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Original Article

Endothelial and Metabolic Function Interactions in Overweight/Obese Children:

The Role of High-Molecular Weight Adiponectin

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***Aim*: Although the underlined mechanisms are still unknown, metabolic/coagulation alterations related to childhood obesity can induce vascular impairments. The aim of this study was to investigate the relationship between metabolic/coagulation parameters and endothelial function/vascular morphology in overweight/obese children.**

***Methods*: Thirty-five obese/overweight children (22 pre-pubertal, mean age: 9.52 ± 3.35 years) were enrolled. Body mass index (BMI), homeostasis model assessment index (HOMA$_{IR}$), metabolic and coagulation parameters, [adiponectin, fibrinogen, high molecular weight adiponectin (HMW), endothelin-1, and vonWillebrand factor antigen] ultrasound early markers of atherosclerosis [flow-mediated dilatation (FMD), common carotid intima-media thickness (C-IMT), and anteroposterior diameter of infra-renal abdominal aorta (APAO)] were assessed.**

***Results*: APAO was related to anthropometric (age: $r=0.520$, $p=0.001$; height: $r=0.679$, $p<0.001$; weight: $r=0.548$, $p=0.001$; BMI: $r=0.607$, $p<0.001$; SBP: $r=0.377$, $p=0.026$) and metabolic (HOMA$_{IR}$: $r=0.357$, $p=0.035$; HMW: $r=-0.355$, $p=0.036$) parameters. Age, height, and systolic blood pressure were positively related to increased C-IMT ($r=0.352$, $p=0.038$; $r=0.356$, $p=0.036$; $r=0.346$, $p=0.042$, respectively). FMD was not related to any clinical and biochemical characteristics of the pediatric population. Age, HOMAIR, fasting glucose levels, and HMW were independent predictors for APAO increase. Each unit decrease in HMW concentrations (1 μg/ml) induced a 0.065 mm increase in APAO.**

***Conclusion*: High molecular weight adiponectin is related to cardiovascular risk in overweight/obese children.**

Key words: Anterior–posterior diameter of infra-renal abdominal aorta, Atherosclerosis, Flow-mediated dilatation of brachial artery, High molecular weight adiponectin, Intima-media thickness of common carotid artery

Introduction

The worldwide prevalence of childhood obesity has greatly increased over the past three decades[1)], causing adverse long-term consequences for health[2)].

Several evidences reported a worldwide increase in children's body mass index (BMI) with a 17.3% prevalence rate of obesity type 3 (BMI ≥ 140% of the 95th percentile or BMI ≥ 40 Kg/m^2) in the Unites States' childhood population[3)]. The consequences of childhood obesity are because of the increase in meta-

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bolic syndrome, cardiovascular disease, type 2 diabetes, obstructive sleep apnea, and polycystic ovarian syndrome[4].

Obesity increases lipid peroxidation and induces persistent platelet activation, affecting the vascular endothelial function and probably conferring premature atherogenicity[5].

Adipose tissue is an endocrine organ that releases adipocytokines both pro-inflammatory, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), raising the C reactive protein (CRP), and anti-inflammatory substances such as adiponectin[6]. Adiponectin protein is probably an insulin modulator and plays a protective role against inflammation and atherosclerosis, stimulating the production of endothelial nitric oxide and inhibiting the accumulation of lipids in macrophages[7-10]. Several studies showed that hypoadiponectinemia is associated with greater BMI, waist circumference, and insulin resistance in obese children[11, 12]. High molecular weight adiponectin acts on cardiac muscular cells protecting them from ischemia/reperfusion damages by improving cyclooxygenases-2 activity and promoting nitric oxide (NO) production; it obstacles the promotion of cardiac fibrosis and the morphological and structural alterations derived from hypertrophic stimuli, while it ameliorates contractile function by inducing calcium influx into myotubes. On vascular beds, it improves endothelial function, inflammation, muscular remodeling, and angiogenesis, i.e., it promotes atheroprotective actions[7]. Abdominal obesity lowers serum levels of adiponectin since childhood[13].

The early endothelial damage associated with obesity is confirmed by the high levels of von Willebrand factor (vWF Ag), D-dimer concentration, thrombin–antithrombin complex (TAT), plasminogen activator inhibitor 1 (PAI-1), and fibrinogen[11, 14]. vWF Ag and PAI-1 are two haemostatic markers of endothelial dysfunction. Increased PAI-1 slows fibrinolysis, exposing the surface of arteries to recurrent micro thrombi[15]. TAT is an index of hypercoagulability and increased thrombin generation; instead, D-dimer concentration correlates with an increased fibrin turnover. Therefore, the excess of adiposity may cause an imbalance in the haemostatic system where more fibrin is produced and deposited, less is degraded or both[11].

Non-invasive techniques can evaluate preclinical atherosclerosis, such as the ultrasound common carotid artery intima-media thickness (C-IMT), flow-mediated dilatation (FMD) of brachial artery, and anteroposterior diameter of infra-renal abdominal aorta (APAO). Some studies showed that obese children have higher endothelium thickness than healthy children, thus becoming more susceptible to cardiovascular events in adult life[16]. Furthermore, other studies have demonstrated that cIMT correlates with high insulin levels in obese patients[17, 18].

Aim

This study aimed to investigate the relationship between childhood obesity and markers of inflammation, endothelial and haemostatic activation.

Methods

We enrolled 35 consecutive outclinic overweight/obese subjects, 20 (57.1%) males (mean age: 9.52 ± 3.35 years), who attended the Department of Pediatrics of University of Bari. Of them, 22 were prepubertal (62.9%).

Exclusion criteria were as follows: (a) secondary obesity (i.e., because of endocrine/genetic syndromes or other identified disorders); (b) concomitant diseases (endocrine, metabolic, renal, hepatic and cardiovascular alterations, allergies, hypertension, and genetic syndromes); (c) a history of inflammatory diseases in the last 30 days; and (d) use of drugs with effects on glucose and/or lipid metabolism and haemostatic parameters.

A written informed consent was obtained from the children's parents or their legal guardians. All the procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation.

All patients underwent a general clinical examination, anthropometric measurements (height in cm, weight in kg, and BMI in kg/m^2) using Italian growth charts[19], and assessment of the pubertal and genital stage according to Tanner criteria[20]. Obesity was defined as a BMI $>95^{th}$ percentile for age and sex, in keeping with Italian growth charts[19]. Waist circumference was also assessed[21].

Both systolic (SBP) and diastolic blood pressure (DBP) were measured in all patients[22]. Blood glucose, insulin, total cholesterol (TC), high (HDL) and low (LDL) density lipoprotein cholesterol, triglycerides (TG), and C-reactive protein (CRP) were measured after overnight fasting in all subjects. Values of TC, LDL, HDL, and TG were considered in the normal range if within the 5^{th} and the 95^{th} percentile[23]. An oral glucose (1.75 g/kg) tolerance test (OGTT) was performed in obese subjects recording basal levels of blood glucose and insulin and after 120 min.

Haemostatic Biomarkers

Total adiponectin and multimeric high-molecu-

lar weight (HMW) subfraction were measured by a commercial ELISA (ELISA 47-ADPH-9755; ALPCO Diagnostics, Salem, Vermont). Endothelin-1 levels were measured by ELISA (R&D System Europe, Lille, France). vWF was measured as vWF antigen by ELISA (Asserachrom Diagnostica, Stago, France). Commercial ELISA method was used to measure D-dimer concentrations (Asserachrom Diagnostica Stago, France), whereas fibrinogen was measured in citrate plasma with a clot-rate assay using ACL 200/IL instrument (Instrumentation Laborator, Milan, Italy).

Vascular Ultrasound Studies

All children underwent high-definition vascular echography according to the following protocols to identify arteries with early atherosclerotic lesions.

Ultrasound measurement of C-IMT

Ultrasonographic echo-color Doppler studies of left and right common carotid arteries were performed bilaterally by the same physician with a Philips Sonos 5500 using a 7.5 MHz high resolution probe. The patients were placed in supine position, with the neck extended and rotated contralaterally by 45°, and the common carotid arteries were examined on the sagittal axis with a lateral view. C-IMT was defined as a low-level echo gray band that does not project into the arterial lumen and was measured during end-diastole according to the method described by Pignoli[24]. The measurements were performed bilaterally 1 cm proximally to the carotid bulb, for three times, and then C-IMT value was calculated as the arithmetical mean of each side. The C-IMT value considered for statistical analyses was the mean of right and left measurements. C-IMT measurements were always performed in arterial segment devoid of the atherosclerotic plaque, i.e., where C-IMT was >1.5 mm or a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding C-IMT value was present[25]. Intraobserver variability was 0.98 according to the intraclass correlation coefficient (ICC; good if >0.80)[26].

FMD of Brachial Artery

Temperature, food, stress, drugs, and sympathetic stimuli influence FMD. The subjects were fasted for at least 8–12 h, then evaluated in a quiet air conditioned room (22–24℃), early in the morning. They were asked not to exercise or take substances like coffee/tea or chocolate for at least 4–6 h before the exam. A preliminary scan to explore the anatomy and identify landmarks was performed, excluding poor quality images, the presence of atherosclerotic plaques, calcifications, and arterial tortuosity or kinking. The scan was performed at the right brachial artery in a long axis projection, 5–10 cm above the elbow, using a 7.0 MHz or higher linear probe. A high resolution ultrasonograph (Philips Sonos 5500) connected to an image analysis system, certified by the CNR of Pisa (MVE II), was used[27] for computing the brachial artery diameter in real-time by analyzing B-mode ultrasound images, setting positivity to the test value at $<5\%$. All the ultrasound examinations were performed by the same physician in order to reduce bias. With the subject in supine position for at least 10 min, the arm was positioned comfortably in such a way as to obtain good images of the humeral artery. After displaying the selected artery segment, a sphygmomanometer cuff was placed in the distal site to the artery. After 1 min baseline acquisition, the artery was occluded by inflating the cuff to a pressure of 200–220 mmHg for exactly 5 min and then it was deflated. The resulting increased shear stress provides the stimulus for dilatation of the humeral artery. The image of the artery was then recorded continuously for 2–3 min after ischemia. Reactive hyperaemia was calculated as the ratio of the change in diameter (maximal dilatation after deflation-baseline) divided by the baseline value, which corresponds to the maximum FMD recovery value. FMD was analyzed as the percentage increase in brachial artery diameter after the application of a pressure stimulus[28]. Intraobserver variability was 0.95 according to ICC (good if >0.80)[26].

Assessment of APAO

To improve image acquisition, subjects were asked to fast for at least 6–8 h and follow a fiber diet for 2 days prior to the examination to reduce intestinal bloating (diet preparation). Ultrasonographic studies of the infra-renal abdominal aorta were performed by a single operator using a single high-resolution vascular ultrasound Philips 5500 equipped with a 3-MHz electronic probe. With the patient in supine position, the electronic probe was placed 1 cm left of the umbilicus. The best image in long-axis projection of the abdominal aorta was then obtained. APAO was defined as the maximal external cross-sectional measurement[29]. It was calculated as the distance between the near and far walls of the abdominal aorta. Measurements were performed 2 cm above and distal to the umbilicus and expressed in centimeters[30, 31]. Intraobserver variability was 0.98 according to ICC (good if >0.80)[26].

Statistical Analysis

The data are given as mean values ± standard deviation (SD) and categorical variables as frequencies

Table 1. Clinical, biochemical and instrumental characteristics of the population

Variables *N* patients = 35	Mean value	Standard deviation
Age (years)	9.52	3.35
Height (cm)	140.94	18.47
Weight (Kg)	55.37	22.27
Body Mass Index (Kg/m^2)	26.59	5.21
Body Mass Index (percentiles)	92.6	7.69
Systolic blood pressure (mmHg)	101.71	11.44
Diastolic blood pressure (mmHg)	67.29	8.77
Fasting glucose (mg/dl)	86.43	6.89
$HOMA_{IR}$	3.49	1.95
D-Dimers (ng/ml)	391.93	221.06
FBG (mg/dl)	280.43	46.09
vWFAg (%)	89.19	21.81
ET-1 (pg/ml)	3.69	1.6
AD (μg/ml)	5.41	1.26
HMW (μg/ml)	2.75	1.31
FMD (%)	7.38	2.22
APAO (cm)	1.34	0.2
C-IMT (mm)	0.47	0.06

AD: adiponectin; APAO: antero-posterior diameter of infrarenal abdominal diameter; C-IMT: carotid intima-media thickness; FBG: fibrinogen; FMD: flow mediated vasodilatation; HMW: high molecular weight adiponectin; $HOMA_{IR}$: homeostatic model assessment; ET-1: endothelin-1; vWFAg: vonWillebrand factor antigen.

and percentage. The Pearson's linear correlation coefficient was used to study the relationship between cardiovascular risk parameters and the continuous variables. Correlation matrix has been calculated for the continuous variables. Multiple regression analysis had been adopted to evaluate the influence of confounding factors on vascular ultrasound parameters. A test F of Snedecor–Fisher has been managed. $P<0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS Statistics 20.

Results

Thirty-five overweight/obese children (mean age: 9.52 ± 3.35 years, BMI percentiles: 92.6 ± 7.69) were consecutively recruited. Among them, 20 male individuals (57.1%) were present and 22 (62.9%) were in their pre-pubertal growth stage according to Tanner criteria[20]. The main clinical, biochemical, and instrumental characteristics of the enrolled population are provided in **Table 1**. The population was normally distributed. We used the Skapiro–Wilk test to analyze the normal distribution of the population. The final results of Skapiro–Wilk test reported the acceptance of the normal distribution of our population.

Table 2 shows the Pearson's correlation coefficients calculated for the analysis of the relationship between instrumental, vascular ultrasound measurements (APAO, FMD, and C-IMT), and the main characteristics of the evaluated population. FMD was not related to clinical and biochemical characteristics of the pediatric population. The evaluation of the endothelial function by means of FMD was not related to any haemostatic parameter: vWFAg, D-dimer concentrations, and fibrinogen. FMD was also not related to to any metabolic parameters such as total adiponectin and its multimeric high-molecular weight, and endothelin-1. When considering C-IMT, only age, height, and systolic blood pressure were significantly and positively related to an increase in C-IMT values ($r=0.352$, $p=0.038$; $r=0.356$, $p=0.036$; $r=0.346$, $p=0.042$, respectively). APAO measurements outlined a strong relationship with anthropometric (age: $r=0.520$, $p=0.001$; height: $r=0.679$, $p<0.001$; weight: $r=0.548$, $p=0.001$; BMI: $r=0.607$, $p<0.001$; SBP: $r=0.377$, $p=0.026$) and metabolic ($HOMA_{IR}$: $r=0.357$, $p=0.035$; HMW: $r=-0.355$, $p=0.036$) parameters. No statistically significant relationships were found according to haemostatic (D-dimers, FBG, and vWFAg) and/or endothelial (ET-1) parameters.

We also compared the values of BMI percentile

Table 2. Pearson's correlation coefficients among cardiovascular risk markers (antero-posterior diameter of infrarenal abdominal diameter [APAO], carotid intima-media thickness [C-IMT], flow mediated vasodilatation [FMD]) and anthropometric/haemostatic parameters

Variables *N* patients = 35		FMD	APAO	C-IMT
Age (years)	*R*	−0.244	0.520	0.352
	p-value	0.158	0.001	0.038
Weight (Kg)	*R*	−0.297	0.679	0.329
	p-value	0.083	0.000	0.054
Height (cm)	*R*	−0.315	0.548	0.356
	p-value	0.065	0.001	0.036
Body mass index (Kg/m^2)	*R*	−0.278	0.607	0.187
	p-value	0.105	0.000	0.282
Body mass index percentile	*R*	−0.074	0.352	−0.017
	p-value	0.665	0.033	0.919
Systolic blood pressure (mmHg)	*R*	−0.148	0.377	0.346
	p-value	0.395	0.026	0.042
Diastolic blood pressure (mmHg)	*R*	−0.014	0.326	0.052
	p-value	0.935	0.056	0.768
Fasting glucose (mg/dl)	*R*	0.128	−0.253	0.141
	p-value	0.464	0.143	0.419
HOMA$_{IR}$	*R*	−0.115	0.357	0.130
	p-value	0.510	0.035	0.455
D-Dimers (ng/ml)	*R*	−0.049	−0.045	−0.055
	p-value	0.780	0.799	0.752
FBG (mg/dl)	*R*	−0.099	0.057	0.012
	p-value	0.573	0.746	0.946
vWFAg (%)	*R*	0.236	0.120	−0.194
	p-value	0.172	0.491	0.265
ET-1 (pg/ml)	*R*	−0.031	0.057	0.091
	p-value	0.866	0.751	0.613
AD (μg/ml)	*R*	0.151	−0.143	−0.216
	p-value	0.402	0.428	0.228
HMW (μg/ml)	*R*	0.243	−0.355	−0.012
	p-value	0.160	0.036	0.945

AD: adiponectin; APAO: antero-posterior diameter of infrarenal abdominal diameter; C-IMT: carotid intima-media thickness; FBG: fibrinogen; FMD: flow mediated vasodilatation; HMW: high molecular weight adiponectin; HOMA$_{IR}$: homeostatic model assessment; ET-1: endothelin-1; vWFAg: vonWillebrand factor antigen.

with the three vascular variables of our study (i.e., FMD, APAO, and IMT). As BMI percentile was not normally distributed, we used the Spearman's correlation coefficient (non-parametric) in order to evaluate such relationship. As outlined in **Table 3**, only APAO was significantly related to BMI percentile ($r = 0.352$, $p = 0.033$). Nevertheless, as further multivariate regression analysis pointed out, this correlation was not proved when considering confounding factors.

To evaluate the influence of confounding factors on these results, we performed a multiple regression analyses. The correlation matrix coefficients among the continuous variables were calculated before performing the multiple regression analysis to exclude

Table 3. Multivariate correlation analysis between antero-posterior abdominal aorta diameter (APAO) [model 1], brachial artery flow-mediated vasodilatation (FMD) [model 2], common carotid intima-media thickness (C-IMT) [model 3] and main population's characteristics

Model 1 (R^2: 0.596) APAO	Coefficient	Standard error	*P*
Age (years)	0.029	0.008	0.001
Fasting glucose (mg/dl)	−0.013	0.004	0.002
HMW (μg/ml)	−0.065	0.018	0.001
$HOMA_{IR}$	0.038	0.015	0.016
Model 2 (R^2: 0.103) FMD			
Body mass index (Kg/m^2)	−0.139	0.072	0.065
vWFAg (%)	0.029	0.017	0.099
Model 3 (R^2: 0.095) C-IMT			
Age (years)	0.007	0.003	0.045

APAO: antero-posterior diameter of infrarenal abdominal diameter; C-IMT: carotid intima-media thickness; FMD: flow mediated vasodilatation; HMW: high molecular weight adiponectin; $HOMA_{IR}$: homeostatic model assessment; vWFAg: vonWillebrand factor antigen.

from the final multiple regression model those variables that were strongly associated with each other (data not showed).

Table 3 gathered the regression models adopted to evaluate the possible confounding factors on APAO, FMD, and C-IMT measurements. Considering APAO, we found that age, $HOMA_{IR}$, fasting glucose levels, and plasma concentrations of HMW were independent predictors for APAO measurements. Age and $HOMA_{IR}$ were positively related to APAO, whereas fasting glucose and HMW concentrations were negatively related. Considering HMW, each unit decrease in HMW concentrations (1 μg/ml) induced an APAO increase equal to 0.065 mm. The adaptation of the model to the results was good (R^2=0.596), thus enforcing the results.

Only age continued to be positively related to C-IMT measurements. Although the final adaptation of the model is quite low (R^2=0.095), it outlined an increase in C-IMT of about 0.007 mm every year in our patients (**Table 3**). No predictors for FMD values were found (**Table 3**).

Discussion

The aim of this study was to evaluate the influence of metabolic (total adiponectin and HMW subfraction) and haemostatic parameters (endothelin-1, vWFAg, and fibrinogen) on cardiovascular risk profile (evaluated by means of instrumental ultrasound parameters such as APAO, C-IMT, and FMD) of overweight/obese pediatric patients.

APAO, C-IMT, and FMD represents the best way to non-invasively assess the health of peripheral vascular system and, indirectly, that of the heart, as already established in the literature[18, 32, 33]. These parameters are *t* related to each other. Lind recently found that FMD was related to atherosclerotic plaque expression in the carotid arteries beyond any influence from cardiovascular risk factors, reaching an odds ratio equal to 0.81[34]. Furthermore, the connection between early markers of atherosclerosis and metabolic conditions was related to the morpho-structural alteration in vascular beds to the systemic dysfunction of the human metabolism. In particular, Jung *et al.*[35] observed that 370 patients (median age: 66 years), followed-up for at least 25 months and without any evidence of carotid atherosclerotic alterations (i.e., increased intima-media thickness and/or carotid plaque) at the enrolment phase, can early develop atherosclerotic plaques at carotid level in relation to the onset of metabolic syndrome. In particular, ΔC-IMT, i.e., the change over time of the intima-media thickness of the carotid, was associated with metabolic syndrome onset at multivariate regression model.

According to the literature, this is the first study that simultaneously evaluated vascular functional and morphological parameters in children and adolescent in relation to their metabolic and haemostatic parameters.

The most attractive feature of our study is the strong relationship between APAO and metabolic parameters. We demonstrated that HOMA$_{IR}$ and HMW were related to APAO when considering Pearson's correlation coefficient (HOMA$_{IR}$: $r=0.357$, $p=0.035$; HMW: $r=-0.355$, $p=0.036$). This relationship was maintained even after adjusting for confounding factors at multivariate regression analysis. Considering adiponectin and its high molecular weight component, we found a direct, negative relationship with APAO: each 1 μg/ml decrease in its concentration was related to a 0.065 mm increase in APAO value. We considered APAO as an expression of vascular impairment because several studies demonstrated its relationship with atherosclerosis and cardiovascular risk since childhood[30, 36]. Strong *et al.*[37] demonstrated that abdominal aorta showed atherosclerotic lesions more often than coronaries; their comparative hystopathological evaluations in autoptic samples pointed out that the susceptibility of aorta to vascular lesions is higher than right coronary arteries. The occurrence of these lesions since childhood led physicians to think about the fundamental role of aorta evaluation in the general assessment of cardiovascular risk profile of individuals. In particular, Laughlin *et al.*[36] validated the use of infrarenal diameter as an early predictor of atherosclerosis. To the best of our knowledge, this is the first study demonstrating a significant relationship between APAO and HMW in a pediatric population. Sarici *et al.*[38] tried to compare the adiponectin levels in the cord blood with abdominal aortic intima-media thickness of 80 healthy, term neonates. Nevertheless, they found no correlation between these two parameters.

Several studies demonstrated a negative relationship between C-IMT and adiponectin blood levels, even considering the latter as more useful in predicting cardiovascular risk as compared with classical risk factors when considered in obese children[39, 40]. Such relationship was maintained even when considering the subtype categories of adiponectin (low-, middle- and high-weight molecular fractions)[41]. Nevertheless, our data did not point out such a statistically significant relationship between C-IMT and adiponectin levels. The linear Pearson's correlation coefficients were all not statistically significant for the relationship between C-IMT and adiponectin/HMW. The lack of significant correlations was observed even according to the other marker of metabolism and coagulation.

When considering the evaluation of endothelial function by means of brachial artery FMD, no relationships were outlined between vascular measurements and coagulation/metabolic parameters. Nevertheless, such results were in line with literature data[10, 42-44]. Singhal *et al.*[42] found no relationship between brachial artery FMD and adiponectin values in 294 young, healthy adolescents (aged 13–16 years), rather observing a direct relationship with HOMA$_{IR}$. Arnaiz *et al.*[10] obtained same results, which demonstrated that adiponectin blood concentrations were related to HOMA$_{IR}$ ($r^2=0.34$, $p<0.0001$) even after adjusting for confounding factors, whereas no relationship with early markers of atherosclerosis where observed (C-IMT and brachial artery FMD). Furthermore, Galler *et al.*[45] revealed that there is no relationship between arterial stiffness and adiponectin values in children and adolescents suffering from type 1 diabetes: this means that low adiponectin values cannot influence the endothelium composition and function. These results may be influenced by the involving of total adiponectin rather than HMW in the evaluations performed in the several studies. HMW has effectively greater sensibility in the general assessment of obese individuals as compared with total adiponectin, as already established by Araki *et al.*[46]. In fact, HMW can better represent the alteration in the metabolism of such individuals rather than adiponectin because a more pronounced decrease in HMW values can be detected in obese individuals as compared with adiponectin blood concentrations; thus, the subtle alterations in vascular walls because of metabolic conditions could be better represented by HMW rather than adiponectin. In fact, Miyazaki *et al.*[47] observed that HMW were directly related to the atherogenic lipoprotein profiles of healthy male individuals, thus relating to the overall cardiovascular risk profile of individuals. Furthermore, a connection between visceral fat accumulation and adiponectin had already been established, this increasing the importance of our previous comments[48].

We believe that our study succeeded in demonstrating the relationship between APAO and metabolic features of overweight/obese children just in relation to the adoption of both total and high-molecular weight adiponectin.

Regarding the coagulation parameters, our study found no relationships with their increase in blood concentrations and early markers of atherosclerosis. D-dimers, fibrinogen, and vWFAg levels did not reach a significant correlation with vascular parameters. These results were in contrast with some previous literature studies,[11, 49-52] which reported the influence of such parameters on endothelial function and on vascular wall morphological structure. In fact, Orenes-Piñero *et al.*[53] pointed out the correlation between BMI and coagulation parameters, observing an increased prothrombotic status in adult subjects suffering from increased body weight.

The findings of this study are really interesting. We demonstrated that in a population of overweight/obese children abdominal aorta is more and earlier involved than other systemic vascular structures. Such alterations are strictly related to metabolic alteration induced by the increased weight and, in particular, HMW seemed to be the best predictor of vascular impairments.

Conclusions

This study demonstrated the association between HMW and APAO. The small sample size of our study forced us to continue the evaluations of such relationship with further improvements and enlargement of the enrolled population.

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Conflicts of Interest

None declared.

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