Low-grade systemic inflammation in overweight children: impact of physical fitness

Running Title: Inflammation in obese children

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Abstract

Obesity as well as low physical fitness and inactivity are associated with an increased incidence of cardiovascular risk factors and coronary artery disease (CAD). Increased inflammation has recently been addressed to play an important role for the relationship between obesity and CAD, as adipose tissue expresses and releases pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). As this relationship is less clear in childhood, we investigated 197 children aged 10-15 years assessing obesity, physical fitness, and a metabolic cardiovascular risk profile including markers of inflammation. Obese children had significantly higher concentrations of inflammatory parameters such as fibrinogen, ferritin, IL-6, and TNF-α than non-obese subjects (P<0.01). When dividing the children into groups regarding obesity (BMI < 22.5 kg/m², BMI ≥ 22.5 kg/m²) and fitness (< 5 MET, ≥ 5 MET), we found that obese, unfit children showed the highest systemic inflammation, whereas fit but obese individuals had as low levels as lean and fit children. These data reveal that even in childhood inflammatory parameters are elevated in obesity and that physical fitness counteracts this association.

Key Words: Obesity, childhood, physical activity, inflammation

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Introduction

Obesity is emerging as one of the major medical problems in Western countries because of its close relationship to cardiovascular risk factors and coronary disease. Recently increased inflammation has been addressed to play an important role in this scenario, as human adipose tissue expresses and releases pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α), thereby possibly triggering or maintaining atherosclerosis (1;2). In contrast, physical fitness has shown to counteract the relationship between obesity and increased cardiovascular risk (3;4;4-6). Likewise obese individuals with good physical fitness assessed by ergometry have a similar risk as non-obese, unfit subjects (4;7). This is explained by improvements in cardiovascular risk factors and particularly serum lipoprotein profile by regular physical activity in obese men and women (7;8). Recently it has been shown that lifestyle intervention by exercise and diet induces both reductions in body weight as well as reductions in systemic inflammatory parameters such as TNF-α and IL-6 (9;10). These findings have led to the hypothesis that the reduced cardiovascular risk in subjects with good physical fitness, high physical activity, or low body weight can also be explained by an improved systemic inflammatory profile (11;12).

These findings came almost exclusively from studies investigating adult subjects (1;13). One large cross-sectional study of children and adolescents (3rd National Health and Nutrition Examination Survey) has investigated the relationship between obesity and increased C-reactive protein (CRP) concentrations and confirmed the findings of adulthood (14). This survey, however, included subjects with increased white blood cell count (WBC) and possibly underlying infection which might have confounded the findings. In addition, physical activity or physical fitness were not evaluated (14). Therefore, we investigated 197 obese and non-obese children and adolescents without evidence for infection assessing several parameters of obesity, physical fitness, and measures of the cardiovascular risk profile including several markers of inflammation.

Methods

Subjects

197 children (104 girls and 93 boys, aged 12.5 ± 2.7 years and 12.3 ± 2.3 years, respectively) were recruited for the study at the Department of Rehabilitative and Preventive Sports Medicine in Freiburg, Germany. They were either referred by local pediatricians to participate at the Freiburg Intervention Trial for obese children (FITOC-Trial) (15), or were routinely screened as part of a preventive screening program of the regional Sports Federation of South-West Germany. Only those children with a normal questionnaire particularly regarding infection, inherited diseases, or metabolic disorders and a normal physical examination, besides obesity, were included into the study. Informed consent for blood analysis and data collection was signed by the parents. The study was approved by the ethical committee of the University of Freiburg.

Anthropometry
Body mass and height were measured using standardized procedures. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of height in metres and was used as an indicator of total body fat (16). In addition, skinfold measurements (Cambridge Scientific Industries, Cambridge MD) were determined. A minimum of three measurements each were obtained at four sites ($SF_{triceps}$, SF_{chest}, $SF_{umbilicus}$, $SF_{scapula}$) on the right side of the body.
Bicycle ergometry was performed after blood was drawn. Heart rate, ECG and blood pressure were monitored during exercise and maximal power was assessed in a stepwise test protocol starting with 25 W with increments of 25 W every 3 minutes until exhaustion. Maximal power was transformed to Metabolic Equivalent (MET).

Laboratory measurements
Blood was drawn and immediately handled for measurements of blood sedimentation rate, blood cell counts, fibrinogen, ferritin, glucose, and lipoprotein measurements. For analysis of IL-6 and TNF-α EDTA plasma was stored at – 80°C until analysis within 3 months.

Inflammation markers
TNF-α and IL-6 were measured by commercially available ELISA kits (human TNF-α Quantikine high sensitivity kit, IL-6 Quantikine HS kit, both R&D Systems). The coefficient of variation within the assays was <8% and between assays <6%. WBC count was assessed using a quantitative, automated haematology analyzer (Coulter Counter, Beckman, Fullerton, CA).

Lipoprotein profile
Cholesterol and triglycerides were measured by automated enzymatic methods. Very-low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were prepared by equilibrium density-gradient ultracentrifugation (17). The apolipoproteins (apo) A-I, B, and A-II were measured by endpoint nephelometry (Boehringer Mannheim, Germany). The coefficient of variation within and between assays was less than 5% (17)

Statistical Analysis
Data of all children were divided into two groups according to BMI (non-obese: BMI < 22.5 kg/m², obese: BMI ≥ 22.5 kg/m²). These groups were then further divided into 2 groups each according to median physical fitness (fit: MET >5, unfit: MET ≤5). Groups were compared for statistical differences by ANOVA to test the hypothesis that inflammatory markers were equal between groups. A conservative multiple comparison test (Scheffé test) was chosen for pair-wise comparisons of means between BMI groups.

All data are expressed as mean ± SD. Data were analyzed using the Statistical Package for the Social Sciences (SPSS/PC, SPSS Inc, Chicago, IL). All *P* values less than 0.05 were considered to indicate statistical significance.

Results

Obese children had significantly higher obesity measures such as skinfold thickness than leaner children (Table 1). In addition, obese children had significantly higher concentrations of inflammatory parameters such as fibrinogen, ferritin, IL-6, and TNF-α (Table 2), despite similar WBC counts. Lipid analysis showed no differences in total cholesterol and triglycerides whereas lipoprotein analysis revealed a more unfavorable lipoprotein profile of significantly lower HDL and higher LDL cholesterol levels in obese children (Table 3).

Table 1.

	BMI < 22.5 kg/m² N=44	BMI ≥ 22.5 kg/m² N=153	P
BMI, kg/m²	20.1 ± 1.4	25.9 ± 3.4	<0.001
$SF_{triceps}$, mm	15 ± 6	25 ± 6	<0.001
$SF_{subscapular}$, mm	13 ± 5	29 ± 9	<0.001
SF_{thorax}, mm	13 ± 5	29 ± 8	<0.001
$SF_{umbilical}$, mm	18 ± 6	35 ± 10	<0.001
HR, min^{-1}	78 ± 13	87 ± 15	<0.05
BP_{sys}, mmHg	126 ± 10	124 ± 21	n.s.
BP_{dia}, mmHg	77 ± 12	82 ± 11	n.s.

Table 1: Anthropometric data of 197 obese and non-obese girls and boys. Body Mass Index (BMI), Skinfolds (SF), Heart Rate (HR), and Blood Pressure (BP). Data are mean ± SD.

Table 2

	BMI < 22.5 kg/m² N=44	BMI ≥ 22.5 kg/m² N=153	P
WBC, $x10^9$ cells/L	6.516 ± 1.556	6.769 ± 1.720	n.s.
BSR, mm	4 ± 3	7 ± 6	<0.05
Fibrinogen, mg/dL	275 ± 52	310 ± 57	<0.01
Ferritin, μg/L	25 ± 17	38 ± 21	<0.01
IL-6, pg/mL	1.2 ± 0.7	1.7 ± 1.3	<0.01
TNF-α, pg/mL	1.4 ± 1.2	1.8 ± 1.2	<0.01

Table 2: Inflammatory parameters in obese and non-obese girls and boys. Blood Sedimentation Rate (BSR), White Blood Cell Count (WBC), Tumor Necrosis Factor-a (TNF-α), Interleukin-6 (IL-6). Data are mean ± SD.

Comparing girls and boys revealed no differences regarding age, BMI, skinfolds, or heart rate. Only maximal power was higher (2.0 ± 0.5 W/kg vs. 1.9 ± 0.3 W/kg, P<0.05) and blood pressure was significantly lower in boys (121 ± 17 mmHg vs. 129 ± 22 mmHg, P<0.05) than girls. Regarding lipids only apoA-II was significantly lower in girls (44.2 ± 6.4 mg/dL vs. 47.3 ± 6.9 mg/dL, P<0.01). Other lipoprotein concentrations were not different. Inflammatory parameter analysis showed similar values for WBC count, fibrinogen, IL-6, and TNF-α. Only BSR in the first hour (8 ± 5 mm vs. 6 ± 6 mm, P<0.05) was higher and ferritin levels lower in girls (32.8 ± 17.9 μg/L vs. 41.3 ± 24 μg/L, P<0.05) than boys.

When dividing the children into 4 groups regarding fitness (5 MET) and obesity (BMI 22.5 kg/m²) we found that obese, unfit children showed the highest values for systemic inflammation parameters (e.g. TNF-α and IL-6), whereas fit but obese individuals had as low levels as lean and fit children. Particularly TNF-α levels were elevated in less fit children independent of obesity measures (Figure 1).

Table 3

	BMI < 22.5 kg/m² N=44	BMI ≥ 22.5 kg/m² N=153	P
Total cholesterol, mg/dL	163 ± 20	172 ± 31	n.s.
Triglycerides, mg/dL	88 ± 38	106 ± 63	n.s.
VLDL cholesterol, mg/dL	22 ± 6	25 ± 12	n.s.
LDL cholesterol, mg/dL	86 ± 18	96 ± 25	<0.05
HDL cholesterol, mg/dL	55 ± 12	50 ± 12	<0.05
ApoA-I, mg/dL	145 ± 20	140 ± 22	n.s.
ApoA-II, mg/dL	41 ± 5	46 ± 7	<0.01
ApoB, mg/dL	84 ± 15	93 ± 24	n.s.
Lipoprotein(a), mg/dL	13 ± 23	18 ± 23	n.s.

Table 3: Lipoproteins and apolipoproteins in mg/dL in obese and non-obese girls and boys. Data are mean ± SD.

Discussion

Our data reveal that even in childhood inflammatory parameters are elevated in obesity and that physical fitness counteracts this association particularly for IL-6. TNF-α, however, seemed to be primarily determined by physical fitness alone. „Low grade systemic inflammation“ in overweight children has been proposed before, based on a large NHANES III survey of 3516 children aged 8 to 16 years (14). CRP and WBC counts were significantly higher in obese children defined as having a BMI or sum of three skinfolds above the gender-specific 85th percentile (14). The present data extend these findings to elevated serum levels of IL-6 and TNF-α in a group of otherwise healthy children with normal WBC count. Furthermore, other acute-phase proteins such as fibrinogen and ferritin were also higher in obese than non-obese children (Table 2), which confirms the hypothesis of low-grade systemic inflammation in childhood obesity (14). These findings cannot be explained by underlying diseases such as infection, respiratory disorders, rheumatoid arthritis, diabetes mellitus, or subclinical cardiovascular disease as proposed in adulthood (18;19) as only clinically healthy children – besides obesity - were investigated in our study.

Other mechanisms such as an increased synthesis and secretion of inflammatory markers from adipose tissue may explain these findings (20-23). Particularly TNF-α plays an important role as it is an important autocrine/paracrine regulator of fat cell size and function, thereby influencing insulin resistance and metabolic disorders (22). In addition, higher concentrations of circulating TNF-α and IL-6 influence hepatic synthesis of acute-phase proteins such as fibrinogen, ferritin and plasminogen activator inhibitor-1 which are independent cardiovascular risk factors themselves (24).

Figure 1

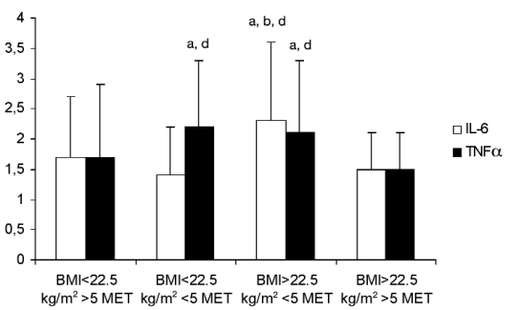

Figure 1: Interleukin-6 (IL-6) and Tumor Necrosis Factor-a (TNF-α) in pg/mL in relationship to obesity (BMI 22.5 kg/m2) and fitness (MET 5) in 4 groups of children.
a significant difference (P<0.05) to group 1
b significant difference (P<0.05) to group 2
d significant difference (P<0.05) to group 3

Interestingly, physical fitness was inversely correlated with levels of inflammatory parameters in our study. Likewise, IL-6 levels were higher in obese, unfit children than in any other group and were as low for obese and fit as for lean children (Figure1). In contrast, TNF-α seemed to be primarily dependent on physical fitness but not obesity as similar levels were found for non-obese as well as obese children with a low physical fitness. From these data it can be hypothesized that exercise capacity and obesity influence inflammatory parameters in different and possibly characteristic ways.

To our best knowledge, these findings have not been shown in children before. Data in adulthood have shown a reduction of inflammatory parameters such as CRP, IL-6, and TNF-α during long-term intervention studies in obese individuals (9;10;21). With a reduction of body mass of 25% by dietary energy restriction-induced weight loss, concordant reductions of CRP by over 30% could be observed (10). Similar findings were demonstrated by Kern *et al*. who found that weight reduction in obese women resulted in concurrent reduction of TNF-α mRNA expression and TNF-α protein reduction in adipocytes to 58% and 46% of baseline values, respectively (21). In addition, a similar weight loss program including exercise revealed that reductions of cytokines were associated with improvements of endothelial function (9).

Physical fitness and activity are known to reduce cardiovascular morbidity and mortality (4;25;26). In addition, cardiovascular mortality is reduced even in obese subjects by maintenance of good physical fitness and the risk is comparable to untrained, sedentary non-obese subjects (7;26). From our data it may be proposed that the reduced risk of obese, physically fit subjects may not only be explained by a more favorable lipid profile as proposed before (8), but may also be explained by their lower levels of inflammatory markers (11).

Conclusion

Our data reveal that childhood obesity is also associated with low-grade systemic inflammation like observed before in adulthood. This is associated with other metabolic risk factors, in particular dyslipoproteinaemia. Whether increased inflammation in association with an unfavorable metabolic risk profile in childhood will predispose to subsequent cardiovascular disease in adulthood remains unclear. However, the Harvard Growth Study investigating the BMI of children and adolescents between the years 1922 and 1935 found a more than two-fold increased risk for cardiovascular disease for obese boys 55 years later (27). In addition, a two- to three-fold increase in risk of myocardial infarction, ischaemic stroke, peripheral artery disease, and coronary heart disease mortality will have to be expected if childhood obesity continues into adulthood (2;28). Therefore, it seems possible that increased inflammation in obese children will predispose to cardiovascular disease in adulthood as observed for middle-aged adult populations (28;29). Increased physical activity and fitness may counteract this association by reducing inflammation and may additionally explain the multifactorial benefit of physical exercise and fitness for prevention strategies in adults and children alike. However, a prospective intervention program in obese children improving their metabolic and inflammatory risk factors will have to be conducted. Follow-up investigations of these children into adulthood are needed to test

the benefit of exercise and weight reduction on body composition, metabolic risk factors, inflammatory markers including CRP, TNFα and IL-6 and endothelial function, as well as subsequent development of atherosclerosis. This will be essential for our understanding of atherogenesis and mechanisms of early life-style intervention in the prevention of cardiovascular disease.

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