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ORIGINAL ARTICLE

Relation of acute-phase reaction and endothelial activation to insulin resistance and adiposity in obese children and adolescents

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Abstract

OBJECTIVES: There is increasing evidence that an ongoing cytokine-induced acute-phase response is closely involved in the pathogenesis of type 2 diabetes and associated complications such as dyslipidemia and atherosclerosis. The aim of this study was to investigate the relationship of inflammation and endothelial activation with insulin resistance in childhood obesity.

METHODS: Two hundred and eleven (122 boys) obese children and adolescents were examined. Fasting levels of ultra-sensitive C-reactive protein (CRP), fibrinogen (FB), interleukin-6 (IL-6), interleukin-1β (IL-1β), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), von Willebrand factor (vWF), glucose, insulin, and HbA1c were determined. Insulin resistance was assessed by the homeostasis method.

RESULTS: HOMA IR correlated significantly with all measures of adiposity as well as with majority of inflammation and endothelial dysfunction markers. After adjustment for age, gender, BMI and fat mass, the correlation with insulin resistance remained significant for CRP, ICAM-1 and von Willebrand factor. There was a trend for association between HOMA IR and IL-6 as well as HOMA IR and fibrinogen.

CONCLUSION: Acute-phase reaction and endothelial activation correlate with insulin resistance in obese youth. It is possible that the cluster of these pro-atherogenic factors may contribute to the accelerated atherosclerosis in obese children.

Introduction

It has been known for many years that obesity is an important risk factor for developing insulin resistance, type 2 diabetes and atherosclerosis. There are increasing evidences that adipose tissue produces different cytokines and other endocrine factors which impair insulin sensitivity and alter glucose and lipid metabolism [13,18,46]. The major cytokine mediator of the hepatic acute-phase reaction is interleukin-6 (IL-6), secreted by immune system as well as adipose tissue, which has been argued to play a main role in the pathogenesis of type 2 diabetes and CVD in the insulin resistance syndrome [13,30,41]. Other cytokines, such as interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α), are central mediators of inflammatory reactions. It is commonly known that cytokines operate as a network in stimulating the production of acute-phase reactants [41]. Elevated level of another inflammatory marker, C-reactive protein (CRP) has been demonstrated to be associated with insulin resistance and cardiovascular disease (CVD), and proposed to be a useful clinical marker of CVD [33, 37,47].

The role of cellular adhesion molecules in the pathogenesis of atherosclerosis has now been clearly demonstrated [6,35]. Inflammation in the vessel wall plays an essential role in the initiation and progression of this disorder [38]. Accumulation of mononuclear cells to the endothelium is one of the earliest events in the formation of an atherosclerosis lesion. After this, mononuclears differentiate into macrophages and foam cells, and infiltrate the vessel wall. Before transformation, monocytes bind to the activated vascular endothelium due to interaction with selectins and cellular adhesion molecules (CAMs) such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [35]. During endothelial activation, soluble forms of CAMs are shed into the circulation. Increased levels of CAMs have been found in patients with CVD [35,39], type 2 diabetes [1], and insulin resistance [5]. So far no study has evaluated an overall interplay of these parameters in obese children.

Childhood obesity is clinically worrisome since it is associated with low levels of physical activity and poor diet leading to increased risk of metabolic and cardiovascular diseases later in life [15]. Obesity an also reflect the presence of disease such as type 2 diabetes mellitus, now increasingly found in children and adolescents [45]. The mechanism linking adipose tissue with pathologic processes of insulin resistance and endothelial dysfunction are still poorly understood.

The objective of the study was to explore the relationship between insulin resistance and acute-phase reaction and endothelial activation markers in obese children and adolescents.

Research design and methods

Study population. The study group comprised 211 obese children and adolescents (122 boys and 89 girls), aged 8–18 years. Obesity was recognized on the basis of body mass index (BMI) value above 97 percentile for age and sex on BMI percentile charts for Warsaw population of children and adolescents [29]. Children with acute or chronic infections, cancers, autoimmunologic diseases, hormonal abnormalities as well as hepatic or renal dysfunction were excluded. None of the subjects was taking any medications or smoking cigarettes. Patients with impaired glucose tolerance or type 2 diabetes were also excluded from the study. Data concerning obesity duration and family history of type 2 diabetes and cardiovascular diseases were obtained from the personal interview.

The protocol of the study was approved by the Ethical Committee of the Pomeranian Medical University.

Anthropometric and clinical measurements. Anthropometric measurements (body height and weight, waist and hip circumference) were performed by trained personnel, with the participants wearing only light underwear and without shoes. Standard, electronic scale and stadiometer were used to determine body weight and height. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m2). Because BMI changes with age, the BMI-SD score was also calculated. The minimal abdominal circumference between the xiphoid process and the iliac crest was measured to define waist circumference. The hip circumference was measured at the widest part of the gluteal region. Waist-to-hip ratio (WHR) was calculated by dividing waist circumference by hip circumference. Percent body fat and fat mass were determined by bioimpedance method (Tanita 131, Japan). Resting blood pressure was measured after subjects had been in a sitting position for a minimum of 10 min. Using a mercury sphygmomanometer, blood pressure was read three times on the right arm. The mean of three measurements was used for this study.

Laboratory analyses. Blood samples were obtained in the morning after an overnight fast. Circulating ICAM-1, VCAM-1, IL-6 and IL-1 beta levels were determined by ELISA with the use of the Quantikine high-sensitivity kit (R & D Systems, Minneapolis, MN, USA). High-sensivity CRP was measured by immunoturbidimetric method, fibrinogen (FB) – by coagulometric method's according to Clauss, and von Willebrand factor (vWF) was determined by ELISA using Asserachrom Diagnostica Stago. HbA1c was measured by immunoturbimetric method. Serum glucose levels were measured by kinetic-colorimetric method, and free insulin concentrations were determined by double-antibody RIA (Pharmacia RIA kit).

The homeostasis model was used to assess insulin resistance (HOMA IR) [18]. The formula for HOMA IR is as follows: insulin resistance (HOMA IR) = (fasting insulin (μU/ml) x fasting glucose (mmol/l)/ 22.5

Statistics. Parameters with skewed distributions were log-transformed. Association of biochemical and clinical characteristics were examined by Pearson's correlation. Biochemical characteristics in HOMA quartiles were compared using two-sided Dunnett's posthoc test.

All statistical analyses were performed with the version 9.0 of the SPSS for Windows software (SPSS Inc., Chicago, USA).

Results

The clinical and biochemical characteristics of the study subjects are presented in *Table 1* and *Table 2*.

Percent body fat, fat mass, and systolic blood pressure were significantly higher in studied girls whereas waist circumference and WHR were higher in boys (*Table 1*).

Fasting insulin, CRP and both CAMs were higher in boys than in girls. There was a trend for higher HOMA IR index, fibrinogen, vWF and IL-6 in boys when compared to girls (*Table 2*).

All measures of adiposity correlated significantly with HOMA IR after adjustment for age, gender, BMI, and fat mass (BMI: r=0.34, p<0.001; BMI-SD score: r=0.28, p<0.001; % fat: r=0.21, p<0.001; fat mass: r=0.32, p<0.001; waist circumference: r=0.35, p<0.001; WHR: r=0.29, p<0.001). BMI, % fat, fat mass, and WHR were also significantly associated with the majority of low-grade inflammation and endothelial activation markers (*Table 3*). Surprisingly, in children without disturbances of carbohydrate metabolism, HbA1c correlated with inflammation and endothelial activation markers, except IL-1β (*Table 3*). After controlling for age, gender, BMI, and fat mass, the correlation became weaker, but remained significant for CRP (r=0.18, p< 0.01), FB (r=0.15, p< 0.05), IL-6 (r=0.28, p< 0.001), ICAM-1 (r=0.29, p< 0.001), VCAM-1 (r=0.16, p< 0.05), and vWF (r=0.18, p< 0.01).

The majority of inflammation and endothelial activation markers correlated significantly with HOMA IR

Table 1: Clinical characteristics of the study subject

Parameter	Male Mean ± SD	Female	p
Age (years)	12.22 ± 2.6	13.6 ± 2.3	=0.5
Body weight (kg)	71.5 ± 19.6	71.0 ± 17.5	NS
Height (cm)	157.5 ± 15.1	155.2 ± 12.2	NS
BMI (kg/m2)	28.2 ± 3.7	29.0 ± 4.0	NS
BMI-SD score	3.19 ± 1.2	3.70 ± 1.4	NS
% FAT	31.4 ± 6.9	36.5 ± 5.0	<0.01
FAT mass (kg)	22.3 ± 8.3	26.3 ± 9.4	<0.01
Waist (cm)	96.4 ±- 11.9	93.6 ± 10.5	<0.05
WHR	0.99 ± 0.05	0.93 ± 0.07	<0.01
Systolic blood pressure (mm Hg)	113.3 ± 10.2	109.1 ± 9.3	=0.05
Diastolic blood pressure (mm Hg)	63,5 ± 7,3	63,8 ± 8,9	NS
Obesity duration (years)	6,6 ± 3,1	6,2 ± 2,7	NS
Family history of type 2 diabetes	53%	40%	NS
Family history of atherosclerosis	48%	37%	NS

Table 2: Biochemical characteristics of the study subjects

Parameter	Male Median (range)	Female	p
Glucose (mmol/L)	4.9 (3.1 – 6.1)	4.8 (3.3 – 7.9)	NS
Insulin (pmol/L)	119,6 (20.3 – 829.8)	109.8 (22.0 – 463.4)	<0.05
HOMA IR	3.6 (0.6 – 28.3)	3.0 (0.1 – 15.3)	=0.05
HbA1c (%)	5.5 (4.3 – 6.4)	5.5 (4.5 – 6.9)	NS
CRP (mg/L)	1.29 (0.0 – 3.8)	0.87 (0.0 – 1.5)	<0.01
Fibrinogen (mmol/L)	9.9 (6.05 – 14.9)	9.1 (6.0 – 14.0)	=0.05
IL-6 (pg/mL)	1,89 (0,1 – 14,8)	1,05 (0,1 – 10,8)	=0.05
IL-1 (pg/mL)	0,45 (0,04 – 11,0)	0,43 (0,8 – 3,84)	NS
ICAM-1 (ug/L)	276 (53 – 654)	246 (45 – 650)	<0.05
VCAM-1 (ug/L)	1331 (145 – 1650)	1272 (176 – 1649)	<0.05
von Willebrand factor (%)	101 (28 – 160)	86 (28 – 160)	<0.01

Table 3: Pearson's correlations between selected biochemical and clinical parameters

	BMI r	% FAT	Fat mass	Waist	WHR	HbA1c	HOMA IR
CRP	0.22**	0.35***	0.28***	0.17*	0.20**	0.31***	0.23***
FB	0.24**	0.36***	0.28***	0.17*	0.05	0.22**	0.19**
IL-6	0.24**	0.33***	0.27***	0.19**	0.19**	0.37***	0.16*
IL-1	0.25*	0.24**	0.24*	0.22*	0.23*	0.13	0.09
ICAM-1	0.14	0.34***	0.17*	0.13	0.36***	0.36***	0.21**
VCAM-1	0.14	0.27**	0.11	0.07	0.36***	0.36**	0.14
vWF	0.14**	0.26***	0.18**	0.10	0.15**	0.24***	0.24***

HOMA IR and all biochemical parameters except CRP, FB, ICAM-1 and VCAM-1 were log-transformed for statistical analysis.
* 0.01<p<0.05 ** 0.001<p<0.01 *** p<0.001

Table 4: Pearson's correlations between inflammation parameters, cytokines and marker of endothelial dysfunction.

	CRP r	FB	IL-6	IL-1	ICAM-1	VCAM-1	vWF
CRP	—	0.38***	0.32***	0.21*	0.41***	0.38***	0.42***
FB	0.38***	—	0.31***	0.13	0.29***	0.25***	0.37***
IL-6	0.32***	0.24***	—	0.32***	0.43***	0.34***	0.23***
IL-1	0.09	0.12	0.11	—	0.06	0.26*	0.01
ICAM-1	0.41***	0.29***	0.53***	0.19	—	0.60***	0.37***
VCAM-1	0.38***	0.30***	0.50***	0.32**	0.62***	—	0.29***
vWF	0.42***	0.37***	0.37***	0.27***	0.13	0.29***	—

All parameters except CRP, FB, ICAM-1 and VCAM-1 were log-transformed for statistical analysis.
* 0.01<p<0.05 ** 0.001<p<0.01 *** p<0.001

(*Table 3*). After adjustment for age, gender, BMI and fat mass, the correlation with insulin resistance remained significant for CRP (r=0.17; p=0.017), ICAM-1 (r=0.17; p=0.018) and von Willebrand factor (r=0.22; p=0.000). There was a trend for association between HOMA IR and IL-6 (r=0.14; p=0.053) as well as HOMA IR and fibrinogen (r=0.13; p= 0.052). It was not found any significant correlation between insulin resistance, expressed as HOMA IR and IL-1β. When the patients were subgrouped according to tertiles of HOMA IR, levels of CRP, IL-6, ICAM-1, VCAM-1, fibrinogen and vWF in the highest HOMA IR tertile were significantly higher than in tertiles 1 and 2.

Several intercorrelations existed between inflammation parameters, cytokines and endothelial dysfunction markers (*Table 4*). After controlling for age, gender, BMI and fat mass, correlation remained significant between CRP and FB (r=0.31, p<0.001), IL-6 (r=0.22, p<0.05), ICAM-1 (r=0.37, p<0.001), VCAM-1 (r=0.32, p<0.001) and vWF (r=0.38, p<0.001).

Notably there was a significant association between IL-6 and ICAM-1 (r=0.33, p<0.001)) and VCAM-1 (r=0.25, p<0.05) and vWF (r=0.20, p=0.05).

Discussion

In this study we attempted to explore the relation of insulin resistance with several markers of both low-grade inflammation and endothelial activation simultaneously in a large sample of children and adolescents with simple obesity. To the best of our knowledge, this study is the first to investigate such relationship in obese children. Data concerning inflammatory markers in obese children are limited [7,28].

The current results provide a strong evidence that inflammatory markers as well as indices of endothelial dysfunction are associated with adiposity in obese children and adolescents. Our data are consistent with others found in obese adults [23,32,44]. Interleukin-6, secreted by immune system and adipose tissue during resting, and muscle during physical activity is major cytokine mediator, stimulating production of so-called acute-phase proteins such as CRP, amyloid A, and fibrinogen by the liver. Growing evidence indicates that adipose tissue is an important source of plasma IL-6 – as much as 30% of circulating IL-6 may originate from adipose tissue [26], and there is a strong positive association between body mass index and both IL-6 [3] and CRP [24]. In the study, conducted by Nemet and co-workers [28], including 30 healthy children, serum IL-6 concentration positively correlated with BMI and fat mass.

Our findings also suggest a possible link between visceral adiposity and inflammation and endothelial activation markers. Waist circumference and WHR have commonly been used as a simple clinical measures of abdominal obesity. Visceral adiposity has been implicated in the regulation of inflammation

[42], as well as other metabolic abnormalities related to insulin resistance [9]. Leinonen et al. [23] found positive correlation between acute-phase reactants and waist circumference in 134 adult patients with type 2 diabetes. However, it is not well-established if this association is caused by enhanced production of proinflammatory cytokines in visceral adipose tissue [47], or if reduced insulin sensitivity in obesity leads to increased hepatic acute-phase proteins production [14].

CRP, at levels below the characteristic of acute inflammation, is a newly recognized risk factor for atherosclerosis [37]. The serum concentration of CRP has been reported to be positively associated with obesity as well as body fat distribution [14,47]. Lemieux et al. [24] studied 159 nondiabetic men of whom over 80% had a BMI greater than 25. All indices of obesity were significantly correlated with CRP. Fat mass was the best correlate, followed by BMI = waist>visceral adiposity, assessed by computed tomography. This study is consistent with our observation that indicators of total body fat correlate more strongly with CRP than does waist circumference and WHR, clinical indicators of fat distribution. However, since IL-6 was not measured in the study by Lemieux et al., a direct comparison with our analysis is not possible. An association between inflammatory markers and fat mass, found in our study, confirms the hypothesis that obesity is a state of chronic, low-grade inflammation [7,36].

In our study, a significant correlation between inflammatory markers and insulin resistance was found. As a surrogate marker of insulin resistance, we used HOMA index, which is relatively simple and generally used in pediatric practice since correlates significantly to insulin resistance estimated by the clamp method [2]. The association between low-grade inflammation and insulin resistance has previously been reported among healthy, glucose-intolerant and diabetic subjects [14,16,44]. Consistent with these studies are results of prospective investigations in which the strong association of elevated levels of acute-phase proteins and proinflammatory cytokines with increased risk of type 2 diabetes was found [41].

There is increasing evidence that an ongoing cytokine-induced acute-phase response (sometimes called low-grade inflammation) is one of the novel risk factors closely involved in the development of type 2 diabetes and its vascular complications [30,20,21]. Several studies have now confirmed that the presence of inflammation predicts the development of type 2 diabetes. The first of these studies by Schmidt et al. [40] showed that the presence of inflammatory markers predicted the future occurrence of type 2 diabetes in adults. A recent paper from the same Atherosclerosis Risk in Communities Study (ARIC) continues this theme by showing that elevated plasma levels of sialic acid, orosomukoid, IL-6 and CRP predict type 2 diabetes [10]. An overall inflammation score based on these four indices as well as the total leukocyte count and plasma fibrinogen concentration, provided an increased risk of 3.7 (when comparing the highest and the lowest quintiles) in white non-smokers subjects for the development of type 2 diabetes. Consistent with these findings are results at least three other prospective investigations confirming the fact that an increase an inflammatory markers at baseline predicts type 2 diabetes and insulin resistance [17,34,41]. Similarly, the correlation between fasting insulin concentrations and CRP concentrations was found [33,47] indicates that insulin resistance and inflammatory processes are related. Among proinflammatory cytokines associated with the risk of type 2 diabetes, the most important are interleukin-6 and interleukin-1β. IL-6 levels have been reported to be lowest in healthy subjects, higher in type 2 diabetic subjects without the metabolic syndrome and highest in patients with type 2 diabetes and the metabolic syndrome [31]. Moreover, the high IL-6 concentrations has been shown to precede the onset of type 2 diabetes. Spranger et al.[41] found elevated IL-6 levels in subjects who were free of type 2 diabetes at baseline and subsequently develop type 2 diabetes during a 2.3-year follow-up period. IL-6 was an independent predictor of type 2 diabetes after adjustment for age, gender, BMI, WHR, physical activity, smoking status, alcohol consumption, education attainment, and HbA1c. It was also found in this study that a combined elevation of IL-1β and IL-6, rather than the isolated elevation of IL-6, independently increases the risk of type 2 diabetes. Such combination of IL-6 and IL-1β increases the production of the acute-phase proteins in the liver as well as produces the characteristics dyslipidemia of the metabolic syndrome, with increased VLDL and decreased HDL. Another possible molecular mechanism how inflammatory process may be involved in the pathogenesis of type 2 diabetes was recently proposed by Hundal and co-workers [19]. They shown that sensitizing of insulin signaling by salicylates is induced via inhibition of the activity of 1kB kinase β. IL-1β is known to activate this enzyme and might thereby induce insulin resistance.

Recently, novel data have appeared showing that the concomitant presence of promoter polymorphism of TNF-α (G-308A) and IL-6 (C-124G) in obese patients with impaired glucose tolerance carry twice the risk of conversion to diabetes type 2 when compared with other genotypes. Moreover, a mutation of the TNF-α promoter is associated with increased plasma TNF-α concentrations and a 1.8 higher risk of developing diabetes compared to non-carriers. A C-124G mutation of the IL-6 promoter increased the risk for insulin resistance [22]. In our study the significant correlation between IL-6 and HOMA IR, after adjustment for age, gender, BMI and fat mass, became weaker but still reached statistical significancy. However, in our study, the genetic background did not determined, so we could not directly confirm the association between IL-6 and insulin resistance.

Two possible mechanisms might be involved in the pathogenesis of inflammation in obesity and type 2 diabetes [7]. There is evidence that glucose intake as well as mixed fast-food meals induce acute oxidative stress and inflammation at the cellular and molecular level for a period of three to seven hours [12,27]. Conversely 48 hours fast leads to a 50% reduction in reactive oxygen species (ROS) generation by leukocytes and a diminution in the expression of NADPH oxidase, the enzyme converting molecular oxygen to the superoxide radical. This superoxide radicals activates the redox sensitive proinflammatory transcription factor, NF-kB, which activates the transcription of most proinflammatory genes. Therefore, it is probable that proinflammatory state of the obese is related to excessive macronutrient intake [7]. Consistent with this hypothesis are studies by Engstrom et al. [11], which found that increased plasma concentrations of inflammation sensitive proteins were associated with future weight gain. Another possible mechanism linking obesity and type 2 diabetes with inflammation is that the insulin resistance state promotes inflammation. Insulin exerts an antyinflammatory effect at the cellular and molecular level both in vitro and in vivo. A low dose infusion of insulin reduces ROS generation by mononuclear cells, suppresses NADPH oxidase expression and intranuclear NF-kB binding, induces IkB expression and suppress plasma intercellular adhesion molecule-1 and monocyte chemotactic protein-1 (MCP-1) concentrations. Insulin also suppresses intranuclear Egr-1, plasma tissue activator and PAI-1 [8]. An interruption of insulin signal transduction would prevent the antyinflammatory effect of insulin from being exerted.

It was also found in our study that indices of endothelial dysfunction i.e. levels of ICAM-1, VCAM-1 and von Willebrand factor correlated significantly with adiposity and body fat distribution. It indicates that in children as well as in adults [4], obesity is associated with endothelial activation. Moreover, in our study ICAM-1 (but not VCAM-1) and von Willebrand factor correlated significantly with HOMA IR. The relationship between insulin resistance and low-grade inflammation and endothelial activation may be partly explained by obesity, but the association remains, though weaker, after adjustment for BMI and fat mass, so other mechanisms must be operative.

In our study several intercorrelation between inflammatory markers, cytokines and CAMs were also found. A probable explanation to the association of IL-6 and ICAM-1 and VCAM-1 in our study is that IL-6 upregulates expression of the CAMs. This is supported by previous studies showing the similar interrelation between circulating levels of ICAM-1 and both CRP and IL-6 [23]. The activation of proinflammatory mechanisms and the accumulation of monocytes and macrophages in the intima (in addition to lipid infiltration) with sheding soluble forms of CAMs into circulation are characteristic for atherosclerosis. Thus, inflammation underlies both insulin resistance and atherosclerosis. One mechanism that might link these features is the anti-inflammatory and potential anti-atherosclerotic effect of insulin, which in the presence of insulin resistance can lead to a proinflammatory state [7].

In summary, our study shows that acute-phase reaction and endothelial activation markers correlate with insulin resistance and adiposity in young patients with simple obesity. Our findings suggest that the cluster of these pro-atherogenic factors may contribute to the accelerated atherosclerosis in childhood obesity.

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