Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 634–641
Journal of Nutritional Biochemistry

The influence of polymorphism of −493G/T MTP gene promoter and metabolic syndrome on lipids, fatty acids and oxidative stress☆

Aleš Žák*, Marie Jáchymová, Eva Tvrzická, Marek Vecka, Ladislava Duffková, Miroslav Zeman, Adolf Slabý, Barbora Staňková

Charles University in Prague, First Faculty of Medicine, 4th Department of Internal Medicine, General Teaching Hospital in Prague, Czech Republic

Received 14 February 2007; received in revised form 28 August 2007; accepted 4 September 2007

Abstract

The aim of this study was to investigate the effect of the microsomal triglyceride transfer protein (MTP) *−493G/T* polymorphism on clinical and biochemical parameters in relation to the presence of metabolic syndrome (MS). A group of 270 participants, 143 men and 127 women [50 men/36 women fulfilled the International Diabetes Federation (IDF) criteria of MS], was categorized on the basis of the MTP *−493G/T* polymorphism: GG homozygotes (Group GG) and carriers of the T allele (Group TT+TG). In men with MS, the presence of the T allele was associated with elevated concentrations of plasma insulin (by 48%, $P<.01$) and nonesterified fatty acids (by 49%, $P<.05$); homeostasis model assessment for insulin resistance index was higher by 64% ($P<.05$). Carriers of the T allele were further characterized by elevated plasma concentrations of total cholesterol (by 14%, $P<.05$) and by increased triglycerides in plasma (by 95%, $P<.01$) and in very low-density lipoprotein (by 106%, $P<.01$). They also had lower concentrations of n-6 polyunsaturated fatty acids in plasma phospholipids (by 3.5%, $P<.05$), lower Δ5-desaturase activities (by 18%, $P<.05$) and elevated concentrations of conjugated dienes in low-density lipoprotein (by 29%, $P<.01$). No significant differences between Groups GG and TT+TG were found in men without MS and in women with and without MS. Our results imply evidence for interactive effects of genetic, metabolic and gender-specific factors on several components of metabolic syndrome, which can increase the risk for cardiovascular disease.

Keywords: Microsomal triglyceride transfer protein; Metabolic syndrome; Atherogenic dyslipidemia; Fatty acid profile; Oxidative stress

1. Introduction

Metabolic syndrome (MS) represents one of the most important health problems in developed countries and the main target for preventive measures [1]. It is characterized by clustering of risk factors (RF) related to cardiovascular (CV) disease and type 2 diabetes mellitus (DM2). As widely accepted, MS includes accumulation of intraabdominal fat, dyslipidemia, hypertension and insulin resistance (IR), which can progress into disturbed glucose homeostasis. Chronic low-grade inflammation, oxidative stress and endothelial dysfunction have recently been added as new components of MS [2,3].

Several genetic and environmental factors were shown to be involved in the etiology of MS. Among the latter, the most important role belongs to excessive energy intake and low physical activity [4].

Fatty acids (FA) composition in plasma phospholipids (PL) and cholesterol esters (CE) reflects both dietary intake of FA over the previous few weeks, as well as endogenous FA metabolism (synthesis of FA de novo, β-oxidation, enzymatic desaturation and elongation as well as lipoperoxidation).

The hallmarks for most pathological stages, including MS as well as IR, are the increased content of saturated FA (SFA), especially that of palmitic acid (PA, 16:0) and the lower content of polyunsaturated FA (PUFA) especially that of linoleic acid (LA, 18:2n-6). Moreover, MS and IR are also accompanied by the increased content of palmitoleic acid (POA, 16:1n-7), γ-linolenic (GLA, 18:3n-6) and dihomo-γ-linolenic (DHGLA, 20:3n-6) acids. Concentrations of arachidonic acid (20:4n-6) remain usually

☆ The study was supported by the research project MSM 0021620820.
* Corresponding author. Tel.: +420 2 2496 2506; fax: +420 2 2492 3524.
E-mail address: azak@vfn.cz (A. Žák).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.09.001

unchanged; increased content was observed only in CE of septuagenarians [5]; its elongation and desaturation products (adrenic acid, 22:4n-6, and docosapentaenoic acid, 22:5n-6) are minor components and mostly not discussed. Our studies were in agreement with these findings, as will be shown thereinafter.

The observed changes in FA composition give evidence for increased activities of Δ9-desaturase (Δ9D) and Δ6-desaturase (Δ6D), as well as for a decreased activity of Δ5-desaturase (Δ5D). Activities of desaturases and elongases are mostly calculated as a ratio of product/substrate. Increased concentrations of POA, GLA and DHGLA caused by changes in enzyme activities are typically associated with IR. FA composition is only partly affected by the composition of dietary fat [6,7].

Elevated concentrations of PA predicted a long-term progression of MS in middle-aged men [7] in all stages of IR, as well as in patients after myocardial infarction (MI) who fulfill the MS criteria [8]. Decreased concentrations of total (Σ) n-6 PUFA are typical of MS in adults [7], children [9] and in men after MI [8]; they can be the predictors for the development of MS in middle-aged and elderly men. The drop in the n-3 PUFA content was a predictor for the development of MS in middle-aged men [5]. Low levels of LA have been associated with the development of impaired glucose tolerance and DM2 [10]. The plasma FA composition in overweight adolescents with MS correlated significantly with IR and with markers of chronic low-grade inflammation [11].

A number of common polymorphisms of the genes that control glucose homeostasis, insulin action, metabolism of lipids and lipoproteins (LP) as well as accumulation and distribution of the adipose tissue have been described in relation to MS [12].

The microsomal triglyceride transfer protein (MTP) has an indispensable role in the synthesis and secretion of very low-density LP (VLDL) and chylomicrons; MTP is a heterodimeric lipid transfer protein that is composed of a unique 97-kDa subunit and a multifunctional enzyme (protein disulphide isomerase). The subunit confers the lipid transfer activity, while protein disulphide isomerase realizes the binding to endoplasmic reticulum. The presence of MTP was demonstrated in hepatocytes and enterocytes, as well as in cells of some other tissues [13].

The absence of the functional MTP causes abetalipoproteinemia, which is a rare autosomal recessive disease causing a deficiency in the assembly process and secretion of VLDL and chylomicrons into the plasma [14]. The gene for the MTP (*4q24*) is polymorphic with a number of genetic variants, which are in linkage disequilibrium [15]. According to some authors, the activity of the minor (variant) *−493T* allele is higher than that of the common (wild) allele *−493G*. The *−493T* allele has been associated with the secretion of large VLDL-1 particles (S_f >400) with a higher triglyceride (TG)/apolipoprotein (apo) B ratio, which can be converted into small dense low-density LP (sd-LDL). On the contrary, a lower amount of the MTP produced in carriers of the common variant *−493G* leads to the secretion of VLDL with a lower content of TG, which results in a higher content of TG in hepatocytes [16,17]. The activity of mRNA MTP is down regulated by insulin [18].

In several studies, the MTP *−493T* allele was associated with decreased serum concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), increased values of body mass index (BMI), increased insulin levels and the secretion of TG-rich LPs (TGRLP) [19–21]. Other studies have proved associations of the *−493T* allele with increased concentrations of TC, LDL-C, TG and apoB [22,23]. No significant associations were found in the Framingham Offspring Study, as well as in a survey of French-Canadian youth [24,25].

The aim of the study was to investigate the effect of the MTP *−493G/T* polymorphism, MS and gender on some clinical and biochemical parameters [indices of IR, composition of plasma LPs, pattern of plasma esterified FA and conjugated dienes in precipitated low-density lipoprotein (CD-LDL), as a marker of oxidative stress]. The participants were categorized according to the gender, the presence or absence of MS and the MTP *−493G/T* polymorphism.

2. Methods and materials

A group of 270 Caucasian subjects (143 men, 127 women) was recruited from outpatients, who were subsequently examined (from December 2004 until August 2006) at the Lipid Clinic of the Fourth Department of Medicine, Charles University, in Prague. Healthy volunteers were also included into the control group. The basic clinical and biochemical characteristics of the whole group are shown in Table 1. Subjects were excluded from the study if they had a history of diabetes mellitus, CV and/or cerebrovascular disease, liver and/or renal disease, or excessive alcohol consumption (>30 g/day) or were treated with antihyperlipidemic medications or supplemented with PUFA and/or antioxidants. Informed consent was obtained from all participants. After the inclusion to the study, the participants were asked to fill 7-day dietary questionnaire. The dietary intake was calculated using the NutriMaster SE software, as described earlier [26]. The study protocol was approved by the Ethical Committee of the First Faculty of Medicine, Charles University, in Prague.

Metabolic syndrome was diagnosed according to the IDF criteria [27]. These criteria were fulfilled in 86 participants—50 men and 36 women.

Basal clinical and anthropometrical data were examined using standard methods [28]. The percentage of body fat was determined according to Durnin and Womersley [29]. Homeostasis model assessment (HOMA) methods—HOMA-R and HOMA-B—were used as indices of IR and β-cell function [30].

Table 1A
Effect of metabolic syndrome on anthropometric parameters, glucose homeostasis, plasma lipids and LPs in the overall group

Parameter	MS (yes) (n=86)	MS (no) (n=184)
Age (years)	54.2±11.5[a,b,***]	41.5±16.1
men/women	50/36	93/91
apoE (ε2/ε3/ε4)[c]	14/129/29	20/292/56
MTP type (TT+TG/GG)	39/47	78/106
BMI (kg.m^{-2})	29.9±4.0 ***	25.0±4.7
Waist (cm)	103±10 ***	86±14
Glucose (mmol/L)	5.8±1.9 ***	4.7±0.6
Insulin (mU/L)	12.3±6.8 ***	8.0±5.1
C-peptide (nmol/L)	1.03±0.34 ***	0.76±0.34
NEFA (mmol/L)	0.59±0.32 *	0.50±0.25
CD-LDL (μmol/L)	72±22 ***	58±20
TC (mmol/L)	6.11±1.24 **	5.58±1.44
TG (mmol/L)	2.88±2.16 ***	1.39±0.71
Apo B (g/L)	1.40±0.34 ***	1.14±0.40
LDL-C (mmol/L)	3.42±0.88	3.22±1.17
HDL-C (mmol/L)	1.36±0.34 **	1.49±0.37

HDL-C, high-density lipoprotein cholesterol.
[a] Mean ± S.D.
[b] Unpaired t test.
[c] Number of alleles in the group.
* P<.05.
** P<.01.
*** P<.001.

Blood samples were taken after 12 h of fasting. Routine biochemical and hematological analyses were performed immediately; samples for special analyses were stored at −70°C until use.

Concentrations of lipids and apolipoproteins were measured using standard methods. Immunoreactive insulin was determined by radioimmunoassay method using two monoclonal antibodies (Insulin IRMA, Imunotech Prague, The Czech Republic). Concentrations of C-peptide were measured by a chemiluminiscence method (ECLIA, Roche Diagnostics, Mannheim, FRG).

Concentrations of CD-LDL were determined spectrophotometrically [31]. FA patterns in main lipid classes of plasma were examined using capillary gas chromatography [32].

The isolation of DNA was performed according to standard desalting procedures, and isoforms of apoE were determined by the method of Hixson and Vernier [33] with our own modification of the analytical procedure [34]. The MTP *−493G/T* polymorphism was determined according to Karpe et al. [35] and Juo et al. [22].

Biomedical Data Processing Program (BMDP Statistical Software, Los Angeles, CA, USA) was used for statistical analysis [36].

3. Results

The basic clinical and biochemical parameters of the studied groups with and without MS are shown in Table 1A; the composition of FA in plasma PL for the respective groups is summarized in Table 1B. The group with MS revealed all hallmarks described above.

The groups with and without MS did not differ in daily energy intake, the energy content of proteins, fats as well as saccharides; moreover, no difference was observed in the intakes of dietary FA–SFA, monounsaturated FA (MFA) and PUFA (calculated as sum of both n-3 and n-6 PUFA) nor dietary fibre and cholesterol (data not presented).

Persons under study were further categorized into groups according to the MTP polymorphism and sex. Table 2 shows clinical, anthropometric and biochemical characteristics of the GG homozygotes (GG) and carriers of the T allele (TT+TG) groups in men with and without MS. In comparison with the group GG, men with MS in the group TT+TG had significantly higher concentrations of insulin, values of HOMA-IR index, concentrations of nonesterified fatty acids (NEFA) and CD-LDL. Carriers of the T allele had elevated concentrations of TC [as a result of higher VLDL cholesterol (VLDL-C)] and of TG. With the only exception of mean age, no significant differences between the groups TT+TG and GG were found in men without MS, not even after the adjustment for age (analysis of covariance).

The basic characteristics of women are presented in Table 3. Carriers of the T allele in women with MS had, in

Table 1B
Effect of metabolic syndrome on fatty acid composition in PLs

Fatty acid	MS (yes) (n=86)	MS (no) (n=184)
14:0[c]	0.27±0.08[a]	0.28±0.11
16:0	29.69±1.59	29.56±1.62
16:1n-7	0.60±0.20[b,*]	0.53±0.20
18:0	14.10±1.32 ***	13.54±1.12
18:1n-9	9.75±1.40	9.70±1.66
18:2n-6	22.31±2.92 ***	24.27±3.09
18:3n-6	0.09±0.04	0.09±0.06
18:3n-3	0.22±0.09	0.23±0.10
20:3n-6	3.29±0.71 ***	2.96±0.66
20:4n-6	11.25±1.91	10.95±1.86
20:5n-3	1.15±0.56 ***	0.93±0.43
22:4n-6	0.30±0.07	0.32±0.06
22:5n-6	0.19±0.05	0.20±0.06
22:5n-3	0.90±0.17	0.90±0.17
22:6n-3	3.71±1.00 **	3.27±0.82
Σ SFA	44.12±1.15 ***	43.43±1.40
Σ MFA	12.06±1.62	12.08±1.86
Σ n-6 PUFA	37.84±2.36 ***	39.16±2.56
Σ n-3 PUFA	5.98±1.41 ***	5.33±1.20
16:1n-7/16:0[c] (Δ9D)	0.020±0.006 ***	0.018±0.006
18:3n-6/18:2n-6 (Δ6D)	0.004±0.002	0.004±0.003
20:4n-6/20:3n-6 (Δ5D)	3.63±1.21	3.89±1.18

Not relevant fatty acids are not included in the table. n, number of carbon atom from the methyl end to the nearest double bond.
Σ SFA, total concentration (the sum) of SFA; Σ MFA, total concentration (the sum) of MFA; Σ n-6 PUFA, total concentration (the sum) of PUFA n-6 family; Σ n-3 PUFA, total concentration (the sum) of PUFA n-3 family.
[a] Mean±S.D.
[b] Unpaired t test.
[c] Shorthand notation of fatty acids:number of carbon atoms:number of double bonds.
* P<.05.
** P<.01.
*** P<.001.

Table 2
Effect of metabolic syndrome and T allele on anthropometric parameters, glucose homeostasis, plasma lipids and LPs in men

Parameter	MS (yes)		MS (no)	
	TT+TG (*n*=27)	GG (*n*=23)	TT+TG (*n*=40)	GG (*n*=53)
Age (years)	52.0±10.5[a]	52.9±11.6	46.2±16.3[b,*]	39.0±14.6
apoE (ε2/ε3/ε4)[c]	2/45/7	4/31/11	6/63/11	3/87/16
Body mass (kg)	97.5±13.6	95.0±12.1	81.1±10.8	82.5±17.1
BMI (kg.m^{-2})	30.6±3.8	30.5±3.4	25.7±3.3	25.8±5.5
Waist (cm)	107±9	106±8	92±10	92±14
Fat mass (kg)	31.0±9.1	30.1±8.7	19.0±6.5	19.8±10.4
Glucose (mmol/L)	6.15±2.10	5.72±1.05	4.81±0.63	4.8±0.57
Insulin (mU/L)	15.56±7.61**	10.53±4.51	7.51±3.32	7.53±4.83
HOMA-IR (ratio)	4.44±3.33*	2.70±1.31	1.61±0.77	1.61±1.05
HOMA-B (ratio)	146±83	116±79	147±186	137±152
C-peptide (nmol/L)	1.10±0.28	1.12±0.42	0.81±0.29	0.77±0.36
NEFA (mmol/L)	0.67±0.38*	0.45±0.29	0.47±0.23	0.40±0.22
CD-LDL (μmol/L)	79±25**	61±17	58±19	59±21
TC (mmol/L)	6.37±1.27*	5.59±1.12	5.68±1.15	5.42±1.68
TG (mmol/L)	4.07±3.26**	2.09±0.82	1.32±0.71	1.51±0.85
apoB (g/L)	1.40±0.36	1.28±0.32	1.21±0.37	1.15±0.50
LDL-C (mmol/L)	3.29±0.9	3.33±0.78	3.24±0.87	3.18±1.42
HDL-C (mmol/L)	1.37±0.37	1.22±0.28	1.47±0.36	1.37±0.31
VLDL-C (mmol/L)	1.25±1.18	0.54±0.31	0.40±0.41	0.42±0.34
VLDL-TG (mmol/L)	2.72±2.21**	1.32±0.68	0.71±0.54	0.94±0.66
VLDL–protein (g/L)	0.647±0.487*	0.384±0.112	0.239±0.119*	0.305±0.165

HOMA-IR: [f-insulin (μU/ml)×f-glucose (mmol/L)/22.5]; HOMA-B, % function of β-cells {20×f-insulin (μU/ml)/[f-glucose (mmol/L) − 3.5]}.
For further abbreviations see Table 1A.

comparison with GG homozygotes, higher values of the HOMA-B index for the function of β cells ($P<.05$). In other parameters, no significant effect of the MTP *−493T* polymorphism could be proven, either in women with or without MS. The groups GG and TT+TG in men and women with and without MS did not differ in the prevalence of *ε2*, *ε3* and *ε4* alleles of the *apoE* gene.

Table 4 shows the FA patterns in plasma PL and the corresponding derived parameters in men. A significant decrease in the content of Σ n-6 PUFA ($P<.05$), as well as in the activity of Δ9D ($P<.05$), was found in male carriers of the T allele, in comparison with GG homozygotes. No differences between the groups GG and TT+TG were found in men without MS.

The FA patterns in plasma PL and the corresponding derived parameters in women are presented in Table 5. No significant differences were observed in relation to the presence or absence of MS and the T allele.

4. Discussion

The most important finding of the present study relates to the effect of the MTP gene *−493G/T* polymorphism (T allele) on several metabolic parameters in middle-aged men with MS. This effect was lacking in male carriers of the common (wild) genotype *−493GG*, in men without MS and in women regardless of the presence or absence of MS and the T allele. To the best of our knowledge, the effect of the MTP *−493G/T* polymorphism on the composition of esterified FA in plasma lipids and on indicators of oxidative stress has not been studied yet.

Carriers of the T allele among men with MS were characterized by more pronounced indicators of IR (elevated plasma concentrations of insulin, C-peptide, NEFA and higher values of the HOMA index), dyslipidemia (higher plasma concentrations of TG, VLDL-C, VLDL-TG), oxidative stress (concentrations of CD-LDL) and changes in the composition of esterified FA related to the presence of MS (decreased concentrations of Σ n-6 PUFA and higher Δ5D activities). In men without MS, as well as in women regardless of the presence of MS, no effect of the T allele could be proved.

The results of clinical and epidemiological studies related to the effects of the MTP *−493G/T* polymorphism on plasma LPs and other components of MS are not consistent. Significantly higher concentrations of sd-LDL, as well as those of TG and VLDL-C, were observed in carriers of the

Table 3
Effect of metabolic syndrome and T allele on anthropometric parameters, glucose homeostasis, plasma lipids and LPs in women

Parameter	MS (yes)		MS (no)	
	TT+TG (*n*=12)	GG (*n*=24)	TT+TG (*n*=38)	GG (*n*=53)
Age (years)	57.4±12.1[a]	56.4±12.4	42.8±17.0	39.9±16.6
apoE (ε2/ε3/ε4)[c]	5/17/2	3/36/9	3/61/12	8/81/17
BMI (kg.m^{-2})	29.1±4.5	29.1±4.4	24.5±4.3	24.5±5.0
Waist (cm)	97±9	97±10	83±13	80±13
Fat mass (kg)	31.7±6.4	31.3±10.4	23.1±8.8	22.0±7.8
Lean body mass (kg)	48.2±8.1	46.1±8.7	43.7±4.6	43.4±6.1
Glucose (mmol/L)	5.26±0.68	5.9±2.63	4.77±0.68	4.61±0.52
Insulin (mU/L)	13.79±8.06	9.49±5.33	9.24±8	8.16±3.76
HOMA-IR (ratio)	3.36±2.30	2.73±1.05	2.06±2.05	1.71±0.91
HOMA-B (ratio)	160±74[b,*]	102±152	148±115	181±117
C-peptide (nmol/L)	1.10±0.44	0.85±0.26	0.80±0.54	0.70±0.19
NEFA (mmol/L)	0.50±0.18	0.66±0.28	0.57±0.26	0.55±0.25
CD-LDL (μmol/L)	76±23	74±21	52±18*	62±20
TG (mmol/L)	2.76±1.08	2.40±1.36	1.33±0.63	1.34±0.59
TC (mmol/L)	6.29±1.22	6.23±1.25	5.63±1.39	5.66±1.42
apoB (g/L)	1.45±0.30	1.48±0.34	1.08±0.33	1.13±0.36
LDL-C (mmol/L)	3.37±0.76	3.66±1.00	3.15±1.12	3.28±1.13
HDL-C (mmol/L)	1.54±0.30	1.41±0.32	1.59±0.35	1.57±0.39
VLDL-C (mmol/L)	0.92±0.56	0.75±0.53	0.32±0.25	0.35±0.27
VLDL–TG (mmol/L)	1.69±0.70	1.49±1.11	0.69±0.47	0.72±0.49
VLDL–protein (g/L)	0.473±0.182	0.400±0.202	0.243±0.106	0.271±0.143

For abbreviations see Tables 1A and 2.

Table 4
Effect of metabolic syndrome and T allele on fatty acid pattern in PLs in men

Parameter	MS (yes)		MS (no)	
	TT+TG (*n*=27)	GG (*n*=23)	TT+TG (*n*=40)	GG (*n*=53)
Σ SFA	44.30±0.97[a]	43.64±1.43	43.68±1.26	43.50±1.39
Σ MFA	12.17±1.84	11.90±1.24	11.71±1.40	11.98±1.54
Σ n-6 PUFA	37.17±2.19[b,*]	38.54±2.19	39.32±2.28	38.90±2.42
Σ n-3 PUFA	6.37±1.42	5.91±1.38	5.29±1.12	5.62±1.46
16:1n-7/16:0[c] (Δ9D)	0.021±0.008	0.019±0.006	0.016±0.005	0.018±0.006
18:1n-9/18:0[c] (Δ9D)	0.703±0.134	0.687±0.087	0.702±0.118	0.718±0.129
18:3n-6/18:2n-6 (Δ6D)	0.004±0.002	0.004±0.002	0.003±0.002	0.004±0.002
20:4n-6/20:3n-6 (Δ5D)	3.452±0.940*	4.227±1.424	4.139±1.512	3.940±1.139

For abbreviations see Table 1B.

T allele among Chinese patients with DM2 [20]. In two Chinese population samples, carriers of the T allele were shown to have elevated plasma concentrations of TC, TG and LDL-C [23]. A longitudinal study of young Afro-Americans showed higher concentrations of TC, LDL-C, apoB and TG in *−493TT* homozygotes [22]. In a group of Swedish healthy men, TT homozygotes had lower numbers of buoyant VLDL particles with a higher proportion of TG [35].

Standard per os fat load in subjects with the GG genotype leads to more than twofold increase in the apoB-48 content in TGRLP (S_f 20–60) [37].

No association between the MTP *−493G/T* polymorphism and lipid levels were found in Japanese men and women [38], as well as in participants of the Framingham Offspring Study [24], and in the survey of French-Canadian youth [25]. On the contrary, other studies showed lower concentrations of LDL-C, low-density lipoprotein (LDL)–TG, VLDL–apoB and LDL–apoB in TT homozygotes, as compared with carriers of the G allele [35]. Similarly, lower concentrations of TG, TC and apoB were found in TT homozygotes recruited from two population cohorts, together with higher values of BMI, waist and insulinemia [21]. The MTP *−493G/T* polymorphism did not significantly influence concentrations of LDL-C in homozygotes of familial hypercholesterolemia; only a trend similar to that in healthy persons was observed [39]. In healthy middle-aged male TT homozygotes, lower concentrations of large LDL particles and of apoB were observed, which probably resulted from a diminished production of intermediate density lipoprotein (d=1.006–1.019g/mL) and LDL [40]. According to some authors, the T allele is associated with an increased risk for coronary heart disease, despite lower lipid levels; the risk can be normalized by statins [41].

In our study, the groups GG and TT+TG of men with MS did not differ in mean age, BMI, waist (as an indicator of intraabdominal fat accumulation) or in the fat mass; these parameters are usually associated with MS [4,42–44].

Our groups GG and TT+TG differed in the degree of IR (higher plasma concentrations of insulin, C-peptide, NEFA and higher values of the HOMA-index), dyslipidemia (higher concentrations of TG, VLDL-TG, VLDL-C), lower concentrations of Σ n-6 PUFA, lower Δ5D activity and higher concentrations of CD-LDL (as an indicator of systemic oxidative stress). All these changes were only present in male carriers of the T allele.

In the Canadian study, combined effects of visceral obesity (or hyperinsulinemia) and the presence of T allele of the MTP *−493G/T* polymorphism resulted in higher plasma concentrations of TC and apoB in LDL, as well as a lower peak particle size of LDL [45].

The relationship between IR and variants of the MTP gene need further studies, as the promoter area of the gene contains negative insulin response elements and insulin can decrease expression of the MTP gene [18].

Elevated plasma concentrations of NEFA have been shown as the dominant pathophysiological change in MS.

Table 5
Effect of metabolic syndrome and T allele on fatty acid pattern in PLs in women

Parameter	MS (yes)		MS (no)	
	TT+TG (*n*=12)	GG (*n*=24)	TT+TG (*n*=38)	GG (*n*=53)
Σ SFA	44.28±0.82[a]	44.28±1.39	43.47±1.82	43.16±1.19
Σ MFA	12.24±1.98	12.01±1.54	12.13±1.61	12.40±2.55
Σ n-6 PUFA	37.58±2.73	38.04±2.42	38.97±2.63	39.41±2.94
Σ n-3 PUFA	5.90±1.54	5.66±1.46	5.43±1.17	5.03±0.97
16:1n-7/16:0[c] (Δ9D[d])	0.021±0.005	0.020±0.005	0.018±0.005	0.020±0.008
18:1n-9/18:0[c] (Δ9D[d])	0.708±0.152	0.697±0.128	0.720±0.145	0.750±0.178
18:3n-6/18:2n-6 (Δ6D[e])	0.005±0.003	0.004±0.002	0.004±0.004	0.004±0.004
20:4n-6/20:3n-6 (Δ5D[f])	3.485±0.867	3.342±1.258	3.955±1.008	3.554±0.984

For abbreviations see Table 1B.

While the levels of NEFA are strictly regulated under physiological conditions, in subjects with IR they remain elevated during 24 h, causing IR in skeletal muscles, liver and pancreas [46].

Accelerated lipolysis during the postabsorptive state (approximately by 70–100%) was demonstrated in subjects with MS, despite two to four times higher plasma concentrations of insulin. An enhanced flux of NEFA from the adipose tissue deteriorates the regulation of the carbohydrate metabolism by insulin in skeletal muscles and in the liver [47,48].

Dysregulation of LP metabolism in MS may be caused by the combined effects of VLDL apoB-100 overproduction, decreased catabolism of LP particles containing apoB-100 and accelerated catabolism of high-density lipoprotein (HDL) particles containing apoA-I [49]. Hypersecretion of VLDL-TG thus appears as the result of combined peripheral and liver IR (accentuated lipogenesis de novo), with the participation of an increased expression of the sterol regulatory element binding protein-1c; together with the elevated activity of MTP and increased flux of NEFA to the liver, these factors stimulate the formation of VLDL apoB and its hepatic secretion [50].

Decreased plasma levels of Σ n-6 PUFA (mainly due to low concentrations of LA) are typical of MS in adults [6] and children [9], as well as in patients after MI [8]. Low concentrations of LA have been shown to predict progression of MS in middle-aged and elderly men [7], as well as progression of impaired glucose tolerance to DM2 [10]. In our study, the most important finding concerning the profile of FA was increased content of SFA and decreased content of n-6 PUFA (especially that of LA) in patients with MS. The abovementioned changes were connected with the higher activity of Δ9D for PA; however, we did not observe changes in activities of Δ6D and Δ5D. It must be stated that none of the cited papers have ever presented all these changes simultaneously. Further categorization of the studied groups with respect to sex and MTP polymorphism led to pronounced decrease in PUFA n-6 content as well as Δ5D activity only in men with MS and carriers of T allele.

We did not find elevated Δ6D activities in PL of male carriers of the T allele with MS. Increased activities of Δ6D have been ascribed to hyperinsulinemia and increased BMI and generally considered as a characteristic feature of IR [9,5]. On the other hand, in the group TT+TG of men with MS, we could demonstrate decreased Δ5D activities, another important feature of MS, which was shown to be independent of BMI and physical activity [51].

A significant effect of the T allele on oxidative stress (plasma concentrations of CD-LDL) was also observed in our study only in men with MS. Apart from being an indicator of oxidative stress, CD-LDL reflect the level of minimally modified LDL, in which only the lipid component of the particle is oxidatively modified [31]. Increased levels of oxidative stress were observed in hypercholesterolemia, hypertriglyceridemia, MS, obesity and DM2 [52–54]. According to the present pathophysiological conceptions, oxidative stress and chronic low-grade inflammation are the main links between the MS or DM2 and the risk for atherosclerosis and CV disease [55]. Adipocytes of the visceral fat play an important role in the pathogenesis of oxidative stress in MS [47]. The extent of oxidative stress can also be assessed by the concentration of urinary 8-*epi*-prostaglandin F_2 that significantly correlates with the size of intraabdominal adipose tissue [56].

In conclusion, the presence of the T allele of the common MTP *−493G/T* polymorphism may play an important role only in middle-aged men with MS. In these men, we have observed deterioration of the IR parameters (higher insulin, C-peptide and HOMA-IR index), dyslipidemia (increased concentrations of NEFA, VLDL-C and VLDL-TG), oxidative stress (raised concentration of CD-LDL) as well as changes in FA profile (higher content of SFA, lower content of n-6 PUFA and decreased activity of Δ5D). None of the above described effects was observed in women.

The *−493T* allele can thus be considered as a RF in the pathogenesis of MS, especially in the interaction with other genetic, nutritional and gender-specific factors.

References

[1] Grundy SM. Drug therapy of the metabolic syndrome: minimizing the emerging crisis in polypharmacy. Nat Rev 2006;5:295–304.
[2] Greenberg AS, McDaniel ML. Identifying the links between obesity, insulin resistance and β-cell function: potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest 2002;32:24–34.
[3] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signalling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002;23:599–622.
[4] Miranda PJ, DeFronzo RA, Califf RM. Metabolic syndrome: definition, pathophysiology, and mechanisms. Am Heart J 2005;149: 33–45.
[5] Warensjö E, Sundström J, Lind L, Vessby B. Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. Am J Clin Nutr 2006;84:442–8.
[6] Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. Ann N Y Acad Sci 2002;967:183–95.
[7] Vessby B. Dietary fat, fatty acid composition in plasma and the metabolic syndrome. Curr Opin Lipidol 2003;14:15–9.
[8] Leskinen MH, Solakivi T, Kunnas T, Alho H, Nikkari ST. Serum fatty acids in post infarction middle-aged men. Scand J Clin Lab Invest 2005;65:485–90.
[9] Decsi T, Csábi G, Török K, Erhardt E, Minda H, Burus I, et al. Polyunsaturated fatty acids in plasma lipids of obese children with and without metabolic cardiovascular syndrome. Lipids 2000;35:1179–84.
[10] Laaksonen DE, Lakka TA, Lakka HM, Nyyssönen K, Rissanen T, Niskanen LK, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med 2002;19:456–64.
[11] Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon Ch. Plasma fatty acid composition is associated with the metabolic syndrome and low grade inflammation in overweight adolescent. Am J Clin Nutr 2005;82: 1178–84.
[12] Laakso M. Gene variants, insulin resistance, and dyslipidemia. Curr Opin Lipidol 2004;15:115–20.

[13] Bakillah A, El Abbouyi A. The role of microsomal triglyceride transfer protein in lipoprotein assembly: an update. Front Biosci 2003;8: 294–305.
[14] Berriot-Varoqueaux N, Aggerbeck LP, Samson-Bouma ME, Wetterau JR. The role of the microsomal triglyceride transfer protein in abetalipoproteinemia. Annu Rev Nutr 2000;20:663–97.
[15] Berthier MT, Houde A, Paradis AM, Couture P, Gaudet D, Després JP, et al. Molecular screening of the microsomal triglyceride transfer protein: association between polymorphisms and both abdominal obesity and plasma apolipoprotein concentrations. J Hum Genet 2004; 49:684–90.
[16] Bernard S, Touzet S, Personne I, Lapras V, Bondon PJ, Berthezène F, et al. Association between microsomal triglyceride transfer protein gene polymorphisms and biological features of liver steatosis in patients with type II diabetes. Diabetologia 2000;43:995–9.
[17] Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, et al. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. J Hepatol 2004;40:853–6.
[18] Lin MC, Gordon D, Wetterau JR. Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. J Lipid Res 1995;36:1073–81.
[19] Gordon DA, Jamil H. Progress towards understanding the role of microsomal triglyceride transfer protein in apolipoprotein-B lipoprotein assembly. Biochim Biophys Acta 2000;1486:72–83.
[20] Chen SP, Tan KC, Lam KS. Effect of the microsomal triglyceride transfer protein *−493 G/T* polymorphism and type 2 diabetes mellitus on LDL subfractions. Atherosclerosis 2003;167:287–92.
[21] Ledmyr H, Karpe F, Lundahl B, Mc Kinnon M, Skoglund-Andersson C, Ehrenborg E. Variants of the microsomal triglyceride transfer protein gene are associated with plasma cholesterol levels and body mass index. J Lipid Res 2002;43:51–8.
[22] Juo SH, Han Z, Smith JD, Colangelo L, Liu K. Common polymorphism in promoter of microsomal triglyceride transfer protein gene influences cholesterol, ApoB, and triglyceride levels in young African American men: results from the coronary artery risk development in young adults (CARDIA) study. Arterioscler Thromb Vasc Biol 2000;20:1316–22.
[23] Li RS, Yin RX, Lin WX, Yang DZ. Relationship between the polymorphism of microsomal triglyceride transfer protein gene and the level of serum lipids in Guangxi Heiyi Zhuang population. Zhuanghua Yi Xue Za Zhi 2005;85:2492–6.
[24] Couture P, Otvos JD, Cupples LA, Wilson PW, Schaefer EJ, Ordovas JM. Absence of association between genetic variation in the promoter of the microsomal triglyceride transfer protein gene and plasma lipoproteins in the Framingham Offspring Study. Atherosclerosis 2000; 148:337–43.
[25] Stan S, Lambert M, Delvin E, Paradis G, O'Loughlin J, Hanley JA, et al. Intestinal fatty acid binding protein and microsomal triglyceride transfer protein polymorphisms in French-Canadian youth. J Lipid Res 2005;46:320–7.
[26] Kunešová M, Hainer V, Tvrzická E, Phinney SD, Štich V, Pařízková J, et al. Assessment of dietary and genetic factors influencing serum and adipose fatty acid composition in obese female identical twins. Lipids 2002;37:27–32.
[27] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome — a new worldwide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006;23:469–80.
[28] Lochman T, Roche A, Martorel R, editors. Standardization of anthropometric measurements. Champaign (Ill): Human Kinetics Publishers; 1989. p. 39–80.
[29] Durnin JV, Womersley J. Body fat assessed from the total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 71 years. Br J Nutr 1974;32: 7–97.
[30] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[31] Ahotupa M, Ruutu M, Mäntylä E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. Clin Biochem 1996;29:139–44.
[32] Tvrzická E, Vecka M, Staňková B, Žák A. Analysis of fatty acids in plasma lipoproteins by gas chromatography-flame ionisation detection. Quantitative aspects. Anal Chim Acta 2002;465:337–50.
[33] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990;31: 545–8.
[34] Jáchymová M, Horký K, Bultas J, Kožich V, Jindra A, Peleška J, et al. Association of the Glu298Asp polymorphism in the endothelial nitric oxide synthase gene with essential hypertension resistant to conventional therapy. Biochem Biophys Res Commun 2001;284: 426–30.
[35] Karpe F, Lundahl B, Ehrenborg E, Eriksson P, Hamsten A. A common functional polymorphism in the promoter region of the microsomal triglyceride transfer protein gene influences plasma LDL levels. Arterioscler Thromb Vasc Biol 1998;18:756–61.
[36] Dixon WJ. BMDP Statistical Software Manual, Vol. 1–2. Los Angeles: University of California Press; 1992.
[37] Lundahl B, Hamsten A, Karpe F. Postprandial plasma ApoB-48 levels are influenced by a polymorphism in the promoter of the microsomal triglyceride transfer protein gene. Arterioscler Thromb Vasc Biol 2002; 22:289–93.
[38] Yamada Y, Ando F, Shimiokata H. Association of microsomal triglyceride transfer protein gene polymorphism with blood pressure in Japanese women. Int J Mol Med 2006;17:83–8.
[39] Lundahl B, Leren TP, Leiv O, Hamsten A, Karpe F. A functional polymorphism in the promoter region of the microsomal triglyceride transfer protein (MTP-493G/T) influences lipoprotein phenotype in familiar hypercholesterolemia. Arterioscler Thromb Vasc Biol 2000; 20:1784–8.
[40] Lundahl B, Skoglund-Andreson C, Caslake M, Bedford D, Stewart P, Hamsten A, et al. Microsomal transfer protein −493 variant reduces IDL+LDL apoB production and plasma concentration of large LDL particles. Am J Physiol Endocrinol Metab 2006;290: E739–45.
[41] Ledmyr H, McMahon AD, Ehrenborg E, Nielsen LB, Neville M, Lithell H, et al. The microsomal triglyceride transfer protein gene *−493T* variant lowers cholesterol but increases the risk of coronary heart disease. Circulation 2004;109:2279–84.
[42] Park YW, Shankuan Z, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome. Prevalence and associated risk factor findings in the US population from the 3rd National Health and Nutritional Examination Survey, 1998 –1994. Arch Intern Med 2003;163:427–36.
[43] Vanhala MJ, Pitkajarvi TK, Kumpusalo EA, Takala JK. Obesity type and clustering of insulin resistance-associated cardiovascular risk factors in middle-aged men and women. Int J Obes Relat Metab Disord 1998;22:369–74.
[44] Reaven GM. Banting Lecture 1988. The role of insulin resistance in human disease. Diabetes 1998;37:1595–607.
[45] St-Pierre J, Lemieux I, Miller-Felix I, Prud'homme D, Bergeron J, Gaudet D, et al. Visceral obesity and hyperinsulinemia modulate the impact of the microsomal triglyceride transfer protein *−493G/T* polymorphism on plasma lipoprotein level in men. Atherosclerosis 2002;160:317–24.
[46] Fonseca VA. The metabolic syndrome, hyperlipidemia, and insulin resistance. Clin Cornerstone 2005;7:61–72.
[47] Lewis GF, Carpentier A, Adeli K, Giacca A. Disorder of fat storage and mobilization in the pathogenesis of insulin resistance and type I diabetes. Endocr Rev 2002;23:201–29.
[48] McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 2002;51: 7–18.

[49] Chan DC, Barrett PH, Watts GF. Recent studies of lipoprotein kinetics in the metabolic syndrome and related disorders. Curr Opin Lipidol 2006;17:28–36.
[50] Avramoglu RK, Basciano H, Adeli K. Lipid and lipoprotein dysregulation in insulin resistant states. Clin Chim Acta 2006;368: 1–19.
[51] Warensjö E, Riserus U, Vessby B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. Diabetologia 2005;48:1999–2005.
[52] Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993;91: 1163–86.
[53] Furukawa S, Fujita K, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–61.
[54] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24:816–23.
[55] Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes 2005;54:1–7.
[56] Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. Circ J 2006;70:1437–42.