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Increased oxidative stress and altered substrate metabolism in obese children

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Abstract

Objective. Pediatric obesity, a major risk factor for cardiovascular diseases and diabetes, has steadily increased in the last decades. Although excessive inflammation and oxidation are possible biochemical links between obesity and cardiovascular events in adults, little information is available in children. Furthermore, effects of gender and fitness on the interaction between dyslipidemia and oxidative/inflammatory stress in children are mostly unknown.

Methods. Therefore, we measured systemic markers of oxidation (F₂-isoprostanes [F₂-IsoP] and antioxidants) and inflammation (interleukin-6 [IL-6] and leukocyte counts) and metabolic variables in 113 peripubertal children (55 obese [Ob] age and gender-adjusted BMI% > 95th, 25 Females [F]; 15 overweight [OW] BMI% 85th-95th, 8 F; 43 normoweight [NW] 25 F).

Results. When compared with NW, Ob displayed elevated F₂-IsoP (99 ± 7 vs. 75 ± 4 pg/mL, p < 0.005), IL-6 (2.2 ± 0.2 vs. 1.5 ± 0.3 pg/mL, p < 0.005), elevated total leukocytes and neutrophils, altered levels of total cholesterol, low- and high-density-lipoprotein cholesterol, triglycerides, free fatty acids, glucose, and insulin (all p < 0.005). This pattern was present in both genders and over a broad range of fitness in Ob.

Conclusions. Our data indicate that alterations in metabolic control and a concomitant increase in inflammation and oxidative stress occur early in life in obese children, likely exposing both genders to a similar degree of increased risk of future cardiovascular diseases.

Key words: Dyslipidemia, inflammation, oxidative stress, childhood obesity

Introduction

The widespread obesity epidemic affecting developed countries is a leading cause of type 2 diabetes, hypertension, stroke, myocardial infarction, and other cardiovascular diseases (1), accounting for more cumulative morbidity and mortality than any other cause (2). Alarmingly, obesity and its complications have especially increased in the last three decades among pediatric populations (1). While some underlying molecular mechanisms remain undefined, the association of cardiovascular disease with overt dyslipidemia and the progression of atherosclerotic lesions are well characterized in both adult and animal models (3). The combination of atherogenic diet, sedentary lifestyle, and genetic makeup in some ethnic groups results in excessive weight gain, dyslipidemia (low high-density-lipoprotein cholesterol [HDL-C], high low-density-lipoprotein cholesterol [LDL-C] and triglycerides), and insulin resistance (4). When elevated, LDL-C infiltrates the arterial endothelium, producing a fatty streak; if dyslipidemia persists, various white blood cell (WBC) subtypes also infiltrate the lipid-laden vascular wall and secrete oxidative molecules and inflammatory cytokines that cause endothelial erosion and damage. In turn, this activates the clotting cascade and platelet aggregation attempting to repair the atheromatous lesion, producing occlusive thrombi and ultimately downstream cellular infarctions and generalized micro- and macro-vascular diseases (4).

These processes are modulated by complex interactions by cytokines and chemokines, such as IL-6, IL-1β, TNF-α, IL-8, MCP-1 (5), and many others. Increased secretion of these molecules by activated
leukocytes, macrophages, and other tissues has been demonstrated in obese, diabetic, and cardiovascular patients (5). Signaling pathways regulating cytokine expression, such as the NF-κB pathway, are also activated by oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS), such as superoxide ion (toxic by-products of oxidative processes), and antioxidant defenses (superoxide dismutase [SOD], catalase, glutathione, glutathione peroxidase [GSH-420] etc.). ROS are also released by activated neutrophils and other leukocytes; indeed, WBC secretion of oxidative and inflammatory mediators surrounding the lipid droplets of the atheromatic core is a crucial biochemical step linking atherosclerotic plaques to thrombotic luminal occlusion (3). Multiple biomarkers of ROS interaction with specific substrates (i.e., DNA, lipids) have been identified; whole-body oxidative stress is best reflected by systemic levels of lipid peroxidation (for which F₂-isoprostanes [F₂-IsoP] are considered the most reliable biomarker) (6).

Despite the rapid global increase and potential devastating impact of pediatric obesity on Western societies in coming decades, very little is known about the progression of obesity complications in children, which may significantly differ from what occurs in adults (7). The overarching hypothesis of this study is therefore that pediatric obesity induces early, comprehensive alterations of substrate metabolism (dyslipidemia, impaired glucose tolerance), paralleled by elevated systemic oxidative/inflammatory status (increased inflammatory cytokines, activated leukocytes, markers of lipid peroxidation, reduced antioxidants), prolonging exposure of patients to the pathogenetic mechanisms associated with long-term development of cardiovascular disease. To elucidate these mechanisms, we performed a comprehensive assessment of inflammatory and oxidative status, leukocyte counts, and components of lipid and carbohydrate metabolism in 113 peripubertal healthy and obese children.

Methods

Participants and preliminary visit

This study was approved by the University of California, Irvine Institutional Review Board (UCI IRB). All 113 children (12.9±0.3 years, 58 Females [F]) and their guardians provided informed consents/assent; none had any history of acute or chronic disease (diabetes, hypertension, hyperlipidemia, other familial dysmetabolic conditions, asthma, other immunologic diseases, recent infection or injury, or use of medication); participants were also nonsmoking, and did not habitually ingest alcohol. Percent body mass index adjusted for age and gender (BMI%) was determined according to current criteria by the Center for Disease Control and Prevention (8). Confirmation that elevated BMI% was due to excess adiposity was obtained via skinfold thickness measurements, with total fat and lean body mass calculated using Slaughter et al. equations for age<16 years and Siri equations for ≥16 years (9,10), or via dual-energy X-ray absorptiometry (DEXA) scanning, utilizing a QDR-4500 Elite Hologic densitometer (Hologic Inc., Bedford, MA). All subjects also completed a validated, standard questionnaire regarding their developmental status to assess pubertal stage (11). While direct physician examination remains the gold standard for pubertal status assessment, an accurate Tanner staging can be obtained through this methodology, which has the advantage of eliminating inter-operator variability and preventing the anxiety that some children may experience from direct physical examination, which in our case may adversely affect the subsequent exercise protocol. For these reasons this mode of Tanner staging has become the method of choice in numerous research studies.

Participants were divided into a normal-weight group (NW, n=43, 25 F, BMI% <85th) and an obese group (Ob, n=55, 25 F, BMI% ≥85th). To maintain a clear separation between groups, children with BMI% ≥85th but <95th BMI% (overweight but not obese) were not included in the main data analysis; however, as a small group of enrolled children were within this category (OW, n=15, 8 F), their data will also be shown for completeness, and results of comparison with other groups by ANOVA will be shown. As physical fitness may independently affect oxidative and inflammatory status, groups were normalized by VO₂ max, measured via a standardized incremental cycle ergometer exercise protocol (Ergoline 800S, Sensors Medic, Yorba Linda, CA) until the limit of exercise tolerance (12). This was possible thanks to the very large pool of pediatric subjects enrolled in the UCI Pediatric Exercise Research Center protocols, which allowed to identify, for each obese child enrolled, an age-matched healthy child with similar fitness; therefore, the comparable average fitness values between healthy and obese children did not only reflect overall across-group similarity, but actual, individual, one-on-one matching of participating children.

Gas exchange was measured breath-by-breath (Sensors medic, Yorba Linda, CA) and VO₂ max was calculated and expressed as mL O₂/min/kg lean body mass.

Collection of blood samples

After an overnight fast, participants presented to the laboratory at ~7:30 am; vital signs were verified, and an intravenous catheter was inserted into a median
cubital vein. To minimize the effect of venipuncture stress on measured variables, subjects rested for 90 min prior to blood draw. Peripheral whole blood was then collected in sterile EDTA tubes, and plasma was isolated and frozen at −80°C until day of assay.

**Laboratory measurements**

The F₂-IsoP assay was performed by the Eicosanoid Core Laboratory in the Division of Clinical Pharmacology at Vanderbilt University, using a gas chromatographic/negative ion chemical ionization mass spectrometric (GC/NICI-MS) method with stable isotope dilution (the Laboratory originally discovered and pioneered precise F₂-IsoP measurement techniques beginning in the early 1990s) (13). The remaining assays were performed by the Pediatric Growth Factor and Cytokine Laboratory of the UCI Institute for Clinical Translational Science (ICTS). SOD and GSH-420 were assayed using ELISA and a kinetic, rate based high-sensitivity colorimetric method (Northwest Life Science Specialties, Vancouver, WA), respectively. High sensitivity ELISA was used for quantification of IL-6 (R&D Systems, Minneapolis, MN) and free insulin (LINCO Research, St. Charles, MO). Plasma glucose was measured with a Beckman Coulter Glucose II Analyzer (Beckman Coulter, Fullerton, CA, USA). Standard ELISA was used for cortisol (Diagnostic Systems Laboratories, Webster, TX) and colorimetric techniques for glycerol (Sigma-Aldrich, St. Louis, MO), free fatty acid (FFA) (Zen-Bio, Research Triangle Park, NC), and triglyceride, total cholesterol, LDL-C, and HDL-C (BioAssay Systems, Hayward, CA).

**Statistical analysis**

Data are shown as group means±standard error (SE). IL-6 required log transformation to normalize the distribution of data prior to statistical analyses. Significant differences between experimental groups were detected using unpaired, two-tailed Student’s t-test with a level of significance set at p<0.05. Interrelated effects of demographic characteristics and metabolic profiles (glucose and lipids) were examined using analysis of covariance (ANCOVA) regression analysis (with stepwise backward selection), and applying a linear regression model to the data set. All statistical procedures were performed using JMP software (SAS Institute, Cary, NC).

**Results**

In Ob, body weight, BMI%, and percent (%) body fat were significantly greater than in NW (Table I), with no difference in mean age, gender distribution, Tanner stage, or VO₂max. Mean weight, BMI%, and % body fat for OW were in between Ob and NW values.

**Fasting systemic concentrations of F₂-IsoP, SOD, GSH-420, IL-6**

Compared with NW, the Ob group had increased concentrations of F₂-IsoP (99±7 vs. 75±4 pg/mL, p<0.005) and IL-6 (2.2±0.2 vs. 1.5±0.3 pg/mL, p<0.005) (Figure 1). The antioxidants SOD and GSH-420 were moderately reduced, albeit not significantly, in Ob children (Figure 1). In OW, F₂-IsoP (87±11 pg/mL) and IL-6 (2.0±0.4 pg/mL) concentrations were in between Ob and NW, SOD and GSH-420 were somewhat lower than either group, with no significant difference detected for any of these variables.

**Lipid profiles, glucose, insulin, cortisol, and leukocytes**

When compared with the NW group, Ob children displayed widespread alterations in their lipid profile, with significantly elevated total cholesterol, LDL-C, LDL:HDL ratio, triglycerides, FFA, and reduced HDL-C (Figure 2). Ob also had elevated plasma glucose and insulin compared with NW, while plasma glycerol and cortisol (Ob, 747±58; NW 706±48

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**Table I. Demographic characteristics of the 2 groups and gender subgroups.**

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI%</th>
<th>% Body Fat</th>
<th>VO₂max (mL/min/lean kg)</th>
<th>Tanner</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ob</td>
<td>12.7±0.3</td>
<td>157.1±1.9</td>
<td>74.2±3.0</td>
<td>97.7±0.2</td>
<td>37.3±1.2</td>
<td>41.8±1.1</td>
<td>3.0±0.2</td>
<td>55</td>
</tr>
<tr>
<td>NW</td>
<td>13.4±0.5</td>
<td>156.2±2.4</td>
<td>49.2±2.2</td>
<td>55.8±3.5</td>
<td>20.1±1.3</td>
<td>43.2±1.5</td>
<td>3.1±0.2</td>
<td>43</td>
</tr>
<tr>
<td>Ow</td>
<td>12.4±0.9</td>
<td>156.4±4.8</td>
<td>60.2±5.7</td>
<td>89.9±0.8</td>
<td>28.2±1.9</td>
<td>46.3±2.0</td>
<td>2.9±0.4</td>
<td>15</td>
</tr>
<tr>
<td>Ob-F</td>
<td>12.8±0.6</td>
<td>154.2±2.3</td>
<td>74.9±4.9</td>
<td>98.0±0.3</td>
<td>37.4±0.9</td>
<td>38.6±1.5</td>
<td>3.3±0.2</td>
<td>25</td>
</tr>
<tr>
<td>NW-F</td>
<td>13.0±0.6</td>
<td>154.8±2.9</td>
<td>48.6±2.6</td>
<td>60.0±4.8</td>
<td>24.8±1.3</td>
<td>43.4±1.4</td>
<td>3.2±0.3</td>
<td>25</td>
</tr>
<tr>
<td>Ob-M</td>
<td>12.6±0.4</td>
<td>159.5±2.9</td>
<td>73.6±3.7</td>
<td>97.5±0.3</td>
<td>37.2±2.0</td>
<td>44.2±1.5</td>
<td>2.7±0.2</td>
<td>30</td>
</tr>
<tr>
<td>NW-M</td>
<td>14.0±0.8</td>
<td>158.2±4.1</td>
<td>50.0±3.8</td>
<td>50.5±5.0</td>
<td>13.6±1.7</td>
<td>42.8±3.1</td>
<td>2.9±0.4</td>
<td>18</td>
</tr>
</tbody>
</table>

Ob, obese; NW, normal weight; OW, overweight; yr, year; F, female; M, male; BMI, body mass index; BMI%, BMI percentile adjusted by age (in months) and gender; *, p<0.05 and †, p<0.005 Ob vs. NW in respective groups. Data are means±SE; SE, standard error.
nmol/L) were similar across groups. Again, values from the OW group mostly fell in between Ob and NW (total cholesterol, 4.5±0.3 mmol/L; LDL-C, 2.5±0.1 mmol/L; LDL-C:HDL-C, 1.4±0.2; triglyceride 0.7±0.1 mmol/L; FFA 512±68 µmol/L; glucose 5.2±0.1 mmol/L; insulin 60±17 pmol/L), with no observed statistical difference.

Total WBC (6.6±0.2 vs. 5.8±0.2, ×10³/µL for all units of leukocytes, p<0.05) and neutrophil (3.6±0.2 vs. 3.1±0.2, p<0.05) counts were elevated in Ob compared with NW (Figure 3), while lymphocytes (2.3±0.1 vs.2.0±0.1) and monocytes (0.48±0.02 vs. 0.43±0.02) were not different between groups (Figure 3).

Gender effects

When the Ob and NW groups were subdivided by gender, the differences observed in the groups

Figure 1. Elevated F₂-isoprostane (F₂-IsoP) and interleukin-6 (IL-6) but not superoxide dismutase (SOD) or reduced glutathione (GSH-420) in Ob. Fasting plasma F₂-IsoP and IL-6 were significantly higher in Ob (grey bar) than NW (white bar); SOD and GSH-420 were greater in NW, although not significantly. Data are mean± standard error (SE). †(p<0.005) are significant differences between Ob and NW.

Figure 2. Elevated fasting lipid profile, glycemia, and insulinemia in Ob. Fasting blood LDL-C, LDL to HDL ratio, triglyceride (Trigly), free fatty acid (FFA), glucose, and insulin were significantly higher in Ob (grey bar) than NW (white bar); HDL-C was lower in Ob than NW. Data are mean± standard error (SE). †(p<0.005) are significant differences between Ob and NW.
as a whole remained substantially present in both genders, as shown in Figure 4. ANCOVA analysis confirmed that gender was not significantly associated with our main outcome variables. When females and males were compared within Ob and NW, no difference was observed for F₂-IsoP (107±12 vs. 91.9 and 80±6 vs. 68±5 pg/mL), IL-6 (2.5±0.3 vs. 1.9±0.2 and 1.8±0.4 vs. 1.1±0.2 pg/mL), SOD (61±9 vs. 56±8 and 69±15 vs. 76±16 ng/mL), or GSH-420 (1.3±0.1 vs. 1.6±0.1 µmol/L and 1.7±0.1 vs. 1.4±0.1) (Ob-F vs. Ob-M and NW-F vs. NW-M, respectively).

Figure 3. Elevated total white blood cells (WBCs) and neutrophils but not lymphocytes or monocytes in Ob. Circulating total WBC and neutrophils were significantly higher in Ob (grey bar) than NW (white bar); lymphocytes and monocytes were also somewhat elevated in OB but not significantly. Data are mean±standard error (SE). *(p<0.05) significant differences between Ob and NW.

Figure 4. Elevated F₂-isoprostane (F₂-IsoP), interleukin-6 (IL-6), and fasting lipid profile between OB and NW within genders. Ob (grey bar) and NW (white bar) were compared within genders. F₂-IsoP, IL-6, LDL-C, triglyceride (Trigly), and free fatty acid (FFA) were significantly higher in Ob females (-F) and Ob males (-M) than NW -F and NW -M, respectively; HDL-C was lower in Ob-F and Ob-M than NW -F and NW -M, respectively. Data are mean±standard error (SE). *(p<0.05) and †(p<0.005) are significant differences between Ob and NW.
Interactions between demographic characteristics, metabolic profiles and indices of inflammation and oxidation

Several factors are known to potentially affect our main inflammatory and oxidative outcome variables. We therefore tested, through a general linear regression model, demographic characteristics (gender, pubertal maturation, fitness, BMI%) and glucose/lipid profiles as predictors of our main outcome variables. There was no significant effect of gender, pubertal status and physical fitness on F2-IsoP and IL-6. After adjusting for gender, pubertal status and fitness, free fatty acid (p=0.001), LDL-C (p=0.003), total cholesterol (p=0.009), and BMI% (p=0.003) were found to significantly affect F2-IsoPs. Triglycerides (p=0.006) and BMI% (p=0.019) were found to significantly affect IL-6. Also, higher HDL (p=0.019) and total cholesterol (p=0.005) were associated with higher SOD, and lower BMI% was associated with higher GSH.

Correlations of direct indices of adiposity with IL-6 and F2-IsoP

While BMI is a very valuable screening tool for overweight or obesity, it only provides an indirect assessment of possible body fat mass, and cannot discriminate between central and peripheral adiposity. To confirm that the observed effects of increased BMI on inflammatory/oxidative markers were mediated by a true increase in body fat mass, we plotted F2-IsoP and IL-6 versus DEXA-derived indices of adiposity (whole-body and abdominal fat, expressed as both fat mass or % of total body mass). F2-IsoP was significantly correlated with all indices, with R values for correlations with DEXA derived variables (0.38–0.41) much greater than with BMI percentile (BMI%tile=0.21). A similar pattern was also observed with IL-6, whose R-values for DEXA variables (0.23–0.28) was also greater than the R-value for the correlation with BMI %tile (0.15).

Discussion

In this study, a cohort of 70 overweight and obese children showed simultaneous elevations in inflammatory and oxidative status, paralleled by marked alterations in carbohydrate and lipid metabolism, when compared with healthy, age-, gender-, and fitness-matched controls. Obese children displayed significantly increased markers of systemic inflammation (IL-6, total WBC, neutrophils), systemic lipid peroxidation (F2-IsoP), and moderately decreased antioxidant defenses. Further, while the female gender commonly displays greater resistance than males against inflammatory and oxidative insults (14), in our study population, obesity-related changes were similar in both genders. Taken together, these observations indicate that at an early age, obesity activates key pathogenetic mechanisms known to induce long-term cardiovascular complications. If left untreated, these complications are likely to occur earlier in life, displaying a more aggressive progression, and disrupting multiple additional aspects of physiological growth and development during childhood and adolescence.

The evolution of the cardiovascular complications of obesity has been clearly associated with exaggerated inflammation in animal and adult human models (3,4). In coronary artery disease, elevated cytokines, chemokines, adhesion molecules and leukocytes have been consistently reported both within the vascular endothelium and in systemic circulation (3,15). Among these biomarkers, one of the best characterized and most consistently defined as proinflammatory is IL-6; secreted primarily by macrophages, T-lymphocytes, adipocytes, skeletal muscle, and osteoblasts, IL-6 is known for its role as an acute phase reactant, inducing fever, modulating innate and adaptive immunity, and activating mobilization of energy substrates (16). Long-term systemic IL-6 elevations have been implicated in atherosclerosis, cardiac events, and all-cause mortality (17). In our study, IL-6 was significantly elevated in obese children, confirming prior observations from our and other laboratories (18,19) and corresponding to results from studies in children with other important pediatric dysmetabolic conditions, such as type 1 diabetes (20,21). An exaggerated inflammatory status in our obese group was also demonstrated by higher leukocyte counts, which also confirm prior observations from our laboratory (22). Importantly, among WBC subtypes, the greatest quantitative increase was in polymorphonuclear cells (PMNs), now identified as an independent risk factor and prognostic indicator for future cardiovascular outcomes regardless of disease status (23).

In the context of the interactions between inflammation and dyslipidemia resulting in vascular atherosclerosis, increasing importance is being attributed to the contributory role of oxidative stress, which independently enhances inflammatory processes via common biochemical pathways (i.e., NF-κB) and accelerates atherosclerotic plaque formation via oxidized LDL molecules (24). Elevations of biomarkers of oxidative stress can be induced by acute hyperlipidemia and hyperglycemia, while chronic elevations have been associated with dyslipidemic states, obesity, poorly-controlled diabetes, and coronary artery disease (25). While considerable analytical challenges rendered biomarkers of systemic oxidation traditionally difficult to measure, a recent major
multi-laboratory study concluded that the most accurate biomarker of systemic oxidative injury in vivo is $\text{F}_2\text{-IsoP}$ (26), the most stably quantifiable of several forms of isoprostanes (IsoPs) formed through non-enzymatic free radical-mediated lipid peroxidation of arachidonic acid (13). $\text{F}_2\text{-IsoP}$ levels parallel systemic lipid peroxidation, and reflect the biological activity of the highly reactive cyclopentenone IsoPs (Cyc-IsoPs, products of an alternate lipid oxidation pathway) (6). Cyc-IsoPs have been shown in vitro and in vivo to induce apoptosis of neural cells and leukocytes and to modulate inflammation by interfering with the NF-κB pathway, and serving as ligands for prostaglandin and PPAR-γ receptors (6). Elevated $\text{F}_2\text{-IsoP}$s have consistently been detected in plasma of cardiovascular patients as well as in atherosclerotic plaques (27,28), and have been identified as an independent prognostic indicator for the development of cardiovascular diseases and diabetes (29). The observed 32% $\text{F}_2\text{-IsoP}$ elevation in our group of 55 obese children seems to indicate that excessive lipid peroxidation, with the associated future risk of cardiovascular events, is already present when obesity is established in the pediatric age. Our results are in agreement with recent studies in smaller groups of overweight and metabolic syndrome children, displaying elevated plasma 8-isoprostaglandin $\text{F}_2\text{α}$ when compared with healthy controls (19). Interestingly, a study on 295 15-year-old normoweight children demonstrated that $\text{F}_2\text{-IsoP}$ levels increase with BMI even within the range of healthy body mass (30). Oxidative stress has also often been associated with reduced concentrations of antioxidant molecules. In this study, concentrations of the two measured antioxidants (SOD and GSH-420) were 10–20% lower in obese children than in controls. While differences did not reach statistical significance, the possibility that these modest changes may reflect an initial weakening of antioxidant defense in our obese group is concordant with prior reports on obese animal models (31), obese adult populations (32), and a previous pediatric Hungarian study in which α-tocopherol, β-carotene, and total antioxidant capacity were significantly reduced in obese versus healthy children (33).

Numerous, often marked gender differences exist in many aspects of lipid and carbohydrate metabolism (34). In animal models, females have been shown to display higher concentrations of antioxidant (35) and greater resistance to oxidative damage (36). Similarly, in human studies, higher glutathione peroxidase (37) and lower lipid and DNA oxidation (38,39) were reported in young women compared with men, while higher glutathione were also reported in newborn baby girls versus boys (40), suggesting that some level of protection against oxidant insults in females may already be established at birth. Differences in metabolic behaviors have also been reported, in both genders, at different stages of sexual maturation. In our study, however, obesity-related alterations in lipid profiles and carbohydrate metabolism, as well as increases in inflammatory and oxidative stress markers, were similar across genders, and in early or late-pubertal children. This suggests that the onset of the pathogenetic pathways triggered by obesity may override gender- and maturational-related mechanisms, equally increasing future risk of cardiovascular events in all conditions.

Physical fitness is known to exert independent positive effects on inflammatory and oxidative homeostasis regardless of adiposity status (41). As overweight and obese subjects tend to be generally less fit than their healthy-weight counterparts, expressing across-group differences without normalizing for fitness levels renders it almost impossible to separate effects of obesity per se from those of differing fitness. In our study, we took great efforts to accurately measure fitness via standardized, incremental exercise tests to exhaustion, and to normalize experimental groups by levels of maximal oxygen uptake per lean body mass. Importantly, within our pool of subjects, fitness was not significantly associated with our main inflammatory and oxidative outcome variables. It should be noted that in our study, not designed to assess fitness effects, fitness levels were not evenly distributed over a wide range, but rather clustered around the group mean values, which happened to roughly coincide with previously reported cut-off points for healthy cardio-respiratory profiles. This is probably the cause of the lack of correlation between fitness and inflammatory/oxidative status in our subjects. This of course does not mean that this correlation does not exist, as repeatedly documented in prior studies, but rather that for a similar fitness level, BMI%tile and lipid concentrations are strong, independent predictors of exaggerated inflammation and oxidative stress.

The small group of children in our study population in the 85th–95th BMI% range was included in our statistical analysis only in an exploratory manner, due to its small sample size; interestingly, mean plasma concentrations of key variables measured in the study ($\text{F}_2\text{-IsoP}$, IL-6, leukocytes, components of the lipid panel) were, in general, intermediate between levels of the control and obese groups, and not statistically different from either. We believe that with a sufficient sample size these differences would have likely become significant. This aspect of our study supports the notion of a dose-dependent relationship between BMI and oxidative/inflammatory stress, with alterations beginning with relatively minor BMI increases. In
fact, by further fractioning our experimental population in smaller groups based on increasing BMI, most key variables appear to increase step-wise; mean plasma F_{2}-IsoP, for instance, was 75 pg/mL in NW, 87 pg/mL in overweight children, 90 pg/mL in children with BMI% 95th–97.5th, 102 pg/mL with BMI% 97.5th–99th, and 105 pg/mL with BMI% 99th. These data indicate the necessity of additional, large scale trials to fully identify optimal BMI% cut-offs for diagnostic, disease management and therapeutic purposes.

In conclusion, the obese children in our study displayed dyslipidemia, altered glucose metabolism, and elevated biomarkers of inflammation and oxidative stress. This pattern was present in both genders and over a broad range of fitness. Our data support the concept that at an early age, obesity activates biochemical mechanisms responsible for long-term cardiovascular complications, thereby considerably increasing, if left untreated, the number of years of expected severe morbidity in patients. Our data underscore the urgent necessity of the immediate implementation of widespread preventive strategies to limit the projected, devastating consequences of pediatric obesity in the upcoming decades.

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