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Gender differences in factors influencing insulin resistance in elderly hyperlipemic non-diabetic subjects

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Abstract

Background: The increase in the prevalence of insulin resistance-related metabolic syndrome, a disorder that greatly increases the risk of diabetes, heart attack and stroke, is alarming. One of the most frequent and early symptoms of metabolic syndrome is hypertriglyceridemia. We examined the gender differences between various metabolic factors related to insulin resistance in elderly non-diabetic men and postmenopausal women of comparable age suffering from hypertriglyceridemia, and compared them with healthy subjects of equal age.

Results: The indexes of insulin resistance HOMA IR and QUICKI were significantly higher in both hyperlipemic men and women than in controls; 95% confidence limits of hyperlipemic subjects did not overlap with controls. In both normolipemic and hyperlipemic men and women serum leptin correlated significantly with insulin resistance, while HDL-cholesterol correlated inversely with HOMA-IR only in women (both normo- and hyperlipemic), and serum tumor necrosis factor α (TNFα) only in hyperlipemic women. According to results of multiple regression analysis with HOMA-IR as a dependent variable, leptin played a significant role in determining insulin resistance in both genders, but – aside from leptin – triglycerides, TNFα and decreased HDL-cholesterol were significant determinants in women, while body mass index and decreased HDL-cholesterol were significant determinants in men. The coefficient of determination (R^2) of HOMA IR by above mentioned metabolic variables was in women above 60%, in men only about 40%.

Conclusion: The significant role of serum leptin in determination of insulin resistance in both elderly men and postmenopausal women of equal age was confirmed. However, the study also revealed significant gender differences : in women a strong influence of triglycerides, TNFα and decreased HDL-cholesterol, in men only a mild role of BMI and decreased HDL-cholesterol.

Background

In association with pandemic obesity the prevalence of the insulin resistance-related metabolic syndrome is constantly growing [1]. As a consequence of this fact, type 2 diabetes mellitus and cardiovascular mortality occurs in much younger age groups [2]. A typical hyperlipemia, consisting of an increase of serum triglycerides and a decrease of serum HDL-cholesterol, is a characteristic and an early symptom of this syndrome [3].

With increasing age, body mass index (BMI) and adiposity, insulin sensitivity declines and the number of cardio-

vascular risk factors increases in both genders [4–6]. It was repeatedly demonstrated that plasma concentration of leptin – a hormone produced mainly by adipose tissue – is substantially higher in all age groups of women than in men [7–10]. This may be caused by different size and/or distribution of fat tissue compartments influenced by hormones: estrogens stimulate, whereas testosterone inhibits leptin secretion. In women subcutaneous fat mass prevails – and during augmentation of overweight it increases – while in men intra-abdominal fat mass prevails [11–13]. Subcutaneous fat in particular serves as a substantial source of tumor necrosis factor α (TNFα), which represents one of the factors that interfere with insulin signal transduction into the cells [14–16]. Leptin, TNFα and some other factors are abundantly expressed in adipose tissue and contribute to the insulin resistance that accompanies overweight and obesity. Leptin correlates positively with hyperinsulinemia, BMI, fat mass and hypertriglyceridemia, respectively, and correlates inversely with HDL-cholesterol and lean body mass [17–25].

The incidence and mortality of ischemic heart disease and of other consequences of atherosclerosis increases with age in both genders, especially after the age of sixty. In premenopausal women, however, the incidence of these disorders is considerably less frequent than in men of appropriate age. After the menopause the prevalence of metabolic syndrome and cardiovascular mortality in women gradually increases, attaining values comparable to men at about the age of 70 [2,26]. Paradoxically, it takes place at the time when serum leptin concentration in women has relatively decreased [27,28].

The aim of this study was to analyze the interrelations between several metabolic variables and factors related to insulin resistance in groups of both normal and hyperlipemic postmenopausal women and men of appropriate age, and to attempt to elucidate the gender differences and some pathophysiologic mechanisms of these differences. We compared homeostatic indexes of insulin resistance HOMA IR and QUICKI, serum lipid and insulin parameters, uric acid, leptin and TNFα between groups of subjects without apparent symptoms of metabolic syndrome, and groups showing mild hypertriglyceridemia with decreased HDL-cholesterol. In addition, serum concentration of the heart fraction of fatty acid binding protein (hFATP) was explored as a factor that might reflect the regulative role of PPAR gamma in lipid homeostasis [29,30], and serum IgG anticardiolipin (ACL-IgG) was investigated as an indirect indicator of oxidized lipid fractions related to atherosclerotic complications [31,32].

Methods

Subjects

The study was carried out on 70 out-patients of the Metabolic Center at the hospital in Sternberk, Czech Republic. From these, 40 patients (20 men and 20 women) were selected with mild hyperlipidemia, i.e. with plasma triglyceride concentration exceeding 2.0 mmol/l, total cholesterol exceeding 6.0 mmol/l, LDL cholesterol exceeding 4.0 mmol/l, and with HDL cholesterol concentration in men under 1.0 mmol/l, and in women under 1.2 mmol/l. These groups were denominated as "hyperlipemic". Two other groups (10 men and 20 women) with approximately normal serum values of these variables were taken as "controls". The average age in men was 59.1 ± 10.6 y, and in women 59.4 ± 10.1 y, respectively. The differences between lipid parameters of hyperlipemic and control groups were highly statistically significant, while the age differences were insignificant (see Table 1). None of the patients had clinically apparent diabetes mellitus, but some of the hyperlipemic patients exerted impaired glucose tolerance or impaired fasting glucose (values between 6.1 and 7.0 mmol/l, or between 6.1 and 7.8 mmol/l, respectively). None of the patients was treated with insulin, peroral antidiabetics or antihyperlipemic drugs; some of them were treated with antihypertensive therapy. No signs of major clinical or laboratory symptoms of other diseases were present in any group of the explored patients. Blood samples were obtained in the morning via a venipuncture after overnight fasting. After clotting the serum was separated and stored at -20° until used. An informed consent was obtained from all probands.

Body mass indexes (BMI), defined as weight in kilograms divided by the square of height in meters, were calculated.

Biochemical methods

Serum leptin concentrations were measured by a sandwich ELISA test kit (Human Leptin ELISA, BioVendor Laboratory Medicine, Inc, Czech Republic). Its sensitivity limit was 0.2 ng/ml, intraassay CV 6.1% at the level of 7.5 ng/m, inter-assay CV 8.5% at the level of 4.8 ng/ml. Tetramethylbenzidine was used as a substrate; quality controls were human based. Several other hormones and peptides were estimated by routine immunochemical tests: insulin, C-peptide, TNFα (IMMULITE, Diagnostic Products Corporation, Los Angeles, CA, U.S.A.), proinsulin intact (DAKO, Denmark), IgG anticardiolipin (ACL-IgG, IMMCO Diagnostics, Buffalo, NY, U.S.A.) and heart fatty acid binding protein (hFABP, Hbt HUMAN H-FABP, HyCult Biotechnology, Uden, the Netherlands). Serum concentration of glucose, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, Apoprotein B and uric acid were measured on a ILAB-600 biochemical analyzer (Instrumentation Laboratory, Lexington, Ma, U.S.A.) using BioVendor sets. All samples were processed and exam-

Table 1: Main characteristics that served in selection of groups of patients under study

	MEN			WOMEN		
	Controls N = 10 Mean ± SD	**Hyperlipemic patients** N = 20 Mean ± SD	**p**	**Controls** N = 20 Mean ± SD	**Hyperlipemic patients** N = 20 Mean ± SD	**p**
Age (years)	60.3 ± 11.0	58.6 ± 10.4	0.69	56.9 ± 13.0	62.0 ± 7.36	0.15
Cholesterol (mmol/l)	5.07 ± 1.06	6.62 ± 0.82	**0.0001**	5.15 ± 0.70	6.92 ± 1.01	**0.0001**
Triglycerides (mmol/l)	1.11 ± 0.44	3.52 ± 1.38	**0.0001**	1.42 ± 0.47 *	2.72 ± 0.92*	**0.0001 KS**
HDL-chol. (mmol/l)	1.43 ± 0.39	0.96 ± 0.24	**0.0003**	1.50 ± 0.21	1.21 ± 0.21***	**0.0001**
LDL-chol. (mmol/l)	3.08 ± 0.83	4.38 ± 1.06	**0.0047**	3.21 ± 0.71	4.74 ± 1.02	**0.0001**

Statistical significance between control and hyperlipemic groups was tested using the unpaired Student's T-test in the case of normal distribution of compared data sets, and using Kolmogorov-Smirnov's test when at least in one of the data sets compared the normal distribution was excluded (marked with $_{KS}$). The significant p values are denoted by thick underlined numbers. With asterisks statistically significant differences between controls and hypelipemic patients of different gender are denoted (*, **, *** = p < 0,05, 0,01 and 0,001, respectively).

ined according to principles of good laboratory practice and under constant intralaboratory and external quality control.

The homeostatic indexes of insulin resistance (HOMA IR and QUICKI) were calculated according to the homeostasis model of assessment [33–35] as follows:

HOMA IR = fasting insulin (μU/ml) * fasting glucose (mmol/l) / 22.5;

QUICKI = 1 / [log fasting insulin (μU/ml) + log fasting glucose (mg/100 ml)].

Statistics

Statistical analysis was performed using the Version 6 SAS/STAT software (SAS Institute, Inc., Cary, NC, U.S.A.). The Shapiro-Wilks tests were used in testing the normality of distribution. Some of the data obtained were not normally distributed. The statistical significance of differences between the means in the hyperlipemic and control groups were evaluated using the unpaired Student's T-test in the case of normal distribution of data sets, and using the Kolmogorov-Smirnov test when at least in one of the data sets the normal distribution was excluded. Spearman's rank-order correlation was used for correlation analysis. Multiple regression analysis was performed using HOMA IR indexes of insulin resistance as dependent variables, and other metabolic and hormonal factors (lipid parameters, BMI, leptin, TNFα, hFABP, ACL-IgG) as independent variables. The so-called step-down regression model was used to select dominant independent variables. Various four-member groups of independent (explanatory) variables were used for the analysis and the non-zero intercept was taken into account. The independent variables were then dropped, one at a time; at each stage one variable making the least contribution to the dependent variable (i.e. that showed the least p-value in the test of the regression coefficient being zero) was excluded. The coefficient of determination R^2, which can be viewed as a percentage explaining the total variance, was simultaneously monitored. A great drop in R^2 after excluding some independent variable enabled selection of those independent variables that could be thought to be the most important determinants of the dependent variable.

Results

Table 1 demonstrates mean parameters in individual groups of subjects matched according to sex, lipid parameters and age. While the age of all four groups did not differ substantially, the concentrations of total serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol differ very significantly in both male and female hyperlipemic groups as compared with controls. In addition, the concentration of triglycerides in control women was significantly higher than in control men, the concentration of triglycerides in hyperlipemic women was lower than in hyperlipemic men, and the concentration of HDL-cholesterol in hyperlipemic women was very significantly higher when compared with hyperlipemic men.

Table 2 shows the values of other metabolic and insulin parameters, factors related to insulin resistance and indexes of insulin resistance, respectively. Body mass indexes and uric acid concentration were significantly higher in hyperlipemic men as compared to controls, but not in hyperlipemic women. Uric acid concentration was substantially lower in hyperlipemic women than in hyperlipemic men. Plasma concentrations of glycemia, insulin and intact proinsulin were significantly higher in both hyperli-

Table 2: Detailed characteristics of the subjects under study

	MEN			WOMEN		
	Controls N = 10 Mean ± SD	**Hyperlipemic** patients N = 20 Mean ± SD	p	**Controls** N = 20 Mean ± SD	**Hyperlipemic patients** N = 20 Mean ± SD	p
BMI	25.91 ± 3.58	28.51 ± 2.60	**0.030**	25.36 ± 3.72	26.73 ± 3.59	0.24
HOMA IR	1.685 ± 0.771	3.137 ± 1.419	**0.002_{KS}**	1.717 ± 0.893	2.694 ± 1.011	**0.001_{KS}**
QUICKI	0,3596 ± 0,0263	0,3266 ± 0,0185	**0,002**	0,3603 ± 0,0281	0,3332 ± 0,0192	**0,0007**
Uric acid (mmol/l)	270.5 ± 65.4	379.9 ± 84.2	**0.003**	236.1 ± 67.0	270.8 ± 58.9***	0.13
Glycemia (mmol/l)	5.31 ± 0.53	6.11 ± 0.95	**0.029_{KS}**	5.26 ± 0.42	5.85 ± 0.70	**0.0027**
Insulin (mIU/l)	6.96 ± 2.80	11.43 ± 4.50	**0.016_{KS}**	7.33 ± 3.80	10.27 ± 3.52	**0.034_{KS}**
Proinsulin intact (pmol/l)	2.68 ± 1.15	5.51 ± 2.75	**0.012_{KS}**	2.51 ± 1.76	4.49 ± 3.11	**0.034_{KS}**
C-peptide (nmol/l)	0.66 ± 0.31	1.11 ± 0.39	**0.0148**	0.75 ± 0.35	0.92 ± 0.34	0.22_{KS}
Leptin (ng/ml)	3.07 ± 3.38	6.21 ± 3.78	**0.012_{KS}**	16.06 ± 13***	17.79 ± 5.9**	0.17_{KS}
TNFα (pg/ml)	14.2 ± 4.15	11.52 ± 2.77	0.063_{KS}	11.65 ± 6.80	12.73 ± 9.15	0.43_{KS}
hFABP (pg/ml)	3.86 ± 2.47	4.53 ± 1.97	0.46	3.09 ± 1.80	3.79 ± 2.00	0.41_{KS}
ACL-IgG (IU/ml)	13.08 ± 6.75	19.63 ± 16.9	0.57_{KS}	12.28 ± 6.38	16.35 ± 16.8	0.99_{KS}

BMI = body mass index, HOMA IR and QUICKI = homeostatic indexes of insulin resistance (see Methods), TNFα = tumor necrosis factor α, hFABP = heart fraction of fatty acid binding protein, ACL-IgG = IgG anticardiolipin. Other designations are the same as in Table 1.

pemic men and women as compared with controls of identical gender, while the concentration of leptin increased only in hyperlipemic men. However, serum leptin concentrations of both control and hyperlipemic women were significantly higher than in corresponding groups of men. Serum concentrations of TNFα, hFABP and ACL-IgG in hyperlipemic groups of both men and women were not significantly different from control groups. On the other hand, the indexes of insulin resistance HOMA IR and QUICKI differed very significantly in hyperlipemic groups of both men and women as compared with corresponding control groups, more distinctly in women.

From Fig. 1, presenting 95% confidence limits of insulin resistance indexes HOMA IR and QUICKI, we concluded that in groups of hyperlipemic patients of both genders the insulin resistance was substantially higher than in control groups; the groups did not overlap each other.

In Table 3 the results of Spearman's correlations between insulin resistance index HOMA IR and various metabolic parameters are presented. In the control group of men, positive significant correlation between HOMA IR and serum leptin concentration, and inverse significant correlation between HOMA IR and ACL IgG, respectively, were found. In the control group of women, the significance of Spearman's correlation between HOMA IR and leptin was more expressive; inverse correlation between HOMA IR and HDL-cholesterol was also present.

Figure 1

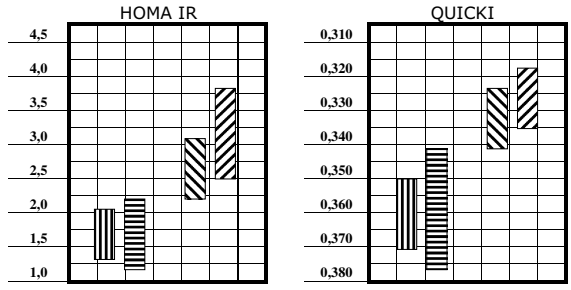

Figure 1
Schematic presentation of 95% confidence limits of insulin resistance indexes HOMA IR and QUICKI in groups of control and hyperlipemic women and men. = Controls (women) = Controls(men) = Hyperlipemic women = Hyperlipemic men

In the hyperlipemic group of men, the significance of the correlation between HOMA IR was more expressive in relation to the control group, and no significant correlation between HOMA IR and ACL IgG was found. In the hyperlipemic group of women, however, the significance of Spearman's correlation between HOMA IR and serum leptin concentration weakened, the inverse correlation between HOMA IR and HDL-cholesterol remained

Table 3: Spearman's correlations between HOMA IR and various metabolic factors studied

CONTROLS								
MEN (n = 10)	**BMI**	**TGL**	**HDL**	**LDL**	**Leptin**	**TNFα**	**ACL IgG**	**FABP**
HOMA IR	S_k = 0,248 p = 0,49	S_k = 0,515 p = 0,13	S_k = 0,006 p = 0,85	S_k = 0,428 p = 0,29	**S_k = 0,658** **p = 0,038**	S_k = -0,119 p = 0,778	**S_k = -0,714** **p = 0,046**	S_k = -0,405 p = 0,319
WOMEN (n = 20)	**BMI**	**TGL**	**HDL**	**LDL**	**Leptin**	**TNFα**	**ACL IgG**	**FABP**
HOMA IR	S_k = 0,354 p = 0,13	S_k = 0,157 p = 0,51	**S_k = -0,499** **p = 0,025**	S_k = 0,393 p = 0,13	**S_k = 0,826** **p = 0,0001**	**S_k = 0,225** p = 0,459	S_k = 0,296 p = 0,325	S_k = 0,134 p = 0.660
HYPERLIPEMIC SUBJECTS								
MEN (n = 20)	**BMI**	**TGL**	**HDL**	**LDL**	**Leptin**	**TNFα**	**ACL IgG**	**FABP**
HOMA IR	S_k = 0,358 p = 0,12	S_k = -0,278 P = 0,24	S_k = -0,180 p = 0,45	S_k = -0,057 p = 0,99	**S_k = 0,574** **p = 0,008**	S_k = -0,235 p = 0,317	S_k = 0,173 p = 0,466	S_k = 0,014 p = 0,952
WOMEN (n = 20)	**BMI**	**TGL**	**HDL**	**LDL**	**Leptin**	**TNFα**	**ACL IgG**	**FABP**
HOMA IR	S_k = 0,308 p = 0,19	S_k = 0,334 p = 0,15	**S_k = -0,554** **p = 0,011**	S_k = -0,111 p = 0,85	**S_k = 0,471** **p = 0,036**	**S_k = 0,447** **p = 0,048**	S_k = 0,079 p = 0,740	S_k = 0,224 p = 0.342

S_k = Spearman's correlation index. Significant values ($p < 0,05$) are denoted with thick numbers.

approximately unchanged, and a positive correlation between HOMA IR and serum concentration of TNFα appeared.

Table 4 shows results of multiple regression analysis, when data from both control and hyperlipemic groups of each gender were judged together. HOMA IR was considered as a dependent variable and differently changed constellations of metabolic and other factors were taken as independent variables.

In men, BMI and leptin seemed to play a main role in influencing the insulin resistance index HOMA IR, while TGL, ACL IgG and LDL-cholesterol didn't play any significant role (see left columns of Table 4). The decreasing of HDL-cholesterol concentration may also have some influence (see a significant drop of R^2 after exclusion of this factor in Table 4A, 4B). But in the presence of leptin in the group of independent factors, the drop of R^2 after exclusion of HDL-cholesterol from these factors was minimal (see Table 4C). On the other hand, after the exclusion of TNFα from the group of independent variables (see Table 4B, 4D) the value of R^2 has unexpectedly risen, which could reflect the interference of TNFα with factors increasing the insulin resistance.

In women (see right columns of Table 4), the maximal values of R^2 were achieved with combination of independent variables containing TGL, leptin and HDL-cholesterol (about 60% influence on HOMA IR! – see Table 4A, 4B, 4C). TNFα seemed to play quite a different role than in men: after exclusion of this factor from the group of independent factors R^2significantly decreased (see Table 4B, 4D). In contrast to men, the role of BMI seemed to be minimal. As in men, the role of ACL IgG and LDL-cholesterol in influencing HOMA IR was negligible, but in contrast to men, hFABP might play a certain role in this process (see Table 4D).

Table 4: Multiple regression analysis of data from men and women (controls and tests).

A		**MEN**						**WOMEN**					
		Interc	**HDL**	**TGL**	**LDL**	**BMI**	**R^2**	**Interc**	**HDL**	**TGL**	**LDL**	**BMI**	**R^2**
HOMA IR	**Par.**	-0,27	-1,07	0,11	-0,06	0,19	0,29	2,74	-1,95	0,31	0,02	0,05	**0,56**
	T = 0	0,91	0,04	0,55	0,79	0,03		0,11	**0,006**	0,09	0,87	0,19	
	Par.	-0,75	-1,68	-0,07		0,19	**0,32**	2,62	-1,83	0,34		0,05	**0,52**
	T = 0	0,73	0,03	0,68		0,016		0,09	**0,007**	0,04		0,16	
	Par.	-0,84	-1,52			0,19	**0,32**	4,11	-1,98	0,38			**0,49**
	T = 0	0,69	**0,02**			0,015		**0,0007**	**0,003**	**0,02**			
	Par.	-2,54				0,18	0,17	6,09	-2,86				**0,42**
	T = 0	0,25				**0,02**		**0,0001**	**0,0001**				

B		**MEN**						**WOMEN**					
		Interc	**BMI**	**TNF α**	**HDL**	**TGL**	**R^2**	**Interc**	**BMI**	**TNFα**	**HDL**	**TGL**	**R^2**
HOMA IR	**Par.**	0,20	0,17	-0,04	-1,46	-0,07	0,27	0,78	0,05	0,05	-0,95	0,41	**0,64**
	T = 0	0,94	0,055	0,62	0,09	0,70		0,62	0,17	**0,0008**	0,14	**0,008**	
	Par.	0,14	0,16	-0,04	-1,29		0,27	2,32		0,05	1,15	0,45	**0,62**
	T = 0	0,95	0,054	0,58	0,08			0,04		**0,0013**	0,07	**0,004**	
	Par.	-0,84	0,18		-1052		**0,32**	0,36		0,06		0,61	**0,58**
	T = 0	0,69	**0,015**		**0,02**			0,31		**0,0002**		**0,0001**	
	Par.	-2,55	0,19				0,17	0,79				0,68	**0,36**
	T = 0	0,25	**0,02**					**0,02**				**0,0001**	

C		**MEN**						**WOMEN**					
		Interc	**BMI**	**Leptin**	**HDL**	**TGL**	**R^2**	**Interc**	**BMI**	**Leptin**	**HDL**	**TGL**	**R^2**
HOMA IR	**Par.**	0,54	0,09	0,13	-1,07	-0,03	**0,39**	1,90	0,01	0,03	-1,14	0,46	**0,61**
	T = 0	0,81	0,31	0,10	0,19	0,85		0,18	0,73	**0,007**	0,077	**0,0056**	
	Par.	0,52	0,09	0,13	-0,99		**0,39**	2,18		0,04	-1,14	0,47	**0,61**
	T = 0	0,81	0,30	0,08	0,14			0,06		**0,002**	0,07	**0,003**	
	Par.	2,62		0,18	-0,80		**0,37**	0,13		0,05		0,64	**0,58**
	T = 0	0,007		**0,005**	0,22			0,68		**0,0001**		**0,0001**	
	Par.	1,59		0,20			**0,33**	0,79				0,68	**0,37**
	T = 0	0,0001		**0,0009**				**0,02**				**0,0001**	

D		**MEN**						**WOMEN**					
		Interc	**Leptin**	**TNF α**	**FABP**	**ACL**	**R^2**	**Interc**	**Leptin**	**TNFα**	**FABP**	**ACL**	**R^2**

Table 4: Multiple regression analysis of data from men and women (controls and tests). ***(Continued)***

HOMA IR	**Par.**	2,48	0,17	-0,04	-0,05	0,004	0,29	0,46	0,04	0,05	0,17	0,002	**0,44**
	T = 0	0,07	**0,02**	0,57	0,70	0,82		0,38	**0,014**	**0,018**	**0,028**	0,82	
	Par.	2,49	0,18	-0,04	-0,04		0,29	0,52	0,04	0,04	0,17		**0,44**
	T = 0	0,06	**0,010**	0,56	0,72			0,25	**0,011**	**0,016**	**0,02**		
	Par.	2,27	0,18	-0,04			0,28	1,25	0,04	0,04			**0,34**
	T = 0	0,04	**0,009**	0,58				0,0012	**0,029**	**0,03**			
	Par.	1,59	0,20				**0,33**	1,36	0,05				0,26
	T = 0	0,0001	**0,0009**					0,0001	**0,0007**				

HOMA IR was taken as dependent variable, various combinations of metabolic factors studied as independent variables. **Par.** = parameter (slope, regression coefficient). **T = 0** = p-value in testing the regression coefficient being zero. **Interc** = intercept. $\mathbf{R^2}$ = coefficient of determination (expressing the percentage of determination of dependent variable by independent variables). A step-down regression model was used to disclose dominant independent variables (see **Statistics**). R^2 of dominant independent variables and statistically significant regression coefficients are denoted by thick numbers.

Generally, the insulin resistance (represented by HOMA IR) was in men much less influenced by metabolic variables than in women; while in women in some combinations of dependent variables R^2 reached 64 %, in men the maximal value of R^2 was only 39 %.

Discussion

In our previous paper [36], the mean value of HOMA IR in healthy subjects of both genders and of age comparable with our controls was 1.57 ± 0.87, and the mean value of index QUICKI 0.366 ± 0.029, respectively. These values, as well as the 95 % confidence limits, correspond to values found in controls in this study.

In accordance with many previous papers, serum concentrations of leptin in women (both control and hyperlipemic) were substantially higher than in men. In the control group of women the correlation between leptin and HOMA IR was highly significant. However, in hyperlipemic women the significance of this correlation lessened, because HOMA IR increased considerably (and significantly) but serum concentration of leptin only slightly (insignificantly). In men the significance of correlations between serum leptin and HOMA IR was high and approximately the same in both the control and hyperlipemic groups, because the values of HOMA IR as well as serum leptin have nearly doubled in hyperlipemic in relation to control groups. In non-hyperlipemic postmenopausal women the high concentration of serum leptin was not associated with higher insulin resistance: HOMA IR did not differ substantially from men. A significant increase of insulin resistance in hyperlipemic women was associated by only slight and insignificant increase of leptin concentration. According to Spearman's correlations, an increase of serum TNFα and/or a decrease of HDL-cholesterol might also play a distinct role in this respect. (see Table 3). In contrast to women, in hyperlipemic men the increase of insulin resistance index was approximately proportional with the increase of leptin concentration.

Multiple regression analysis affirmed the importance of leptin serum in increasing of insulin resistance in both genders. In men, only BMI and HDL-cholesterol from other factors studied seemed to play a certain role, but the maximal values of influencing HOMA IR reached only 39%, with leptin and BMI being the more important factors. On the other hand, in women the maximal determination of HOMA IR as high as 60% was registered in combination of serum leptin, TGL and decreased HDL-cholesterol as independent factors; the role of BMI was insignificant.

It is not known how leptin is regulated. A strong correlation between plasma leptin and fasting insulin undoubtedly exists, but hyperleptinemia in both obese and lean humans is not likely the result of hyperinsulinemia [37]. A relationship between leptin and insulin dependent on sex or BMI was reported, but relationship between triglyceride concentrations and leptin was independent of sex, BMI, and insulin [18,24]. Hyperleptinemia, as an early sign of obesity, was closely linked to subcutaneous fat mass [39,40]. Percentage of body fat has been shown to be the strongest predictor of leptin levels even in lean women [41]. Leptin was highly correlated with percentage of body fat and with fat mass in adults irrespective of gender and age; however, the mean determinant of leptin plasma concentration in men and postmenopausal women was BMI, while in premenopausal women it was only the fat mass [42]. These findings contrast with our results showing minimal influence of BMI on HOMA IR in postmenopausal women.

All factors mentioned are connected with fat tissue: leptin and TNFα are directly produced chiefly by adipocytes, BMI growth is obviously accompanied by fat mass increase, and the typical hypertriglyceridemia associated with a decrease of HDL-cholesterol goes along with obesity and fat mass growth. The gender differences in circulating leptin were best explained by percentage of body fat

and – inversely – by lean body mass [25]. In both genders the intra-abdominal fat correlated with insulin resistance, while the subcutaneous fat correlated with circulating leptin [11,12]. In men obesity led to a prevalent increase of intra-abdominal fat, while in women of subcutaneous fat [13]. Influences of different compartments of adipose tissues could elucidate the variability of correlations between insulin resistance and high leptin concentrations in lean and obese subjects of both genders [43]. In our non-hyperlipemic postmenopausal women the content of subcutaneous fat mass might be higher than in non-hyperlipemic men of appropriate age, which indicated a higher serum concentration of leptin. However, the insulin resistance – related to intra-abdominal fat mass – did not differ from men. The significant increase of insulin resistance and leptin concentration in hyperlipemic men might reflect the growing content of both subcutaneous and intra-abdominal fat mass (see the significant increase of BMI). In hyperlipemic women the significant increase of insulin resistance accompanying only minimal insignificant increase of leptin could be caused by prevalent growing of intra-abdominal fat mass.

In elderly postmenopausal women, an association between leptin and plasma lipoprotein concentration was found which depended on adiposity [17], and inverse correlations between serum leptin and HDL-cholesterol were described [44]. In our study, insulin resistance in women seemed to be more notably than in men influenced by lipid disorders, i.e. positively by serum triglycerides and inversely by HDL-cholesterol. These findings might be important in considering the concept of treatment of insulin resistance-related disorders in postmenopausal women.

The significant role of TNFα in insulin resistance, caused by inhibiting the transduction of insulin signaling and by down-regulation of glucose transporter GLUT-4 and insulin receptor substrate-1, has been repeatedly confirmed [45–48]. Our results supported these findings unambiguously only in women, while in men TNFα seemed paradoxically to interfere with other factors – mainly BMI and leptin – in influencing insulin resistance, thus playing a quite different role. Previously it was found [46] that correlation between serum TNFα on the one side, and insulin, HOMA IR, serum triglycerids, respectively, on the other side, was substantially more significant in women than in men. Serum concentration of TNFα in patients with type 2 diabetes of both genders correlated only with the quantity of intra-abdominal fat compartment [50]. Visceral obesity correlated with plasmatic aldosterone and with insulin resistance only in premenopausal women, but not in men [51].

From all these data we might support our above mentioned conclusion – that rising of insulin resistance in hyperlipemic women was associated with an increase of intra-abdominal fat, because this fat mass in particular is a source of TNFα, which interfered with insulin sensitivity only in women. We came to this conclusion irrespective of the finding that the increase of serum TNFα in hyperlipemic women was statistically insignificant; results of Spearman's correlation (Table 3) and multiple regression analysis confirm a distinct role of this factor. In hyperlipemic men not only the serum concentration of TNFα has decreased instead of increasing, but according to multiple regression analysis it played a quite different role in influencing insulin sensitivity, interfering with factors that determined insulin resistance (leptin and BMI).

In the control group of men IgG anticardiolipin was inversely correlated to HOMA IR. The significance of this finding is not clear. These antibodies indicate vascular and thrombotic complications and oxidative modification of lipoproteins [52,53] and may represent an increased risk of atherogenic and inflammatory complications. In this case, however, their growing might be connected with an increase in insulin sensitivity. Anyway, ACL IgG evidently did not participate significantly in influencing the increase of insulin resistance associated with hyperlipidemia, although other anti-cardiolipin correlations could be masked by the relatively large inter-individual variations in this parameter.

Neither serum concentration of hFABP, a factor ensuring transmembrane transport and oxidative metabolisation of long-chain fatty acids [54,55], was significantly changed in hyperlipemic and insulin resistant subjects of both genders. This factor was very weakly associated only with HOMA IR in women (see Table 4D), indicating that enhanced metabolisation of fatty acids in cells might to some degree contribute to insulin resistance.

Conclusions

In postmenopausal women as well as in men of approximately equal age serum leptin plays a significant role as an important determinant of insulin resistance. In addition to this factor, in women the grade of insulin resistance is very considerably influenced by serum triglycerides, tumor necrosis factor alpha, and by decreased concentration of HDL-cholesterol, while in men only a mild influence of BMI and decreased HDL-cholesterol is observed. These findings are explained as a consequence of gender-related differences in adipose tissue composition and/or distribution in both normal-weight and over-weight subjects and should be taken into account in treatment of patients with metabolic risk factors of cardiovascular diseases.

List of abbreviations

HDL-Cholesterol = high-density cholesterol

LDL-cholesterol = low-density cholesterol

HOMA IR = Homeostasis Assessment of Insulin Resistance

= fasting insulin (μU/ml) * fasting glucose (mmol/l) / 22,5

QUICKI = 1 / [log fasting insulin (μU/ml) + log fasting glucose (mg/100 ml)]

TNFα = tumor necrosis factor alpha

BMI = body mass index

R^2 = coefficient of determination

hFABP = heart fatty acid binding protein

ACL-IgG = IgG fraction of anticardiolipin

TGL = triglycerides

GLUT-4 = glucose transporter-4

PPARγ = Peroxisome Proliferator-Associated Receptor gamma

CV = coefficient of variation

Authors' contributions

Dr. Radka Lichnovská collected the clinical material, performed analysis of biochemical values and edited the manuscript.

Dr. Simona Gwozdziewiczová performed analysis of clinical and biochemical data and edited the manuscript.

Prof. Jirí Hrebícek initiated the study, participated in its design and coordination, and wrote and edited the manuscript.

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References

1. Ford ES, Giles WH, Dietz WH: **Prevalence of the metabolic syndrome among US adults.** *JAMA* 2002, **287:**356-359
2. Isomaa B, Almgren P, Tuomi T, Forsén B, Nissén M, Taskinen MR, Groop L: **Cardiovascular morbidity and mortality associated with the metabolic syndrome.** *Diabetes Care* 2001, **24:**683-689
3. Reaven GM: **Banting Lecture 1988. Role of insulin resistance in human disease.** *Diabetes* 1988, **37:**1596-1607
4. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G: **Insulin resistance and hypersecretion in obesity.** *J Clin Invest* 1997, **100:**1166-1173
5. Moran A, Jacobs DR, Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR: **Insulin resistance during puberty: results from clamp studies in 357 children.** *Diabetes* 1999, **48:**2039-2044
6. Meisinger C, Thorand B, Schneider A, Stieber J, Doring A, Lowel H: **Sex differences in risk factors for incident type 2 diabetes mellitus: the MONICA Augsburg cohort study.** *Arch intern Med* 2002, **162:**82-89
7. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, *et al*: **Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects.** *Nature Med* 1995, **1:**1155-1161
8. Hickey NS, Israel RG, Gardiner SN, *et al*: **Gender differences in serum leptin levels in humans.** *Biochem Mol Med* 1996, **59:**1-6
9. Haffner SM, Gingerich RL, Miettinen H, Stern MP: **Leptin concentrations in relation to overall adiposity and regional fat distribution in Mexican Americans.** *Int J Obes* 1996, **20:**904-908
10. Zimmet P, Hodge A, Nicholson M, Straten M, De Courten M, Moore J, Morawiecki A, Lubina J, Collier G, Alberti G, Dowse G: **Serum leptin concentration, obesity, and insulin resistance in Western Samoans: Cross sectional study.** *BMJ* 1996, **313:**965-969
11. Harmelen VV, Reynisdottir S, Eriksson P, Thörne A, Hoffstedt J, Lönnquist F, Arner P: **Leptin secretion from subcutaneous and visceral adipose tissue in women.** *Diabetes* 1998, **47:**913-917
12. Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S: **Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution.** *Diabetes* 1997, **46:**342-347
13. Tai ES, Lau TN, Ho SC, Fok ACK, Tan CE: **Body fat distribution and cardiovascular risk in normal weight women. Association with insulin resistance, lipids and plasma leptin.** *Intern J of Obesity* 2000, **24:**751-757
14. Hotamisligil GS, Arner P, Caro JF, Atkinson L, Spiegelman BM: **Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance.** *J Clin Invest* 1995, **95:**2409-2415
15. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB: **The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss and relationship to lipoprotein lipase.** *J Clin Invest* 1995, **95:**2111-2119
16. Moller DE: **Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes.** *Trends Endocrinol Metab* 2000, **11:**212-217
17. Couillard C, Meuriége P, Prud'homme D, Nadeau A, Tremblay A, Bouchard C, Després J-P: **Plasma leptin concentrations: gender differences and associations with metabolic risk factors for cardiovascular disease.** *Diabetologia* 1997, **40:**1178-1184
18. Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, Garvey WT: **The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin sensitivity, and energy expenditure.** *J clin Endocrinol Metab* 1997, **82:**1293-1300
19. Erikkson J, Valle T, LindströM j, Haffner S, Louhernta A, Uusitupa M, Tuomilehto J: **Leptin concentrations and theit relation to body fat distribution and weight loss – A prospective study in individuals with impaired glucose tolerance.** *Horm Metab Res* 1999, **31:**616-619
20. Hattori A, Uenura K, Miura H, Ueda M, Tamaya N, Iwata F, Muraguchi M, Ohmoto Y, Iguchi A: **Gender-related diferrence in relationship between insulin resistance and serum leptin level in Japanese type 2 diabetic and non-diabetic subjects.** *Endocrine journal (Japan)* 2000, **47:**615-621
21. Doucet E, St-Pierre S, Alméras N, Mauriége P, Després J-P, Richard D, Bouchard C, Tremblay A: **Fasting insulin levels influence plasma leptin levels independently from the contribution of adiposity: Evidence from both a cross-sectional and intervention study.** *J Clin Endocrinol Metab* 2000, **85:**4231-4237
22. Baumgartner RN, Ross RR, Waters DL, Brooks WM, Morley JE, Montoya GD, Garry PJ: **Serum leptin in elderly people: associations with sex hormones, insulin, and adipose tissue volumes.** *Obesity Research* 1999, **7:**141-149

23. Ruige JB, Dekker JM, Blum WF, Stehouver CD, Nijpels G, Mooy J, Kostense PJ, Bouter LM, Heine RJ: **Leptin and variables of body adiposity, energy balance, and insulin resistance in a population-based study. The Hoorn Study.** *Diabetes Care* 1999, **22:**1097-1104
24. Marshall JA, Grunwald GK, Donahoo WT, Scarbro S, Shetterly SM: **Percent body fat and lean mass explain the gender difference in leptin: Analysis and interpretation of leptin in Hispanic and non-Hispanic whitw adults.** *Obesity Research* 2000, **8:**543-552
25. Assmann G, Carmena R, Cullen P, Fruchart J-C, Jossa F, Lewis B, Mancini M, Paoletti R: **Coronary heart disease: Reducing the risk.** *Nutrition, metabolism and cardiovascular disease* 1998, **8:**205-271
26. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Galagher D, Chu F, Leibel RL: **Effects of gender, body composition, and menopause on plasma concentrations of leptin.** *J Clin Endocrinol Metab* 1996, **81:**3424-3427
27. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD: **Gender differences in plasma leptin concentrations.** *Nature Med* 1996, **2:**949-950
28. Schoonjans K, Martin G, Staels B, Auwerx J: **Peroxisome proliferator-activated receptors, orphans with ligands and functions.** *Current Opinion in Lipidology* 1997, **8:**159-166
29. Martin G, Schoonjans K, Staels B, Auwerx J: **PPAR activators improve glucose homeostasis by changing fatty acid partitioning.** *In: Atherosclerosis XI (Edited by: Jacocot B, Mathé D, Frichart J-C) Elsevier Science, Singapore* 1998, 35-47
30. Wu R, Nityanand S, Berglund L, Lithell H, Holm G, Lefvert AK: **Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction.** *Arteriosclerosis, Thrombosis, and Vascular Biology* 1997, **17:**3159-3163
31. Erkkilä AT, Närvänen O, Lehto S, Uusitupa MIJ, Ylä-Herttuala S: **Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease.** *Arteriosclerosis, Thrombosis, and Vascular Biology* 2000, **20:**204-209
32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RL: **Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentration in man.** *Diabetologia* 1985, **28:**412-419
33. Haffner SM, Miettinen H, Stern MP: **The homeostasis model in the San Antonio heart study.** *Diabetes Care* 1997, **20:**1087-1092
34. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ: **Quantitative insulin sensitivity check Index: a simple, accurate method for assessing insulin sensitivity in humans.** *J Clin Endocrinol Metab* 2000, **85:**2402-2410
35. Hrebícek J, Janout V, Malincíková J, Horáková D, Cízek L: **Detection of insulin resistance by simple quantitative sensitivity check index QUICKI for epidemiological assessment and prevention.** *J Clin Endocrinol Metab* 2002, **87:**144-147
36. Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M: **Plasma leptin and insulin relationship in obese and nonobese humans.** *Diabetes* 1996, **45:**695-698
37. Considin RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW: **Serum immunoreactive-leptin concentrations in normal-weight and obese humans.** *N Engl J Med* 1996, **334:**292-295
38. Caprio S, Tamborlane WV, Silver D, Robinson C, Leibel R, McCarthy S, Grozman A, Belous A, Maggs D, Sherwin RS: **Hyperleptinemia: an early sign of juvenile obesity. Relations to body fat depots and insulin concentrations.** *Am J Physiol* 1996, **271:**623-630
39. Ryan AS, Elahi D: **The effects of acute hyperglycemia and hyperinsulinemia on plasma leptin level: its relationships with body fat, visceral adiposity, and age in women.** *J Clin Endocrinol Metab* 1996, **81:**4433-4438
40. Sumner AE, Falkner B, Kushner H, Considine RV: **Relationship of leptin concentration to gender, menopause, age, diabetes, and fat mass in African Americans.** *Obesity Research* 1998, **6:**128-133
41. Cnop M, Landchild MJ, Vidal J, Havel PJ, Knowles NG, Carr DR, Wang F, Hull RL, Boyko EJ, Retzlaff BM, Walden CE, Knopp RH, Kahn SE: **The concurrent accumulation of intra-abdominal and subcutanous fat explains the association between insulin resistance and plasma leptin concentrations.** *Diabetes* 2002, **51:**1005-1015
42. Mendoza-Núnez VM, García-Sanchéz A, Sánchéz-Rodrigéz M, Galván-Duarte RE, Fonseca-Yerena ME: **Overweight, waist circumference, age, gender, and insulin resistance as risk factors for hyperleptinemia.** *Obesity Research* 2002, **10:**253-259
43. Hotamisligil GS, Shargill NS, Spiegelman BM: **Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance.** *Science* 1993, **259:**87-91
44. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: **IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha and obesity-induced insulin resistance.** *Science* 1996, **259:**87-91
45. Hotamisligil GS: **Mechanisms of TNFα-induced insulin resistance.** *J Exp Clin Endocrinol Diabetes* 1999, **107:**119-125
46. Stephens JM, Lee JL, Pilch PF: **Tumor necrosis factor-alpha induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT 4 expression without a loss of insulin receptor-mediated signal transduction.** *J Biol Chem* 1997, **272:**971-976
47. Hoffstedt J, Eriksson P, Hellström L, Rössner S, Rydén M, Arner P: **Excessive fat accumulation is associated with the TNFα-308 G/A promoter polymorphism in women but not in men.** *Diabetologia* 2000, **43:**117-120
48. Bertin E, Nguyen P, Guenounou M, Durlach V, Potron G, Leutenegger M: **Plasma levels of tumor necrosis factor-alpha (TNF-alpha) are essentially dependent on visceral fat amount in type 2 diabetes mellitus.** *Diabetes Metab* 2000, **26:**178-182
49. Goodfriend TL, Kelley DE, Goodpaster BH, Winters SJ: **Visceral obesity and insulin resistance are associated with plasma aldosterone levels in women.** *Obes Res* 1999, **7:**355-362
50. Schlame M, Hostetler KY: **Cardiolipin synthase from mammalian mitochondria.** *Biochimica and Biophysica Acta* 1997, **1348:**207-213
51. Vaarala O: **Antiphospholipid antibodies and myocardial infarction.** *Lupus* 1998, **7(Suppl 2):**S132-S134
52. Bonen A, Luiken JJ, Liu S, Dyck DJ, Kiens B, Kristiansen S, Turcotte LP, Van Der Vusse GJ, Glatz JF: **Palmitate transport and fatty acid transporters in red and white muscles.** *Am J Physiol* 1998, **275(3 Pt 1):**E471-E478
53. Memon RA, Feingold KR, Moser AH, Fuller J, Grunfeld C: **Regulation of fatty acid transport protein and fatty acid translocase mRNA levels by endotoxin and cytokines.** *Am J Physiol* 1998, **274(2 Pt 1):**E210-E217