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### Adolescent Obesity Predicts Cardiovascular Risk

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#### 1. Introduction

Rapid increase in the prevalence of obesity among children and adolescents has become a major worldwide health issue. Several large epidemiological studies have demonstrated that childhood and adolescent obesity is a significant independent predictor of metabolic disorders, such as hypertension, dyslipidemia and insulin resistance, which have a major impact on the premature development of atherosclerosis and cardiovascular morbidity and mortality in adulthood (Bibbins-Domingo et al., 2007; Dietz & Robinson, 2005; Franks et al., 2010). The cluster of metabolic disorders including abdominal obesity, insulin resistance, dyslipidemia and elevated blood pressure has been defined as metabolic syndrome (Alberti et al., 2006; Aggoun, 2007, Han & Lean, 2006). It is now generally accepted that overweight or obese children and adolescents are at increased risk for some or all of the metabolic syndrome (MS) features (Carnethon et al., 2004; Franks et al., 2010; Magnussen et al., 2010; Zimmet et al., 2007). The data from the Third National Health and Nutrition Examination Survey (NHANESIII, 1988-1994) have demonstrated that about 4 % of the whole population and almost 30% of the overweight or obese 12 to 19-y-old adolescents met the criteria of MS (Cook et al., 2003). The early occurrence of the MS in childhood and at the pubertal age was also found to have a major impact on the development of atherosclerosis, a life-time risk of cardiovascular disease (Aggoun, 2007), and an increased rate of premature death (Franks et al., 2010; Nieto, 1992). Excessive body weight in childhood and adolescence is considered a strong predictor of adult obesity (Wang et al. 2008) and obesity-related health consequences including diabetes and heart disease (Carnethon et al., 2004; Must et al., 1992; Morrison et al. 2007) regardless of whether the parents were obese. Notably, parental obesity was found to double the risk of being obese in adulthood (Shengxu et al., 2003; Whitaker et al., 1997).

The main reason for undertaking the present study was to evaluate the prevalence of obesity among 15-y-old adolescents and to estimate the risk for adverse health outcomes in this population sample. Another goal was to find out relatively simple biochemical markers that could be suitable for the early diagnosis of metabolic disorders and cardiovascular risk, and would help to implement prevention programs targeted at risk groups.

#### 2. Methods

#### 2.1 Population sample

The data were obtained from 505 adolescents, middle school pupils (264 boys and 241 girls) aged 15 years, who were enrolled into a cross- sectional health screening study undertaken by the municipal health care authorities of Katowice (Poland) - in collaboration with our group. In the morning, after an overnight fast, the participants reported to the hospital to undergo a brief general medical examination, including assessment of anthropometric parameters, measurement of blood pressure, and collection of venous blood samples for biochemical analyses. The participants, boys and girls separately, were classified into one of the four groups, according to their Body Mass Index (BMI) score, using the age and gender-specific cut-off points of the BMI percentiles established for 15-years old Polish youth by Palczewska & Niedźwiecka (2002): group A of the underweight (<25 c), group B of the normal weight (25-75c), group C of the overweight (75-97 c), and group D of the obese (>97 c) individuals (Table 1). Criterion for diagnosis of obesity at BMI≥ 97<sup>th</sup> percentile was consistent with the WHO references for children 5 to 19 years old (available at http://www.who.growthref). Informed consent was obtained from all adolescents and their parents prior to participation in the study, the protocol of which was approved by the local Ethics Committee.

	Variable		Percer	ntile ranges (c)	
Gender		Underweight (<25 c) X±SD	Normal weight (25-75 c) X±SD	Marginally overweight (75-90 c) and overweight (90-97 c) X±SD	Obese (>97 c) X±SD
	No. of cases	N=55	N=98	N=54	N=57
	Body height, m	1.65*±0.08	1.71±0.07	1.69±0.09	1.70±0.08
Boys	Body mass, kg	45.9***±5.2	57.7±6.0	70.3***±7.93	82.7***±11.6
Bo	BMI	16.7***±0.9	19.7±1.1	24.6***±0.9	28.6***±3.4
	SBP, mm Hg	118.8±10.6	124.9±14.3	132.8*±14.8	135.7**±15.2
	DBP, mm Hg	71.3±8.5	71.6±11.1	73.4±9.2	73.7±10.2
	No. of cases	N=58	N=81	N=46	N=56
	Body height, m	$1.65 \pm 0.08$	1.66±0.08	1.65±0.09	1.64±0.08
Girls	Body mass, kg	46.1***±5.1	54.1±6.5	67.2***±7.8	79.4***±11.8
Ë	BMI	17,0***±0.9	19.6±1.2	24.7***±0.8	29.3***±3.2
	SBP, mm Hg	118.7±12.9	124.8±12.4	124.7±15.7	132.5*±15.3
	DBP, mm Hg	69.4±0.08	74.4±9.2	72.9±11.1	74.8±11.1

Table 1. Anthropometric characteristics, and systolic (SBP) and diastolic (DBP) blood pressure of 15-year-old Polish adolescents. Significance of differences *vs.* normal weight individuals: \*p<0.05, \*\*\* p<0.001

#### 2.2 Analytical procedures

Concentrations of serum glucose, total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG) were assessed by the enzymatic methods using commercially available diagnostic kits (BioMaxima cat.no. 1-033-0400, 1-023-0400, 1-029-0200, 1-053-0400, respectively). Concentrations of low-density lipoprotein cholesterol (LDL-C) were calculated from TC, HDL-C, and TG using the Friedewald formula (Friedewald et al., 1972). Serum insulin was measured by the immunoradiometric method (Insulin IRMA IM3210, Immunotech SA, France), sensitivity was 2.0 mIU/ml, and autoantibodies against oxidized LDL (oLAb) in serum samples were evaluated using ELISA assay (oLAb-ELISA, BI-20032; Biomedica GmbH, Wien, Austria) according to Tatzber & Esterbauer (1995).

To evaluate risk for vascular disease, the lipid ratios (TC/HDL-C, LDL-C/HDL-C, TG/HDL-C) and atherogenic index of plasma [AIP= log<sub>10</sub>(TG/HDL-C) with TG and HDL-C expressed in molar concentrations] (Dobiášová & Frohlich, 2001, 2004, 2011) were calculated. The homeostatic model assessment (HOMA) was used to estimate insulin resistance according to the method described by Matthews (1998). Insulin resistance score computed using following formula: (HOMA-IR) was the HOMA-IR=glucose (mmol/L)\*insulin (mIU/L)/22.5, with the cut-off point for adolescents less than or equal to 3.16, as suggested by Keskin et al. (2005). Moreover, in 231 individuals (121 boys and 110 girls) serum adiponectin was measured by an immunoenzymatic method (Human Adiponectin ELISA, BioVendor, cat. no. RD195023100), and serum insulin-like growth factor (IGF-1) was evaluated in 80 adolescents (40 boys and 40 girls) using a commercially available diagnostic kit (IGF-I ELISA, Immunodiagnostic Systems GmbH, sensitivity 3.1  $\mu$ g/L, reference range for 12 to 15 y old subjects 142-525  $\mu$ g/L).

#### 2.3 Statistics

All statistical analyses were performed with STATISTICA 7.1 software (StatSoft, Tulsa, OK, USA). The data reported as means ± SD were tested for homogeneity of variances by using the Levene test, then two-way ANOVA was performed to analyze the effect of gender and the degree of overweight/obesity on the studied variables, followed where appropriate, by the Tukey post-hoc comparisons. Spearman's rank order correlation analysis was performed to assess relationships between selected variables. Additionally, Pearson's Chi-square test of independence was used to evaluate the impact of the degree of overweight/obesity on SBP, serum triglycerides and surrogate markers of insulin resistance (HOMA-IR, TG/HDL-C). To predict the relative contribution of selected metabolic variables to prevalence of over normal SBP, insulin resistance (as assessed by HOMA-IR) and atherogenic potential of plasma (as assessed by AIP) a stepwise multiple regression analysis was applied. The level of significance of P<0.05 was chosen for all statistical comparisons.

#### 3. Results

Physical characteristics of individual groups of the participants are presented in Table 1. In the evaluation of between-group differences, the data obtained in 113 adolescents (55 boys and 58 girls) with underweight, in 100 individuals (54 boys and 46 girls) with a tendency to overweight or overweight, and in 113 obese subjects (57 boys and 56 girls) were compared to the data obtained in 179 adolescents (98 boys and 81 girls) with normal weight. Mean

values and percentiles of BMI were similar among the groups of boys and girls with various degree of overweight, while the mean values of BMI in both groups of normal-weight teens were very close to the 50 percentile of BMI distribution for 15 year old boys and girls (Palczewska & Niedźwiecka, 2001; WHO Growth reference data for 5 to 19 years, available at http://www.who.growthref).

The readings of systolic and diastolic blood pressure (SBP and DBP) in all groups of adolescents, stratified according to gender and degree of overweight, were compared with the data of the updated blood pressure charts for children and adolescents according to the child's age and height percentile. For the purposes of this study, the following criteria were adopted. The adolescents were classified as normotensive if their systolic BP was <90th percentile, i.e. <130 mm Hg. The measured SBP >90th percentile, which could indicate prehypertension or hypertension, was classified as "over normal". These criteria were conform to the recommendations of the National High Blood Pressure Education Program The Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents, (2004), and to the guidelines of the IDF 2007 Consensus Statement (Zimmet et al., 2007; Alberti et al., 2006, 2007). There were numerous cases, in particular in overweight or obese boys and in obese girls, when SBP exceeded 136 mm Hg, the level suggesting occurrence of significant hypertension according to blood pressure criteria established by the Task Force report (Blood pressure charts for children and adolescents by age and height percentile, 2005; Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents. A Working Group report from the National High Blood Pressure Education Program, 1996). Notably, more than a half of obese teens, especially girls, had over normal systolic BP.

Results of biochemical tests obtained in all groups stratified by gender and the degree of overweight are presented in Table 2. Except for a very limited number of cases, fasting blood glucose level, in all groups of boys and girls, did not exceed the upper limit of the normal range (100 mg/dL), while fasting insulin concentration tended toward higher values with growing degree of overweight to reach, in several cases, levels exceeding the upper limit of the reference range of the method used (2,6 - 22  $\mu$ U/mL) (Table 2, Fig.1). As expected, the prevalence of insulin resistance, as assessed by the HOMA-IR index, increased significantly with the degree of overweight as measured by BMI. In the prevailing number of cases, the fasting serum levels of total cholesterol, HDL-C, LDL-C and calculated lipid ratios (TC /HDL-C, LDL-C / HDL-C) were within the specific reference ranges, while the concentration of TG and the TG/HDL-C ratio were found to reach the markedly higher values only in both groups of obese individuals (Table 2, Fig.1). Except for serum HDL-C, which was higher (p<0.05) in girls, there were no significant inter-gender differences for the remaining tested variables. There was a clear tendency toward higher levels of SBP, insulin, HOMA-IR, triglycerides, and TG/HDL-C ratio with increased degree of overweight (Fig.1) in adolescent boys and girls, and most of these measures were affected significantly by the degree of overweight (Table 2). It is also worth to note that the degree of overweight/obesity significantly affected the atherogenic index of plasma (AIP), which adopted the lowest (most favorable) levels in the underweight groups, and then increased progressively with rising body mass index (Table 2, Fig.1). No between-group differences were found in titers of autoantibodies against oxidized LDL (oLAb), while individual oLAb titers were very variable, reaching over normal levels (>600 mU/mL) (Pincemail et al., 2000) in several subjects from each group. Similarly, no between-group differences were observed

			Perce	entile ranges (c)		
Gender	Variables	Underweight (<25 c) X±SD	Normal	Marginally overweight (75-90 c) and overweight (90-97 c) X±SD	Obese (>97 c) X±SD	Main effect: degree of overweight P value
	No. of cases	N=55	N=98	N=54	N=57	N=264
	Cholesterol (TC), mg/dL	169.4±25.3	167.2±26.0	170.5±25.1	174.1±24.3	P<0.05
	HDL-C, mg/ dL	54.5±5.3	53.5±5.3	54.8±6.1	54.9±4.8	NS
	LDL-C, mg/ dL	93.3±22.9	92.1±22.0	93.2±22.8	93.0±24.6	NS
	oLAb, mU/mL	921.6±817.8	947.6±726.9	845.3±849.8	821.1±672.4	NS
	TG, mg/ dL	108.0±27.3	108.0±22.9	112.3±29.0	130.7**±50.0	P<0.005
	TC/HDL-C	3.12±0.44	3.13±0.41	3.12±0.40	3.18±0.42	NS
Boys	LDL-C/HDL-C	1.72±0.41	1.72±0.38	1.71±0.40	$1.70\pm0.44$	NS
g	TG/HDL-C	2.00±0.55	2.02±0.42	2.07±0.59	2.41*±0.98	P<0.005
	AIP	$-0.074 \pm 0.114$	-0.063±0.090	-0.059±0.111	-0.007*±0.117	P<0.01
	Glucose, mg/ dL	84.2±11.9	82.2±11.8	83.7±11.8	81.5±10.2	NS
	Insulin, mIU/L	9.4±7.8	11.6±8.0	13.7±12.3	16.1*±9.5	P<0.005
	HOMA-IR	1.97±1.61	2.38±1.80	2.89±2.82	3.24±1.94	P<0.05
	Adiponectin, ng/mL (N=121)	14.3**±19.9	9.5±7.7	9.9±6.4	9.4±5.3	P<0.005
	IGF-1, ng/mL (N=40)	287.8±118.6	500.3±261.4	432.8±127.6	420.8±123.3	NS
	No. of cases	N=58	N=81	N=46	N=56	N=241
	Cholesterol (TC), mg/dL	173.7±23.2	167.0±26.1	175.5±23.9	171.4±22.5	NS
	HDL-C, mg/dL	56.7±6.1	55.03±5.486.3	55.23±5.14	55.65±5.91	NS
	LDL-C, mg/dL	95.8±20.9	89.1±23.4	96.8±20.0	89.5±20.7	NS
	oLAb, mU/mL	877.3±800.0	790.5±688.7	980.5±800.8	787.5±705.2	NS
	TG, mg/dL	106.0±25.2	114.7±29.0	117.3±25.4	131.6*±51.6	P<0.05
	TC/HDL-C	3.08±0.44	3.05±0.46	3.18±0.34	3.10±0.44	NS
Ś	LDL-C/HDL-C	1.71±0.39	1.63±0.43	1.75±0.32	1.62±0.39	NS
Sirls	TG/HDL-C	1.89±0.51	2.10±0.54	2.13±0.44	2.41±1.07	P<0.005
-	AIP	-0.097±0.110	-0.051±0.105	-0.041±0.089	-0.009±0.151	P<0.01
	Glucose, mg/ dL	81.2±9.7	80.8±10.4	83.7±12.6	83.3±10.8	NS
	Insulin, mIU/L	8.4±4.2	12.6±12.9	15.5±13.3	18.3±15.1 <sup>P=0.06</sup>	P<0.005
	HOMA-IR	1.69±0.90	2.65±2.86	3.29±3.08	3.74±3.11	P<0.05
	Adiponectin, ng/mL (N=110)	14.2**±10.1	8.3±4.7	9.0±4.5	11.3±5.5	P<0.01
	IGF-1, ng/mL (N=40)	385.0±76.5	387.5±170.5	398.0±98.5	388.3±60.8	NS

Table 2. Serum biochemical parameters in 15-year-old Polish adolescents. Significance of differences vs. normal weight individuals: \*p<0.05, \*\* p<0.005, NS-non significant

in serum IGF-1, there was only a slight tendency toward higher IGF-1 levels in boys and the lowest values in underweight individuals, although both effects did not reach statistical significance. Serum adiponectin in both groups of overweight or obese individuals was close to the levels recorded in normal weight teens, but the highest concentrations of this adipokine, significantly (p<0.05) different from the values recorded in normal weight individuals, were found in the underweight groups (Table 2, Fig1).

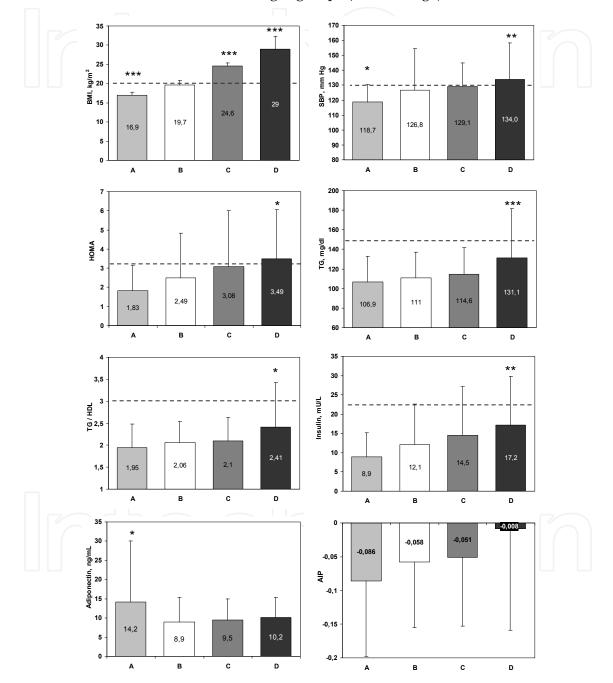


Fig. 1. The impact of the degree of overweight/obesity on systolic blood pressure (SBP), HOMA-IR, serum concentration of triglycerides (TG), TG/HDL ratio, insulin and adiponectin levels, and atherogenic index of plasma (AIP) in 15-year-old Polish adolescents with underweight (A), normal weight (B), overweight (C) and obesity (D). Significance of differences *vs.* individuals with normal weight: \*p<0.05,\*\* p<0.01, \*\*\*p<0.001

The analysis carried out to assess the prevalence (%) of adolescents with over normal values of SBP (>130 mm Hg), serum triglycerides (> 150 mg/dL) (Zimmet et al., 2007), HOMA-IR (>3.16) (Keskin et al., 2005), and TG/HDL-C ratio (>3.0) (McLaughlin et al., 2003; Hannon et al., 2006) revealed a rising number of individuals fulfilling these criteria with an increasing degree of overweight (Table 3). Noteworthy, Pearson's chi-square test showed significant increases in the prevalence (%) of individuals with over normal levels of these parameters with raising BMI.

Prevalence (%) of individuals with over normal values of SBP, HOMA-IR, TG, and TG/HDL-C ratio						Pearson's Chi-square (df=3)		
Gro Boys (B) and	-	ls (G)	Underweight (<25 c)	Normal weight (25-75 c)	Marginally overweight (75-90 c) and overweight (90-97 c)	Obese (>97c)	X2	P value
SBP	B+G	34.5	13.4	31.1	40.0	56.9	48.5	<10-5
>130	В	36.2	13.0	32.3	50.0	52.8	24.1	<10-4
mmHg	G	32.8	13.8	29.6	28.3	60.7	30.1	<10-5
HOMA-IR	B+G	22.5	8.0	19.6	25.5	39.3	33.2	<10-5
>3,16	В	21.7	10.9	20.1	22.6	33.3	8.4	< 0.05
	G	23.4	5.2	18.5	28.9	45.5	27.5	<10-5
TG	B+G	10.7	6.2	6.7	7.0	24.8	30.3	<10-5
>150	В	9.5	7.3	3.1	7.4	24.6	20.4	< 0.0005
mg/dL	G	12.0	5.2	11.1	6.5	25.0	12.9	< 0.005
TG/HDL-	B+G	6.3	4.4	3.4	5.0	14.2	15.3	< 0.005
С	В	7.2	5.5	2.1	7.4	17.5	13.3	< 0.005
> 3.0	G	5.4	3.5	5.0	2.2	10.7	4.5	NS

Table 3. Prevalence (%) of 15 year-old boys (B) and girls (G) with over normal values of systolic blood pressure (SBP), HOMA-IR, triglycerides (TG), and TG/HDL-C.

In order to assess the impact of the degree of overweight on selected biochemical parameters and biomarkers of atherogenic risk, the Spearman rank correlation coefficients were computed, and selected statistically significant associations are summarized in Tables 4 and 5. As expected, the BMI and serum triglycerides correlated positively with systolic and diastolic blood pressure, insulin, and HOMA-IR. Serum adiponectin correlated negatively with lipid ratios (TG/HDL-C, TC/HDL-C, LDL-C/HDL-C), while it was positively associated with HDL-C. The oLAb titers correlated positively with serum LDL-C and LDL-C/HDL-C ratio (Table 4).

The important finding was that the atherogenic index of plasma (AIP) was positively correlated with the BMI, systolic and diastolic blood pressure, TG, total cholesterol, common lipid ratios (LDL-C/HDL-C, TC/HDL-C), insulin, HOMA-IR, but negatively associated with HDL-C and adiponectin (Table 5). Although these relationships do not infer casual

dependence between the variables studied, they may suggest that there is a cluster of metabolically linked risk factors that, along with the hypertension and degree of obesity, determines the risk of atherosclerotic vascular disease. This hypothesis was fully supported by our results of a stepwise multivariate regression analyses that have shown that the main predictors of insulin resistance, as assessed by HOMA-IR, were concentrations of insulin and TG or TG/HDL-C ratio (R<sup>2</sup>=0.96, p<0.0001), while concentrations of TG, LDL-C and of total cholesterol (TC) accounted for 95% (R<sup>2</sup>=0.95, p<0.0001) of atherogenic potential of plasma (AIP) variance, and the most important predictors of systolic blood pressure (SBP) were BMI, TG and LDL/HDL ratio (R<sup>2</sup>=0.16, p<0.001).

Variables	Ν	R	р
BMI & SBP	498	0.332	P<10-6
BMI & DBP	498	0.128	P<0.005
BMI & insulin	502	0.377	P<10-6
BMI & HOMA-IR	502	0.360	P<10-6
BMI & TG	505	0.189	P<10-4
BMI & TG/HDL-C	505	0.174	P<10-4
TG & SBP	498	0.184	P<10-4
TG & DBP	498	0.170	p<0.0005
TG & insulin	502	0.272	P<10-6
TG & cholesterol	505	0.343	P<10-6
TG & HOMA-IR	502	0.266	P<10-6
TG/HDL-C & HOMA-IR	502	0.262	P<10-6
Adiponectin & HDL-C	231	0.308	P<10-4
Adiponectin & TG/HDL-C	231	-0.194	P<0.005
Adiponeectin & TC/HDL-C	231	-0.208	P<0.005
Adiponectin &LDL/HDL-C	231	-0.198	P<0.005
Adiponectin & oLAb	231	-0.169	P<0.05
oLAb & LDL-C	438	0.106	P<0.05
oLAb & LDL-C/HDL-C	438	0.129	P<0.01

Table 4. Spearman's rank order correlation coefficients between selected anthropometric and biochemical variables in 15-year- old Polish adolescents.

Variables	N	R	р
AIP & BMI	505	0.174	P<0.0001
AIP & SBP	498	0.139	P<0.005
AIP & DBP	498	0.104	p<0.05
AIP & TG	505	0.907	P<10-6
AIP & HDL-C	505	-0.297	P<10-6
AIP & INS	502	0.272	P<10-6
AIP & HOMA-IR	502	0.262	P<10-6
AIP & adiponectin	231	-0.194	P<0.005

Table 5. Spearman's rank order correlation coefficients between atherogenic index of plasma (AIP) and selected physical and biochemical variables in 15-year- old Polish adolescents.

#### 4. Discussion

Multiple studies (Aggoun, 2007; Carnethon et al., 2004; Davis et al., 2001; Dietz, 1998; Dietz & Robinson, 2005; Franks et al., 2010; Han & Lean, 2006; Magnussen et al., 2010; Morrison et al., 2007; Must et al., 1992; Shengxu et al., 2003; Wang et al., 2008; Whitaker et al., 1997) have demonstrated that obesity at the developmental age poses an important risk factor for the development of cardiovascular diseases in youth and adulthood. The main objective of this cross-sectional study was screening for cardiovascular risk factors in a locally living urban population of 15-year old adolescents that could be easily applied in the early diagnosis of metabolic disorders in overweight and obese youth. The following independent risk factors were taken into consideration : degree of overweight/obesity, over normal blood pressure, insulin resistance, dyslipidemia, and adiponectin level (Aggoun, 2007; McLaughlin et al., 2003; Hannon et al., 2006; Kaelber & Pickett, 2009; McNiece et al., 2007; The Fourth report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, 2004).

The important finding of this study is that over normal SBP and DBP values were strongly associated with body mass index (BMI). Namely, the prevalence of over normal SBP (i.e.>130 mmHg) was twice as high in the obese, compared with normal-weight teens, and four times higher in the obese than in the underweight groups, but there was no significant difference in the prevalence of over normal SBP between the two sexes. The marked increase of prevalence of over normal SBP with the degree of overweight in the participants of our study strongly supports the view that obesity is becoming a significant health issue in the young Polish population. Our observations fully support previous studies among various ethnic and racial groups that have shown the higher prevalence of abnormally high blood pressure in obese, compared with non-obese children and adolescents (Mc Niece et al. , 2007; Sorof &Daniels, 2002; Verma et al., 1994; Macedo et al., 1997).

Obesity-related elevated blood pressure in adolescence, is known to increase the risk of hypertension in adulthood (Cook et al., 2003; The Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents, 2004; Williams et al., 2002). Increased blood pressure is also considered the independent predictor of atherosclerosis, hyperplasia and hypertrophy of vascular smooth muscle, the factors affecting arterial stiffness (Aggoun, 2007).

There is a large body of evidence that obesity is associated with metabolic pathology such as impaired glucose tolerance, insulin resistance, and dyslipidemia. Studies using specimens collected at autopsy (McGill, 1997; Berenson et al., 1998), or using non-invasive assessment of carotid intima-media thickness (IMT) by ultrasonography (Berenson, 2002; Davis et al., 2001; Raitakari et al., 2003; Shengxu et al., 2003) demonstrated that the atherosclerotic process begins in childhood and adolescence and may track into adulthood. In this regard, the occurrence of highly statistically significant positive correlations between body mass index (BMI) and serum levels of triglycerides and insulin, or surrogate markers of insulin resistance (HOMA-IR and TG/HDL-C) fully confirmed the view that obesity occurring during developmental age raises the risk of developing type 2 diabetes and cardiovascular disease in the young.

In the present study, the assessment of risk for CVD and insulin resistance in 15-year old subjects was based on the analysis of serum biomarkers, such as total cholesterol (TC) and

its lipoprotein fractions (LDL-C and HDL-C), triglycerides (TG), insulin, and glucose. Interestingly, no between group differences in serum concentrations of total cholesterol, LDL- and HDL cholesterol, lipid ratios (TC/HDL-C and LDL-C/HDL-C) and glucose were found. The mean values of these variables did not exceed the cut-off points recommended by the American Heart Association (Kavey et al., 2003) set at 170 mg/dL for TC, 110 mg/dL for LDL-C and 35 mg/dL for HDL-C. In this respect, the results of the present study are consistent with those previously reported by Lee at al. (2009), who found that the BMI percentiles do not provide effective discrimination for distinguishing children with abnormal TC and LDL-C levels. On the contrary, compared with the control groups of normal weight teens, the mean concentration of serum TG was significantly higher in the obese boys and girls, although it did not exceed the borderline level (150 mg/dL) as defined by the IDF guidelines (Alberti et al., 2006, 2007; Zimmet et al. , 2007). A similar trend was observed for TG/HDL-C ratio, considered a simple metabolic marker for identification of overweight individuals who are insulin resistant (McLaughlin et al., 2003; Hannon et al., 2006; Brehm et al., 2004).

The usefulness of the TG/HDL-C ratio as a marker of insulin resistance was additionally supported by its significant correlation with HOMA-IR. It should be stressed, however, that not all overweight and obese teens could be diagnosed as insulin resistant. Noteworthy, the prevalence of individuals with HOMA-IR exceeding the cut-off point (3.16) for adolescents (Keskin et al., 2005) rose with degree of overweight, and reached the highest levels in the obese groups, but only in about 40% of obese teens it exceeded the borderline level. Noteworthy is that the risk of becoming insulin resistant was higher among obese girls than among obese boys. Less marked increases were observed for TG or TG/HDL-C levels, as only in about 25% or 15 % of obese individuals they exceeded the respective cutoff points. Significantly higher serum levels of TG in obese individuals may indicate increased risk for atherosclerosis and obesity-related insulin resistance. This hypothesis is strongly supported by our finding of significant correlations between TG and atherogenic index of plasma (AIP), total cholesterol (TC), insulin, and HOMA-IR. Existing evidence suggests that serum TG is a strong predictor of CVD (Gotto, 1998), although it was found that TG can regulate lipoprotein interactions, but is not an independent risk factor.

This opinion is supported by the evidence that hypertriglyceridemia is associated with predominance of more atherogenic small dense LDL particles (sdLDL) (Packard, 1996). The increased risk may be related to easier penetration of sdLDL into the sub-endothelial space, their lower binding affinity to the LDL receptor and a higher susceptibility to oxidation. Although various techniques are available for direct measurement of LDL particle size distribution (Superko, 1996), an indirect (surrogate) method may be used for evaluation of the atherogenicity of plasma lipoproteins based on the assessment of the Atherogenic Index of Plasma (AIP), calculated as log(TG/HDL-C) (with TG and HDL-C expressed in molar concentrations) (Dobiášová & Frohlich , 2001). It is well documented that changes in AIP may predict LDL particle size, and provide reliable information about the atherogenicity of plasma (Dobiášová & Frohlich, 2001; Dobiášová, 2004; Dobiášová et al., 2011; Onat et al., 2010). One of the most important findings of the present study is that there was a statistically significant trend toward higher values of AIP with raising degree of overweight, which supports the view that obesity is the main predictor of the CVD risk, and that atherogenic index of plasma (AIP) is a suitable tool to evaluate the risk.

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It has been suggested that accelerated atherosclerosis in diabetes may be due to an enhanced oxidative modification of LDL, and of sdLDL in particular ( Uusitupa et al., 1996). It was also suggested that LDL modified by glycation, as it may occur in diabetic patients, may be more susceptible to oxidation ( Jenkins et al., 2004). Oxidatively modified LDL (oxLDL) that are strongly atherogenic may induce the immune response associated with enhanced production of specific autoantibodies against oxLDL (oLAb) (Steinerová et al., 2001; Shoenfeld et al., 2004). In the present study, the oLAb titers, the mean values of which were within the reference range set by Pincemail et al. (2000) for the method used in our study, appeared to be independent of the degree of obesity and HOMA-IR estimated insulin resistance but, as could be predicted, they were correlated with serum LDL-C and the LDL-C/HDL-C ratio. These results are fully consistent with those reported by Uusitupa et al (1996) and Jenkins et al. (2004) who found that autoantibodies against oxLDL indicate the presence of oxidatively modified LDL *in vivo*, but their titers do not seem to predict cardiovascular morbidity or carotid IMT.

Research into the mechanisms and mediators of obesity-related pathologies also called attention to the involvement of the adipose tissue in the regulation of energy balance by a number of adipose tissue -derived peptide hormones (adipocytokines), such as leptin, adiponectin and resistin, all seem to be involved in insulin resistance associated with obesity. We focused our attention on adiponectin, and we found that the highest circulating levels of this adipocytokine were found in the underweight groups of teens. This observation is consistent with the previously described effects of weight-loss on increases in serum adiponectin (Elloumi et al., 2009). Interestingly, high levels of serum adiponectin have been reported in anorectic patients (Nedvidkova et al., 2005; Pannacciulli et al., 2003). Adiponectin is recognized for its beneficial antiatherogenic, antidiabetogenic, and anti inflammatory action, mainly due to its ability to improve insulin sensitivity (Körner et al., 2007; Cruz et al., 2004; Nedvidková et al., 2005), to inhibit TNF-α mediated adherence of monocytes, to reduce their phagocytic activity, to suppress the accumulation of modified lipoproteins in the vascular wall , and to stimulate endothelial NO production (Ekmekci & Elmekci, 2006). Statistically significant positive correlation between serum adiponectin and HDL-cholesterol, and the negative associations with common lipid ratios (TC/HDL-C and LDL-C/HDL-C) and AIP seem to confirm the beneficial antiatherogenic effect of this hormone, and suggest low risk of dyslipidemia in adolescents with low body weight. Of note, the associations of adiponectin with HOMA-IR, LDL-cholesterol, triglycerides and insulin were also negative, but they did not reach statistical significance, most likely due to the limited number (N=231) of adiponectin data.

In the present study we have also made a preliminary attempt to investigate the association between serum IGF-1 and obesity-related insulin resistance in adolescent subjects. The data from the literature suggest that IGF-1 may have beneficial effect on glucose homeostasis, due to its glucose lowering and insulin sensitizing actions (Kabir et al., 2010). Adult studies suggest that lower IGF-1 level in childhood predict increased risk for developing insulin resistance and type 2 diabetes (Dunger et al., 2003). Moreover, previous studies reported that circulating fasting free IGF-1 were higher in obese subjects compared with normal weight controls, whereas total IGF-1 were not significantly different between the groups (Nam et al., 1997). Our results on obesity- related changes in serum total IGF-1, that found neither between group differences nor significant associations between total IGF-1 and selected indices of insulin resistance, are consistent with the latter findings.

The main limitation of this cross-sectional study was that it did not allow to validate the hypothesis of tracking the obesity-related cardiovascular risk into adulthood . Therefore, a follow up study of adolescent subjects diagnosed as being at higher risk for CVD would be necessary. However, given a large body of evidence provided by numerous previously mentioned prospective studies substantiating this assumption, early detection and awareness of CVD risk in adolescent subjects may allow the implementation of preventive strategies at a stage when intervention, such as personal advice for lifestyle improvement or well targeted drug therapy, may reverse damage.

#### 4.1 Conclusions

We seem to be authorized to conclude that:

- 1. Overweight and obesity in adolescents strongly predispose them to a range of adverse health outcomes, including insulin resistance, hypertension and cardiovascular morbidities, despite the absence of evident symptoms of impaired glucose tolerance or dyslipidemia, as they are rarely reported for this age group.
- 2. Obese adolescents are at significant risk for becoming obese adults.
- 3. The most suitable metabolic markers and predictors of cardiovascular disease risk during adolescence and adulthood are HOMA-IR , TG/HDL-C ratio, and atherogenic index of plasma (AIP).
- 4. High serum adiponectin concentration and low HOMA-IR or TG/HDL-C ratio are good prognostic markers of low risk for insulin resistance and atherosclerotic vascular disease.

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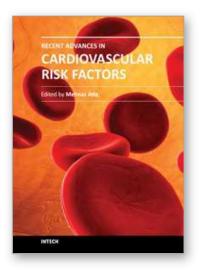
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