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Waist circumference is related to low-grade inflammation in youth

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Abstract

Objectives: To examine markers of inflammation in 9- and 15-year olds with high waist circumference and compare these with controls, and to examine the relationships between inflammatory markers and cardiovascular disease (CVD) risk factors. **Methods:** Cross-sectional analysis of data from 2299 Norwegian 9- and 15-year-olds participating in the "Physical activity among Norwegian Children Study". In each sex and age group, the 10 participants with the highest waist circumference (HW) were selected (n=40) for analyses, and a random sample of 40 participants within the same groups were included as controls. Inflammatory markers included C-reactive protein (CRP), leptin, adiponectin, plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor- α (TNF-α), hepatocyte growth factor (HGF), resistin and interleukin-6 (IL-6). The CVD risk factors included blood pressure, glucose, insulin, triglycerides and high density lipoprotein cholesterol. **Results:** HW participants had elevated levels of CRP (mean difference 1.50 mg/l; 95% confidence interval (CI) 0.33 to 2.66), PAI-1 (mean difference 13.3 ng/ml; 95% CI 4.1 to 22.5) and HGF (mean difference 0.29 ng/ml; 95% CI 0.07 to 0.51) compared with controls. All CVD risk factors differed between the HW group and controls. The CVD risk factors were not associated with TNFα or IL-6, but CRP, HGF and PAI-1 were related to the metabolic risk score. **Conclusions:** Low grade systemic inflammation is already present in youth with high waist circumference. CRP, HGF and PAI-1 may be related to the adverse overall metabolic risk profile observed in these children and adolescents.

Key words: *Cardiovascular disease, cytokine, obesity, waist circumference, youth*

Introduction

Childhood obesity is a growing problem reaching epidemic proportions worldwide (1). Adipose tissue is more than a storage depot—it also serves as an endocrine organ that secretes cytokines and growth factors (2;3), including tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), adiponectin, plasminogen activator inhibitor-1 (PAI-1) (4;5) and hepatocyte growth factor (HGF) (6). These inflammatory cytokines may affect metabolism, and prospective studies in adults have shown that chronic low-grade inflammation may contribute to the development of insulin resistance, atherosclerosis, diabetes and cancer (7). It has been hypothesized that an elevated level of TNF-α causes insulin resistance, and one of the key mechanisms for the adverse effects of abdominal fat on cardiovascular disease (CVD) risk factors could be the production of TNF-α in fat tissue (8).

C-reactive protein (CRP) is another inflammatory marker that has received considerable attention. CRP is a non-specific marker of inflammation, and may be regulated by cytokines, primarily IL-6 and TNF-α. Elevated levels of CRP are found in individuals with abdominal obesity (9), and the CRP level is an important predictor of the development of type 2 diabetes and CVD (10). Although patients with CVD typically become symptomatic after the age of 40 years, atherosclerotic changes start early in life (11). Moreover, inflammation has been associated with early arterial changes in both healthy (12) and obese children (13).

Given the evidence that a high proportion of children and adolescents have single or multiple adverse CVD risk factors (14-16) there is growing interest in the function of inflammatory markers in youth. Based on findings from the adult population, inflammatory markers have been hypothesized to be important contributors to the development of clustered metabolic risk in childhood. Several studies have aimed to determine whether these inflammatory markers

differ between obese and non-obese children and adolescents (17-20), and the possible relationship between these factors and components of the metabolic syndrome (21-23). Although these studies have shown that obese youth have an adverse metabolic risk profile, elevated levels of TNF-α and IL-6 and decreased adiponectin concentrations have not been found consistently in obese youth, indicating a need for better understanding of the interrelationship between inflammation and components of the metabolic syndrome. In addition, few studies have examined other inflammatory markers such as HGF and PAI-1 in children and adolescents. The aims of this study were: 1) to examine markers of inflammation in 9- and 15-year-old youths with high waist circumference and compare these with values in controls, and 2) to examine the relationships between inflammatory markers and CVD risk factors in these youths.

Subjects and methods

Subjects

Data collected as part of the "Physical Activity among Norwegian Children Study" conducted in 2005 and 2006 (24) were included in this cross-sectional data analysis. A total of 63 randomly selected schools from all regions of Norway were included in the study. Of 2,818 subjects invited to participate in the study, 2,299 accepted, giving an overall participation rate of 82% (89% and 74% for the 9- and 15-year-olds, respectively). The present analyses included 10 participants from each sex and age group (9- and 15-year-olds) with the highest waist circumference (HW) (n=40), and a random sample of 40 participants from the rest of the cohort that were included as controls. The randomization was performed with the random number generators function in SPSS statistical software, v. 15.0 (SPSS Inc., Chicago, IL., USA). Valid analyses of cytokines and measures of pubertal stage were available in 70 subjects (36 HW and 34 controls). The study was carried out according to the Helsinki

Declaration and was approved by the Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services. Each participant's parent or guardian provided written informed consent, and all subjects agreed to participate.

Measures

Measures of anthropometry, pubertal stage, blood pressure and aerobic fitness (VO_{2peak} in $ml \cdot min^{-1} \cdot kg^{-1}$) and a fasting blood sample were obtained in the school setting by trained research assistants.

Anthropometry and sexual pubertal stage

Body height (nearest mm) and body weight (nearest 0.1 kg) were measured in light clothing using an electronic scale (SECA 770 GmbH, Hamburg, Germany). Body mass index (BMI) was calculated as weight (kg) divided by the height squared (m^2). On the basis of BMI, we classified children and adolescents as overweight or obese according to the age and sex adjusted cut-offs described by Cole et al. (25). Waist circumference (nearest mm) was measured with anthropometric tape around the umbilicus at the end of a normal expiration. Identification of pubertal status was assessed by trained personnel according to Tanner`s classification (26) and was based on breast development in girls, and genitalia development in boys.

Blood pressure

After the participant was seated for at least 5 minutes, blood pressure was measured automatically five times at 2 minute intervals (Omega^{TM} Non-invasive blood pressure monitor, Invivo Research, Inc., Orlando, FL, USA). The mean value of the last three measurements was used in the analyses.

VO_{2peak} *measurement*

Aerobic fitness was assessed during a progressive cycle test to exhaustion using an electronically braked cycle ergometer (Ergomedic 839E; Monark, Varberg, Sweden). The test is described in detail elsewhere (24). The criteria for maximal exhaustion were met if there was a subjective judgement by the tester that the subject showed signs of intense effort (e.g., facial flushing or difficulties in keeping up pedal frequency) and if the heart rate was ≥ 185 beats per minute or the respiratory exchange ratio was ≥ 0.99.

Blood analysis

After an overnight fast, venous serum blood samples were collected between 8 and 10 AM. The samples were spun for 10 minutes at 2500 × *g* and separated within 30 minutes. The concentrations of high-density lipoprotein cholesterol (HDL-c) triglycerides (TG) and glucose were analysed by colorimetry on a Cobas Integra analyser (F. Hoffmann-La Roche Ltd, Basel, Switzerland). Insulin was measured by fluoroimmunoassay using an automatic immunoassay system (AutoDELFIA® Insulin, PerkinElmer, Turku, Finland). Homeostasis model assessment (HOMA) was calculated as the product of fasting glucose (mmol/l) and insulin (μU/ml) divided by the constant 22.5 (27). CRP was measured on a Hitachi 917 automatic analyzer (Hitachi, Tokyo, Japan) using a high-sensitive latex-enhanced turbidimetric assay from Roche Diagnostics, Mannheim, Germany (range 0.1–20 mg/l, intra-assay coefficient of variation <2% at 0.5 mg/l.) The serum levels of various adipokines were quantified using the Human Serum Adipokine, panel A (adiponectin, resistin and PAI-1(total)) and panel B (TNF-α, IL-6, HGF, and leptin) kits (Linco Research, Inc. St. Charles, MI) and the Luminex-100 system (Luminex Corporation, Austin, TX, USA). The samples were randomly analysed twice and the acquired fluorescence data were analysed using STarStation software (Version 2.0; Applied Cytometry Systems, Sheffield, UK).

Metabolic risk score

A continuous score representing a composite CVD risk factor profile was derived by computing standardized residuals (z-score) by age and sex for the HOMA score, waist circumferences, TG, systolic blood pressure and HDL-c. The z-scores of the individual risk factors were summed to create the metabolic risk score. These variables were chosen because they are used as clinical criteria for diagnosing the metabolic syndrome in adults (28) and youth (29). Waist circumference was omitted from the metabolic risk score used in the analysis of bivariate associations.

Statistical analysis

The data were analysed using SPSS statistical software, v. 15.0 (SPSS Inc., Chicago, IL., USA).Values are expressed as mean (SD) unless otherwise stated. We used chi-square analysis to identify differences in the distribution of sexual maturity status between the HW and control groups. The distribution of each variable was tested for Gaussian distribution. TG, HOMA, CRP, leptin and HGF were transformed using the natural logarithm for all analyses. A general linear model was used to compare groups after adjusting for sex, age and pubertal stage. Partial correlation adjusted for sex, age and pubertal stage was used to examine bivariate associations. With the sample size of 35 subjects in each group we have an effect size of 0.3 to detect differences between the HW group and controls.

Results

Table 1 shows the anthropometric data for the HW and control groups. The two groups had similar age and height, and equal pubertal stage distribution. The HW group had a higher waist circumference, (27 cm difference), body weight and BMI compared with the randomly

selected controls. All subjects in the HW group were classified as overweight (n=11) or obese (n=25).

Inflammatory markers

Differences in the inflammatory markers between the two groups are shown in Table 2. CRP concentration was significantly higher in the HW group than in the control group (mean difference 1.50 mg/l). In the HW group, 44%, 42% and 14% had CRP values < 1, 1 to 3 and > 3 mg/l, respectively. In the control group, these percentages were 85%, 6% and 9%. Levels of leptin, PAI-1 and HGF were higher in the HW group than in the control group. TNF-α, IL-6, adiponectin and resistin levels did not differ between the two groups (Table 2).

CVD risk factors

Table 3 displays the differences in the variables related to the metabolic syndrome between the two groups. The HW group had significantly higher HOMA score, systolic blood pressure and TG than controls. HDL-c and $VO_{2\ peak}$ were significantly lower in the HW group than in the controls.

Correlations between inflammatory markers and CVD risk factors

The relationship between inflammatory markers and CVD risk factors are summarized in Table 4. Partial correlations controlling for sex, age and pubertal stage revealed that CRP, HGF and PAI-1 correlated positively with waist circumference and metabolic risk score and negatively with $VO_{2\ peak}$. TNF-α and IL-6 did not correlate significantly with any other variables, except that TNF-α and PAI-1 were significantly correlated. Finally, HGF correlated with all inflammatory markers and CRP correlated with leptin.

Discussion

We studied two groups of 9- and 15-year-olds assigned to different groups according to their waist circumference. The HW group showed unfavourable levels of all CVD risk factors and the presence of adverse adipokines. These variables were not associated with TNF-α or IL-6, but CRP, HGF and PAI-1 were related to the metabolic risk score.

Our results confirm other reports showing that obesity is associated with enhanced low-grade systemic inflammation as indicated by CRP levels (30-32). Our results are consistent with previous findings (23;32-34), showing that elevated CRP levels in children and adolescents are associated with several metabolic risk factors. Applying cut-off values of CRP concentration (35) to distinguish between low (< 1 mg/l), moderate (1 to 3 mg/l) and high risk (> 3 mg/l) for future CVD, showed that about half of the HW group had CRP levels in the moderate or high risk range. There is no current consensus on clinical cut-off values for CRP concentration in young people, although Guran et al. (33) found that CRP concentration > 1.04 mg/l predicted CVD risk factors in children and Soriano-Gullièn et al. (36) showed that CRP concentration could be useful in the early detection of CVD risk in obese children and youth.

To our knowledge, this is the first study to report elevated HGF levels in apparently healthy children and youth with high waist circumference. We found elevated levels of leptin and PAI-1 and slight but significant increased HGF levels in the HW group, and these correlated with CVD risk factors. It is known that these are important factors that stimulate blood vessel formation during fat mass expansion (37). Differences in serum HGF levels found in the present study correspond well with findings that have been reported among obese and lean adults (38). Rehman et al. (6) found, however, a threefold higher HGF level in obese

compared to lean subjects. Thus, it is difficult to interpret the clinical implications of the HGF variations we found. However, there is a growing body of evidence suggesting that elevated HGF levels may play an important role linking obesity and the metabolic syndrome in adults (39). In addition, we also found a correlation between PAI-1 and HGF levels. Endothelial dysfunction is considered the earliest stage in the atherosclerotic process. Our findings might be of interest because PAI-1 levels have shown to correlate positively with markers of endothelial dysfunction (40). Leptin is another cytokine that has been reported to affect the vessel wall (4), and we found an association between leptin and both PAI-1 and HGF. Taken together these data suggest that elevated concentrations of PAI-1, HGF and leptin are related to endothelial dysfunction and that these elevated levels may contribute to the low grade systemic inflammation and furthermore, play a role in the clustering of CVD risk factors observed in the HW group.

Conversely, the HW group did not have elevated levels of TNF-α or IL-6 even though these subjects had high waist circumference, higher HOMA and a more adverse metabolic risk score. We also found no association between waist circumference, metabolic risk score and TNF-α and IL-6. This does not necessary exclude TNF-α and IL-6 as possible contributors to future CVD risk. One might speculate that the increase in the levels of these cytokines requires a more severe state of obesity or more time for the associations to develop. We acknowledge that the concentrations of these markers were measured in blood and thus may be influenced by the fact that these are sensitive markers which are metabolized quite fast.

Our study showed a significantly lower aerobic fitness level in the HW group and that the concentration of CRP, HGF and PAI-1 were negatively associated with aerobic fitness. Reduction in the levels of inflammatory markers have been shown to be related to weight loss

(4) and greater amounts of physical activity (41). Although the results in the present study suffer from lack of validity for a causal relationship, the following pathway for the relationship between aerobic fitness and inflammatory markers could be plausible. Increased levels of aerobic fitness might induce a decrease in visceral adipose tissue resulting in reduced secretion of inflammatory markers. This could favourably change a broad number of CVD risk factors and, hence, possibly protect against development of future CVD.

One strength of this study is the inclusion of a number of components that are related to the metabolic syndrome and several markers of inflammation. The inclusion of a young sub-population allowed us to examine the differences and relationships between variables before these subjects may develop manifest diseases.

This study has some limitations. Firstly, the cross-sectional nature does not allow explanations of causality. Secondly, the study sample was relatively small and may introduce lack of statistical power. However, associations giving r-values > 0.23 were statistically significant, and weaker associations may not have biological importance. The selection of subjects with high waist circumference and the comparison with randomly selected controls may have shown differences that are of clinical relevance. In addition, the correlations coefficients may have been influenced by the inclusion of one extreme group (HW) and a randomly selected control group in the analysis of the bivariate associations.

In conclusion, low-grade systemic inflammation is already present in youth with high waist circumference and CRP, HGF, PAI-1 and leptin may be related to the adverse overall metabolic risk profile observed in these children and adolescents. Our findings suggest that

CRP, PAI-1 and HGF may be important biomarkers in assessing CVD risk associated with childhood obesity.

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Table 1. Characteristics of the study population (mean and range).

	High waist		Control	
	9-year (n= 17)	15-year (n=19)	9-year (n= 16)	15-year (n=18)
Age (years)	9.7 (9.1−10.4)	15.6 (15.0−16.2)	9.6 (8.9−10.2)	15.5 (14.9−16.1)
Height (cm)	147.0 (133.0−160.5)	173.4 (161.0−182.0)	140.4 (130.0−153.0)	173.1 (156.0−191.0)
Weight (kg)	56.5 (43.1−66.8)	91.5 (67.3−132.4)	32.6 (26.1−43.7)	62.3 (51.2−76.0)
BMI (kg/m^2)	26.2 (22.4−30.1)	30.3 (24.6−40.0)	16.5 (14.1−19.0)	20.8 (18.2−24.1)
Waist circumference (cm)	89.8 (82.0−100.0)	99.1 (89.0−122.0)	61.1 (52.0−69.9)	73.3 (64.8−85.0)
Pubertal stage* Stage 1/ 2-4/ 5 (number)	11/6/0	0/3/16	13/3/0	0/3/15

* Based on Tanner`s 5 stages

Table 2. Inflammatory markers in high waist and controls (mean SD).

	High waist (n=36)	Control (n=34)	Mean difference	95% CI	*P*-value*
CRP (mg/l)	2.21 (3.11)	0.72 (1.41)	1.50	0.33-2.66	<0.001
TNF-α (pg/ml)	4.51 (2.09)	4.02 (1.48)	0.49	-0.38-1.36	0.213
Leptin (ng/ml)	27.8 (14.6)	5.1 (4.7)	22.6	17.4-27.9	<0.001
Adiponektin (µg/ml)	24.2 (13.8)	30.1 (15.5)	-6.0	-13.0-1.0	0.082
PAI-1 (ng/ml)	56.3 (19.6)	43.0 (18.9)	13.3	4.1-22.5	0.004
IL-6 (pg/ml)**	5.23 (5.23)	4.02 (3.97)	1.21	-1.12-3.54	0.328
Resistin (pg/ml)	12.7 (4.7)	11.5 (5.0)	1.2	-1.1-3.5	0.287
HGF (ng/ml)	1.10 (0.53)	0.81 (0.36)	0.29	0.07-0.51	0.017

CRP, c-reactive protein; HGF; hepatic growth factor; IL-6; interleukin-6; PAI-1, plasminogen activator inhibitor-1, tumor necrosis factor-α (TNF-α).

* All *p* values are adjusted for sex, age and pubertal stage

** Six subjects (3 from HW and 3 controls) had IL-6 levels 5 SD above the mean and were therefore excluded from the analysis.

Table 3. CVD risk factors in high waist and controls (mean SD).

	High waist (n=36)	Control (n=34)	Mean difference	95% CI	*P*-value*
SBP (mmHg)	118.9 (12.0)	109.6 (9.7)	9.4	4.2-14.6	<0.001
HDL-c (mmol/l)	1.28 (0.27)	1.67 (0.35)	-0.39	-0.54- -0.25	<0.001
TG (mmol/l)	1.23 (0.58)	0.62 (0.24)	0.61	0.40-0.83	<0.001
HOMA	3.67 (2.19)	1.88 (3.12)	1.80	0.52-3.07	<0.001
VO_{2peak} ($ml \cdot min^{-1} \cdot kg^{-1}$)	32.9 (5.5)	48.4 (8.2)	-15.5	-18.8- -12.2	<0.001

HDL-c, high density lipoprotein cholesterol; HOMA, homeostasis model assessment (insulin resistance); SBP, systolic blood pressure; TG, triglycerides.

* All *p* values are adjusted for sex, age and pubertal stage

Table 4. Partial correlation (r) adjusted for sex, age and pubertal stage between inflammatory markers, HOMA, waist, VO_{2peak} and metabolic risk (z-score).

	Waist	BMI	HOMA	VO_{2peak}	CRP	HGF	Leptin	PAI-1	IL-6	TNF-α	Z-score
Waist	1.00										
BMI	**0.95****	1.00									
HOMA	**0.62****	**0.62****	1.00								
VO_{2peak}	**-0.85****	**0.82****	**-0.56****	1.00							
CRP	**0.63****	**0.60****	**0.33***	**-0.58***	1.00						
HGF	**0.37***	**0.30****	**0.29***	**-0.36***	**0.33***	1.00					
Leptin	**0.84****	**0.83****	**0.63****	**-0.84****	**0.56****	**0.32***	1.00				
PAI-1	**0.41****	**0.37****	0.14	**-0.33***	0.12	**0.34***	**0.29***	1.00			
IL-6	0.09	0.09	-0.00	-0.09	**0.25***	**0.27***	0.19	0.20	1.00		
TNF-α	0.22	0.22	0.18	-0.20	0.17	**0.29***	0.21	**0.41***	0.13	1.00	
Z-score	**0.72****	**0.70****	**0.86****	**-0.64****	**0.42****	**0.38***	**0.71****	**0.27***	-0.01	0.15	1.00

BMI, body mass index; CRP, c-reactive protein; HGF; hepatic growth factor; HOMA, homeostasis model assessment (insulin resistance); IL-6; interleukin-6; PAI-1, plasminogen activator inhibitor-1; tumor necrosis factor-α (TNF-α).

**$P < 0.01$;* $P < 0.05$