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Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children

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Abstract

Obesity has been associated with low-grade chronic systemic inflammation, potentially leading to insulin resistance. This study was designed to examine relationships between cardiovascular risk factors, insulin resistance, and simultaneously measured inflammatory parameters in obese children. We examined serum inflammatory parameters in 115 obese children and 30 normal-weight controls; 62 obese children were followed longitudinally in a 1-year obesity intervention study. Serum concentrations of adipose tissue hormones adiponectin and resistin as well as adipocytokines were assessed. Cross-sectional analysis showed significant correlations between standard deviation score body mass index and resistin ($P = .0004$) as well as monocyte chemoattractant protein–1 (MCP-1, $P = .04$). Increased homeostasis model assessment of insulin resistance index greater than 95th percentile was present in 32% of obese patients, correlating with adiponectin ($r = -0.40$, $P = .0007$). Significant correlations were found between adiponectin and several mediators of inflammation (interleukins [ILs] IL-1β, IL-6, and IL-8 and tumor necrosis factor–α). In longitudinal analysis, substantial weight loss (change standard deviation score body mass index >0.5) observed after intervention in 29 children was associated with a significant decrease in blood pressure, homeostasis model assessment of insulin resistance index, and serum concentrations of insulin and IL-1β, IL-8, and MCP-1, but increase of adiponectin (all Ps < .05). In 33 children without substantial weight loss, resistin and MCP-1 levels increased after 1 year. Changes in IL-1β correlated positively with changes of weight status, interferon-γ, IL-6, IL-8, and tumor necrosis factor–α (all Ps < .01). Our study demonstrates significant correlations between different metabolic risk factors at baseline and after changes of weight status and that weight loss in obese children reduces low-grade inflammation, insulin resistance, and blood pressure.

1. Introduction

Obesity is a common risk factor for what is known as the *metabolic syndrome*, a cluster of potentially life-threatening pathologies including insulin resistance, hypertension, and type 2 diabetes mellitus [1,2]. A common cause of many of the metabolic comorbidities of obesity might be the low-grade systemic inflammation that has been associated with increased fat mass [3], characterized by chronically increased levels of inflammatory cytokines such as tumor necrosis factor (TNF)–α and interleukin (IL)-6. A primary source of many of these cytokines and chemokines is the immune cells located within expanded adipose tissue, as well as the adipocytes themselves [3]. Expansion of adipose tissue associated with obesity results in more blood vessels, more connective tissue fibroblasts, and especially more macrophages. Beside adipocytes, endothelial cells, fibroblasts, and immune cells located in adipose tissue can contribute to secretion of interleukins and inflammatory cytokines. In particular, visceral adipose tissue is known to release more vascular endothelial growth factor, resistin, IL-6, plasminogen activator inhibitor–1, transforming more growth factor–β1, IL-8, and IL-10 per gram of tissue than does abdominal subcutaneous adipose tissue [4]. Many of

Institutional approval: The study protocols were approved by the local standing committees for clinical studies and the committees on ethical practice of the Universities Witten/Herdecke, Bonn, Germany, as well as the institutional review board, Seattle Children's Hospital (12858).

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these cytokines, including TNF-α and IL-6, have been shown to induce insulin resistance, both locally and systemically [3-6]. Similarly, the chemokine monocyte chemoattractant protein (MCP)–1 that is secreted by adipocytes reduces insulin sensitivity [7]. Inflammatory activation of tissue resident immune cells, such as macrophages, has also been shown to affect the physiologic function of adipocytes. For example, the expression of anti-inflammatory adipose tissue hormone adiponectin is reduced in response to TNF-α signaling from immune cells [8].

Enzyme-linked immunosorbent assays have long been the common method for quantitative analysis of cytokines, but are limited in that only one analyte can be measured at a time. The simultaneous measurement of different biomarkers in a single sample has been facilitated through multiplex immunobead technology [9]. The purpose of this study was to use multiplex bead assays for the simultaneous, quantitative measurement of cytokines to test the hypothesis that systemic markers of inflammation correlate with degree of overweight and insulin resistance in obese children, and that weight loss will reduce both systemic inflammation and insulin resistance.

2. Methods and procedures

2.1. Patients

The study protocols were approved by the local standing committees for clinical studies and the committees on ethical practice of the Universities Witten/Herdecke, Bonn, Germany, as well as the institutional review board, Seattle Children's Hospital (12858). Written parental consent and/or patient assent was obtained, and the investigations were conducted according to the principles expressed in the Declaration of Helsinki.

We examined anthropometric markers, fasting glucose, insulin, and inflammatory markers in 115 obese white children and in 30 normal-weight healthy white children of similar age, sex, and pubertal stage (Table 1) who presented either at the Department of Pediatrics, University of Bonn, or at the Department of Pediatric Nutrition Medicine, Vestische Kinderklinik, Datteln, Germany (ClinicalTrials.gov NCT00435734).

Table 1
Baseline parameters of 145 (30 normal-weight, 115 obese) children studied in cross-sectional study

	Normal-weight children	Obese children	*P* value
n	30	115	
Age (y)	11.4 ± 0.44	10.7 ± 0.29	.231
Sex	59% female	49.6% female	.181
Pubertal stage	49% prepubertal	46% prepubertal	.831
BMI (kg/m^2)	21.6 ± 0.75	29.4 ± 0.48	<.0001
SDS-BMI	0.86 ± 0.29	2.67 ± 0.04	<.0001
Insulin (mU/L)	12.8 ± 2.1	16.4 ± 1.2	.034
HOMA-IR	2.3 ± 0.3	3.3 ± 0.2	.073

Data as mean and standard error.

Inclusion criteria included age range between 6 and 17 years, and obesity (body mass index [BMI] >97th age-related percentile) or normal weight (BMI 10th-90th percentile) [10,11]. Exclusion criteria were any medications, smoking, alcohol consumption, endocrine disorders, syndromal obesity, or premature adrenarche. Sixty-two of the 115 obese children (45% male, 44% prepubertal) participated in the 1-year German obesity intervention program "Obeldicks." Briefly, this outpatient intervention program for obese children was based on increased physical activity, nutrition education, and behavior therapy including individual psychological care of the child and his or her family as described in more detail in previous studies [5,11]. Thirty-three (age 11 ± 0.4 years, 42% prepubertal) of the 62 participants did not lose weight, whereas 29 patients (age 11 ± 0.5 years, 45% prepubertal) lost weight effectively. The nutritional intervention consisted of a targeted reduction in fat and sugar intake. Using the L (lambda), M (mu), S (sigma) calculation method described in detail previously [5,10-12], substantial weight loss in the course of 1 year was defined as a reduction of standard deviation score (SDS)–BMI greater than 0.5. The rationale for this categorization was that no improvement in insulin resistance and cardiovascular risk factors can be measured in obese children with a weight loss of less than 0.5 SDS-BMI [5,11].

2.2. Anthropometric data

Obesity was defined according to the International Task Force of Obesity using population-specific data [10]. Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. Standard deviation scores SDS-height, SDS-weight, and SDS-BMI were calculated according to German percentiles as described in detail previously [5,10-12]. Pubertal developmental stage was assessed using the standards of Marshall and Tanner and categorized into 2 groups (prepubertal: boys with pubic hair and gonadal stage I, girls with pubic hair stage and breast stage I; pubertal: boys with pubic hair or gonadal stage ≥II, girls with pubic hair stage or breast stage ≥II).

Systolic and diastolic blood pressures (SBP and DBP, respectively) were measured according to guidelines [13] at the right arm twice after a 10-minute rest in the supine position by using a calibrated sphygmomanometer and then averaged. The cuff size was based on the length and circumference of the upper arm and was as large as possible without having the elbow skin crease obstruct the stethoscope [13]. The intraoperator variability was less than 5% for SBP and DBP.

2.3. Blood parameters

Blood sampling was performed in the fasting status at 8:00 AM, and the samples were centrifuged immediately. Serum specimens for adiponectin, resistin, and cytokines were frozen at −80°C and thawed only once for multiplex

analysis, whereas all blood glucose and insulin determinations were performed directly. Serum insulin concentrations were measured by microparticle enhanced immunometric assay (MEIA; Abbott, Wiesbaden, Germany). Serum glucose levels were determined by colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargmuend, Germany). Intra- and interassay coefficients of variation were less than 5% for both glucose and insulin measurements. Homeostasis model assessment of insulin resistance (HOMA-IR) was assessed from the fasting glucose and insulin concentrations using the following formula: HOMA-IR = (insulin [in microunits per liter] × glucose [in millimoles per liter])/22.5 [14]. We used sex- and age-related HOMA-IR percentiles published by Allard et al [15] for representative childhood HOMA-IR values. Values greater than the 95th percentile were considered as elevated.

Luminex xMAP technology (Luminex, Austin, TX) was used to simultaneously measure serum levels of adiponectin, resistin, or cytokines IL-1β, IL-6, IL-8, interferon (IFN)-γ, TNF-α, and MCP-1 (Millipore, Billerica, MA). The intraassay coefficient of variation was determined by replicate analysis (n = 8) of 2 different samples, the results being 7.8% and 7.5%, respectively. All assays were measured in blinded fashion, and samples before and after intervention were measured in same runs to minimize variations.

2.4. Statistical analyses

Statistical analyses were performed using the Winstat software package (Fitch Software, Bad Krozingen, Germany). Data are presented as mean and standard deviation. In cross-sectional analyses, HOMA-IR and SDS-BMI were correlated to inflammatory markers by Spearman rank correlations because all inflammatory markers were not normally distributed as tested by Kolmogorov-Smirnov test. As cytokine values were not normally distributed, all values were log-transformed prior analysis. Multiple linear regression analyses with log-transformed inflammatory markers as dependent variables were performed with the independent variables age, sex, pubertal stage, SDS-BMI, and all other log-transformed inflammatory markers that had shown a significant correlation to the dependent variable in univariate analysis. Sex and pubertal stage were used as categorical markers in all these models. Correlations in cross-sectional analyses were corrected for multiple tests using the Bonferroni method, and P values < .00625 were considered as statistically significant (see values in bold in Table 2).

For longitudinal analyses, changes were expressed as Δ variable and calculated as variable measured at baseline minus variable 1 year later. Changes in the concentrations of inflammatory markers were correlated to changes of SDS-BMI by Spearman rank correlation. Furthermore, changes in the concentrations of log-transformed inflammatory markers were correlated to changes of HOMA-IR and resistin by partial regression analysis adjusted to changes of SDS-BMI. In addition, children with and without substantial weight loss were compared concerning their concentrations of insulin, adiponectin, resistin, and inflammatory markers. Student t test for paired and unpaired observations of normally distributed values and paired Wilcoxon and Mann-Whitney test for nonnormally distributed values were used as appropriate. A P value < .05 was considered as significant.

3. Results

3.1. Cross-sectional analyses

Thirty-two percent of patients had increased HOMA-IR greater than 95th percentile, which correlated negatively with adiponectin ($r = -0.40$, $P = .0007$). Obese children had significantly higher resistin (35.1 [20.1/56.4] vs 22.6 [16.9/49.1] μg/mL, $P = .034$), higher MCP-1 levels (222 [157/288] vs 184 [112/243] pg/mL, $P = .009$), and slightly though

Table 2
Spearman correlation coefficients of insulin resistance and inflammatory parameters in fasting serum of 145 children (significant results corrected for multiple comparisons are in bold)

		Adiponectin	Resistin	IFN-γ	IL-1β	IL-6	IL-8	MCP-1	TNF-α
HOMA-IR	r	−0.40	−0.252	0.008	0.068	−0.017	−0.058	−0.248	−0.135
	P	**.0007**	**.001**	.4631	.2108	.4220	.2479	**.0015**	.0547
Adiponectin	r	–	0.83	−0.287	−0.427	−0.404	−0.407	0.128	−0.224
	P		**<.0001**	.383	**<.0001**	**<.0001**	**<.0001**	.090	.009
Resistin	r		–	−0.233	−0.318	−0.128	−0.056	0.458	0.008
	P			**.0029**	**.0001**	.0681	.2566	**<.0001**	.4635
IFN-γ	r			–	0.463	0.335	0.157	−0.207	0.158
	P				**<.0001**	**<.0001**	.0312	.0066	.0309
IL-1β	r				–	0.573	0.491	−0.027	0.408
	P					**<.0001**	**<.0001**	.3756	**<.0001**
IL-6	r					–	0.783	0.138	0.292
	P						**<.0001**	.0510	**.0002**
IL-8	r						–	0.241	0.393
	P							**.0019**	**<.0001**
MCP-1	r							–	0.277
	P								**.0004**

not significantly higher TNF-α (15.3 [8.0/28.3] vs 12.2 [6.6/21.7] pg/mL, $P = .11$); but values for IFN-γ (4.9 [1.4/18.0] vs 15.4 [1.4/45.0] pg/mL, $P = .25$), IL-1β (1.44 [0.71/5.0] vs 1.40 [0.83/3.32] pg/mL, $P = .40$), IL-6 (21.7 [2.2/77.2] vs 19.8 [4.9/58.1] pg/mL, $P = .43$), and IL-8 (18.9 [7.4/55.8] vs 15.6 [6.4/53.5] pg/mL, $P = .44$) were not different compared with normal-weight children. Median and interquartile ranges are given for these comparisons. At baseline, univariate Spearman correlation analyses of all 145 children showed significant positive correlations between degree of overweight (as SDS-BMI) and resistin ($P = .0004$) and MCP-1 ($P = .04$), but not IFN-γ, IL-1β, IL-6, IL-8, and TNF-α. Adiponectin correlated negatively to HOMA-IR, IL-1β, IL-6, IL-8, and TNF-α (Table 2). There were strong correlations between different cytokines and chemokines: IFN-γ correlated with IL-1β, IL-6, IL-8, MCP-1, TNF-α, and resistin (Table 2). In multiple regression analyses adjusted to age, sex, pubertal stage, and degree of overweight, all cytokines were log-transformed. Interferon-γ correlated to IL-1β (β coefficient, 0.85 ± 0.22; $P < .001$; $r^2 = 0.21$) and IL-6 (β coefficient, 0.70 ± 0.23; $P = .004$; $r^2 = 0.15$). Interleukin-1β correlated to TNF-α (β coefficient, 0.56 ± 0.11; $P < .001$; $r^2 = 0.49$). Interleukin-6 correlated to IL-8 (β coefficient, 0.77 ± 0.06; $P < .001$; $r^2 = 0.78$) and MCP-1 (β coefficient, 0.16 ± 0.07; $P = .022$; $r^2 = 0.26$). Interleukin-8 correlated to TNF-α (β coefficient, 0.75 ± 0.16; $P < .001$; $r^2 = 0.46$). The children participating in the lifestyle intervention did not differ significantly to the children not participating in the lifestyle intervention in respect to age, sex, stage of puberty, degree of overweight (SDS-BMI), insulin, glucose, insulin resistance index (HOMA), or any inflammatory marker measured in this study.

3.2. Longitudinal analyses

At baseline, sex, pubertal stage, SDS-BMI, inflammatory markers, insulin, and glucose did not differ significantly between the children with and without substantial weight loss (Table 3). In the 29 children with substantial weight loss, a significant reduction of insulin concentrations, HOMA-IR, as well as SBP and DBP was observed. Furthermore, concentrations of IL-1β, IL-8, and TNF-α decreased significantly and adiponectin increased significantly (Fig. 1); but no significant changes were found for IL-6, IFN-γ, and MCP-1. In the 33 children without substantial weight loss, a significant increase in resistin (Fig. 1) and MCP-1 (before, 249 ± 18; after, 290 ± 23 pg/mL; $P = .04$) was observed.

3.3. Correlations of the magnitude of changes between different parameters

Regarding all 62 patients who participated in the lifestyle intervention program, changes of SDS-BMI correlated positively with changes in resistin ($r = 0.20$, $P = .0004$), IL-1β ($r = 0.314$, $P = .0066$), and IL-8 ($r = 0.30$, $P = .009$) and negatively with adiponectin ($r = -0.27$, $P = .018$). Changes of resistin correlated positively with changes of SBP ($r = 0.20$, $P = .03$) and IL-6 ($r = 0.24$, $P = .03$). There were strong correlations between changes of different inflammatory markers (Table 4). Changes of IL-1β correlated significantly with changes of IL-6, IL-8, IFN-γ, and TNF-α (Table 4). Changes of IL-6 correlated significantly with HOMA-IR, resistin, IFN-γ, IL-1β, IL-8, and TNF-α (Table 4).

In partial regression analyses adjusted to changes of SDS-BMI, changes of HOMA-IR were significantly correlated to changes of adiponectin ($r = -0.31$, $P = .02$). Changes of IFN-γ correlated to changes of IL-1β ($r = 0.57$, $P < .001$) and IL-6 ($r = 0.47$, $P < .001$). Changes of IL-1β correlated to changes of IL-6 ($r = 0.48$, $P < .001$), IL-8 ($r = 0.25$, $P = .024$), and TNF-α ($r = 0.56$, $P < .001$). Changes of IL-6 correlated to changes of IL-8 ($r = 0.63$, $P < .001$) and TNF-α ($r = 0.33$, $P = .004$). Changes of IL-8 correlated significantly to changes of TNF-α ($r = 0.48$, $P < .001$). In Spearman rank correlations,

Table 3
Changes of weight status, blood pressure, fasting serum insulin, glucose, as well as insulin resistance index HOMA in obese children with and without substantial weight loss in the course of 1 year

	Substantial weight loss			No substantial weight loss		
n	29			33		
Age (y)	11 ± 0.5			11 ± 0.4		
Sex	34% male			55% male		
Pubertal stage	45% prepubertal			42% prepubertal		
	At baseline	1 y later	*P* value[a]	At baseline	1 y later	*P* value[a]
BMI (kg/m^2)	28.7 ± 2.9	24.2 ± 2.6	<.001	29.4 ± 5.2	29.6 ± 5.5	.354
SDS-BMI	2.3 ± 0.4	1.7 ± 0.4	<.001	2.43 ± 0.48	2.38 ± 0.57	.17
SBP (mm Hg)	118 ± 2	109 ± 1	.001	122 ± 4	115 ± 2	.045
DBP (mm Hg)	64 ± 2	57 ± 2	.009	66 ± 3	64 ± 2	.569
Insulin (mU/L)	15 (9-18)	11 (6-15)	.028	17.0 (12.0-23.5)	18.3 (11.3-23.9)	.701
Glucose (mmol/L)	4.7 (4.4-4.9)	4.6 (4.3-4.9)	.498	4.6 (4.3-4.8)	4.5 (4.2-4.8)	.787
HOMA-IR	3.1 (1.8-4.0)	2.4 (1.3-3.2)	.029	3.6 (2.5-4.7)	2.9 (2.9-4.3)	.924

Data as mean and standard error or median and interquartile range if variable was not normally distributed.
[a] Baseline vs 1 year later; values before and after lifestyle intervention were analyzed by paired tests.

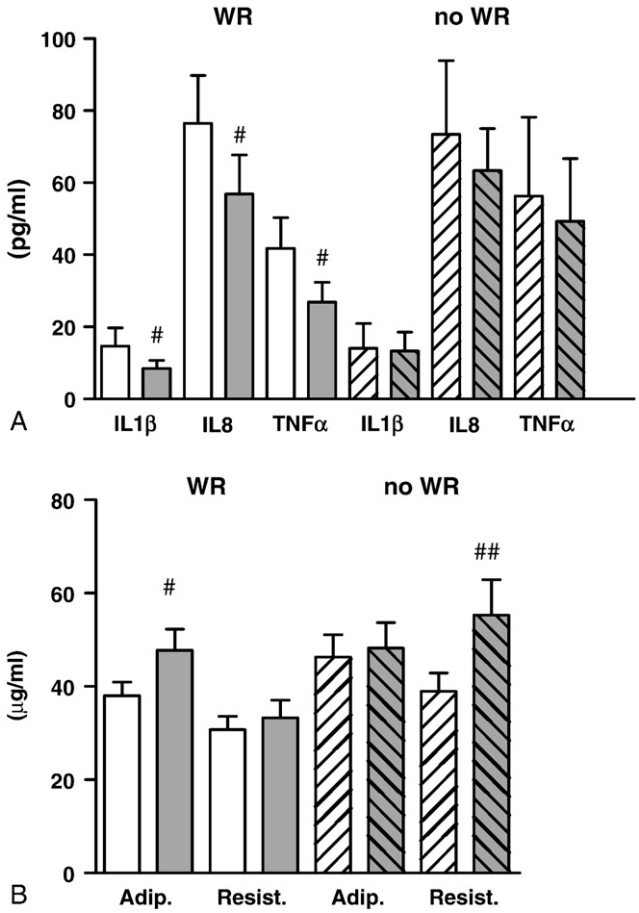

Fig. 1. A and B, Inflammatory markers before after intervention. Serum IL-1β, IL-8, TNF-α, adiponectin, and resistin are shown from patients before (white bars) and after (gray bars) intervention. Groups of significant reduction of overweight (WR, unstriped bars) vs unsuccessful weight reduction (no WR, striped bars) were examined. Pre- vs posttreatment values were compared. $^{\#}P < .05$; $^{\#\#}P < .01$.

changes of SDS-BMI correlated significantly to changes of IL-1β ($r = 0.31$, $P = .006$) and IL-8 ($r = 0.30$, $P = .009$).

4. Discussion

In this study, we examined serum markers of inflammation and their correlations to degree of overweight and insulin resistance in normal-weight vs obese children. To the best of our knowledge, this is the first study where the multiplexed immunobead method has been used to quantify cytokines in both cross-sectional and longitudinal analyses to assess the complex interactions of different adipose tissue signaling peptides and adipocytokines in obese children. Significant correlations were found not only among several inflammatory parameters, but also between inflammatory parameters and clinical markers of overweight and insulin resistance. In cross-sectional analyses, higher resistin and MCP-1 levels were found in obese compared with normal-weight children; and significant correlations could be demonstrated between degree of overweight (as SDS-BMI), adiponectin, resistin, and MCP-1. There were also strong correlations between different cytokines and chemokines, such as IFN-γ, which correlated with IL-1β, IL-6, IL-8, MCP-1, and TNF-α. In longitudinal analyses, BMI reduction was accompanied by reduction of blood pressure, insulin, HOMA-IR values, IL-1β, IL-8, and TNF-α. Weight reduction in obese children led to favorable changes of some inflammatory markers, thereby suggesting an important role in reducing the risk for developing complications of obesity such as type 2 diabetes mellitus. Strong reduction of blood pressure was achieved after lifestyle intervention to a greater degree in patients with weight reduction vs those with no weight reduction. Methods in ascertaining blood pressure were similar at both time points before and after intervention.

Table 4
Spearman correlation coefficients of HOMA-IR, inflammatory parameters, and BMI changes in fasting serum of 62 obese children before and after 1-year lifestyle intervention

		Δ IFN-γ	Δ IL-1β	Δ IL-6	Δ IL-8	Δ MCP-1	Δ TNF-α	Δ SDS-BMI
Δ HOMA-IR	*r*	0.049	0.353	0.039	0.106	0.303	0.146	0.295
	P	.357	.003	.0316	.212	.009	.150	.011
Δ IFN-γ	*r*	–	0.379	0.617	0.466	−0.004	0.224	0.083
	P		.0015	<.0001	.0001	.4871	.0441	.293
Δ IL-1β	*r*		–	0.463	0.506	0.072	0.395	0.313
	P			.0001	<.0001	.2893	.0007	.006
Δ IL-6	*r*			–	0.634	−0.025	0.216	0.024
	P				<.0001	.4230	.0474	.426
Δ IL-8	*r*				–	0.169	0.499	0.300
	P					.0948	<.0001	.008
Δ MCP-1	*r*					–	0.334	0.194
	P						.0040	.066
Δ TNF-α	*r*						–	0.229
	P							.046

Increased physical activity might explain that reduction of blood pressure was also observed even in patients without significant weight reduction.

Resistin is an adipocyte-secreted protein that has been shown to reduce insulin action in mice [16]. Resistin's function in humans is still unclear, but its proinflammatory properties indicate a role in inflammatory processes [17]. Recent animal models show that insulin is not the major regulator of resistin [18]. Two longitudinal adult human studies reported serum resistin changes to be positively correlated with changes in fat mass or weight loss [19,20], yet another adult study reported no correlations [21]. Resistin serum levels studied in obese children after 1 year of weight loss did not show any differences between lean and obese children, and no significant changes after weight loss [22].

Interleukin-1β has been known to impair insulin signaling and action by targeting the insulin receptor substrate [23], and TNF-α has been shown to prevent the normal development of the murine 3T3-L1 preadipocyte cell line to fully differentiated adipocytes and promote an inflammatory adipocyte phenotype [24]. Proper functioning of adipocytes is important in the regulation of insulin action. Of the children who participated in our lifestyle intervention program, changes in SDS-BMI correlated positively to changes of resistin and the inflammatory cytokines IL-1β and IL-8. Previous studies have shown that exposure to inflammatory factors down-regulates the insulin-responsive glucose transporter, GLUT4, expression in adipocytes [25] and that exercise training up-regulates GLUT4 expression in adipocytes and skeletal muscle [26]. As also previously found by Balagopal et al [27], the reduction of inflammation markers by lifestyle intervention observed in this study may reflect the improvement of inflammation that is accompanied by a restoration of GLUT4 expression in adipose tissue.

In the children that did not have substantial weight loss, a significant increase in MCP-1 was observed. Monocyte chemoattractant protein–1 has been found to be involved in attracting monocytes to adipose tissue, which are then able to secrete large amounts of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β in response to cytokines such as IFN-γ. The abundance of adipose tissue macrophages and insulin resistance has been closely correlated [28], which supports the possible role that adipose tissue macrophages may play in obesity-induced adipocytes dysfunction and metabolic disorders [29]. Our cross-sectional correlations between IFN-γ, TNF-α, IL-1β, IL-6, and IL-8 support these previously reported results.

Overweight in children is associated with higher C-reactive protein concentrations and higher white blood cell counts, but lower adiponectin levels compared with normal weight [30,31]. In the present study, we analyzed correlations of weight status and insulin resistance to adipocyte-derived hormones adiponectin and resistin and 6 cytokines. All 6 cytokines, which are expressed and secreted by adipose tissue and released into circulation, are known as factors that are involved in low-grade inflammation leading to cardiovascular risk [32]. Levels of anti-inflammatory peptide adiponectin correlated negatively to HOMA-IR, IL-1β, IL-6, IL-8, and TNF-α. Resistin correlated to SDS-BMI and MCP-1 and inversely to HOMA-IR, IFN-γ, and IL-1β in cross-sectional analysis. Changes of resistin correlated positively with changes of SBP and IL-6. We did not find a reduction of IL-6 levels in the successful weight reduction group, confirming results from Olson et al [33], who did not find changes of IL-6 and C-reactive protein after weight reduction in overweight women participating in a 1-year dietary and fitness program. The possibility exists that a decrease in inflammatory IL-6 levels due to reduction of overweight was counterbalanced by increased muscular injury and inflammation caused by increased physical activity [34], an integral part of the lifestyle intervention "Obeldicks." It is also possible that the Luminex multiplex system may not be ideal to sensitively detect changes for all serum inflammatory molecules.

Standard deviation score–BMI correlated to MCP-1 in cross-sectional analysis, and IL-8 showed significant correlations to all other cytokines in cross-sectional and most cytokines in longitudinal analysis. In a previous large multicenter study in adults with and without coronary heart disease, levels of both MCP-1 and IL-8 correlated with coronary heart disease risk; and both are preceding factors of type 2 diabetes mellitus [35] after adjustment for age, sex, and survey with hazard ratios (95% confidence intervals). There is solid evidence to suggest that the chemokine MCP-1 plays an important role in the development of adipose tissue inflammation in wild-type mice [7]. Other chemotactic agents that might be involved in the recruitment of immune cells into adipose tissue include complement factors 3a and 5a, or leukotrienes such as LTB_4 [36].

Our study has a few potential limitations. The first limitation is that SDS-BMI but not body composition was used to classify study groups as overweight. Although SDS-BMI is a good measure for overweight, one needs to be aware of its limitation as an indirect measure of fat mass. The subjects studied were not morbidly obese; and therefore, even without changes in BMI and/or weight, it is possible that there was an undetected positive redistribution of body composition. Second, the HOMA-IR model is only an assessment of insulin resistance. Clamp studies are actually the criterion standard for analyzing insulin resistance [37]. Third, we compared only prepubertal (Tanner I) vs pubertal (Tanner II-V) children. Potentially, we might have missed differences in cytokine levels that are related to different pubertal stages. Fourth, although we excluded patients with any evidence of infection, subclinical infection is especially common in childhood and could have caused unspecific activation of inflammatory pathways. Compared with the study published by Balagopal et al [27], IL-6 levels measured in our study were much higher. This might be explained by the different immunoassays used in both studies, but it is also possible that the higher values found in few subjects may be due to physiologic differences between the subjects. We are aware that there are relatively high

variations of cytokine levels in serum samples from children and that there are significant heritabilities for circulating cytokines and pleiotropy between cytokines and obesity traits [38].

In summary, our data showed that multiplexed bead assays are useful for the simultaneous evaluation of inflammatory biomarkers to broadly assess the risk for chronic inflammation, which is emerging as a major factor for the development of cardiovascular disease in childhood obesity. Significant correlations were found between several inflammatory parameters. For future studies, if a selection of key cytokines/chemokines is necessary, measurement of IL-1β and IL-8 should be included, as they showed strong correlations with changes of weight status and other inflammatory markers. We found that weight reduction in obese children led to reduction of insulin resistance and blood pressure and was also accompanied by favorable changes in some inflammatory markers. The simultaneous measurement of different inflammatory biomarkers is recommended in future studies of childhood obesity, as it facilitates the assessment of a biochemical risk profile for cardiovascular disease as well as the above-mentioned pathologies associated with obesity.

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