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Associations between circulating free fatty acids, visceral adipose tissue accumulation, and insulin sensitivity in postmenopausal women

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Abstract

The aims of the study were to evaluate the contribution of visceral adipose tissue (AT) accumulation and insulin sensitivity to the determination of circulating free fatty acid (FFA) concentrations measured during a 2-hour euglycemic-hyperinsulinemic clamp and to verify whether elevated FFAs are associated with other components of the metabolic syndrome in postmenopausal women. This cross-sectional study included 115 postmenopausal women (46-68 years old). Visceral AT was estimated by computed tomography. Insulin sensitivity was assessed by a 2-hour euglycemic-hyperinsulinemic clamp. Free fatty acid concentration was measured in the fasting state and every 30 minutes during the clamp. Fasting plasma glucose and 2-hour plasma glucose were measured by an oral glucose tolerance test. Visceral AT was associated positively and insulin sensitivity negatively with FFA area under the curve (AUC) measured during the clamp. Women with high visceral AT accumulation and low insulin sensitivity had higher FFA AUC than women with high visceral AT accumulation and high insulin sensitivity or women with low visceral AT combined with either low or high insulin sensitivity. Free fatty acid AUC was positively associated with triglyceride ($r = 0.25$, $P < .05$), fasting plasma glucose ($r = 0.26$, $P < .01$), 2-hour plasma glucose ($r = 0.27$, $P < .01$), and diastolic blood pressure ($r = 0.21$, $P < .05$) independently of visceral AT and insulin sensitivity. In postmenopausal women, the presence of both high visceral AT and low insulin sensitivity is needed to observe an elevated FFA AUC. Moreover, FFA AUC is associated with some components of the metabolic syndrome, independently of visceral AT and insulin sensitivity.

1. Introduction

Menopausal transition is associated with a body fat redistribution leading to preferential abdominal adipose tissue (AT) accumulation [1,2]. High visceral AT accumulation has been associated with a deteriorated metabolic profile such as high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) concentrations in postmenopausal women [3,4]. The link between visceral AT accumulation and deteriorated metabolic profile may be explained in part by high lipolytic action of visceral AT leading to an increased flux of free fatty acids (FFAs) in portal circulation to liver that increases intracellular availability of TG and stimulates the assembly and secretion of very low-density lipoprotein (VLDL) particles (reviewed by Wajchenberg [5]). In addition, insulin resistance enhances the impact of visceral AT on the elevation of plasma FFA levels. In normal conditions, insulin has the ability to suppress plasma FFA concentration [6]; however, individuals with insulin resistance are resistant to the suppressive effect of insulin on fatty acids even at supraphysiologic concentrations of insulin (reviewed by Julius [7]). Moreover, visceral fat is more resistant to the antilipolytic effect of insulin than subcutaneous fat [8]. Consequently, in the presence of insulin resistance, the antilipolytic function of insulin is disturbed, therefore facilitating the liberation of FFA from the visceral AT depot. However, few studies have examined the respective contribution of visceral AT accumulation and insulin resistance to the determination of circulating FFA concentrations.

Free fatty acids may play a central role in the metabolic syndrome. In fact, a high FFA concentration has been shown to stimulate hepatic glucose production and inhibit insulin-

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stimulated glucose uptake, leading to high blood glucose concentrations (reviewed by Miranda et al [9]). A high FFA concentration may also stimulate secretion of VLDL particles and contribute to increase plasma TG concentration (reviewed by Wajchenberg [5]). Furthermore, it has been demonstrated recently that an acute elevation of plasma FFA levels increased systolic blood pressure [10]. However, it remains not fully understood whether FFA acts directly on these variables or is simply a marker for visceral AT accumulation and/or insulin resistance. Therefore, the aims of the present study were (1) to determine the respective contribution of visceral AT, insulin sensitivity, and their interaction to the regulation of FFA and (2) to determine whether the elevation in FFA is associated with other variables of the metabolic syndrome independently of visceral AT and insulin sensitivity in a group of postmenopausal women not taking hormone therapy.

2. Patients and methods

2.1. Patients

This cross-sectional study was conducted in a sample of 115 postmenopausal women (aged between 46 and 68 years) recruited through the local newspapers of the Quebec City metropolitan area. Postmenopausal status was determined by the absence of menses for at least 1 year and by a measure of the follicle-stimulating hormone between 28 and 127 UI/L. All women included in our study were free from metabolic disorders, were not using any type of hormone therapy, and were not under treatment of cardiovascular disease, diabetes, dyslipidemia, or endocrine disorders (except stable thyroid disease). None of the participants had received a diagnosis of type 2 diabetes mellitus before the study. All participants signed an informed consent document before entering the study, which was approved by the Laval University Medical Center and Laval University Research Ethics Committees.

2.2. Anthropometry and computed tomography

Height and body weight were measured using standardized procedures as reported previously [11]. Measurements of abdominal AT areas (subcutaneous and visceral) were performed between the L4 and L5 vertebras by computed tomography with a GE High Speed Advantage computed tomography scanner (General Electric Medical Systems, Milwaukee, WI) as previously described [12].

2.3. Oral glucose tolerance test

A 75-g oral glucose tolerance test was performed in the morning after an overnight fast to determine the glucose tolerance status of women. Blood samples were collected through a venous catheter from an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes for the determination of plasma glucose and insulin concentrations. Plasma glucose was measured enzymatically, whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation as previously described [3]. Glucose tolerance status was defined according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Normal glucose tolerance* (*NGT*) was defined as a fasting plasma glucose (FPG) less than 6.1 mmol/L and 2-hour plasma glucose (2 hPG) less than 7.8 mmol/L, impaired glucose tolerance (IGT) as FPG less than 7.0 mmol/L and 2 hPG between 7.8 and 11.1 mmol/L, and type 2 diabetes mellitus as FPG of at least 7.0 mmol/L or 2 hPG of at least 11.1 mmol/L [13]. According to these criteria, 64 subjects had NGT, 33 had IGT, and 18 had type 2 diabetes mellitus.

2.4. Euglycemic-hyperinsulinemic clamp

Insulin sensitivity was determined with a 2-hour euglycemic-hyperinsulinemic clamp as previously described [3]. After a 12-hour overnight fast, an antecubital arm vein was cannulated with a catheter for infusion of insulin and glucose (20% dextrose). A hand vein from the contralateral arm was cannulated to permit blood sampling. Fasting blood sample was drawn for baseline measurements. A primed continuous infusion of insulin (Humulin R, Eli Lilly Canada, Toronto, Canada) (40 $mU \cdot m^{-2} \cdot min^{-1}$) was then started. Adjustments in glucose infusion rate were performed to reach the FPG value and a steady state of about 5.5 mmol/L for women with FPG above the reference range (≥6.1 mmol/L). Once the steady state of glucose concentration was reached, the insulin infusion was continued for a total of 2 hours. Blood samples were collected from time −15 minutes and then every 5 minutes during the test to measure blood glucose concentrations. Plasma insulin concentrations were monitored from blood samples collected every 10 minutes. The insulin-stimulated glucose disposal rate (*M* value) was calculated as the glucose infusion rate divided by kilograms of body weight during the last 30 minutes of the clamp. Insulin sensitivity was determined as the *M* value divided by the mean insulin concentration during the last 30 minutes of the clamp, as described previously [14]. Plasma FFA concentration was measured in the fasting state and every 30 minutes of the clamp (Wako Chemicals, Neuss, Germany). Free fatty acid area under the curve (AUC) during the 2-hour euglycemic-hyperinsulinemic clamp was determined by the trapezoidal rule. The percentage of suppression of FFA during the clamp was calculated with the following formula : {[(fasting plasma FFA concentration − 2-hour plasma FFA concentration)/fasting plasma FFA concentration]∗100}.

2.5. Plasma lipoprotein lipid profile

Blood samples were collected in the morning after a 12-hour overnight fast from an antecubital vein. Cholesterol and TG concentrations were determined enzymatically in plasma and lipoprotein fractions with a Technicon RA-500 analyzer (Bayer, Tarrytown, NY). Plasma lipoprotein fractions (low-density lipoprotein) and HDL were isolated by sequential ultracentrifugations that have been previously described [15]. Apolipoprotein B (apo B) was measured by

Table 1
Characteristics of postmenopausal women

Variables	Means ± SD
Age (y)	56.9 ± 4.4
Time since menopause (y)	6.4 ± 5.2
Blood pressure	
Systolic blood pressure (mm Hg)	130 ± 15
Diastolic blood pressure (mm Hg)	82 ± 8
Anthropometric variables	
Body mass index (kg/m^2)	28.6 ± 5.8
Visceral AT (cm^2)	140.2 ± 56.3
Subcutaneous AT (cm^2)	370.5 ± 130.2
Lipid/lipoprotein variables	
Total cholesterol (mmol/L)	5.44 ± 0.91
HDL-C (mmol/L)	1.42 ± 0.35
LDL-C (mmol/L)	3.56 ± 0.82
TG (mmol/L)	1.25 ± 0.62
Apo B (g/L)	1.00 ± 0.21
Fasting FFA (mmol/L)	0.53 ± 0.17
FFA AUC	15.2 ± 7.7
FFA suppression (%)	95.4 ± 5.8
Glucose/insulin variables	
FPG (mmol/L)	5.6 ± 0.8
2 hPG (mmol/L)	7.9 ± 2.8
Insulin sensitivity (mg·kg^{-1}·min^{-1}·(pmol/L)$^{-1}$)	0.0106 ± 0.0050

LDL-C indicates low-density lipoprotein cholesterol.

nephelometry (BN ProSpec; Dade Behring, Newark, NJ) in plasma and lipoprotein fractions with reagents provided by this company (N Antisera to Human Apolipoprotein B).

2.6. Other measurements

Systolic blood pressure and diastolic blood pressure were measured in the right arm of seated participants, as previously described [16]. Time since menopause was self-reported by women.

2.7. Statistical analyses

Statistical analyses were performed using the SAS software (Version 8.2; SAS Institute, Cary, NC). The interaction between visceral AT and insulin sensitivity on the determination of FFA levels was evaluated by analyses of covariance using the general linear model procedures. Included in the model were visceral AT, insulin sensitivity, and the interaction term. The source of variation in metabolic parameters was computed using the type III sum of squares that applies to unbalanced study designs and quantifies the effects of an independent variable after adjusting for all other variables included in the model. The total sample of women was stratified into 4 groups according to the median of visceral AT distribution (135.4 cm^2) and the median of insulin sensitivity distribution (0.01015 mg·kg^{-1}·min^{-1}·(pmol/L)$^{-1}$). We compared the FFA AUC between the 4 groups by analysis of variance. In the presence of significant effects, Scheffe multiple comparison test was used to determine precisely the location of significant differences. Pearson correlation coefficients were calculated to quantify the univariate associations between fasting plasma FFA concentration, FFA AUC, FFA suppression, and other variables. Partial Pearson correlations were performed to control for the effect of visceral AT and/or insulin sensitivity. Some variables were not normally distributed (FFA AUC, body mass index, FPG, and TG). For these variables, analyses were done on their log-transformed values. The critical P value for significance was set at .05.

3. Results

Characteristics of subjects are presented in Table 1. Postmenopausal women had a mean age of 56.9 years and a mean body mass index of 28.6 kg/m^2. Mean levels for FPG and 2 hPG were 5.6 and 7.9 mmol/L, respectively.

Associations between FFA parameters and age, time since menopause, anthropometric variables, as well as insulin sensitivity are shown in Table 2. Free fatty acid AUC was positively associated with age, time since menopause, body mass index, visceral AT, and subcutaneous AT, whereas FFA AUC was negatively associated with insulin sensitivity. Similar associations were found for fasting plasma FFA concentration, whereas FFA suppression was negatively associated with anthropometric variables and positively associated with insulin sensitivity and was not significantly associated with age or with time since menopause. Moreover, FFA AUC was positively associated with visceral AT independently of insulin sensitivity (r = 0.20, P = .04), whereas FFA AUC was negatively associated with insulin sensitivity independently of visceral AT (r = −0.36, P = .0001). Women with type 2 diabetes mellitus had higher fasting plasma FFA concentration as well as higher FFA AUC than women with a normal glucose tolerance (0.49 mmol/L vs 0.60 mmol/L and 12.3 mmol·min/L vs 23.1 mmol·min/L, respectively; P < .05). To illustrate the interaction between visceral AT and insulin sensitivity, the sample of postmenopausal women was stratified according to median values of visceral AT distribution (135.4 cm^2) and median values of insulin sensitivity distribution (0.01015 mg·kg^{-1}·min^{-1}·(pmol/L)$^{-1}$) (Fig. 1). Thirteen percent of women with a normal glucose

Table 2
Associations between FFA parameters and age, time since menopause, anthropometric parameters, and insulin sensitivity in postmenopausal women

	Fasting FFA	FFA AUC	FFA suppression
Age	0.25**	0.28**	−0.08
Time since menopause	0.22*	0.28**	−0.17
Body mass index	0.29**	0.38****	−0.20*
Visceral AT	0.22*	0.41****	−0.19*
Subcutaneous AT	0.29**	0.33***	−0.20*
Insulin sensitivity	−0.24*	−0.47****	0.22*

* Significant P < .05.
** Significant P < .01.
*** Significant P < .001.
**** Significant P < .0001.

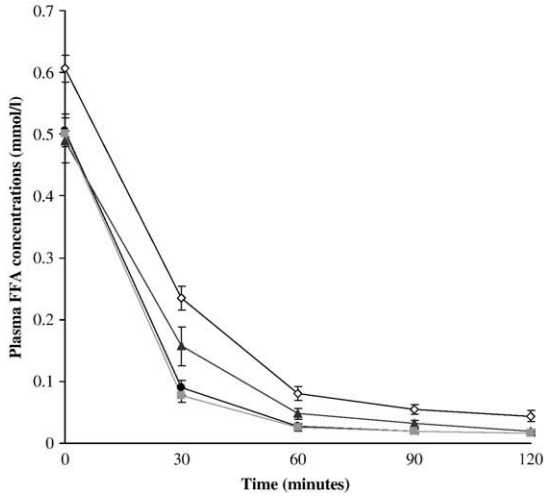

Fig. 1. Plasma FFA concentrations during a 2-hour euglycemic-hyperinsulinemic clamp in subgroups formed on the basis of median values of insulin sensitivity distribution (0.01015 $mg \cdot kg^{-1} \cdot min^{-1} \cdot (pmol/L)^{-1}$) and median values of visceral AT distribution (135.4 cm^2) in postmenopausal women. Black circles represent low visceral AT/high insulin sensitivity, black triangles represent low visceral AT/low insulin sensitivity, gray squares represent high visceral AT/high insulin sensitivity, and white diamonds represent high visceral AT/low insulin sensitivity. Bars represent standard error.

tolerance, 36% of women with an IGT, and 83% of women with type 2 diabetes mellitus were classified in the group characterized by high visceral AT accumulation and low insulin sensitivity. Postmenopausal women characterized by both high visceral AT accumulation and low insulin sensitivity had higher FFA AUC (20.9 ± 8.5) than women characterized by high visceral AT and high insulin sensitivity (11.4 ± 5.5) or those with low visceral AT and either low (15.0 ± 7.2) or high insulin sensitivity (12.0 ± 4.9, $P < .0001$) (Fig. 2). No other between-group differences

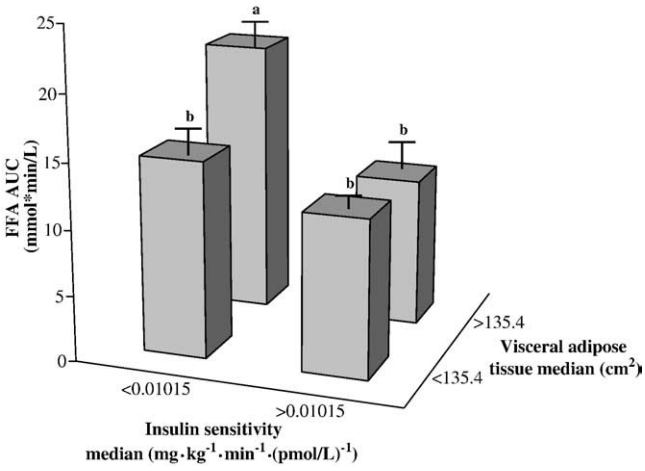

Fig. 2. Free fatty acid AUC during a 2-hour euglycemic-hyperinsulinemic clamp in subgroups formed on the basis of median values of insulin sensitivity distribution and median values of visceral AT distribution in postmenopausal women. Bars with different superscript letters are significantly different ($P < .05$). Bars represent standard error.

Table 3
Associations between FFA AUC and metabolic variables

	FFA AUC			
	Nonadjusted	Adjusted for visceral AT	Adjusted for insulin sensitivity	Adjusted for insulin sensitivity and visceral AT
Blood pressure				
Systolic blood pressure	0.31***	0.23*	0.19*	0.17
Diastolic blood pressure	0.33***	0.26**	0.20*	0.21*
Lipid/lipoprotein variables				
Total cholesterol	0.13	0.16	0.15	0.13
HDL-C	−0.34***	−0.22*	−0.22*	−0.13
LDL-C	0.08	0.08	0.08	0.04
Apo B	0.27**	0.22*	0.23*	0.16
TG	0.47****	0.37***	0.33***	0.25*
Glucose/insulin variables				
FPG	0.45****	0.36***	0.31***	0.26**
2 hPG	0.52****	0.41****	0.33***	0.27**

* Significant $P < .05$.
** Significant $P < .01$.
*** Significant $P < .001$.
**** Significant $P < .0001$.

were observed. Further analyses also revealed a significant interaction between visceral AT and insulin sensitivity on the determination of FFA AUC variance (F = 4.62, $P = .03$) (result not shown).

Table 3 shows associations between FFA AUC and metabolic variables. Free fatty acid AUC was positively associated with blood pressure, apo B, TG, FPG, and 2 hPG and negatively associated with HDL-C concentrations. After adjustments for visceral AT or insulin sensitivity, all these associations remained significant. After further adjustments for both visceral AT and insulin sensitivity, associations with systolic blood pressure, HDL-C, and apo B were no longer significant, whereas FFA AUC remained significantly associated with diastolic blood pressure, TG, FPG, and 2 hPG.

4. Discussion

Principal results from our study are that women characterized by the combination of high visceral AT and low insulin sensitivity had higher FFA AUC than women characterized by high visceral AT and high insulin sensitivity as well as women with low visceral AT with either low or high insulin sensitivity. This was supported by a statistically significant interaction between visceral AT and insulin sensitivity on the determination of FFA AUC. Moreover, FFA AUC was associated with diastolic blood pressure, plasma TG, FPG, and 2 hPG levels, independently of visceral AT and insulin sensitivity.

The associations between plasma FFA concentration and anthropometric variables have been well documented. For

example, Lovegrove et al [17] have found a positive correlation between fasting nonesterified fatty acids and adiposity measures such as body mass index and waist circumference in postmenopausal women not taking hormone therapy. More specifically, we found that fasting plasma FFA concentration was positively associated with visceral AT, but to a lesser extent than with subcutaneous AT. Rendell et al [18] have shown that fasting plasma FFA was closely related to subcutaneous AT in early postmenopausal women but failed to find an association between fasting plasma FFA and intraabdominal fat. In fact, upper-body subcutaneous fat has been identified as the major contributor to systemic FFA [19]. However, even if subcutaneous AT is the major contributor to plasma FFA concentration, it is well recognized that visceral AT is less responsive to the antilipolytic effect of insulin than subcutaneous AT [8]. Kurioka and colleagues [20] reported that subjects with type 2 diabetes mellitus with high suppression of plasma FFA during a 2-hour euglycemic-hyperinsulinemic clamp had lower visceral AT, whereas no association was found between the suppression of plasma FFA and subcutaneous AT. Similarly, our results showed that FFA AUC was more strongly associated with visceral AT than with subcutaneous AT. Thus, subcutaneous AT seems to be related more closely to fasting plasma FFA concentration than visceral AT, whereas visceral AT seems to be related more closely to FFA AUC than subcutaneous AT.

We found that insulin sensitivity was associated negatively with both fasting plasma FFA concentration and FFA AUC. Insulin resistance has been associated with a decrease in skeletal muscle glucose uptake [21]. Adipose tissue can also become resistant to the effects of insulin [7]. In fact, it has been demonstrated that insulin resistance leads to a diminution in the antilipolytic effect of insulin and consequently could enhance lipolysis and reduce FFA uptake and esterification, leading to an increase flux of plasma FFA (reviewed by Adeli et al [22]). On the other hand, this elevated plasma FFA concentration could be implicated in the exacerbation of insulin resistance [23,24], as several clinical studies have found a deterioration of insulin sensitivity after an experimental acute elevation of plasma FFA [24,25].

Kurioka et al [20] found that type 2 diabetes mellitus subjects with high suppression in plasma FFA during a 2-hour euglycemic-hyperinsulinemic clamp had lower visceral AT and higher insulin sensitivity in 10 men and 10 women. The present study supports these associations in a population of postmenopausal women and extends these results because we found that the combination of high visceral AT area and low insulin sensitivity was essential to observe high FFA AUC, whereas high visceral AT alone or low insulin sensitivity alone was not associated with increased FFA AUC. In support of that, we reported a significant interaction effect between high visceral AT and low insulin sensitivity on the determination of elevated FFA AUC. As indicated above, we observed that the presence of high visceral AT accumulation in combination with increased insulin sensitivity was not associated with elevated FFA AUC. This last result is concordant with a study from Karlsson et al [26] on the effects of rosiglitazone treatment in obese patients with newly diagnosed type 2 diabetes mellitus. In that study, insulin resistance measured during a hyperinsulinemic-euglycemic clamp decreased in response to rosiglitazone treatment; and this was accompanied by a decrease in FFA concentrations measured during the clamp. This decrease in FFA concentrations was observed despite the fact that obese subjects studied did not show any decrease in their body weight.

We have also demonstrated that FFA AUC was positively associated with diastolic blood pressure, TG, FPG, and 2 hPG independently of visceral AT and insulin sensitivity. Our results may therefore suggest that elevation of FFA plays a central role in the deterioration of the metabolic profile and could be one of the mechanisms linking a high visceral AT/low insulin sensitivity and other parameters of the metabolic syndrome. Specifically, a high plasma FFA concentration has been shown to stimulate hepatic glucose production and inhibit insulin-stimulated glucose uptake, leading to a high blood glucose concentrations (reviewed by Miranda et al [9]). Moreover, a high FFA concentration may increase intracellular availability of TG and stimulate secretion of VLDL particles (reviewed by Wajchenberg [5]).

Visceral obesity, insulin sensitivity, and FFA metabolism are closely interrelated. However, the links between these variables are not perfectly understood. Even if associations between FFA AUC and some metabolic parameters were independent of insulin sensitivity and visceral AT, the cross-sectional design of our study does not allow us to draw causal-effect link between these variables. Moreover, this study included only postmenopausal women; and results could not be applicable to other populations. It remains to be determined whether FFA plays a direct role in the deterioration of the metabolic profile or if it is a simple innocent bystander of another critical physiologic mechanism.

In conclusion, our study suggests that the combination of high visceral AT accumulation and low insulin sensitivity seems to be essential to observe an elevated FFA AUC. Moreover, FFA AUC was associated with a deteriorated metabolic profile, independently of visceral AT and insulin sensitivity, in our sample of postmenopausal women.

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References

[1] Morita Y, Iwamoto I, Mizuma N, et al. Precedence of the shift of body-fat distribution over the change in body composition after menopause. J Obstet Gynaecol Res 2006;32:513-6.

[2] Ozbey N, Sencer E, Molvalilar S, et al. Body fat distribution and cardiovascular disease risk factors in pre- and postmenopausal obese women with similar BMI. Endocr J 2002;49:503-9.

[3] Piche ME, Weisnagel SJ, Corneau L, et al. Contribution of abdominal visceral obesity and insulin resistance to the cardiovascular risk profile of postmenopausal women. Diabetes 2005;54:770-7.

[4] Hernandez-Ono A, Monter-Carreola G, Zamora-Gonzalez J, et al. Association of visceral fat with coronary risk factors in a population-based sample of postmenopausal women. Int J Obes Relat Metab Disord 2002;26:33-9.

[5] Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000;21:697-738.

[6] Carpentier A, Patterson BW, Leung N, et al. Sensitivity to acute insulin-mediated suppression of plasma free fatty acids is not a determinant of fasting VLDL triglyceride secretion in healthy humans. Diabetes 2002;51:1867-75.

[7] Julius U. Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. Exp Clin Endocrinol Diabetes 2003;111: 246-50.

[8] Jensen MD. Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model Obesity (Silver Spring) 2006;14 (Suppl 1):20S-4S.

[9] Miranda PJ, DeFronzo RA, Califf RM, et al. Metabolic syndrome: definition, pathophysiology, and mechanisms. Am Heart J 2005;149: 33-45.

[10] Riksen NP, Bosselaar M, Bakker SJ, et al. Acute elevation of plasma non-esterified fatty acids increases pulse wave velocity and induces peripheral vasodilation in humans in vivo. Clin Sci (Lond) 2007;113: 33-40.

[11] Lohman T, Ram R. Standardization of anthropometric measurements. Champaign: Human kinetics Publishers; 1988. p. 39-80.

[12] Ferland M, Despres JP, Tremblay A, et al. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr 1989;61:139-48.

[13] Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26(Suppl 1):S5-S20.

[14] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.

[15] Couillard C, Despres JP, Lamarche B, et al. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. Arterioscler Thromb Vasc Biol 2001;21:1226-32.

[16] Tremblay AJ, Despres JP, Piche ME, et al. Associations between the fatty acid content of triglyceride, visceral adipose tissue accumulation, and components of the insulin resistance syndrome. Metabolism 2004; 53:310-7.

[17] Lovegrove JA, Silva KD, Wright JW, et al. Adiposity, insulin and lipid metabolism in post-menopausal women. Int J Obes Relat Metab Disord 2002;26:475-86.

[18] Rendell M, Hulthen UL, Tornquist C, et al. Relationship between abdominal fat compartments and glucose and lipid metabolism in early postmenopausal women. J Clin Endocrinol Metab 2001;86: 744-9.

[19] Koutsari C, Jensen MD. Thematic review series: patient-oriented research. Free fatty acid metabolism in human obesity. J Lipid Res 2006;47:1643-50.

[20] Kurioka S, Murakami Y, Nishiki M, et al. Relationship between visceral fat accumulation and anti-lipolytic action of insulin in patients with type 2 diabetes mellitus. Endocr J 2002;49:459-64.

[21] DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. Diabetes Care 1992;15:318-68.

[22] Adeli K, Taghibiglou C, Van Iderstine SC, et al. Mechanisms of hepatic very low-density lipoprotein overproduction in insulin resistance. Trends Cardiovasc Med 2001;11:170-6.

[23] Frias JP, Macaraeg GB, Ofrecio J, et al. Decreased susceptibility to fatty acid–induced peripheral tissue insulin resistance in women. Diabetes 2001;50:1344-50.

[24] Homko CJ, Cheung P, Boden G. Effects of free fatty acids on glucose uptake and utilization in healthy women. Diabetes 2003;52:487-91.

[25] Carpentier A, Mittelman SD, Lamarche B, et al. Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. Am J Physiol 1999;276:E1055-66.

[26] Karlsson HK, Hallsten K, Bjornholm M, et al. Effects of metformin and rosiglitazone treatment on insulin signaling and glucose uptake in patients with newly diagnosed type 2 diabetes: a randomized controlled study. Diabetes 2005;54:1459-67.