ASSOCIATION OF METABOLIC AND GENETIC FACTORS WITH CHOLESTEROL ESTERIFICATION RATE IN HDL PLASMA AND ATHEROGENIC INDEX OF PLASMA IN A 40 YEARS OLD SLOVAK POPULATION.

Katarína Rašlová[1], Milada Dobiášová[2], Jaroslav A Hubáček[3,4], Dominika Bencová[5], Daniela Siváková[5], Zuzana Danková[5], Jana Franeková[6], Antonín Jabor[6], Juraj Gašparovič[1], Branislav Vohnout[1,7]

1. National Reference Centre for Familial Hyperlipoproteinemias, Slovak Medical University, Limbova 12, 833 03 Bratislava, Slovakia
2. Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
3. Institute for Clinical and Experimental Medicine, Prague, Czech Republic
4. 3rd Dept. of Medicine, 1st School of Medicine, Charles University, Prague, Czech Republic
5. Department of Anthropology, Faculty of Sciences, Comenius University, Bratislava, Slovakia
6. Department of Laboratory Methods, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
7. Department of Physiological and Clinical Nutrition, Faculty of Nursing and Health Professional Studies, Slovak Medical University, Bratislava, Slovakia

Author for correspondence:
Assoc. Prof. Katarína Rašlová, MD, PhD
National Reference Centre for Familial Hyperlipoproteinemias,
Slovak Medical University
Limbova 12, 833 03 Bratislava
Slovakia
E-mail: katarina.raslova@yahoo.com

Short title: Risk factors and FER_{HDL} and AIP in 40 years old

Summary

Background: We assessed association between novel biomarkers of cardiovascular disease and conventional factors in 40 years old subjects (208 men and 266 women) from the general population of Slovakia. ***Methods:*** FER_{HDL} (cholesterol esterification rate in HDL plasma), AIP- Atherogenic Index of Plasma [Log(TG/HDL-C)] as markers of lipoprotein particle size, and *CILP2, FTO and MLXIPL* polymorphisms, were examined in relation to biomarkers and conventional risk factor**s**. ***Results:*** Univariate analyses confirmed correlation between AIP, FER_{HDL} and the most of measured parameters. Relations between AIP and *CILP2*, *FTO* and *MLXIPL* were not significant. However, *CILP2* was significantly related to FER_{HDL} in both genders. In multivariate analysis BMI was the strongest correlate of AIP levels. In multivariate model variability of FER_{HDL} was best explained by AIP (R^2=0.55) in both genders with still significant effect of *CILP2* SNP in men. In a model where AIP was omitted, TG levels explained 43% of the FER_{HDL} variability in men, while in women HDL-C was the major determinant (42%). ***Conclusion:*** FER_{HDL} and AIP related to the known markers of cardiovascular risk provide means to express their subtle interactions by one number. Our novel finding of association between *CILP2* polymorphism and FER_{HDL} supports its role in lipid metabolism.

Keywords: Fractional esterification rate of cholesterol (FER_{HDL}), Atherogenic index of plasma (AIP), biomarkers of CVD, *CILP2*, *FTO*, *MLXIPL*,

Introduction

Slovakia belongs to European countries with the highest cardiovascular mortality (Müller-Nordhorn *et al.* 2008). In 2003 we performed a large population study aimed to screen lipid disorders in subjects 40 years old in the year of the examination (Gašparovič *et al.* 2007). This age was chosen with respect to relatively low cardiovascular risk in that category and thus very effective possibility to diagnose, treat and prevent future cardiovascular event. In this study we assessed conventional risk factors (RF) of cardiovascular disease (CVD) such as plasma lipids, glycaemia and anthropometric parameters. This age homogenous population sample enabled to investigate how the novel biomarkers – fractional esterification rate of cholesterol in LDL/VLDL depleted plasma (FER_{HDL}) and atherogenic index of plasma (AIP), markers of lipoprotein particle size, are related to the metabolic and genetic risk factors.

FER_{HDL} has been shown to be the strongest predictor test of positive findings on coronary angiography (Frohlich and Dobiášová 2003) and one of best indicators of changes in the progression of coronary artery disease (CAD) after treatment with statins and antioxidants (Brown *et al.* 2001, Dobiášová et al. 2011). Its predictive potential consists in differently sized HDL cholesterol (HDL-C) subpopulations which regulate the rate of cholesterol esterification by lecithin cholesterol acyltransferase (LCAT). The size of lipoprotein particles is crucial not only for cholesteryl esters production but also for their destination (Dobiášová 2004). Differently sized lipoprotein particles play a protective (buoyant HDL-C and LDL-C particles) or an atherogenic role (small HDL-C and LDL-C particles) in cardiovascular disease (Austin *et al.* 1990, Drexel *et al.* 1992). Thus FER_{HDL} as a marker of lipoprotein particle size serves as a functional test of lipoprotein quality.

Logarithmically transformed ratio TG/HDL-C (Atherogenic Index of plasma - AIP) highly correlates with FER_{HDL} (Dobiášová and Frohlich 2001) and it is closely associated with

particle size of HDL-C, LDL-C and VLDL (Dobiášová *et al.* 2005, 2011). Thus FER_{HDL} and AIP may represent biomarkers of cardiovascular risk based on the lipoprotein particle size composition.

Recently, new identified loci and genes have been related to lipid concentrations and obesity. MLXIPL was primarily associated with triglyceride (TG) concentrations and CILP2 with LDL cholesterol (Kathiresan *et al.* 2008), variants in FTO (fat mass and obesity associated) gene with body mass index (BMI) (Scuteri *et al.* 2007, Hubáček *et al.* 2009).

The objective of the current study was to examine association of FER_{HDL} and AIP with metabolic factors and recently identified polymorphisms at novel lipid and obesity related loci (Kathiresan *et al.* 2008, Scuteri *et al.* 2007) .

Methods

Screening of 40-year-old men and women was done in eight cities covering major part of Slovak regions in cooperation with MEDPED lipid clinics and 142 general practitioners (GPs) in 2003 (Gašparovič *et al.* 2007). For the current study we have randomly selected 474 subjects (208men, 266 women) out of the population study consisting of 2323 subjects. Fasting blood drawing, blood pressure and standard anthropometric measures**.** were carried out in GPs' offices. The questionnaire covering personal and family history as well as smoking status filled by subjects was checked and confronted with medical records. The study was approved by the ethics committee of the Slovak Medical University. All study subjects agreed to participate by written informed consent.

Total cholesterol (TC), triglycerides (TG), HDL-C, apolipoprotein B (apoB), apolipoprotein AI (apoAI), alanine aminotransferase (ALT) and glucose levels were analyzed using enzymatic autoanalyzer methods. We considered ALT for the current study due to its suggestive role as a biomarker for the risk of metabolic and cardiovascular diseases (Goessling *et al* 2008, Yilmaz 2010). LDL-C was calculated using the Friedewald formula.

ApoB and apoAI were measured using automatic immunoturbidimetric assay for ApoA-I and ApoB (Abbott Architect system ci8200). Remaining plasma was stored in liquid nitrogen. DNA was isolated from peripheral blood leukocytes by the phenol extraction method and stored at -80°C.

The assay of FER_{HDL} has been previously described in detail (Dobiášová and Frohlich 1996). Briefly, apo B-containing lipoproteins were precipitated from EDTA plasma by phosphotungstic acid and $MgCl_2$. To the supernatant which contains plasma with HDL only (HDL plasma), was added a filter paper disk containing a trace of 3-H cholesterol. After an overnight incubation at 4°C the disk was removed, and the plasma with homogenously labeled HDL-C was incubated at 37°C for 30 min. Plasma lipids were extracted by ethanol, separated by thin layer chromatography. Spots of CH and CHE visualized by iodine were cut, transferred to vials with scintillation solution and measured. The fractional esterification rate was calculated from the ratio of radioactivity of free and esterified cholesterol. As this esterification only concerns the HDL-C, it was called fractional esterification rate in HDL-C (FER_{HDL}) and its values were percentages of HDL-cholesterol esterified per hour.

AIP was calculated as the logarithmically transformed ratio of the molar concentration of TG to HDL-cholesterol (Dobiášová and Frohlich 2001).

DNA samples and results of genotypes of *rs16996148* CILP2 (CILP), *rs17817449* FTO (FTO) and *rs3812316* MLXIPL (MLX) polymorphisms were available in 407 (183 males, 224 females), 439 (191 males, 248 females) and 431 (191 males, 240 females) subjects, respectively.

SNPs within the MLXIPL (Vráblik *et al.* 2008) and FTO (Hubáček *et al.* 2009) genes were determined as described elsewhere.

Rs16996148 variant near CILP/PBX4 genes was analysed using PCR/RFLP. Briefly, oligonucleotides CILP-F 5` ctcttgtccactggccacatcccc and CILP-R 5` ttctcccatgcctccaggcccccaag were used. PCR product (135 bp) was cleaved by restriction

enzyme Hin1II (Fermentas) and separated on 10% PAA gel (Day and Humphries 1994). The minor allele T is characterized by fragments of 82 + 53 bp, while an uncut fragment represents the major G allele.

Genotype frequencies (n, %) were as follows: CILP – 345 (84.77%), 57 (14%), 5 (1.23%), FTO – 135 (30.75%), 203 (46.24%), 101 (23.01%), and MLX 335 (77.72%), 88 (20.42%), 8 (1.86%) for genotypes 11, 12 and 22 (with 1 representing the major allele and 2 representing the minor allele for each SNP). The frequencies of the alleles were determined by genotype count and compared with the values predicted on the basis of the assumption of Hardy-Weinberg equilibrium.The genotype distribution was in Hardy-Weinberg equilibrium for all 3 polymorphisms.

Risk factors were defined as follows: *dyslipidemia* if at least one of the TC>5.0 mmol/l, LDL-C>3.0 mmol/l, HDL-C <1.0 mmol/l in men and HDL-C<1.2 mmol/l in women, or TG>1.7 mmol/l, *obesity* if at least one of the BMI>30 kg/m^2 or waist >102 cm in men and waist>88 cm in women, *glucose* >6.0 mmol/, *blood pressure* (BP) if at least one of the systolic BP>140 mmHg or diastolic BP>90 mmHg (De Backer et al. 2003), *current smoking* and *positive family history* of premature cardiovascular disease at age <60.

AIP risk categories were created according to published epidemiological data: Low risk < 0.11, Intermediate 0.11-0.21 and High >0.21 (Dobiášová *et al.* 2008, www.biomed.cas.cz/fgu/aip).

Statistics

Five outliers for glucose and two for ALT were eliminated and HDL-C, triglyceride, FER_{HDL}, ALT and BMI levels were log transformed to normalize their positively skewed distribution. Qualitative variables were analysed using $\chi 2$, with Fisher exact test where appropriate and quantitative variables were analysed by ANOVA (proc GLM). Major determinants of AIP and FER_{HDL} were assessed applying multivariable linear regression

analysis (Procedure REG in SAS). The analysis strategy was as following: (a) simple linear regression or ANOVA (for categorical variables) were used to identify variables associated with AIP or FER_{HDL} at the level $P < 0.1$; (b) all the variables identified in the univariate analysis were inserted in a full multivariable linear regression model. To avoid collinearity problems, we checked for multicollinearity, measuring the variance inflation factor for each variable and the condition number for the full model. A variable whose variance inflation factor value is greater than 10 indicates the presence of collinearity; also, a large condition number, 100 or more, is an indication of the global instability of the regression coefficients. In our multivariate linear regression analyses on FER_{HDL} we excluded LDL-C variable due to collinearity observed and we did not evaluate LDL-C in the AIP models because it was calculated with Friedewald formula from HDL-C and TG. Mean values are reported as mean ± standard deviation or as geometric mean and 95% confidence interval for log transformed data. P value of less than 0.05 was considered to indicate statistical significance. All computations were carried out with the SAS statistical package (SAS System for Windows V8, SAS institute Inc. Cary, N.C.; SAS Institute Inc., 1989).

Results

Basic characteristics of the population are in the Table 1. Men significantly differed in almost all parameters from women, with more favourable risk pattern in women. There was no difference in presence of family history of premature CVD between males and females, and only few subjects suffered from CVD in both genders.

We observed positive relationship between number of risk factors and both FER_{HDL} and AIP values in both genders (Table 2).

AIP determinants

TC, FER_{HDL}, ApoA1, ApoB, ALT, BMI, waist, systolic blood pressure, diastolic blood pressure and smoking were all associated with AIP levels in both men and women in

univariate analysis (Table 3). Glucose was significantly associated with AIP in women, but not in men.

In multivariate analysis, in men, AIP levels were associated only with BMI, ApoA1, ApoB and smoking (Table 3). In women, AIP was associated with BMI, ApoA1, ApoB, total cholesterol, ALT and systolic blood pressure. In this model (model 1) BMI participated on 20,8% out of 35% of total variability explained by the model in men and on 23,4% out of 43% in women, respectively (Table 3). As expected, when also FER_{HDL} was included in the analyses (model 2), the FER_{HDL} explained almost all of the variability observed (Table 3), both in men (52%) and women (54%).

FER_{HDL} determinants

In univariate analyses all the parameters except of *FTO* genotype and diastolic BP in women and glucose, ALT, *FTO* and *MLX* genotypes in men were significant determinants of FER_{HDL} (Tables 3 and 4). In multivariate analysis (Table 3), in men, FER_{HDL} levels were associated with HDL-C, TG, TC and waist in model 1. In women, it was, associated with HDL-C, TG, TC, apoB and ALT. However, in contrast to men, where TG levels explained 43 percent of the FER_{HDL} variability, in women HDL-C was the major determinant (42%). When AIP levels were considered instead of HDL-C and TG in model 2, in both sexes, AIP levels explained more than 55% out of more than 65% of total FER_{HDL} variability explained by models 2.

CILP, FTO and MLX polymorphisms´ relation with AIP and FER_{HDL}

We did not find any significant association of genotypes of the three polymorphisms with AIP (Table 3 and 4). When we considered also recessive and dominant effect of the polymorphisms (Table 4), recessive model of *FTO* in males and dominant model of *CILP* in females were significantly related to AIP in univariate (Table 4) but not in multivariate analysis (data not shown).

In univariate analyses, *CILP* genotype in both men and women, recessive model of *CILP* in men and dominant models of *CILP* and *MLX* in women were associated with FER_{HDL} levels (Table 4). However, *CILP* genotype remained a significant predictor of FER_{HDL} in multivariate analysis only in men (in model 2) but not in women (Table 3). In the same line with this finding were highly significant associations of recessive effect of *CILP* on FER_{HDL} in both model 1 ($p=0.0007$, partial $R^2=0.0256$) and model 2 ($p=0.0004$, partial $R^2=0.0303$) in men. Dominant models of *CILP* and *MLX* in women did not remain significant predictors of FER_{HDL} levels in multivariate analysis (data not shown).

Men and women stratified according to AIP risk into low, medium and high risk categories.

The data of men and women stratified according to the severity of AIP risk (Table 5) corresponded with criteria established for the assessment of the cardiovascular risk. Higher AIP risk was associated with significantly increased levels of FER_{HDL}, lipid and lipoprotein parameters, but also BMI, waist circumference, blood pressure, and number of smokers, the latter in men only. Different distribution of AIP risk was found in men and women; 81% of women while only 57% of men belonged to low risk category, and 31% of men and only 11% of women came to the group of the highest AIP risk (Table 5).

Discussion

In this study a relation between the novel biomarkers of cardiovascular diseases FER_{HDL} and AIP and conventional risk factors was assessed in 40 years old subjects of the general Slovak population. We also evaluated components of variability of FER_{HDL} and AIP and association of three recently identified polymorphisms related to them, blood lipids and BMI.

Univariate analyses demonstrated that both, FER_{HDL} and AIP, which strongly correlated with each other, are determined not only by lipoprotein parameters comprised of

plasma lipids, apoAI and apoB but also by BMI, blood pressure and smoking. Moreover, also by glycaemia and ALT, in women. AIP levels were positively associated by half of total variability with BMI (cca 20% out of 40%) if FER_{HDL} (as the marker of lipoprotein quality) was not included in the multivariate analyses. However, if FER_{HDL} was included in the model it explained almost all of the AIP variability observed (cca 53%). The major determinant of FER_{HDL} was AIP explaining most of the FER_{HDL} variability in the model. However, when TG and HDL-C levels were considered instead of AIP (Table 3), TG levels explained 43 percent (HDL-C 14%) of the FER_{HDL} variability in men while in women HDL-C was the major determinant (42%) compared to 17% of TG. The high impact of BMI on AIP variability and low impact of total cholesterol on variability of both biomarkers suggest their relatively independent role in the assessment of CVD risk.

Fasting levels of TG and HDL-C are well established risk factors for CVD and elevated TG levels together with low HDL-C levels are characteristic components of the atherogenic dyslipidemia (Grundy 1995). However, combining the contradictory parameters into one AIP [log(TG/HDL-C)] has shown to be significantly better in subjects with risk factors for CVD including male gender (Dobiášová and Frohlich 2001). Measurement of HDL-C concentration refers to the mass of cholesterol within high-density lipoproteins but this does not reflect the number of HDL-C particles and does not provide functional information (Genest 2008). On the other hand FER_{HDL} and AIP as markers of HDL-C, LDL-C and VLDL particle sizes (Dobiášová *et al*. 2011) might serve as a functional test of lipoprotein quality. We have analysed data separately in men and women and have shown that the unified age of forty years in our population (as pre-menopausal status in women) even more strengthened the differences in the cardiovascular risk markers between genders. Eighty one percent of women in the study have been stratified into the category of the lowest risk according to AIP (Table 5) while only 11% into the high risk one. On the other hand, men belonged by 31 per cent to the high risk category and 57 per cent to the low one. Similar

distributions were reported for men and women of control groups of survivors of myocardial infarction (Dobiášová *et al.* 2001). When pooled data of both men and women were analyzed in the regression models, gender was a significant determinant of AIP, but not of FER_{HDL} (data not shown). With the increase of AIP risk category gradually increased also markers of atherogenic lipoprotein profile, especially FER_{HDL} and apoB levels (especially in men) contrary to LDL-C (Table 5). We have also shown the close relation of FER_{HDL} and AIP to overweight and glycemia in women (Table 3) – markers of metabolic syndrome. Both markers were also able to reflect the delicate metabolic differences such as life-style between families of survivors of myocardial infarction and their controls (Dobiášová *et al.* 2001 *).* The role of FER_{HDL} and AIP as markers of insulin resistance was earlier reported (Tan *et al*. 1998). Also the ratio of the plasma concentrations of triglyceride to high-density lipoprotein cholesterol was proposed to be the best predictor of insulin resistance and LDL particle diameter (McLaughlin *et al.* 2005). It has been shown that this ratio independently predicted all-cause mortality in women (Bittner *et al.* 2009). However, Tan et al. (Tan *et al.* 2004) demonstrated by using normal probability plots and correlations between residual error and expected residual error terms that AIP is preferable to the TG/HDL ratio for use in statistical analysis.

We have found that the *CILP* polymorphism was under a general effects model associated with FER_{HDL} in both sexes and this association remained significant after adjustment for other variables related to FER_{HDL} in men. We have also demonstrated a highly statistically significant association of the minor allele with lower FER_{HDL} under a recessive model of inheritance in men even after adjustment for other variables. However, we did not find significant association between *CILP* polymorphism and AIP though AIP levels were distributed according to CILP genotypes in a pattern similar to FER_{HDL}. Relatively smaller sample size might play a role in this finding. On the other hand, even despite a high correlation between AIP and $FER_{HDL,}$ this correlation is not absolute and we cannot exclude a

real different effect of CILP on FER_{HDL}. In the line with this finding, with exception of dominant model of inheritance in women on TG levels (p=0,037, univariate analysis), neither general, recessive nor dominant model of inheritance of the *CILP* polymorphism was associated with TG or HDL-C levels (data not shown). Only scant and inconsistent information exists so far on association of this polymorphism and blood lipids. Associations with LDL-C, HDL-C and TG were observed in some, but not all studies (Kathiresan *et al.* 2008, Tai *et al.* 2009, Nakayama *et al.* 2009). Although we did not find an association with AIP, our novel observation of statistically significant association of the minor allele with FER_{HDL} under a recessive model of inheritance is consistent with the finding of recessive effect of the minor allele on HDL-C levels recently published by Tai et al. (Tai *et al.* 2009). However, the exact role of the SNP or related SNPs on lipid metabolism still needs to be investigated.

In conclusion, FER_{HDL} and AIP related to the known markers of cardiovascular risk provide means to express their subtle interactions by one number. AIP as can be readily calculated from the routine lipid profile offers a simple marker of the risk and possible control of effectiveness of the therapy in clinical practice. Our novel finding of association between *CILP* polymorphism and FER_{HDL} supports its role in lipid metabolism.

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Table 1. Basic characteristics of the population

	MEN (n=208)	*WOMEN* (n=266)	<*P*
TC (mmol/l)	5.2±1.0	5.0±0.9	0.002
LDL-C (mmol/l)	3.2±0.9	2.9±0.7	0.0001
*HDL-C (mmol/l)**	1.26 (1.21-1.30)	1.48 (1.44-1.53)	0.0001
*TG (mmol/l)**	1.46 (1.35-1.57)	1.05 (0.98-1.12)	0.0001
AIP	0.064±0.310	-0.150±0.306	0.0001
*FER $_{HDL}$**	14.01 (13.21-14.84)	10.61 (10.08-11.17)	0.0001
ApoA1	1.3±0.3	1.4±0.3	0.005
ApoB	0.9±0.2	0.8±0.2	0.0001
Glucose (mmol/l)	5.1±0.6	4.8±0.6	0.001
*ALT**	0.44 (0.42-0.47)	0.27 (0.25-0.28)	0.0001
*BMI (kg/m^2)**	26.1 (25.6-26.7)	24.1 (23.6-24.6)	0.0001
Waist (cm)	93.7±9.4	80.4±11.6	0.000
Systolic BP (mmHg)	122±14	116±15	0.000
Diastolic BP (mmHg)	79±9	75±10	0.000
Smoking	74 (35.75 %)	68 (25.56 %)	0.020
CVD (CHD,MI, stroke, PVD)	4 (1.92 %)	3 (1.13 %)	NS
Family history of CVD	63 (30.58 %)	83 (31.20 %)	NS

Data for continuous variables presented as mean±SD or * Geometric mean and 95% CI, TC – total cholesterol, TG – triglycerides, AIP – atherogenic index of plasma, ALT - alanine aminotransferase, BP – blood pressure, CVD – cardiovascular diseases

Table 2. Men and women stratified according to number of risk factors and their FER_{HDL} and AIP values

N. of risk factors	MEN (n=208)	*FER_{HDL}*	*AIP*	WOMEN (n=266)	*FER_{HDL}*	*AIP*
		Geom.mean (95%CI)	Mean±SD		Geom.mean (95%CI)	Mean±SD
0	18 (8.9 %)	8.91 (7.38-10.76)	-0.207±0.164	45 (17.3 %)	8.03 (7.26-8.88)	-0.334±0.209
1	68 (33.7 %)	12.78 (11.58-14.11)	-0.0407±0.293	97 (37.3 %)	9.50 (8.87-10.18)	-0.232±0.241
2	66 (32.7 %)	15.48 (13.97-17.16)	0.153±0.294	70 (26.9 %)	11.39 (10.54-12.31)	-0.097±0.285
3	33 (16.3 %)	14.57 (12.59-16.85)	0.149±0.277	35 (13.5 %)	13.85 (12.36-15.52)	0.059±0.288
4	15 (7.4 %)	18.20 (14.81-22.37)	0.227±0.234	12 (4.6 %)	17.80 (14.62-21.67)	0.170±0.299
5	2 (1.0 %)	28.65 (16.29-50.38)	0.483±0.183	1 (0.4 %)	26.71 (13.91-51.29)	1.221 (n=1)
P		<0.0001	<0.0001		<0.0001	<0.0001

Table 3. Univariate and multivariate linear regression analysis of metabolic, anthropometric, and genetic correlates of AIP and FER_{HDL} in men and women

AIP	*MEN*					*WOMEN*				
	Univariate	***Model 1****	Total R^2=0.3532	***Model 2****	Total R^2=0.5923	Univariate	***Model 1****	Total R^2=0.4332	***Model 2****	Total R^2= 0.5964
	p	p	Partial R^2	p	Partial R^2	p	p	Partial R^2	p	Partial R^2
TC	0.0137	-		-		<0.0001	0.0225	0.0159	0.0456	0.0088
logFER$_{HDL}$	<0.0001	NA		<0.0001	0.5233	<0.0001	NA		<0.0001	0.5403
ApoB	0.0014	0.0204	0.0199	-		<0.0001	<0.0001	0.0628	-	
ApoA1	<.0001	<0.0001	0.0708	0.0389	0.0103	0.0001	<0.0001	0.0745	-	
Glucose	0.2674	NA		NA		0.0040	-		-	
logALT	0.0330	-		0.0199	0.0128	0.0895	0.0367	0.0136	0.0220	0.0114
logBMI	<0.0001	<0.0001	0.2087	0.0054	0.0196	<0.0001	<0.0001	0.2339	0.0016	0.0227
Waist	<0.0001	-		0.0439	0.0094	<0.0001	-		-	
Systolic BP	0.0053	-		-		0.0063	0.0184	0.0176	-	
Diastolic BP	0.0016	-		-		0.0172	-		-	
Smoking	0.0037	0.0030	0.0338	0.0212	0.0130	0.0756	-		-	
CILP	0.2141	NA		NA		0.0825	-		-	
FTO	0.1375	NA		NA		0.5286	NA		NA	
MLX	0.9282	NA		NA		0.3768	NA		NA	

logFER$_{HDL}$	*MEN*					*WOMEN*				
	Univariate	***Model 1$^{\&}$*** total R^2=0.6646		***Model 2$^{\$}$*** total R^2=0.6534		Univariate	***Model 1$^{\&}$*** total R^2= 0.6765		***Model 2$^{\$}$*** total R^2=0.6634	
	p	p	Partial R^2	p	Partial R^2	p	p	Partial R^2	p	Partial R^2
TC	0.0015	<0.0001	0.0599	-		<0.0001	<0.0001	0.0400	-	
logHDL	<0.0001	<0.0001	0.1397	NA		<0.0001	<0.0001	0.4235	NA	
logTG	<0.0001	<0.0001	0.4317	NA		<0.0001	<0.0001	0.1702	NA	
AIP	<0.0001	NA		<0.0001	0.5509	<0.0001	NA		<0.0001	0.5589
ApoA1	<0.0001	-		0.0017	0.0232	<0.0001	-		0.0006	0.0268
ApoB	<0.0001	-		0.0108	0.0160	<0.0001	0.0025	0.0169	<0.0001	0.0589
Glucose	0.76	NA		NA		0.008	-		-	
logALT	0.26	NA		NA		0.003	0.0176	0.0110	0.0500	0.0073
logBMI	<0.0001	-		-		<0.0001	-		-	
Waist	<0.0001	0.0049	0.0179	<0.0001	0.0460	<0.0001	-		-	
Systolic BP	0.01	-		-		0.02	-		-	
Diastolic BP	0.01	-		-		0.08	-		-	
Smoking	0.03	-		-		0.03	-		-	
CILP	0.0007	-		0.0329	0.0103	0.02	-		-	
FTO	0.17	NA		NA		0.83	NA		NA	
MLX	0.87	NA		NA		0.04	-		-	

NA – variables not included in multivariable analysis due to p›0.1 in univariate or when only in one of the models used (AIP and $logFER_{HDL}$), R^2 the coefficient of determination, * model 1 – multivariate analysis without *$logFER_{HDL}$*, $^{\#}$ model 2 – multivariate analysis with *$logFER_{HDL}$*, $^{\&}$ model 1 – multivariate analysis without *AIP*, $^{\$}$ model 2 – multivariate analysis with *AIP*

TC – total cholesterol, TG – triglycerides, AIP – atherogenic index of plasma, ALT - alanine aminotransferase, BP –blood pressure, CILP - *rs16996148* CILP2 polymorphism, FTO - *rs17817449* FTO polymorphism, MLX - *rs3812316* MLXIPL polymorphism

Table 4. Mean levels of FER_{HDL} and AIP according to genotypes

	FER_{HDL} *		*AIP*	
	MEN	*WOMEN*	*MEN*	*WOMEN*
CILP	Geom.mean (95%CI)	Geom.mean (95%CI)	Mean±SD	Mean±SD
11	14.98 (14.04-15.98)	10.84 (10.27-11.44)	0.091±0.302	-0.152±0.292
12	14.10 (12.07-16.47)	8.74 (7.59-10.07)	0.091±0.315	-0.280±0.291
22	6.15 (3.93-9.63)	8.99 (4.31-18.73)	-0.220±0.045	-0.053±0.075
p	0.0007	0.02	0.21	0.0825
p-rec	0.0002	0.68	0.079	0.58
p-dom	0.078	0.005	0.62	0.0492
FTO				
11	13.72 (12.25-15.36)	10.89 (9.94-11.93)	0.054±0.338	-0.120±0.319
12	13.64 (12.46-14.94	10.51 (9.75-11.33)	0.039±0.303	-0.168±0.291
22	15.81 (13.83-18.08)	10.57 (9.54-11.71)	0.152±0.283	-0.168±0.322
p	0.17	0.83	0.14	0.53
p-rec	0.061	0.88	0.048	0.68
p-dom	0.56	0.55	0.66	0.26
MLX				
11	14.35 (13.36-15.42)	10.96 (10.34-11.61)	0.075±0.301	-0.142±0.312
12	13.85 (12.02-15.96)	9.29 (8.31-10.38)	0.076±0.337	-0.211±0.312
22	16.21 (6.92-37.98)	10.98 (7.78-15.49)	0.145±0.260	-0.139±0.208
p	0.87	0.037	0.93	0.38
p-rec	0.77	0.83	0.70	0.90
p-dom	0.70	0.016	0.92	0.19

* p values are from log transformed FER_{HDL} analyses, p-rec – p value for a model of recessive effect, p-dom – p value for a model of dominant effect, CILP - *rs16996148* CILP2 polymorphism, FTO - *rs17817449* FTO polymorphism, MLX - *rs3812316* MLXIPL polymorphism

Table 5. Men and women stratified according to AIP risk into Low, Intermediate and High risk categories.

	MEN				***WOMEN***			
AIP Risk	< 0.1	0.10 - 0.21	> 0.21	<*P*	< 0.1	0.10 - 0.21	> 0.21	<*P*
n	118	26	64		216	21	29	
%	56.7	12.5	30.8		81.2	7.9	10.9	
TC	5.09± 1.03	5.39±0.94	5.48±0.95	0.05	4.85±0.77	5.53±1.01	5.49±0.99	0.0001
LDL-C	3.14± 0.92	3.48±0.83	3.20±0.96	NS	2.81±0.69	3.52±0.67	3.05±0.79	0.0001
HDL-C	1.43 (1.38-1.48)	1.13 (1.05-1.22)	1.03 (0.98-1.08)	0.0001	1.58 (1.54-1.62	1.18 (1.08-1.29)	1.09 (1.02-1.18)	0.0001
TG	1.01 (0.95-1.07)	1.66 (1.45-1.88)	2.73 (2.52-2.97)	0.0001	0.87 (0.83-0.92)	1.72 (1.46-2.04)	2.90 (2.52-3.34)	0.0001
FER_{HDL}	10.97 (10.31- 11.67)	16.18 (14.18- 18.45)	20.94 (19.23- 22.82)	0.0001	9.49 (9.09- 9.91)	16.34 (14.27- 18.71)	17.72 (15.73- 19.97)	0.0001
ApoA1	1.35± 0.27	1.21±0.23	1.17±0.24	0.0001	1.38±0.28	1.24±0.25	1.23±0.29	0.005
ApoB	0.81±0.22	0.89±0.21	0.94±0.28	0.005	0.73±0.21	0.96±0.22	0.90±0.22	0.0001
ApoB/ApoA1	0.61±0.19	0.76±0.21	0.82±0.24	0.0001	0.54±0.15	0.79±0.16	0.74±0.17	0.0001
TC/HDL-C	3.59±0.88	4.78±0.89	5.35±1.08	0.0001	3.11±0.70	4.63±0.54	5.05±1.17	0.0001
Glucose	5.05±0.63	5.04±0.75	5.16±0.61	NS	4.80±0.52	4.94±0.97	5.01±0.78	NS
ALT	0.41 (0.37- 0.45	0.47 (0.39- 0.57)	0.49 (0.44- 0.56)	0.05	0.26 (0.24- 0.27)	0.38 (0.32- 0.45)	0.27 (0.23- 0.31)	0.0001
BMI	24.84 (24.30- 25.40)	27.36 (26.11- 28.67)	28.15 (27.32- 29.00)	0.0001	23.26 (22.77- 23.77)	27.80 (25.95- 29.78)	28.21 (26.60- 29.91)	0.0001
WAIST	90.4±8.5	98.3±7.8	97.9±9.2	0.0001	78.0±9.8	91.9±14.6	90.5±11.9	0.0001
Systolic BP	118±14	128±12	125±12	0.0001	114±15	124±17	120±17	0.01
Diastolic BP	77±9	84±8	80±9	0.0001	75±9	79±10	79±10	0.05
Smoker	26.5%	46.1%	48.4%	0.01	24.5%	14.3%	41.4%	NS

AIP Risk: Low - values < 0.1, Intermediate - values 0.10 - 0.21, High - values > 0.21, NS – not significant, TC – total cholesterol, TG – triglycerides, AIP – atherogenic index of plasma, ALT - alanine aminotransferase, BP – blood pressure