activation M1 is caused by the cytokine IFNγ in combination with lipopolysaccharide (LPS), whereas the alternative macrophage activation (M2) is caused by the cytokines IL-4 and IL-13 [12–14]. Recently, it was shown both in vitro and in vivo that pomegranate polyphenols directly suppress macrophage inflammatory responses and promote macrophage phenotype switch from M1 to M2 [15]. Understanding the mechanisms of macrophage plasticity and resolving functional characteristics of distinct macrophage phenotypes should help in the development of new strategies for treatment of chronic inflammation in atherosclerosis [16, 17]. Paraoxonase 2 (PON2) is an intracellular enzyme that is widely expressed in almost every tissue including macrophages [18, 19]. Several studies indicate a major role for PON2 in attenuation of atherosclerosis development [20–23]. PON2's antiatherogenic properties include protection of arterial wall cells from oxidative stress and apoptosis [18, 19, 24–26] and also from triglyceride accumulation [27]. PON2 is expressed also in immune cells, and it hydrolyzes 3OC (12)-HSL, a quorum-sensing molecule produced by

gram-negative microbial pathogens [28, 29]. PON2 plays an important role in hepatic insulin signalling and underscores the influence of macrophage-mediated inflammatory response on hepatic insulin sensitivity [30]. The mechanisms contributing to the generation of proinflammatory or anti-inflammatory macrophage phenotype during atherosclerosis development are not fully understood. Paraoxonase 1 (PON1), another member of the paraoxonase gene family that protects against atherosclerosis development [31], is not expressed in macrophages [18], and it is present in the circulation associated with HDL. A recent study, using peritoneal macrophages or bone marrow-derived macrophages from PON1 transgenic mice which express human PON1, an artificial nonphysiological status, demonstrated that PON1 reduces the inflammatory response to M1 stimulation [32]. Since PON2 possesses different antiatherogenic properties than PON1 and since PON2 is normally expressed in human and mouse macrophages, the aim of the present study was to assess the direct effect of PON2 on the polarization of macrophages. For this purpose we used MPM from PON2KO mice in comparison to control C57BL/6 MPM. In addition, we transfected human PON2 into the PON2KO MPM. The effect of PON2 on both M1 and M2 activation was analyzed.

2. Materials and Methods

2.1. Chemicals. Phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS) (heat-inactivated at 56°C for 30 min), penicillin, streptomycin, L-glutamine, and sodium pyruvate were from Biological Industries (Beth Haemek, Israel). 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) and lipopolysaccharide (LPS) from *Salmonella typhimurium* were from Sigma Aldrich (St. Louis, MO, USA). Recombinant murine interferon-gamma (IFNγ) and interleukin-4 (IL-4) were from PeproTech (Rocky Hill, NJ, USA).

2.2. Animals. Five-week-old male C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). The PON2-deficient mice on the C57BL/6 background were generated as previously described [20] and were a generous gift from Dr. Srinivasa T. Reddy, Atherosclerosis Research Unit, Division of Cardiology, Department of Medicine at UCLA, Los Angeles, CA, USA. We used only male mice in our study. The mice were bred under pathogen-free conditions in the animal facility of the Faculty of Medicine (Technion-Israel Institute of Technology, Haifa, Israel). The research was conducted in conformity with the Public Health Service Policy on Human Care and Use of Laboratory Animals and approved by the Committee for Supervision of Animal Experiments, the Technion-Israel Institute of Technology, Haifa, Israel.

2.3. Mouse Peritoneal Macrophages (MPM). MPM were prepared from mice that were sacrificed by overanesthesia, and MPM were harvested prior to removal of the aorta, from the peritoneal fluid, 3 days after intraperitoneal injection into each mouse of 3 mL of thioglycolate (24 g/L) in saline. The cells (10–20 $\times$ 10^6/mouse) were washed and centrifuged three times with phosphate buffered saline (PBS) at 1000 g for 10 min and then resuspended to 10^9/L in DMEM containing 15% horse serum (heat-inactivated at 56°C for 30 min), 0.1 U/L penicillin, 100 mg/L streptomycin, and 2 mmol/L-glutamine. The cell suspension was dispensed into 35 mm plastic Petri dishes and incubated in a humidified incubator (5% CO_2, 95% air) for 2 h. The dishes were washed once with 5 mL DMEM to remove nonadherent cells, and the monolayer was then incubated under similar conditions for 18 h, prior to the beginning of the experiment.

2.4. Proinflammatory (M1) and Anti-Inflammatory (M2) Activation. Cells were activated with either LPS (100 ng/mL) and IFNγ (20 ng/mL) or IL-4 (20 ng/mL) for increased periods of times up to 30 hours. Cytokine secretion reached a maximal level after 16 hours. Thus, incubation for 16 hours was employed in all experiments for measuring cytokine secretion and mRNA expression.

2.5. Cytokine Secretion. The levels of cell-released TNFα, IL-6, and IL-10 were measured in the collected incubation medium and determined by using DuoSet ELISA development systems (R&D Systems, Inc., Minneapolis, MN, USA) following manufacturer's instructions. All reactions were run at room temperature. Optical density was determined and analyzed by the KC4 microplate reader (BIO-TEK, Instruments Inc., Winooski, VT, USA).

2.6. Cytokines, Arginase I, and Arginase II mRNA Expression. RNA was extracted from cells using MasterPure RNA purification kit (Epicentre Biotechnologies, Madison, WI, USA). cDNA was prepared using Verso cDNA kit (Thermo Scientific, Epsom, UK). Primers and probes for genes were designed by Primer Design, Southampton, UK, using ABsolute Blue QPCR ROX mix (Thermo Scientific), and expression was determined by quantitative real-time PCR with a Rotor-Gene 6000 amplification detection system following manufacturer's instructions.

2.7. Assessment of Phagocytosis. Phagocytosis tests were performed using 2 μm fluorescent blue-green latex beads (Sigma Aldrich, St. Louis, MO, USA). Latex beads were opsonized by incubation in 1 : 1 ratio of latex bead and mice serum for 2 hours at 37°C. Opsonized latex beads were then suspended in 1 mL DMEM supplemented with 5% FCS and added to macrophages for 4 h at 37°C. Macrophages were washed twice with PBS to remove nonphagocytosed material, scraped, and then analyzed for the uptake of FITC-coupled beads by FACS.

2.8. Detection of Reactive Oxygen Species (ROS) Production. ROS production was assayed through the oxidation of DCFH-DA. MPM cells were seeded on 12-well plate in DMEM supplemented with 5% FCS and incubated at 37°C/5% CO_2 until reaching 50% confluency. M1 activation cells were washed with PBS, suspended in 100 μL of PBS, and incubated with 10 μmol/L DCFH-DA for 30 min at 37°C. Reaction was stopped by washing the cells with PBS. Seven

measurements of cellular fluorescence determined by FACS were done at 510 to 540 nm, after excitation of the cells at 488 nm with an argon ion laser. Ten thousand events were registered for each experiment. Cellular fluorescence was measured using FACS analysis with a FACS Calibur flow Cytometer (Becton-Dickenson, San Jose, CA), and data were analyzed using CellQuest software (BD Biosciences, San Jose, CA, USA).

2.9. Transfection of MPM with Human PON2. MPM from PON2-deficient mice were transfected with 2 μg/mL of plasmid DNA (human PON2 (hPON2) gene in pcDNA3.1+ plasmid or with the empty pcDNA3.1+ plasmid, a generous gift from Dr. Dragomir Draganov, University of Michigan, Ann Arbor, MI) in Dulbecco's modified Eagle's medium containing 3 μL/mL of FuGene 6 reagent (Roche).

2.10. Statistical Analysis. Each experiment was performed in triplicate, and each individual experiment was replicated three times ($n = 3$) in order to achieve statistical significance meaning. Statistical analyses used Student's t-test for comparing differences between two groups and one-way ANOVA, followed by the Student-Newman-Keuls test, for comparing differences between multiple groups. Results are given as mean ± SD.

3. Results

3.1. MPM from PON2-Deficient Mice (PON2KO) Display Enhanced Proinflammatory Activation. In MPM harvested from PON2KO mice, spontaneous secretion of TNFα and IL-6 (Figure 1(a)) and basal mRNA expression (Figure 1(b)) of TNFα and IL-6 were significantly elevated by 1.3-, 2-, 2.8-, and 4.5-fold, respectively, in comparison to MPM from C57BL/6 control mice. M1 activation induced by IFNγ and LPS stimulated TNFα and IL-6 secretion (Figure 1(c)) and expression (Figure 1(d)) in comparison to unstimulated cells (Figures 1(a) and 1(b)) in MPM from both mice groups. However, both TNFα and IL-6 secretion (Figure 1(c)) and mRNA (Figure 1(d)) expression in response to M1 activation were significantly increased by 1.5- and 1.3-fold and by 3- and 1.4-fold, respectively, in PON2KO MPM in comparison to control MPM.

In addition, arginase II mRNA expression (expressed by M1 macrophages) was 4.5-fold higher in unstimulated PON2KO MPM cells as compared to MPM obtained from control mice (Figure 1(e)). M1 activation of MPM from both mice groups stimulated arginase II mRNA expression in comparison to unstimulated cells. However, in M1-activated PON2KO MPM, arginase II mRNA expression increased 2.2-fold as compared to control MPM. Collectively, these results suggest that PON2 expression in macrophages is associated with protection against proinflammatory stimuli.

3.2. MPM from PON2KO Display Reduced Anti-Inflammatory Activation. Next, MPM harvested from control and PON2KO mice were subjected to M2 activation induced by IL-4. Spontaneous IL-10 secretion (Figure 2(a)) and basal mRNA expression (Figure 2(b)) were significantly reduced by 67% and 50%, respectively, in PON2KO MPM in comparison to control MPM. M2 activation stimulated IL-10 secretion (Figure 2(c)) and mRNA expression (Figure 2(d)) in comparison to unstimulated cells in MPM from both mice groups (Figures 2(a) and 2(b)). However, in response to M2 activation, both IL-10 secretion (Figure 2(c)) and mRNA expression (Figure 2(d)) were significantly lower in PON2KO MPM versus control MPM by 49% and 33%, respectively.

In parallel, the basal arginase I mRNA expression (Figure 2(e)) in PON2KO MPM was significantly lower 1.5-fold as compared to control MPM. After M2 stimulation it was 3-fold lower in PON2KO MPM compared to the level of expression in control MPM. Taken altogether, these results indicate that PON2 expression by macrophages not only inhibits macrophage response to classical M1 activation, but also promotes macrophage polarization toward the M2 alternative phenotype.

3.3. PON2 Modulates Macrophage M1 Functional Phenotype. Next, we determined whether PON2 affects macrophage functions that are operative in M1, including ROS production and phagocytosis. We measured the phagocytosis of FITC-labeled latex beads by M1- or M2-activated MPM from control and PON2KO mice. Figure 3(a) demonstrates an increment in latex particle phagocytosis in PON2KO MPM compared to control MPM in unstimulated cells as well as M1-activated macrophages by 1.3- and 1.7-fold, respectively. Following M2 activation, macrophage phagocytosis ability was also enhanced in PON2KO MPM versus control MPM by 1.24-fold and was similar to unstimulated cells (Figure 3(a)). In parallel, the level of ROS production (Figure 3(b)) from resting unstimulated macrophages was 1.7-fold elevated in PON2KO MPM compared to control MPM. M1 activation induced a remarkable increase in ROS production in comparison to resting unstimulated macrophages. ROS production in M1 stimulated MPM increased 1.6-fold in PON2KO MPM as compared to control MPM (Figure 3(b)). M2 activation did not affect MPM ROS production as compared to unstimulated cells. These results suggest that macrophage PON2 inhibits M1-induced ROS formation and phagocytosis.

3.4. Human PON2 (hPON2) Promotes Macrophage Polarization toward an Anti-Inflammatory M2 Phenotype. To further confirm the direct inhibitory effect of PON2 on macrophage inflammatory response, we reintroduced PON2 to PON2KO MPM by transfecting them with a vector containing the hPON2 plasmid or with an empty plasmid (EP) as a control. The expression of hPON2 in the transfected cells was confirmed by quantitative PCR (inset of Figure 4(a)). After transfection (48 hours), the cells were untreated or activated toward M1 with LPS + IFNγ or toward M2 with IL-4, and we measured M1 and M2 markers accordingly. Compared to EP, transfection of hPON2 to PON2KO MPM significantly inhibited MPM inflammatory responses as reflected by a decrease in IL-6 (Figure 4(a)) and in arginase II mRNA expression (Figure 4(b)), in unstimulated cells by 89% and

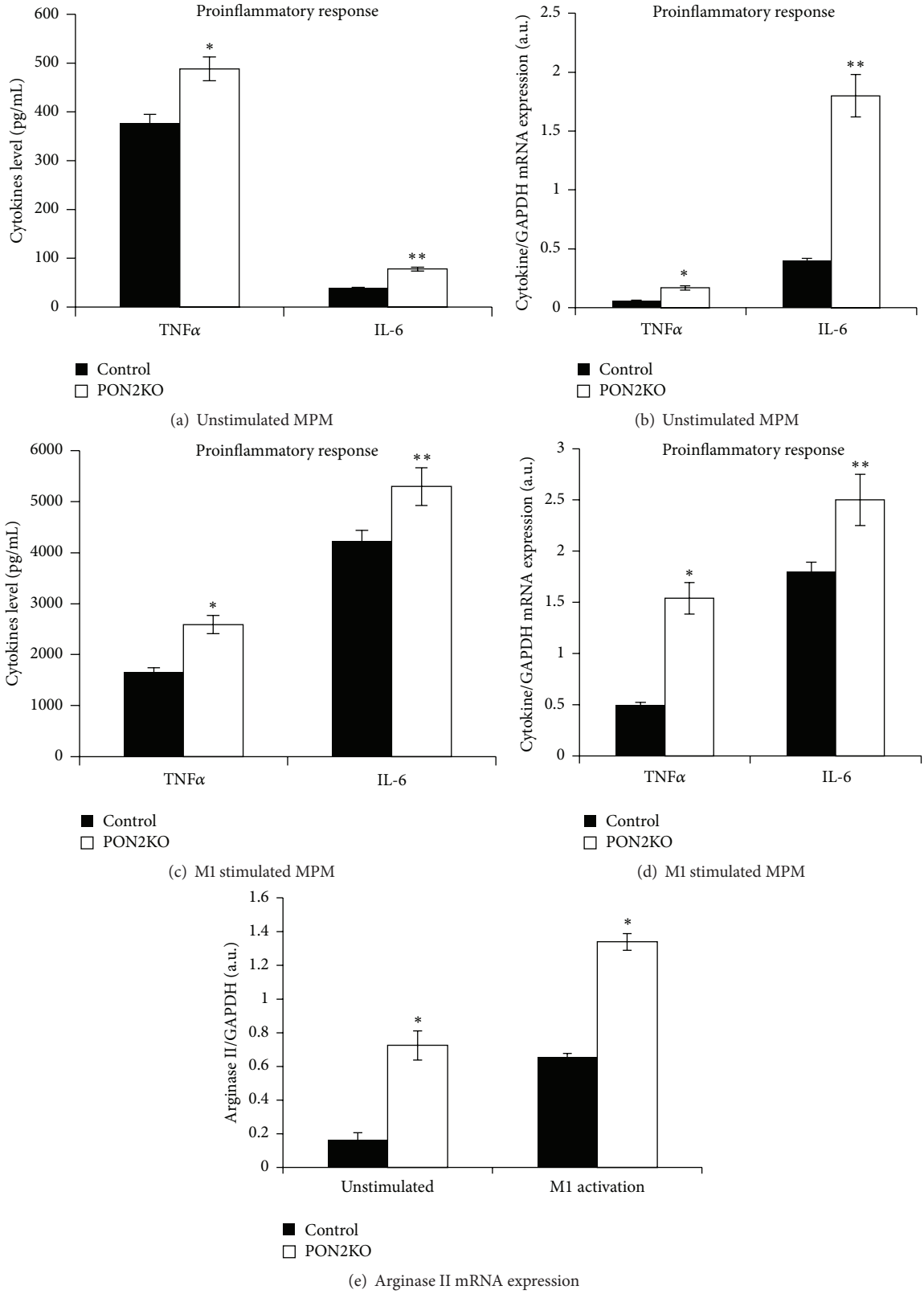


Figure 1: MPM from PON2KO display increased proinflammatory activation. MPM were harvested from 3-month-old control (C57BL/6) male mice and from age matched PON2KO mice. The cells were stimulated toward M1 activation with LPS (100 ng/mL) and IFNγ (20 ng/mL) for 12 hours. Secretion of TNFα and IL-6 to the medium was measured under basal (a) or M1 stimulation (c) conditions. TNFα and IL-6 mRNA expression, analyzed by quantitative PCR and normalized to GAPDH, were measured in the cells under basal (b) or M1 stimulation (d) conditions. Arginase II mRNA expression was also measured under basal or M1 stimulation conditions (e). Results are expressed as mean ± SD ($n = 3$), $^{*}P < 0.03$, PON2KO versus control MPM.

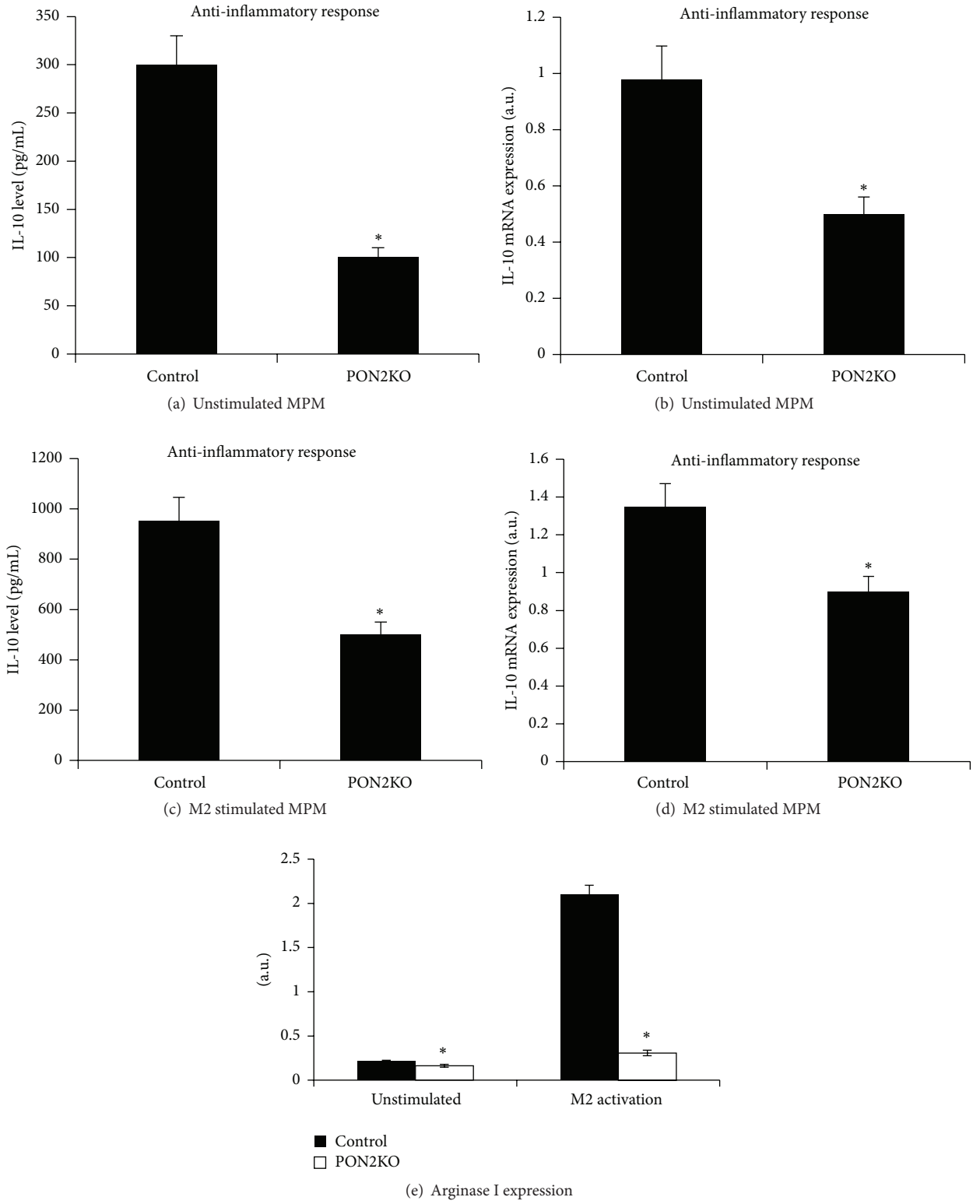
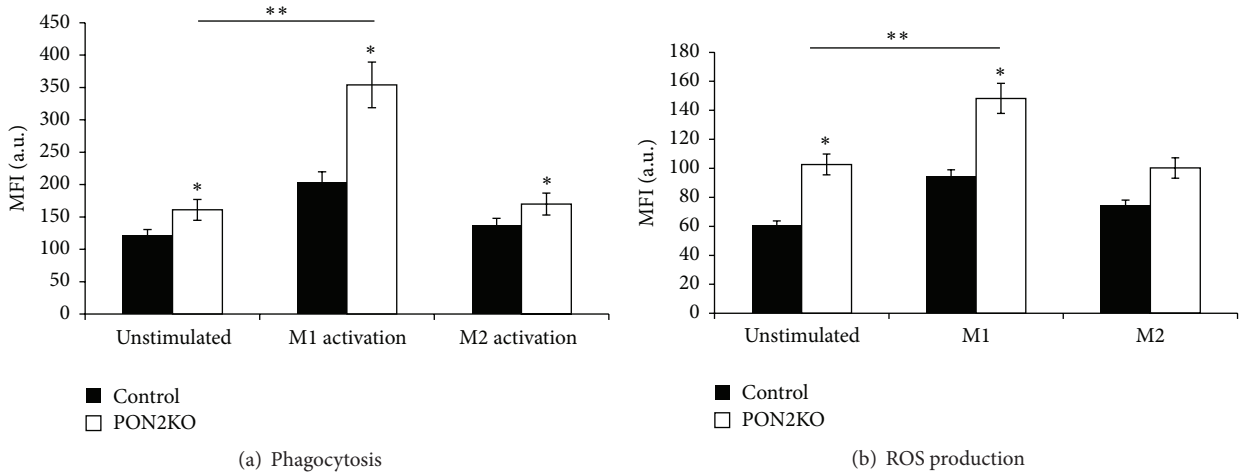
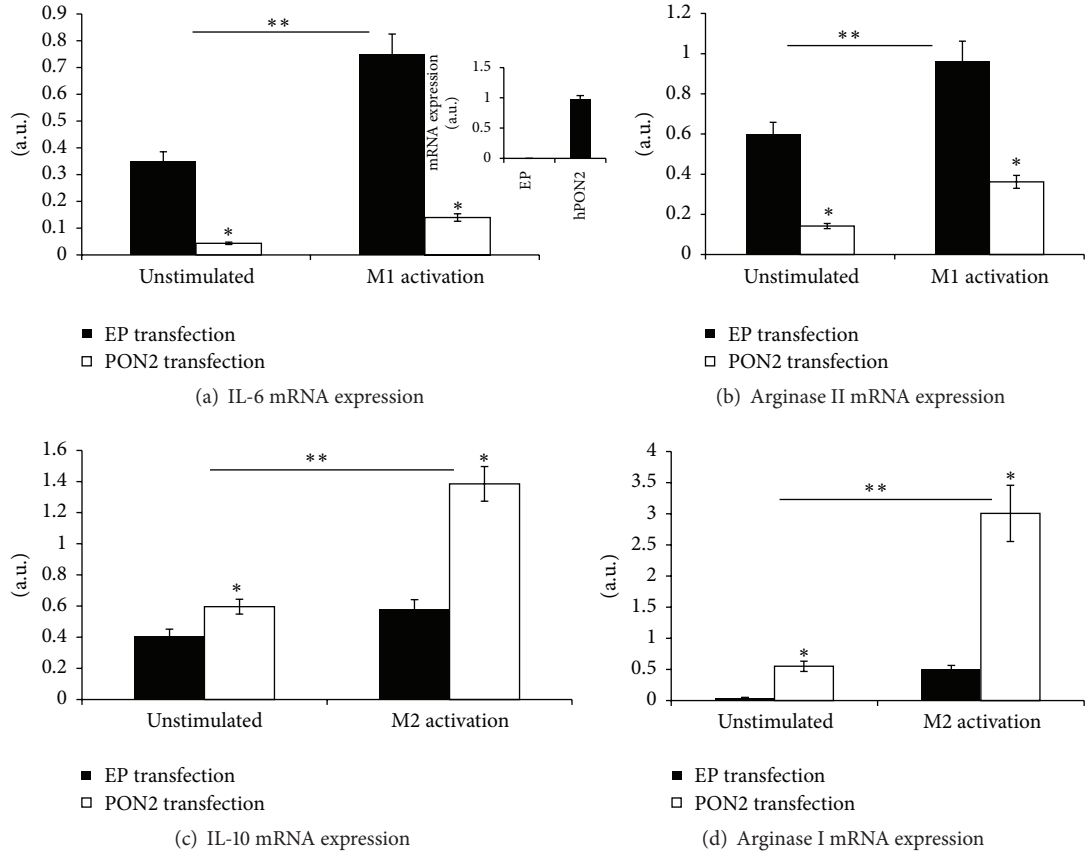


Figure 2: MPM from PON2KO display reduced anti-inflammatory activation. MPM were harvested from 3-month-old control (C57BL/6) male mice and from age matched PON2KO mice. The cells were stimulated toward M2 activation with IL-4 (20 ng/mL) for 12 hours. Secretion of IL-10 to the medium was measured under basal (a) or M2 stimulation (c) conditions. IL-10 mRNA expression, analyzed by quantitative PCR and normalized to GAPDH, was measured in the cells under basal (b) or M2 stimulation (d) conditions. Arginase I mRNA expression was also measured under basal or M2 stimulating conditions (e). Results are expressed as mean ± SD ($n = 3$), $^{*}P < 0.01$, PON2KO MPM versus control MPM.



(a) Phagocytosis (b) ROS production

Figure 3: M1 functional phenotype in PON2KO MPM versus control mice MPM. MPM from control or PON2KO mice were either nontreated or activated to the M1 or M2 phenotype. (a) Phagocytosis was determined in cells that were incubated with FITC-conjugated latex beads for 4 hours and analyzed using FACS. Results are given as mean fluorescence intensity (MFI). (b) ROS production was analyzed by FACS and is expressed as MFI. Results are expressed as mean ± SD ($n = 3$), $^{*}P < 0.05$, PON2KO MPM versus control MPM; $^{**}P < 0.01$, M1 stimulated versus unstimulated cells.



(a) IL-6 mRNA expression (b) Arginase II mRNA expression

(c) IL-10 mRNA expression (d) Arginase I mRNA expression

Figure 4: Direct effect of human PON2 (hPON2) on the inflammatory and anti-inflammatory responses in PON2KO MPM. MPM were harvested from 3-month-old PON2KO male mice. The cells were transfected with pcDNA3.1+ empty plasmid (EP) or with pcDNA3.1+ plasmid containing hPON2 for 4 hours at 37°C. Cells were then washed and cultured with DMEM medium + 10% fetal calf serum for 48 hours. The cells were cultured for additional 12 hours unstimulated and then either M1-activated with IFNγ/LPS or M2-activated with IL-4. IL-6 (a), arginase II (b), IL-10 (c), and arginase I (d) mRNA expressions were determined by quantitative PCR and results were normalized to GAPDH expression. The inset of (a) shows hPON2 mRNA expression in PON2-deficient MPM transfected with EP and PON2KO MPM transfected with hPON2. Results are expressed as mean ± SD ($n = 3$), $^{*}P < 0.01$, PON2KO MPM versus control MPM; M1 stimulated versus unstimulated, $^{**}P < 0.001$, M1, M2 stimulated versus unstimulated cells.

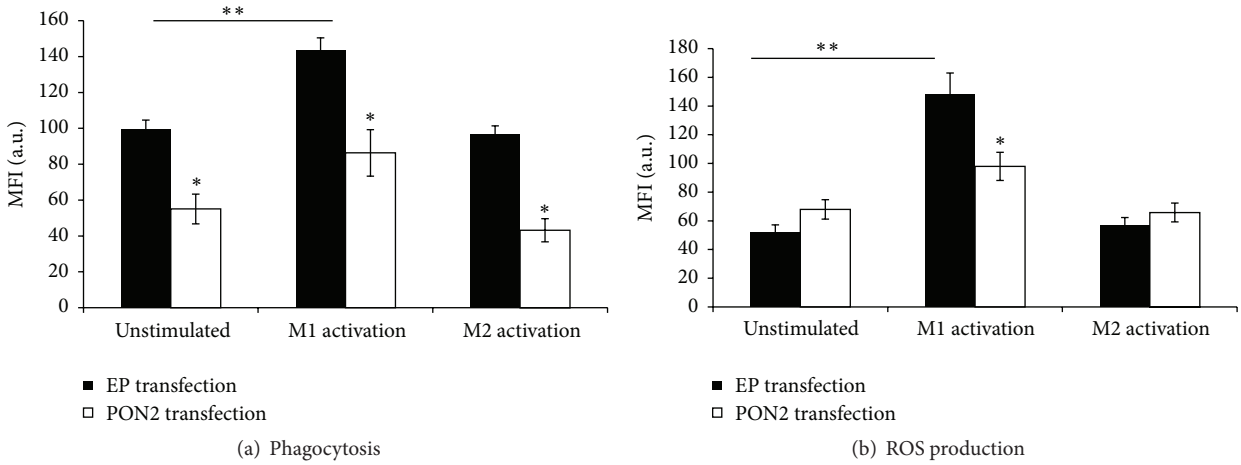


Figure 5: Direct effect of hPON2 on M1 functional phenotype in PON2KO MPM. MPM obtained from 3-month-old PON2KO male mice were transfected with pcDNA3.1+ empty plasmid (EP) or with pcDNA3.1+ plasmid containing hPON2 for 4 hours at 37°C. Cells were then washed and cultured with DMEM medium + 10% fetal calf serum for 48 hours. The cells were cultured for an additional 12 hours either unstimulated or stimulated with IFNγ/LPS or IL-4. (a) Phagocytosis was determined in cells that were incubated with FITC-conjugated latex beads for an additional 4 hours and analyzed using FACS. Results are given as mean fluorescence intensity (MFI). (b) ROS production was measured after 45 minutes of incubation with DCFH by FACS and is expressed as MFI. Results are expressed as mean ± SD ($n = 3$), $^{*}P < 0.01$, PON2KO MPM versus control MPM; $^{**}P < 0.01$, M1 stimulated versus unstimulated cells.

by 75%, respectively, and after M1 activation by 80% and 65%, respectively. In parallel, compared to EP, hPON2 transfection to PON2KO MPM showed an enhanced MPM anti-inflammatory response reflected by increased IL-10 (Figure 4(c)) and arginase I mRNA expression (Figure 4(d)) in unstimulated cells 1.5- and 11-fold, respectively, and in M2 activation 2.8- and 6-fold, respectively.

These results indicate that indeed PON2 not only inhibits macrophage response to classical M1 activation, but also promotes macrophage polarization toward the M2 anti-inflammatory phenotype.

3.5. hPON2 Modulates Macrophage M1 Functional Phenotype. Compared to EP, transfection of hPON2 to PON2KO MPM inhibited macrophage phagocytosis in unstimulated cells, as well as M1-activated cells and M2-activated cells by 43%, 38%, and 54%, respectively (Figure 5(a)). PON2 transfection to PON2KO MPM had no statistically significant effect on the macrophages' ability to generate ROS, as compared to MPM transfected with EP. However, in M1 stimulated cells, macrophage ROS production was significantly attenuated upon hPON2 transfection by 32% as compared to cells transfected with an EP (Figure 5(b)). No statistically significant effect of hPON2 transfection on MPM ROS production was noted under M2 activation versus unstimulated cells or versus cells with EP transfection.

4. Discussion

In the present study we demonstrate that macrophage PON2 directly attenuated the proinflammatory phenotype, in both unstimulated and M1 stimulated peritoneal macrophages. In addition, PON2 induced a phenotypic switch in macrophage polarization favoring an M2 anti-inflammatory phenotype.

We used in our study peritoneal macrophages from PON2KO mice in comparison to MPM from control C57BL/6 mice, which express PON2. M1 classic activation was induced by IFNγ + LPS following determination of TNFα, IL-6, and arginase II expression. All these parameters were significantly higher in the PON2KO versus control MPM. The M2 alternative activation was induced by IL-4 following determination of IL-10 and arginase I expression. All these parameters were significantly lower in the PON2KO versus control MPM. Functionally, PON2 deficiency was associated with increased ROS formation, in both unstimulated and M1 stimulated MPM in accordance with previous studies [19, 24, 25], and with enhanced latex particles phagocytosis.

Direct role of macrophage PON2 in the macrophage inflammatory responses was assessed by transfecting human PON2 into PON2KO MPM. This procedure resulted in a significant decrement in TNFα, IL-6, and arginase II expression and in parallel a significant increment in IL-10 and arginase I expression. The reverse of these responses by transfecting PON2KO macrophages with the human PON2 gene clearly indicates that PON2 plays an important role in polarization of macrophages from M1 toward the M2 phenotype. It could be that PON2-induced reduction in macrophage oxidative stress leads to the observed changes in the expression of the proinflammatory versus anti-inflammatory cytokines.

In a previous study it was shown that PON1 reduces macrophage inflammatory response [32]. PON1 unlike PON2 is not expressed in macrophages [18], and unlike PON2 it is present in the circulation associated with HDL. In that study [32] they used macrophages (MPM of BMDM) from human PON1 transgenic mice. These macrophages express human PON1, but this is artificial state, not physiological one. In addition the authors used recombinant PON1 incubation

with J774A.1 macrophages, but there is no free PON1. PON1 can contribute to HDL anti-inflammatory activity. In that study [32] the authors did not measure arginase I and II expression or used M2 activation like we did. The novelty of our study is the use PON2KO MPM and PON2KO MPM transfected with human PON2.

PON1 and PON2 were both shown to protect from atherosclerosis development [31], secondary to HDL-associated PON1 antiatherogenic effects in the circulation and in the arterial wall and to PON2 antiatherogenic effects only in the arterial wall. Both enzymes were shown to reduce oxidative stress [24], but PON2 protects also from triglyceride accumulation [27], whereas PON1 protects from cholesterol accumulation, by inhibiting macrophage cholesterol biosynthesis [33] and by stimulating of HDL-mediated cholesterol efflux [34]. In both PON2KO MPM and C57BL/6 MPM, cellular cholesterol metabolism was similar, as noted by similar macrophage cholesterol mass and similar rates of cholesterol biosynthesis and of HDL-mediated cholesterol efflux [35]. In PON2KO MPM versus C57BL/6 MPM, there was a significant increase in the cellular triglyceride content and in the rate of macrophage triglyceride biosynthesis [35].

PON1 was also shown to protect from diabetes development in mice [36], and PON2 was shown to affect hepatic insulin signaling [31] and protected macrophages from high glucose-induced oxidative stress and triglyceride accumulation [37].

The progression of the atherosclerotic plaque in terms of size and nature is mediated by the inflammatory status of the macrophages in the vascular wall. Advanced atherosclerotic lesions are characterized by activated macrophages dominated by the M1 subset in all stages [4, 8] whereas in early lesions macrophages express markers corresponding to the M2 phenotype [9]. In addition, M2 macrophages were suggested to be involved in inflammation remission [11].

In summary, our results support the role of PON2 in protecting against atherosclerosis development by shifting the polarization of macrophages toward the M2 anti-inflammatory phenotype. Understanding the mechanisms of macrophage plasticity and resolving functional characteristics of distinct macrophage phenotypes should help in the development of new strategies for treatment of chronic inflammation in atherosclerosis.

Conflict of Interests

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

References

[1] R. Ross, "Atherosclerosis—an inflammatory disease," *The New England Journal of Medicine*, vol. 340, no. 2, pp. 115–126, 1999.

[2] Y. V. Bobryshev, "Monocyte recruitment and foam cell formation in atherosclerosis," *Micron*, vol. 37, no. 3, pp. 208–222, 2006.

[3] M. Fernández-Velasco, S. González-Ramos, and L. Boscá, "Involvement of monocytes/macrophages as key factors in the development and progression of cardiovascular diseases," *Biochemical Journal*, vol. 458, no. 2, pp. 187–193, 2014.

[4] Z.-Q. Yan and G. K. Hansson, "Innate immunity, macrophage activation, and atherosclerosis," *Immunological Reviews*, vol. 219, no. 1, pp. 187–203, 2007.

[5] S. Colin, G. Chinetti-Gbaguidi, and B. Staels, "Macrophage phenotypes in atherosclerosis," *Immunological Reviews*, vol. 262, no. 1, pp. 153–166, 2014.

[6] A. Mantovani, C. Garlanda, and M. Locati, "Macrophage diversity and polarization in atherosclerosis: a question of balance," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 10, pp. 1419–1423, 2009.

[7] C. D. Mills, "Anatomy of a discovery: M1 and M2 macrophages," *Frontiers in Immunology*, vol. 6, article 212, 2015.

[8] K. J. Moore, F. J. Sheedy, and E. A. Fisher, "Macrophages in atherosclerosis: a dynamic balance," *Nature Reviews Immunology*, vol. 13, no. 10, pp. 709–721, 2013.

[9] J. Khallou-Laschet, A. Varthaman, G. Fornasa et al., "Macrophage plasticity in experimental atherosclerosis," *PLoS ONE*, vol. 5, no. 1, Article ID e8852, 2010.

[10] D. A. Chistiakov, Y. V. Bobryshev, N. G. Nikiforov, N. V. Elizova, I. A. Sobenin, and A. N. Orekhov, "Macrophage phenotypic plasticity in atherosclerosis: the associated features and the peculiarities of the expression of inflammatory genes," *International Journal of Cardiology*, vol. 184, pp. 436–445, 2015.

[11] M. Peled and E. A. Fisher, "Dynamic aspects of macrophage polarization during atherosclerosis progression and regression," *Frontiers in Immunology*, vol. 5, article 579, 2014.

[12] D. M. Mosser and J. P. Edwards, "Exploring the full spectrum of macrophage activation," *Nature Reviews Immunology*, vol. 8, no. 12, pp. 958–969, 2008.

[13] F. O. Martinez, A. Sica, A. Mantovani, and M. Locati, "Macrophage activation and polarization," *Frontiers in Bioscience*, vol. 13, no. 2, pp. 453–461, 2008.

[14] F. O. Martinez and S. Gordon, "The M1 and M2 paradigm of macrophage activation: time for reassessment," *F1000Prime Reports*, vol. 6, article 13, 2014.

[15] S. Aharoni, Y. Lati, M. Aviram, and B. Fuhrman, "Pomegranate juice polyphenols induce a phenotypic switch in macrophage polarization favoring a M2 anti-inflammatory state," *BioFactors*, vol. 41, no. 1, pp. 44–51, 2015.

[16] H. J. Medbury, V. James, J. Ngo et al., "Differing association of macrophage subsets with atherosclerotic plaque stability," *International Angiology*, vol. 32, no. 1, pp. 74–84, 2013.

[17] H. J. Medbury, H. Williams, and J. P. Fletcher, "Clinical significance of macrophage phenotypes in cardiovascular disease," *Clinical and Translational Medicine*, vol. 3, no. 1, article 63, 2014.

[18] M. Rosenblat, D. Draganov, C. E. Watson, C. L. Bisgaier, B. N. La Du, and M. Aviram, "Mouse macrophage paraoxonase 2 activity is increased whereas cellular paraoxonase 3 activity is decreased under oxidative stress," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 3, pp. 468–474, 2003.

[19] C. J. Ng, D. J. Wadleigh, A. Gangopadhyay et al., "Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein," *Journal of Biological Chemistry*, vol. 276, no. 48, pp. 44444–44449, 2001.

[20] C. J. Ng, N. Bourquard, V. Grijalva et al., "Paraoxonase-2 deficiency aggravates atherosclerosis in mice despite lower apolipoprotein-B-containing lipoproteins: anti-atherogenic role for paraoxonase-2," *The Journal of Biological Chemistry*, vol. 281, no. 40, pp. 29491–29500, 2006.

[21] C. J. Ng, S. Y. Hama, N. Bourquard, M. Navab, and S. T. Reddy, "Adenovirus mediated expression of human paraoxonase 2 protects against the development of atherosclerosis in apolipoprotein E-deficient mice," *Molecular Genetics and Metabolism*, vol. 89, no. 4, pp. 368–373, 2006.

[22] A. Devarajan, V. R. Grijalva, N. Bourquard et al., "Macrophage paraoxonase 2 regulates calcium homeostasis and cell survival under endoplasmic reticulum stress conditions and is sufficient to prevent the development of aggravated atherosclerosis in paraoxonase 2 deficiency/apoE$^{-/-}$ mice on a Western diet," *Molecular Genetics and Metabolism*, vol. 107, no. 3, pp. 416–427, 2012.

[23] A. Devarajan, N. Bourquard, S. Hama et al., "Paraoxonase 2 deficiency alters mitochondrial function and exacerbates the development of atherosclerosis," *Antioxidants and Redox Signaling*, vol. 14, no. 3, pp. 341–351, 2011.

[24] M. Aviram and M. Rosenblat, "Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development," *Free Radical Biology and Medicine*, vol. 37, no. 9, pp. 1304–1316, 2004.

[25] H. Hagmann, A. Kuczkowski, M. Ruehl et al., "Breaking the chain at the membrane: paraoxonase 2 counteracts lipid peroxidation at the plasma membrane," *The FASEB Journal*, vol. 28, no. 4, pp. 1769–1779, 2014.

[26] S. Altenhöfer, I. Witte, J. F. Teiber et al., "One enzyme, two functions: PON2 prevents mitochondrial superoxide formation and apoptosis independent from its lactonase activity," *The Journal of Biological Chemistry*, vol. 285, no. 32, pp. 24398–24403, 2010.

[27] M. Rosenblat, R. Coleman, S. T. Reddy, and M. Aviram, "Paraoxonase 2 attenuates macrophage triglyceride accumulation via inhibition of diacylglycerol acyltransferase 1," *Journal of Lipid Research*, vol. 50, no. 5, pp. 870–879, 2009.

[28] J. B. Kim, Y.-R. Xia, C. E. Romanoski et al., "Paraoxonase-2 modulates stress response of endothelial cells to oxidized phospholipids and a bacterial quorum-sensing molecule," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 11, pp. 2624–2633, 2011.

[29] A. Devarajan, N. Bourquard, V. R. Grijalva et al., "Role of PON2 in innate immune response in an acute infection model," *Molecular Genetics and Metabolism*, vol. 110, no. 3, pp. 362–370, 2013.

[30] N. Bourquard, C. J. Ng, and S. T. Reddy, "Impaired hepatic insulin signalling in PON2-deficient mice: a novel role for the PON2/apoE axis on the macrophage inflammatory response," *Biochemical Journal*, vol. 436, no. 1, pp. 91–100, 2011.

[31] C. J. Ng, D. M. Shih, S. Y. Hama, N. Villa, M. Navab, and S. T. Reddy, "The paraoxonase gene family and atherosclerosis," *Free Radical Biology and Medicine*, vol. 38, no. 2, pp. 153–163, 2005.

[32] S. Aharoni, M. Aviram, and B. Fuhrman, "Paraoxonase 1 (PON1) reduces macrophage inflammatory responses," *Atherosclerosis*, vol. 228, no. 2, pp. 353–361, 2013.

[33] O. Rozenberg, D. M. Shih, and M. Aviram, "Human serum paraoxonase 1 decreases macrophage cholesterol biosynthesis: possible role for its phospholipase-A2-like activity and lysophosphatidylcholine formation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 3, pp. 461–467, 2003.

[34] M. Rosenblat, L. Gaidukov, O. Khersonsky et al., "The catalytic histidine dyad of high density lipoprotein-associated serum paraoxonase-1 (PON1) is essential for PON1-mediated inhibition of low density lipoprotein oxidation and stimulation of macrophage cholesterol efflux," *The Journal of Biological Chemistry*, vol. 281, no. 11, pp. 7657–7665, 2006.

[35] M. Rosenblat, N. Volkova, and M. Aviram, "Pomegranate juice (PJ) consumption antioxidative properties on mouse macrophages, but not PJ beneficial effects on macrophage cholesterol and triglyceride metabolism, are mediated via PJ-induced stimulation of macrophage PON2," *Atherosclerosis*, vol. 212, no. 1, pp. 86–92, 2010.

[36] M. Koren-Gluzer, M. Aviram, E. Meilin, and T. Hayek, "The antioxidant HDL-associated paraoxonase-1 (PON1) attenuates diabetes development and stimulates β-cell insulin release," *Atherosclerosis*, vol. 219, no. 2, pp. 510–518, 2011.

[37] E. Meilin, M. Aviram, and T. Hayek, "Paraoxonase 2 (PON2) decreases high glucose-induced macrophage triglycerides (TG) accumulation, via inhibition of NADPH-oxidase and DGAT1 activity: studies in PON2-deficient mice," *Atherosclerosis*, vol. 208, no. 2, pp. 390–395, 2010.