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Insulin resistance and endothelial function in children and adolescents

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ABSTRACT

Aims: Insulin resistance (IR) impairs cellular response to insulin due to a dysfunction in glucose metabolism, associated with an increased cardiovascular risk. The aim of our study was to investigate the relationship among homeostasis model assessment index (HOMA index), endothelial function and vascular morphology in order to better stratify cardiovascular risk in children and adolescents.

Methods: A total of 150 children and adolescents (55 pre-pubertal, mean age 10.4 ± 3.1 years) were enrolled. Anthropometric [body mass index (BMI), waist circumference (WC)], laboratory [blood lipids, inflammatory markers, insulinemia, glycemia], HOMA index and ultrasound parameters [flow-mediated dilatation (FMD), common carotid intima–media thickness (cIMT) and antero-posterior diameter of infra-renal abdominal aorta (APAO)] were assessed.

Results: cIMT was positively related to age ($r = 0.274$, $p < 0.01$), BMI ($r = 0.318$, $p < 0.01$), WC ($r = 0.315$, $p < 0.01$) and triglycerides ($r = 0.230$, $p < 0.01$). APAO measurements showed a linear positive correlation with age ($r = 0.435$, $p < 0.01$), BMI ($r = 0.505$, $p < 0.01$), WC ($r = 0.487$, $p < 0.01$), triglycerides ($r = 0.280$, $p < 0.01$), C-reactive protein ($r = 0.209$, $p < 0.05$), fasting insulin ($r = 0.378$, $p < 0.01$) and HOMA index ($r = 0.345$, $p < 0.01$). FMD was inversely related to age ($r = -0.251$, $p < 0.01$), rough BMI ($r = -0.318$, $p < 0.01$), WC ($r = -0.340$, $p < 0.01$), fasting insulin ($r = -0.281$, $p < 0.01$) and HOMA index ($r = -0.282$, $p < 0.01$). Multiple regression analysis found no influence of HOMA index on APAO and cIMT. HOMA index was an independent predictor for brachial artery FMD worsening after the statistical adjustment.

Conclusion: HOMA index increase induced a worsening in endothelial function since childhood.

1. Introduction

Insulin resistance (IR) is a metabolic condition characterized by an impaired ability of plasma insulin at normal concentrations to promote peripheral glucose clearance, suppress gluconeogenesis in the liver, and inhibit very low density lipoprotein (VLDL) output [1]. In addition to regulation of glucose homeostasis in target tissues, as skeletal muscle and adipose tissue, insulin has important physiological functions in heart and vessels, modulating the release of vasodilator substances, such as nitric oxide and prostaglandins, from vascular endothelium, by both stimulating and inhibiting the sympathetic nervous system and by protecting smooth muscle cells in blood vessel from apoptosis induced by oxidative stress [2]. Thus, the vasodilatory and antioxidant effects of insulin are depressed in case of insulin deficiency and IR conditions. IR plays a major patho-physiological role in type 2 diabetes and is tightly associated with major public health problems including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome [3,4]. The increase of childhood obesity has determined the increased incidence and prevalence of IR and its cardiovascular complications [5]. Furthermore, childhood obesity has been reported associated with biochemical and inflammatory factors (i.e. interleukin-6, tumor necrosis factor-alpha) that affect vascular endothelial function and that might confer a premature atherogenicity [6]. Visceral fat is also responsible for low serum levels of adiponectin, a molecule with anti-inflammatory and anti-atherosclerotic properties, whose deficiency is implicated in the pathogenesis of impaired insulin sensitivity [7]. The atherosclerotic process seems to start in childhood and proceeds silently over a long period of time before clinical manifestations [8]. Carotid artery intima–media thickness (cIMT) is considered a significant predictive marker of generalized atherosclerosis because of its correlation with coronary artery disease and it may predict future

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cardiovascular events in adults [8]. It is also recommended by the American Heart Association as a noninvasive imaging parameter for detecting atherosclerosis [9,10]. Previous studies reported that cIMT correlates with high insulin levels (a sign of IR) in obese patients and with high fasting glucose levels (a sign of relative insulin deficiency) in subjects with type 1 diabetes [11]. Moreover, other ultrasound parameters have been associated with cardiovascular risk in obese subject [12].

This study aimed to evaluate the presence of early signs of atherosclerosis in a group children and adolescents by evaluating three ultrasound parameters, flow-mediated dilatation (FMD) of brachial artery, intima–media thickness of the common carotid artery (C-IMT) and antero-posterior diameter of infra-renal abdominal aorta (APAO). We tried to understand if IR parameters in children and adolescents can influence structural and functional vascular changes.

2. Materials and methods

We enrolled 150 consecutive outclinic subjects, 74 (49%) males, mean age 10.4 ± 3.1 years who attended the Department of Pediatrics of University of Bari. Of them, 55 were pre-pubertal (36.4%).

Exclusion criteria were as follows: (a) secondary obesity (i.e., due to endocrine/genetic syndromes or other identified disorders); (b) concomitant diseases (endocrine, metabolic, renal, hepatic and cardiovascular alterations, allergies, hypertension, 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 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, BMI expressed in kg/m^2) using Italian growth charts [13], and assessment of the pubertal and genital stage, according to Tanner criteria [14]. Both systolic (SBP) and diastolic blood pressure (DBP) were measured in all patients [15], while waist circumference was assessed only in obese subjects [16]. 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 5th and the 95th percentile [17]. 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.

2.1. 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 the 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 a 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 et al. [18]: by focusing and freezing images on the distal wall of the common carotid artery on the lengthwise axis, the IMT was the distance between the leading borders of the first hyperechoic line and of the second hyperechoic line, separated by a hypoechoic space ("double-track pattern"). The measurements were performed bilaterally 1 cm proximally to the carotid bulb, for three times, and then 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. IMT measurements were always performed in arterial segment devoid of atherosclerotic plaque, defined according to Mannheim carotid intima–media thickness consensus (2004–2006) as IMT greater than 1.5 mm or a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value [19]. Intra-observer variability was 0.99 according to the ICC (intraclass correlation coefficient, good if >0.80) [20].
- *FMD of brachial artery*
  Temperature, food, stress, drugs and sympathetic stimuli influence the FMD. Therefore, the study was performed with the subjects fasting for at least 8–12 hours, in a quiet air conditioned room (22–24 °C), early in the morning. Moreover, the subjects were asked not to play exercise, or take exciting substances like coffee/tea, chocolate which could impair endothelial function and for at least 4–6 hours before the exam. It is necessary to perform a preliminary scan to explore the anatomy and identify landmarks, paying particular attention to poor quality images, the presence of atherosclerotic plaques, calcifications, arterial tortuosity or kinking. The scan was done at the right brachial artery in a long-axis projection between 5 and 10 cm above the elbow using a 7.0-MHz or higher linear probe. The study was performed using a high-resolution ultrasonograph (Philips Sonos 5500) connected to an image analysis system, certified by the CNR of Pisa (MVE II) [21], for computing the brachial artery diameter in real-time by analyzing B-mode ultrasound images, setting positivity to the test value at less than 5%. All the ultrasound examinations were performed by the same physician in order to reduce observer bias. With the subject in a supine position for at least 10 min, the arm was positioned comfortably in such a way as to get good images of the humeral artery. The selected artery segment to be displayed was above the antecubital fossa in the longitudinal plane, identifying the part where the anterior and posterior intimal interfaces between the lumen and vessel wall were clear. A sphygmomanometer cuff was placed in the distal site to the artery, in cases of a humeral artery on the forearm. After 1 min of flow image baseline acquisition, the artery was occluded by inflating the cuff to a pressure of 200–220 mm Hg for exactly 5 min. When the cuff is deflated, it induces a short state of high flow (reactive hyperemia in the forearm microcirculation) through the brachial artery to adjust to the reduced downstream resistance caused by the ischemia-induced dilatation. The resulting increased shear stress provides the stimulus for dilatation of the humeral artery. Within 15 seconds from the end of ischemia, the flow rate was measured and then the degree of hyperemia. The image of the artery was then recorded continuously for 2-3 min after ischemia. Reactive hyperemia 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 [22]. Intra-observer variability was 0.93 according to the ICC (intraclass correlation coefficient, good if >0.80) [20].
- *Assessment of APAO*
  To improve the image acquisition, subjects were asked to keep fasting for at least 6–8 hours and follow a fiber diet for the 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 a 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. The APAO was defined as the maximal external cross-sectional measurement [23]. It was calculated as the distance between the near and the far walls of the abdominal aorta. Measurements were performed 2 cm above and distal to the umbilicus and expressed in centimeters [24].

Intra-observer variability was 0.97 according to the ICC (intraclass correlation coefficient, good if >0.80) [20].

3. Statistical analysis

The data are given as mean values ± standard deviation (SD), and categorical variables as frequencies 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 in order to evaluate the influence of confounding factors on vascular ultrasound parameters. An *F* test of Snedecor–Fisher has been managed. A $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS Statistics 20.

4. Results

A total of 150 (74 male, mean age 10.4 ± 3.1 years) consecutive children and adolescent were recruited and visited for the study. Among them, 55 (36.7%) were in their pre-pubertal growth stage according to the criteria of Tanner et al.[14]. All the main characteristic of the population studied have been gathered in Table 1.

Table 2 outlined linear correlation analyses between the instrumental measurements (APAO, FMD and cIMT) and the main characteristics of the evaluated population. Results pointed out that cIMT was positively related to age ($r = 0.274$, $p < 0.01$), the rough value of BMI ($r = 0.318$, $p < 0.01$), waist circumference ($r = 0.315$, $p < 0.01$) and plasma concentrations of triglycerides ($r = 0.230$, $p < 0.01$). These meant that only these features were able to increase cIMT, while no statistically significant influence of serum cholesterol levels (total, HDL and LDL cholesterol), inflammatory systemic markers (i.e., C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]) and glucose metabolism parameters (fasting insulin and glycemia and HOMA index) was detected for cIMT progression.