Biomarkers of cardiometabolic risk in obese/overweight children: effect of lifestyle intervention.

Vrablík M *, Dobiášová M **, Zlatohlávek L *, Urbanová Z *, Češka R ***

*3rd Dept. of Medicine, 1st Medical Faculty, Charles University and General Teaching Hospital, Prague, Czech Republic, **Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, *** The Children's Clinic, 1st Medical Faculty, Charles University Prague, Czech Republic.

Short title: Markers of cardiometabolic risk in obese children

Corresponding author: Milada Dobiášová, PhD, DSc, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague 4, 142 20, Czech Republic

Tel: +420 777 175 527

Fax: +420 24062488

E-mail: dobias@biomed.cas.cz

ABSTRACT

Vrablík M, Dobiášová M, Zlatohlávek L, Urbanová Z and Češka R. Biomarkers of cardiometabolic risk in obese/overweight children: effect of lifestyle intervention.

Background and Aims: Obesity is a strong cardiometabolic (CM) risk factor in children. We tested potential CM risk in obese/overweight children and the effect of an intensive lifestyle intervention using newer CM markers: atherogenic index of plasma AIP [Log(TG/HDL-C)], apoB/apoAI ratio and a marker of insulin resistance HOMA-IR. **Methods and Results:** The participants (194 girls, 115 boys, average age 13) were enrolled in an intensive, one-month, inpatient weight reduction program. The program consisted of individualised dietary changes and the exercise program comprised aerobic and resistance training. Anthropometrical and biochemical parameters in plasma and CM risk biomarkers - (AIP, apoB/apoAI ratio and HOMA-IR) were examined before and after the intervention. AIP and HOMA-IR significantly correlated with BMI while apoB/apoAI ratio did not. Only AIP and HOMA-IR showed systematic increases according to the level of obesity by BMI quartiles. Lifestyle intervention significantly improved anthropometrical and biochemical values and the biomarkers too. The response of lipid parameters to the intervention was considerably higher in boys than in girls. The children were stratified into three risk categories according to AIP, where 13.8% of boys and 5.3% of girls fell into high risk category. **Conclusions:** The monitored biomarkers may complement each other in the prognosis of CM risk. AIP was strongly related to obesity and to lipid and glycid metabolism, while the relationship of the apoB/apoAI ratio to obesity and glycid metabolism was not significant. The obese children benefited from the intensive lifestyle intervention which improved the anthropometrical and biochemical parameters and CM risk biomarkers.

Key words:

AIP [Log(TG/HDL-C)] -- apoB/apoAI ratio -- HOMA-IR (insulin resistance) -- cardiometabolic risk markers -- intensive lifestyle intervention -- Overweight/obese children

Introduction

It is necessary to pay close attention to the prevention of cardiovascular diseases as early as childhood. In children the principal warning signals are obesity and dyslipidemia (Kwiterowitch Jr. 1991, Canas *et al*. 2013). In the Czech Republic, 6% of children suffer from obesity, 9% are overweight, 23% have dyslipidemia (Kobzová *et al*. 2003, Šamánek and Urbanová 2008). However, typical atherogenic risks, such as increased concentration of cholesterol (TC), and triglycerides (TG) and a low high density cholesterol (HDL-C) concentration are not manifested in children as considerably as in adults. Characteristic differences in reduced HDL-C concentration between males and females are not manifested in children either. Only in post-pubescent girls (≥ 15 years), the ratio of the large cardioprotective lipoprotein HDL_{2b} particles to HDL lipoproteins increases first, while remains unchanged in boys (Dobiášová *et al.* 1998). Therefore, it is justifiable to look for more sensitive biomarkers for the future prognoses of children.

Recently, the predictive importance of AIP (Dobiášová and Frohlich 2001, Frohlich and Dobiášová 2003**,** Onat *et al*. 2010, Dobiášová *et al.* 2011) and apoB/apoAI ratio (Walldius *et al*. 2001, Thompson and Danesh 2006) as biomarkers of cardiovascular diseases has been confirmed, reflecting the balance between risk and protective biomarkers (TG and HDL-C in AIP; LDL and HDL in apoB/apoAI ratio). Both biomarkers correspond to specific sub-populations of HDL and LDL lipoproteins that vary in density, and size and different atherogenic potentials. Small dense LDL particles are distinctively atherogenic (Campos *et al.* 1992) relative to large LDL particles. In contrast, large particles are strongly atherogenic among the VLDL sub-populations. Sub-populations of HDL also have different atherogenic potentials:

large particles are more protective than the smaller ones (Jeyarajah *et al*. 2006). The atherogenic profile of plasma is thus defined by a summary of risk factors reflected in the structure of lipoprotein sub-populations and, thus, also in the AIP and apoB/apoAI biomarkers. Therefore, both AIP and apoB/apoAI carry greater predictive value than the concentrations of individual lipid parameters and can be used as risk biomarkers in the case of relatively normal concentrations of plasmatic lipids. Insulin resistance (as indicated HOMA-IR – homeostatic model assessment- insulin resistance]) together with obesity represents another respected and significant CM marker (Reaven 2012).

The objective of this study was to determine the success of the CM biomarkers, AIP, apoB/apoAI ratio and HOMA-IR in overweight/obese children and to identify the types of relationships among these biomarkers and other CM risk parameters. Another area of focus was the frequency of risk values of those biomarkers in a monitored population of girls and boys who had been through an intensive lifestyle intervention.

Patients and Methods

Patients

Between June 2009 and March 2011, 309 overweight/obese children between 8 and 15 years of age participated in the study. Overweight was defined as a BMI ≥85th - <95th percentile and obesity was defined as a BMI ≥ 95th percentile for children of the same age and sex. Exclusion criteria comprised known diabetes mellitus, arterial hypertension, smoking, use of any medications and contraindication of prescribed physical activity. All participants were of Caucasian ethnicity, and written informed consent was given by the parents/guardians of all individuals. The study was approved

by the institutional ethics committee and conducted according to good clinical practice guidelines. Patients, intervention and measurements were described in more detail previously (Motykova *et al*. 2011).

Intervention, Anthropometric measurements

The intervention program consisted of individualised diet changes made to achieve a caloric intake of 5,000 kJ for the age category of 8 to 10 years old and 7,000 kJ for those 11 to 15 years old. The exercise program consisted of aerobic and resistance training (5 units, daily, 50 minutes each) complemented with ball games, swimming, dancing and fast walking. All participants underwent a thorough physical examination. Body weight was measured with a calibrated electronic weight scale. Height was measured to the nearest 0.5 cm. Waist and hip circumferences were also measured with an accuracy of 0.5 cm. BMI (kg per m^2) was calculated from obtained measurements. Diastolic and systolic blood pressures were measured after 10 minutes in a sitting position with an automated blood pressure unit (Automated sphygmomanometer BP-203 NA, Nippon Colin co., Ltd). Total body fat was determined by impedance analysis using a Bodystat analyser (1500 MDD, Bodystat, Isle of Man., UK).

Biochemical analyses

Venous blood was collected after 12 hours of fasting and plasma lipid levels, insulin and glycaemia were assessed by enzymatic methods using automated analysers (Hitachi, Japan). The LDL-C level was calculated by the Friedewald equation [LDL-C = TC - (HDL-C) – TG/2.2].

Measurement of the CM risk biomarkers AIP, apoB/apoAI and HOMA-IR.

AIP was calculated as a common logarithm of the ratio of molar concentrations of plasma triglycerides and HDL cholesterol AIP was used to stratify children according

to their atherogenic risk level. Optionally, as previously described (Rašlová *et al.* 2011, **www.biomed.cas.cz/fgu/aip**). AIP values under 0.1 were defined characteristic of low risk, while values corresponding to for medium risk ranged from 0.11 to 0.21 and values over 0.21 were considered characteristic of high risk. ApoB/apoAI ratio is a biomarker of cardiovascular risk introduced as a result of a prospective study called AMORIS by Walldius and others (Walldius *et al.* 2001). The Insulin Resistance HOMA-IR was calculated as ratio of plasma insulin to glycaemia divided by 22.5 (Tresaco *et al.* 2005).

Statistical methods. Statistical analysis including the following evaluations, was performed using SPSS 15.0. The data are presented as the means ± SD both before and after intervention for each group. For descriptive purposes, the differences between measurements taken before and after treatment were tested by paired t –test. Differences between genders were tested - by independent-samples T-test of initial variables with a 95% confidence interval. To investigate the correlations between variables, we used Pearson's correlation tested for the equality of two interdependent coefficients by Bonferroni´s correction method of significance levels. The variables use to define AIP, apoB/apoAI and HOME-IR were not included in correlation analysis to avoid co-linearity problems.

Results.

Characteristics of the group and influence of intensive lifestyle intervention. Statistically important differences in anthropometric values were found between boys and girls (Table 1). As expected, boys were considerably taller and heavier than girls (+8.7 centimetres and +13.7 kilograms). The boys also had higher BMI values (31.7) than girls (30.1). The initial value of systolic pressure was slightly significantly higher

in boys, while the values of diastolic pressure did not vary significantly. Waistline, abdomen- and hipline were larger in boys. Initial biochemical values did not vary depending on sex, and only one significance was found – lower apoAI and HDL-C in boys (Table 2).

The lifestyle intervention resulted in a significant reduction of all anthropometric parameters (Table 1). Weight and BMI were reduced in both sexes by 8%, and the waistline, abdomen- and hipline measurements were reduced by approximately 6%. The average percentage of fat was reduced by 15% in boys, but only by 7% in girls. Both systolic and diastolic pressure were significantly lowered.

Statistically significant changes occurred in biochemical parameters (Table 2). The test of differences before and after the intervention was significant in the most tested variables ($p<0.001$), with the exception of glycaemia in both sexes and the apoB/apoAI ratio in girls. TC, TG, LDL-C, and apoB levels were reduced by 15-20% in girls and by 25-30% in boys. The concentrations of HDL-C and apoAI were reduced in all children after the intervention. In both sexes, C-peptide dropped by 13% and, insulin by 23%, however, there was no change in glycaemia. The AIP values were reduced in both sexes, while the apoB/apoAI biomarker was statistically significantly reduced only in boys. The reduction of HOMA-IR was significant in both sexes (Table 2).

The relation between obesity and biochemical parameters.

The correlation analysis (Table 3) showed a strong relation among the initial and final values of BMI and the concentration of plasma TG, HDL-C and AIP. Associations between BMI and total cholesterol, LDL-C and apoprotein B levels were not statistically significant. The inverse correlation between BMI and apoAI was significant only between the values after the intervention. Despite the important

connection found between BMI and C-peptide, and insulinemia and HOMA-IR, glycaemia was not influenced.

AIP strongly correlated with BMI, C-peptide, insulin and HOMA-IR, both apoproteins and apoB/apoAI ratio. Unlike the apoB/apoAI ratio, AIP was not correlated with the concentration of TC or the initial values with LDL-C. In contrast, the apoB/apoAI ratio was very strongly correlated with the lipid components, namely with TC, LDL-C and TG. HOMA-IR was significantly correlated with obesity, TG, HDL-C and AIP, while its relationships to TC and LDL-C were not significant. The relationships between the BMI and CM markers confirmed the distribution of values into BMI quartiles, where AIP and HOMA-IR in the BMI quartiles showed a systematic increase according to the obesity grad, while differences in the apoB/apoAI ratio bordered on statistical discrepancy (Figure 1).

Stratification of children according to the AIP criteria.

The children's initial data were divided into three AIP categories, according to the importance of atherogenic risk (Figure 2) specified for the adult population (Methods). A total of 84.7% of girls and 74.1% of boys fell into the low-risk category (AIP≤0.1), 10% of girls and 12.1% of boys fell into the medium-risk category (AIP between 0.11-0.21), and the high-risk category (AIP> 0.21) included 5.3% of girls and 13.8% of boys.

Discussion

The objective of the study was to examine cardiometabolic risk in overweight/obese children using the CM biomarkers AIP, apoB/apoAI ratio and the HOMA-IR. The children took part in an intensive lifestyle intervention project during a month-long spa treatment. In addition to the usually examined anthropometrical and biochemical

parameters, we analysed the influence of sex on the initial values and on the effect of the intervention. We studied the relationships among the children's obesity, both biomarkers and insulin resistance that plays an important role in pathogenesis of coronary heart disease in non-diabetic individuals (Reaven 2012). Boys had higher values of anthropometrical parameters than girls, with the exception of diastolic pressure (Table 1). In contrast, the initial biochemical values of girls and boys exhibited no significant differences, with the exception of slightly higher concentrations of apoAI and HDL-C in girls than in boys. During the intervention, the typically measured anthropometrical parameters improved, and the blood pressure decreased slightly but significantly. However, the intervention produced a significant difference between the sexes because the reaction of certain variables to diet and exercise was stronger in boys than in girls. The reduction of TG, LDL-C, apoB was significantly higher in boys, while the reductions of HDL-C and insulin were at the same level (Table 2). The intervention also caused a reduction in the concentration of HDL-C and apoAI because of the substantial reduction of the total cholesterol, Glycaemia remained unchanged, while C-peptide exhibited a reduction of 13% and insulin and HOMA-IR decreased by 23-25% in both sexes.

Three CM biomarkers whose importance had been confirmed by a number of studies in adults were studied in parallel within the project. The importance of the association of TG with HDL-C in relation to obesity and CM risk in children had already appeared in the study by Bogalusa (Kikuchi *et al*. 1992) and other studies had also confirmed the importance of the TG/HDL-C ratio and insulin resistance for the identification of CM risk (Musso *et al*. 2011). As "an estimate of small, dense low-density lipoprotein cholesterol" TG/HDL-C was described as „an independent determinant of arterial stiffness in adolescents and young adults, especially in obese youth" (Murguía-

Romero *et al.* 2013). In our studies, AIP is used as a logarithmically transformed TG/HDL-C ratio because the transformed value produces higher correlations and normal probability plots (Tan *et al.* 2004). Thus, it is more suitable from the statistical perspective than the simple TG/HDL-C ratio (Urbina *et al.* 2011).

Another important CM marker – apoB/apoAI ratio reported in the AMORIS study was also described in children as a predictor of cardiovascular risk (Sellers *et al.* 2009) and, insulin resistance (Sierra-Johnson *et al*. 2007) as a predictor of carotid media thickness and brachial endothelial function in adulthood (Juonala *et al.* 2008).

In our study, we compared children´s obesity with selected biomarkers as a primary CM risk factor. AIP was significantly correlated with BMI (initial $r=0.265$, $p<0.0003$, final $r=0.422$, $p<0.0000$), and the categorisation into BMI quartiles indicated a systematic increase of AIP and HOMA-IR according to the obesity grades (Figure 1). AIP showed an equally strong association with elements of both lipid and glycid metabolism when the correlation coefficient between AIP and HOMA-IR was high (initial $r=0.406$, final $r=0.517$ $p<0.0000$). The correlation between AIP and the studied parameters was also significant (Table 3). In children, a significant relation of AIP was not found among the main predictors of CM risk in adults, i.e., TC and LDL-C, even if the relation to apoB (a LDL component) was significant.

The associations between the apoB/apoAI ratio and BMI and HOMA-IR were not statistically significant, while the correlations with TC, LDL-C and TG were highly significant. However, the relationship between the AIP and apoB/apoAI ratio biomarkers before and after the intervention ($p<0.0000$) was very strong, possibly because both biomarkers are related to lipoprotein particle size. The values of the apoB/apoAI ratio between the BMI quartiles (0.67-0.74) were lower than in a study examining the levels of lipids and apolipoproteins in adult Swedish, Iranian and

American populations showed that the average normal value of apoB/apoAI ratio ranged between 0.80 and 0.92 (Solphour *et al.* 2009). In our study, the relation of apoB/apoAI to glycid metabolism exhibited lower intensity than in other populations (Sierra-Johnson *et al*. 2007) because the children had lower concentrations of TC and LDL-C than the adult population.

Insulin resistance HOMA-IR - a strong CM risk factor– varied from 2.4 to 4.5 with increasing obesity from in the last BMI quartile (Figure 1) within our group, and the lifestyle intervention reduced the index from 3.5 to 2.6. Those values correspond well to earlier reports in children, where values close to 3 are adequate for the paediatric population (Kurtoglu *et al.* 2010). The associations of HOMA-IR with the lipoprotein components, including inverse associations with HDL-C and apoAI, and positive associations with TG and (naturally AIP) were highly significant within our group, but we did not prove the significance of the relationships between BMI to TC, LDL-C and the apoB/apoAI ratio.

The question of how many obese children had probably higher CM risk with respect to TG/HDL-C ratio or apoB/apoAI ratio is, currently, resolvable using sequestration according to AIP. A total of 13.8% of boys and only 5.3% of girls within the entire group had high-risk values (Figure 2). Of those high-risk children, 50% belonged to the 4^{th} BMI quartile. When a normal Slovak population, aged 40 years, was examined for CM risk factors, 10% of women and 30% of men fell into the high-risk AIP category (Rašlová *et al*. 2011). In another group examined for clinical cardiovascular problems, 41% of women and 58% of men with positive angiological diagnoses had high risk (Frohlich and Dobiášová 2003). Evidently, the degree of risk increases with age and increases to respective risk factors. Other studies also show that AIP can be used to detect CM disorders even at relatively normal lipid levels (Dobiášová *et al*.

2001). *Conclusions.* AIP, apoB/apoAI and HOMA-IR are complementary in CM risk prognosis among overweight/obese children. AIP may have higher prognostic value due to its strong relation to both glycid metabolism and lipoprotein metabolism. Using biomarkers in overweight/obese children enables us to provide a more detailed description of the CM risk profile in plasma and to monitor the effects of various interventions. Applying a proper dietary and exercise regime during a spa treatment, even a short-term one, could substantially influence the future prognosis of the children with respect to both obesity reduction and CM risk.

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Figure Legends

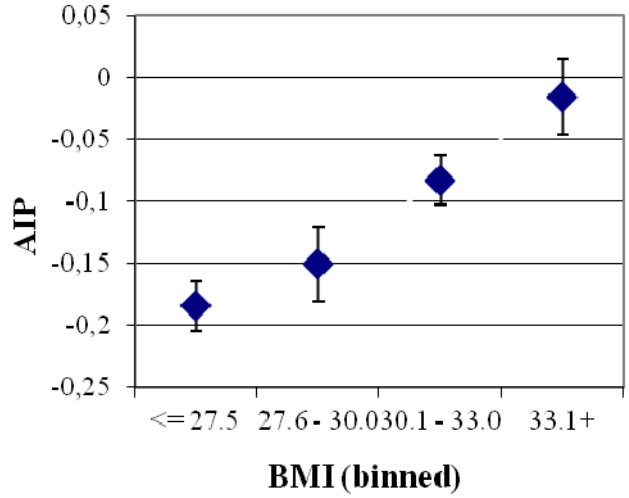
Figure 1. Initial values (Mean ± S.E.) of AIP, apoB/apoAI ratio and HOMA-IR in BMI quartiles.

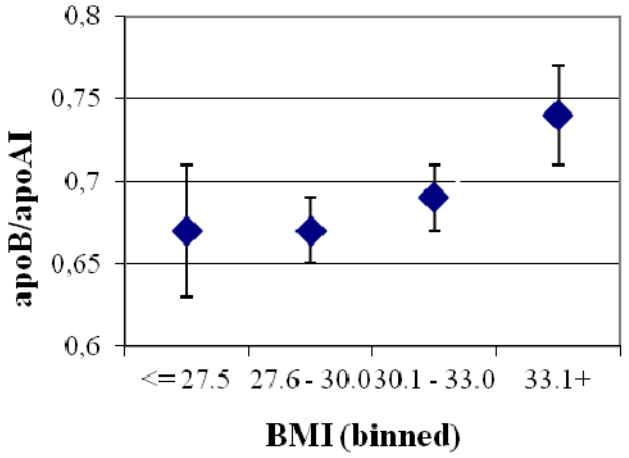
Figure 2. Classifying children into risk categories according to AIP in %.

AIP risk: Low ≤0.10, Medium 0.11 - 0.21, High >0.21.

Girls - full columns, boys - open columns.

Fig. 1:


0,05
0
-0,05
-0,1
-0,15
-0,2
-0,25
AIP
<=27.5
27.6 - 30.0
30.1 - 33.0
33.1+
BMI (binned)


0,8
0,75
0,7
0,65
0,6
apoB/apoAI
<=27.5
27.6 - 30.0
30.1 - 33.0
33.1+
BMI (binned)

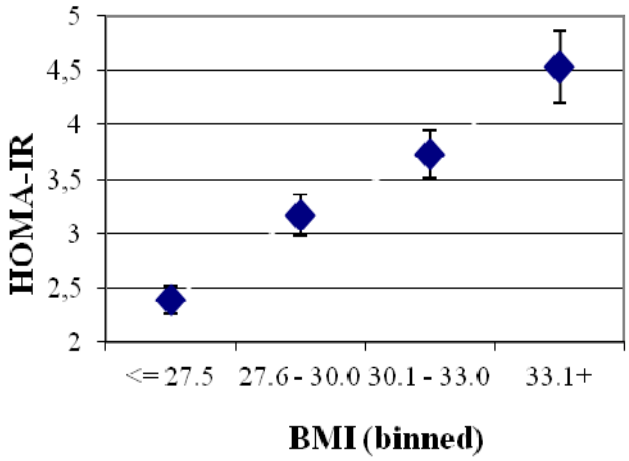

5
4,5
4
3,5
3
2,5
2
HOMA-IR
<=27.5
27.6 - 30.0
30.1 - 33.0
33.1+
BMI (binned)

Fig. 2:

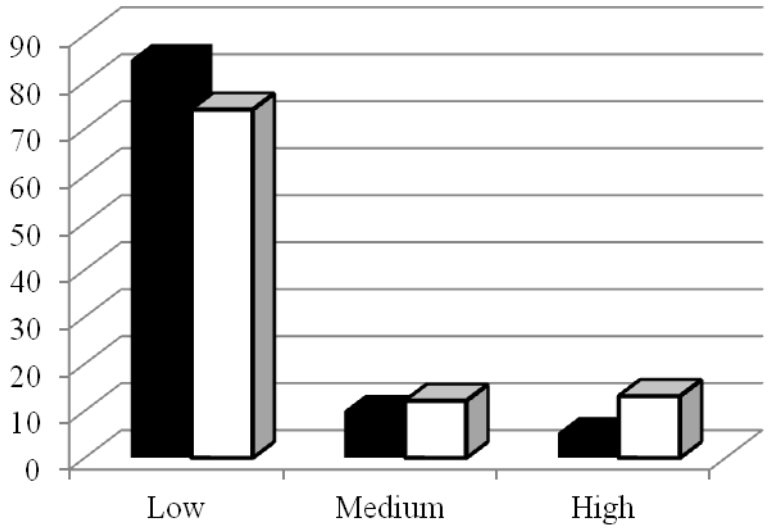


Table 1. Anthropometric characteristics of the children before (1) and after (2) lifestyle intervention: Gender differences.

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	GIRLS			**BOYS**			**G. vs. B.**
	n	Mean ± SD	Red. %	n	Mean ± SD	Red. %	p<
Age, years	194	13.0±2.1		115	13.5±1.9		*
Height, cm	197	158.6±9.8		118	167.3±11.3		***
Weight, kg 1	197	75.3±17.3		118	89.0±21.1		***
Weight, kg 2	190	69.9±16.3***	-7.5	111	81.6±18.3***	-8.2	***
BMI, kg/m^2 1	195	30.1±4.4		117	31.7±4.3		**
BMI, kg/m^2 2	187	27.9±4.1***	-7.4	112	29.2±4.2***	-8.0	*
$BP_{Systolic}$, mm/Hg 1	192	120.9±15		117	125.1±13.2		*
$BP_{Systolic}$, mm/Hg2	187	117.1±13***	-2.9	112	121.8±14.6**	-2.5	**
$BP_{Diastolic}$, mm/Hg 1	192	76.8±10.9		117	78.2±10.8		
$BP_{Diastolic}$, mm/Hg 2	187	73.7±10***	-3.8	112	73.1±8.4**	-6.3	
Waist circ., cm 1	189	86.4±10.6		110	95.9±11.1		***

Waist circ., cm 2	183	81.0±10.4***	-6.1	104	88.9±10.1***	-7.1	***
Abdominal c., cm 1	196	98.9±11.1		118	107.1±12.1		***
Abdominal c., cm 2	190	93.1±11.2***	-5.8	112	99.8±11.1***	-6.6	***
Hip, cm 1	196	101.4±11.4		118	106.0±10.4		***
Hip,cm 2	190	96.1±11.3***	-5,3	112	99.7±10.2***	-6.0	**
Total body fat, % 1	183	33.2±13.2		107	32.0±10.2		
Total body fat, % 2	161	30.1±12.9***	-7,2	92	26.6±9.7***	-15.0	*

Mean ± SD, Significance of differences between initial (1) and final (2) values: * $p<0.05$, ** $p< 0.01$, *** $p< 0.001$, Red.% … Reduction of the initial values in per cent (%), G. vs, B. …. significant differences of values between girls and boys.

Table 2. Biochemical variables of girls and boys before (1) and after (2) lifestyle intervention: Gender differences.

	GIRLS			BOYS			G. vs. B.
	n	Mean±SD	Red. %	n	Mean±SD	Red. %	
TC, mmol/L 1	194	4.65±1.01		118	4.44±0.81		
TC, mmol/L 2	184	3.79±0.81***	-18.1	110	3.33±0.67***	-24.8	***
TG, mmol/L 1	190	1.05±0.47		117	1.11±0.60		
TG, mmol/L 2	182	0.84±0.32***	-18.5	109	0.78±0.31***	-30.1	
LDL-C, mmol/L 1	193	2.85±0.82		118	2.71±0.71		
LDL-C, mmol/L 2	185	2.24±0.69***	-21.4	110	1,87±0.60***	-30.5	***
HDL-C, mmol/L 1	193	1.31±0.28		118	1.24±0.28		*
HDL-C, mmol/L 2	185	1.16±0.23***	-11.6	110	1.08±0.26***	-12.8	*
Glycaemia, mmol/L 1	191	4.93±0.40		117	5.03±0.46		
Glycaemia, mmol/L 2	184	4.96±0.37	0.5	109	4.94±0.37	-2.0	
C-peptid, ng/mL 1	181	0.86±0.32		117	0.88±0.31***		
C-peptid, ng/mL 2	170	0.76±0.27***	-11.7	101	0.76±0.29	-13.8	

Insulin, mlU/L 1	180	15.59±8.73		117	15.30±8.01		
Insulin, mlU/L 2	168	11.89±6.44***	-23.6	101	11.59±7.57***	-23.5	
apoAI, mmol/L 1	130	1.34±0.19		73	1.28±0.17		*
apoAI, mmol/L 2	120	1.17±0.17***	-12.2	66	1.06±0.17***	-17.0	***
apoB, mmol/L 1	129	0.90±0.26		73	0.88±0.22		
apoB, mmol/L 2	119	0.76±0.22***	-15.0	66	0.65±0.19***	-27.2	**
CM markers							
AIP 1	190	-0.124±0.212		117	-0.084±0.257		
AIP 2	182	-0.159±0.183**		109	-0.161±0.221***		
apoB1/apoAI 1	130	0.67±0.21		73	0.70±0.20		
apoB2/apoAI 2	120	0.65±0.21		66	0.62±0.19***		
HOMA-IR 1	115	3.48±2,17		115	3.46±0.92		
HOMA-IR 2	162	2.64±1.53***		162	2.56±1.84***		

Mean ± SD, Significance of differences between initial (1) and final (2) values: * $p<0.05$, ** $p< 0.01$, *** $p< 0.001$, Red .% …

Reduction of the initial values in per cent (%), G. vs, B. …. significant differences of values between girls and boys.

Table 3. Pearson's correlations between BMI, CM markers AIP, apoB/apoAI, HOMA-IR and biochemical values in all subjects; initial (1) and final (2) examinations.

	BMI			**AIP**			**apoB/apoAI**			**HOMA-IR**		
	r	n	p Bonf	r	n	p Bonf	r	n	p Bonf	r	n	p Bonf
BMI 1	1.000	305		0.265	303	**0.0003**	0.130	198	1.0000	0.395	291	**0.0000**
BMI 2	1.000	290		0.422	283	**0.0000**	0.180	180	1.0000	0.448	260	**0.0000**
TC 1	0.001	303	1.0000	0.088	306	1.0000	0.647	201	**0.0000**	0.031	294	1.0000
TC 2	0.033	284	1.0000	0.120	286	1.0000	0.632	183	**0.0000**	0.006	261	1.0000
TG 1	0.210	303	**0.0250**	NI	306		0.514	201	**0.0000**	0.377	294	**0.0000**
TG 2	0.299	284	**0.0000**	NI	286		0.457	183	**0.0000**	0.464	261	**0.0000**
LDL-C 1	0.008	303	1.0000	0.101	306	1.0000	0.743	201	**0.0000**	0.048	294	1.0000
LDL-C 2	0.038	284	1.0000	0.214	286	**0.0288**	0.744	183	**0.0000**	0.016	261	1.0000
HDL-C 1	-0.209	303	**0.0269**	NI	306		0.242	201	0.0581	0.204	294	**0.0466**
HDL-C 2	-0.346	284	**0.0000**	NI	286		0.245	183	0.0896	0.290	261	**0.0002**
Glycaemia 1	0.020	301	1.0000	0.105	304	1.0000	0.081	200	1.0000	NI	294	
Glycaemia 2	0.069	282	1.0000	0.196	284	0.0970	0.088	182	1.0000	NI	261	

C-peptid 1	0.459	290	**0.0000**	0.399	293	**0.0000**	0.214	189	0.3357	0.644	293	**0.0000**
C-peptid 2	0.376	261	**0.0000**	0.397	261	**0.0000**	0.248	159	0.1752	0.588	260	**0.0000**
Insulin 1	0.416	290	**0.0000**	0.427	293	**0.0000**	0.150	189	1.0000	NI	293	
Insulin 2	0.486	259	**0.0000**	0.521	259	**0.0000**	0.176	158	1.0000	NI	260	
apoAI 1	0.125	199	1.0000	0.391	202	**0.0000**	NI	201		0.132	191	1.0000
apoAI 2	0.307	181	**0.0028**	0.443	182	**0.0000**	NI	183		0.296	159	**0.0164**
apoB 1	0.069	198	1.0000	0.332	201	**0.0002**	NI	201		0.078	190	1.0000
apoB 2	0.014	180	1.0000	0.297	182	**0.0050**	NI	183		0.014	159	1.0000
CM markers												
AIP 1	0.265	303	**0.0003**	1.000	306		0.503	201	**0.0000**	0.406	294	**0.0000**
AIP 2	0.422	283	**0.0000**	1.000	286		0.515	182	**0.0000**	0.517	260	**0.0000**
apoB1/apoAI 1	0.135	198	1.0000	0.520	201	**0.0000**	1.000	201		0.178	190	1.0000
apoB2/apoAI 2	0.180	180	1.0000	0.515	182	**0.0000**	1.000	183		0.151	159	1.0000
HOMA-IR 1	0.395	291	**0.0000**	0.406	294	**0.0000**	0.129	190	1.0000	1.000	294	
HOMA-IR 2	0.447	260	**0.0000**	0.517	260	**0.0000**	0.151	159	1.0000	1.000	262	

r…correlation coefficient. p Bonf. ...Significance after Bonferroni correction. NI ... Not included due to co-linearity of individual variables with biomarkers. **bold values**.. statistically significant.