Title

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Journal

International Journal of Obesity, 30(6)

ISSN

0307-0565

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Publication Date

2006-06-01

DOI

10.1038/sj.ijo.0803227

International Journal of Obesity (2006) 30, 906–911
 0307-0565/06 $30.00
www.nature.com/ijo

ORIGINAL ARTICLE

Body fat and circulating leukocytes in children

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Objective: To determine the effects of obesity on baseline levels of circulating granulocytes, monocytes, and lymphocyte subtypes in otherwise healthy children.
Design: Two group comparison of leukocytes in normal weight control and overweight children.
Subjects: In total, 38 boys and girls, ages 6–18 years, divided in two groups: normal weight, (NW, BMI < 85th %tile, $n = 15$) and overweight (OW, body mass index (BMI) > 85th %tile, $n = 23$).
Measurements: BMI obtained from direct measures of height and body mass. Body fat was assessed by DEXA. Complete blood counts (CBC) were obtained by standard clinical hematology methods and surface antigen staining by flow cytometry.
Results: The OW group compared to the NW group had increased total leukocytes counts ($P = 0.011$), neutrophils ($P = 0.006$), monocytes ($P = 0.008$), total T (CD3) lymphocytes ($P = 0.022$), and Helper T ($CD4^+$) cells ($P = 0.003$). Significant correlations were evident between leukocytes, and BMI percentile, BMI, or percent body fat. Neither lean body mass nor VO_{2peak} per unit lean body mass were significantly related to any of the leukocytes. Percent body fat and BMI percentile were positively correlated ($P < 0.05$) to total T cells (CD3) and/or helper T cells ($CD4^+$).
Conclusion: A group of 23 overweight children displayed elevated counts in most types of circulating immune cells, suggesting the presence of low-grade systemic inflammation, a known pathogenetic mechanism underlying most long-term complications of obesity. Our data provide an additional rationale for the importance of avoiding or correcting pediatric obesity.
International Journal of Obesity (2006) **30,** 906–911. doi:10.1038/sj.ijo.0803227; published online 17 January 2006

Keywords: neutrophils; monocytes; granulocytes; lymphocytes; natural killer cells

Introduction

In the last decade, obesity has become one of the most significant public health crises in the United States for both adults and children.[1] Obesity, during adulthood, has been associated with coronary artery disease, diabetes, hypertension, cancer, and joint disease, just to name a few. Although, obesity is associated with numerous medical complications in adults, the implications of obesity in the growing child are not clearly defined.

Research has shown that obese adults, ($BMI \geqslant 30\,kg/m^2$), have elevated total leukocytes.[2,3] The majority of the elevation of leukocytes appears to be related to monocytes[4]; however, neutrophils, eosinophils, and lymphocytes may be elevated.[5] In addition, several groups have observed that obese adults have elevated levels of many proinflammatory cytokines.[6–10] Taken together, these studies suggest that changes in the number of circulating leukocytes in adults reflect some stimulation of stress/inflammatory mediators, such that obesity should be considered a low-level inflammatory condition.[11–13]

Studies have shown that proinflammatory cytokines are positively associated with BMI in both boys and girls.[14–16] However, these studies did not evaluate the influence of BMI on the leukocyte profile. Visser *et al.*[16] did observe an elevation in total leukocyte count in obese youth; however, they did not present any breakdown of the specific cells contributing to the elevation. Knowledge of which of the leukocytes are elevated is important, since monocytes and lymphocytes (T cells) are known to contribute to the cytokine population. Furthermore, knowing the circulating levels of neutrophils and eosinophils as well as monocytes and lymphocytes, in obese children may be important in understanding evolution of inflammation and diseases.

Exercise influences the circulating levels of anti and proinflammatory mediators in both children and adults. Exercise training in adults resulting in improved aerobic power (VO_2max), is related to lower levels of inflammatory

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Received 5 February 2005; revised 18 November 2005; accepted 29 November 2005; published online 17 January 2006

mediators in the circulation.[17–19] The reduction in inflammatory mediators may be one way exercise training may protect against cardiovascular disease and reduced sensitivity to insulin. In children, exercise training and aerobic power are associated with increases in circulating cytokines levels.[14,20] These authors did not present any information regarding the leukocyte populations, which is important since the leukocytes are one of the primary sites of cytokine production. Thus, the purpose of this study was to determine the effects of obesity and aerobic power on resting leukocyte profiles. We hypothesized that circulating levels of leukocytes are altered in overweight compared to normal weight children.

Methods

Subjects

In total, 38 boys and girls (19 of each gender) ages 6–18 years, were recruited for this study, which was conducted at the UC Irvine General Clinical Research Center (Table 1). Exclusion from participation included history of any chronic medical conditions or use of any medications. The Institutional Review Board at the University of California, Irvine approved this study and written informed consent and assent was obtained by all participants and their parents upon enrollment into the study.

Based on NHANES age- and gender-specific norms, they were divided into a normal weight group (NW, BMI percentile <85th; $n=15$) and overweight group (OW, BMI percentile >85th; $n=23$).[21] Within the overweight group, seven children (4 m/3 f) had a BMI between the 85th and 95th %tile, and the remaining 16 (8 m/8 f) a BMI > the 95th %tile. Whereas the small size and uneven distribution of these two subgroups did not allow for meaningful subgroup comparisons, for completeness the sorted data are presented as ancillary findings of the study at the end of the results section.

Tanner staging to assess pubertal status could not be performed in this study. However, an approximate evaluation of the subjects' pubertal status could be derived by the distribution of their age with respect to the average age of sexual maturation stages. Children were considered prepubertal if <11years (boys) or <10 3/12 years (girls), whereas mid-puberty was estimated at ~13.5 years (boys) and ~12.5 years (girls). According to this criteria (provided by consultation with the UC Irvine pediatric endocrinology clinic), a similar proportion of children was estimated to be considered prepubertal (20% in NW, 17% in OW), early pubertal (40% in NW, 32% in OW) and late pubertal (40% in NW, 52% in OW) in the two experimental groups.

Table 1 Mean ± s.d. for the physical characteristics of the subjects in the normal weight (BMI <85th%tile) and overweight (BMI >85th%tile) groups

Variable	*Normal weight group (n = 15)*	*Overweight group (n = 23)*
Age (year)	12.4 ± 3	12.3 ± 3
Gender		
Female	7	12
Male	8	11
Ethnicity		
Asian	6	4
Black	1	2
Caucasian	6	9
Hispanic	0	4
Other[a]	2	4
Height (cm)	153 ± 17	157 ± 14
Body mass (kg)	44.2 ± 14	74.0 ± 24*
BMI (kg/m^2)	18.5 ± 2	29.5 ± 7*
BMI percentile	44.8 ± 27	95.8 ± 4*
Body fat (%)	19.9 ± 6	37.8 ± 9*
Lean body mass (kg)	34.9 ± 12	42.9 ± 10*
VO_{2peak} (ml/kg/min)	32.1 ± 7	23.5 ± 7**
VO_{2peak} (ml/kg_{LBM}/min)	40.5 ± 6	38.5 ± 7

*$P \leqslant 0.0006$ normal weight group vs overweight group. **$P < 0.04$ normal weight group vs overweight group. [a]Ethnicity could not be assigned to a single racial group.

Anthropometric measurements

Standard, calibrated scales and stadiometers were used to determine height, body mass, and body mass index (body mass index (BMI) = kg/m^2). As BMI changes with age, we calculated BMI percentile for each child using the recently published standards from the Centers for Disease Control, National Center for Health Statistics.[21] Subjects were classified as normal weight (NW = <85%tile) or overweight (OW = >85%tile). Assessment of body fat was made by DEXA, using a Hologic QDR 4500 densitometer (Hologic, Inc. Bedford, MA, USA.) and pediatric software. On the days of each test the DEXA machine was calibrated using the procedures provided by the manufacturer.

Peak aerobic power

To prevent the confounding effect between groups of a different level of fitness, a well-known modulator of circulating leukocyte counts, experimental groups were normalized through prestudy assessment of aerobic power. Each subject performed a ramp-type progressive exercise test to volitional fatigue on a cycle ergometer to measure peak oxygen uptake (VO_{2peak}). Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath-by-breath and the VO_2 computed using a Sensor Medics metabolic system (Sensor Medics Corporation, Anaheim, CA, USA).

Blood sampling and analysis

Blood samples were drawn into a Vacutainer containing sodium heparin using standard phlebotomy. Hematocrit levels were determined using standard technique. Complete

blood counts (CBC) were obtained by standard methods from the clinical hematology laboratory at UCI.

Surface antigen staining

The following monoclonal antibodies (mABs) directed against human cell surface markers were used: CD3 (Total T cells), CD4 (helper T cells), CD8 (cytotoxic T cells), CD19 (B Cells), and CD16/56 (natural killer (NK) cells). All mABs and appropriate isotype controls were purchased from Pharmingen/Becton Dickinson (San Diego, CA, USA). Surface antigen-specific fluorescent-conjugated mABs were added to labeled 12×75 mm tubes, within 2 h of blood collection, 100 μl of blood from each sample was added to the tubes. The tubes were mixed well and incubated in the dark at room temperature for 30 min. FACS Lysing Solution (Becton Dickinson, San Jose, CA, USA) was then added to the lysed red cells. The tubes were mixed gently and incubated for 10 min at room temperature in the dark. Cell suspensions were then centrifuged at 500 *g* for 5 min. The supernatant was removed whereas being careful not to disturb the cell pellet. Cells were washed using 2 ml wash buffer ($1 \times$ PBS containing, 5% bovine serum albumin and 0.1% sodium azide) and centrifuged at 500 *g* for 5 min. Cells were fixed in 500 μl of 1% paraformaldehyde in $1 \times$ PBS. Samples were analyzed using a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA). CaliBRITE beads and FACSComp software were used for setting the photomultiplier tube (PMT) voltages and the fluorescence compensation, as well as checking instrument sensitivity prior to use. A forward scatter threshold was used to acquire 100 000 events for each prepared sample. Data analysis was accomplished using CellQuest software (version 3.2.1).

Statistical analysis

Data analysis was perfomed by the UCI GCRC Biostatistics Core. The NW and OW groups were compared using *t*-tests with Bonferroni corrections for multiple comparisons. Linear correlations were computed between the leukocytes and body fat, lean body mass, and peak aerobic power. Data are presented as mean $\pm$ s.d. SAS statistical software (SAS, Cary, NC, USA) was used for all analyses.

In addition, for exploratory purposes only, the data from the OW group were further subdivided by BMI (BMI = 85–95 percentile, $n = 7$ and BMI > 95th percentile; $n = 16$), and compared to the normal weight group. Although the original study design was not powered to assess these differences, they are reported for completeness with the intent to address the potential curiosity of some readers on this issue.

Results

Based on the NHANES age- and gender-specific norms data, 15 children fell into the NW group and 23 children were classified as OW (Table 1). Both groups had similar age and gender distribution (χ^2; $P > 0.37$). The OW group had a mean BMI percentile of 96 and a body fat content of $\sim$38%. The BMI of the NW group averaged 45th percentile, whereas body fat was $\sim$20%. The OW group was taller, weighed more, and had a higher lean body mass content than the NW group. The peak aerobic power (VO_{2peak}) of the NW group was significantly greater than the OW group when expressed per kilogram body mass (ml/kg/min), but was similar ($P = 0.373$) when expressed per kilogram lean body mass (ml/kg_{LBM}/min). Since the ages and gender distribution were similar for both groups, further leukocyte analyses used intact NW and OW groups.

The leukocyte data are presented in Figure 1 and Table 2. The OW group had an overall increase in total leukocytes counts ($P = 0.011$). Significant increases in the cell numbers were noted in neutrophils ($P = 0.006$), monocytes ($P = 0.008$), and in total T lymphocytes ($P = 0.022$) comparing the OW group with the NW group. Based on surface maker analyses (Table 2), the increase in total T cells in the OW group was a result of an increase in Helper T (CD4) cells ($P = 0.003$).

Modest, but significant correlations were evident when comparing leukocyte numbers, BMI percentile, BMI, and percent body fat (Table 3). In addition, neither lean body mass nor VO_{2peak} per unit of lean body mass, were significantly related to any of the leukocytes. However, the correlation between total granulocytes or neutrophils and VO_{2peak} expressed in ml/kg body mass/min were significant.

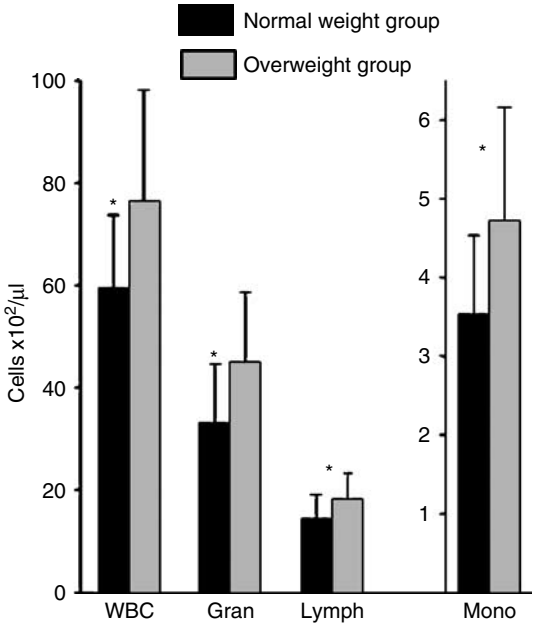

Figure 1 Circulating levels of total white blood cells (WBC), total granulocytes (Gran), total lymphocytes (Lymph), and monocytes (Mono) in 23 overweight children (BMI > 85th %tile) and 15 normal weight children (BMI < 85th %tile). Data are presented as group mean $\pm$ s.d. *$P < 0.02$–0.01 between groups.

Table 2 Mean ± s.d. leukocyte subpopulation breakdown for the normal weight and overweight groups

Leukocytes ($\times 10^9$/l)	*Normal weight (n = 15)*	*Overweight (n = 23)*	P-*value*
Total count	5951 ± 1422	7641 ± 2176	**0.011**
Granulocytes	3310 ± 1138	4481 ± 1376	**0.010**
Neutrophils	3094 ± 1171	4287 ± 1264	**0.006**
Eosinophils	207 ± 134	186 ± 153	0.668
Lymphocytes			
Monocytes	353 ± 101	473 ± 144	**0.008**
Lymphocytes	2168 ± 458	2689 ± 755	**0.022**
Total B cells ($CD19^+$)	344 ± 158	480 ± 272	0.090
Total T cells ($CD3^+$)	1444 ± 347	1808 ± 505	**0.020**
Helper T cells ($CD3^+CD4^+$)	753 ± 167	1023 ± 296	**0.003**
Cytotoxic T cells ($CD8^+$)	541 ± 157	660 ± 267	0.128
NK cells ($CD16/56^+$)	317 ± 186	318 ± 134	0.989

Significant *P*-values in bold.

Correlations were also computed between the lymphocyte subsets and measures of fitness and fatness (Table 4). Few of these correlations were significant ($P<0.05$); however, BMI percentile and percent body fat were significantly correlated to total T cells and/or helper T cells.

Exploratory analysis by BMI subgroups

When further subdivided by BMI (<85th%, 85–95th% >95th%), our study population displayed significant overall differences for total counts (BMI <85th%: 5950 ± 1422; BMI 85–95th%: 7036 ± 1978; BMI >95th%: 7906 ± 2265 $\times 10^9$/l), neutrophils (BMI <85th%: 3094 ± 11172; BMI 85–95th%: 3679 ± 848; BMI 85–95th%: 4554 ± 1345 $\times 10^9$/l; BMI < 85th% vs BMI >95th, $P<0.01$), and monocytes (BMI <85th%: 353 ± 101; BMI 85–95th%: 503 ± 95; BMI >95th: 460 ± 162 $\times 10^9$/l: BMI <85th% vs BMI 85-95th%, $P<0.03$). There were no significant differences between the three subgroups for eosinophils (BMI <85th%: 207 ± 134; BMI 85–95th%: 160 ± 133; BMI >95th: 197 ± 163 $\times 10^9$/l) or lymphocytes (BMI <85th%: 2168 ± 458; BMI 85–95th%: 2778 ± 837; BMI >95th 2650 ± 741 $\times 10^9$/l). The majority of lymphocyte subsets were also not significantly different between the three subgroups, except Helper T cells (BMI <85th%: 753 ± 167; BMI 85–95th%: 932 ± 291; BMI >95th: 1064 ± 299 $\times 10^9$/l; BMI <85th% vs BMI >95th, $P<0.01$).

Discussion

Our data supports the observation that overweight children have higher levels of circulating leukocytes, when compared to normal weight children. Thus, our data concurs with previous observations by Visser *et al*.[16] and Bao *et al*.,[22] who noted that overweight children have elevated total circulating leukocyte counts. Our study extends the findings of these two studies, showing that the increase in circulating leukocytes is related to increase neutrophils, monocytes, and lymphocytes. Increased adiposity appears to increase leukocytes within the circulation.

The increases in leukocytes observed in our overweight children are similar to results that have been reported in adults.[2–5] In adults, a high leukocyte count has been shown to be an independent risk factor for coronary heart disease,

Table 3 Correlation matrix for pairwise correlations between measures of anthropometry (BMI, BMI percentile, % body fat, lean body mass) or fitness (VO_{2peak}: ml/kg/min, ml/kg_{LBM}/min) and leukocytes

Variable	*Total count*	*Granulo cytes*	*Neutro-phils*	*Eosino-phils*	*Lympho cytes*	*Mono cytes*
BMI	0.312**	0.399*	0.426*	−0.129	0.124	0.314
BMI%tile	0.391*	0.431*	0.469*	−0.209	0.333**	0.410*
Body fat (%)	0.339**	0.478*	0.502*	−0.059	0.115	0.322**
Lean body mass	0.063	0.100	0.120	−0.155	−0.066	0.004
VO_{2peak} (ml/kg/min)	−0.301	−0.459*	−0.483*	0.070	−0.110	−0.254
VO_{2peak} (ml/kg_{LBM}/min)	−0.069	−0.174	−0.186	0.041	0.001	−0.109

*$P\leqslant 0.01$; **$P\leqslant 0.05$.

Table 4 Correlation matrix for pairwise correlations between measures of anthropometry (BMI, BMI percentile, % body fat, lean body mass) or fitness (VO_{2peak}: ml/kg/min, ml/kg_{LBM}/min) and measured lymphocyte subsets

Variable	*T cells total ($CD3^+$)*	*T cells helper ($CD4^+$)*	*T cells cytotoxic ($CD8^+$)*	*B cells total ($CD19^+$)*	*NK cells ($CD16/56^+$)*
BMI	0.103	0.291	−0.028	0.041	0.088
BMI%tile	0.374**	0.469*	0.212	0.276	−0.201
Body fat (%)	0.187	0.366**	0.005	0.021	−0.182
Lean body mass	−0.095	−0.044	−0.004	−0.050	−0.045
VO_{2peak} (ml/kg/min)	−0.127	−0.261	0.023	−0.186	0.213
VO_{2peak} (ml/kg_{LBM}/min)	−0.016	−0.032	−0.180	−0.202	0.338#

*$P\leqslant 0.01$; **$P<0.025$; #$P<0.05$.

such that a 1 billion reduction in total leukocyte count results in a 14% decreased in risk of CHD.[23,24] The mechanism for this effect is still speculative, but is probably related to the granuolcytes and monocytes release of free radical, procoagulants, and proteolytic enzymes that can exacerbate arteriosclerosis and clotting. In addition, monocytes release tissue necrosis factor-α (TNF-α) that has been related to insulin resistance.[25,26] We noted that in our overweight youth neutrophils (39%) and monocytes (34%) increased more than lymphocytes (24%). Since the granulocytes and monocytes appear to be related to CHD,[27,28] our results suggest that these detrimental processes may start very early on in life. Thus, childhood obesity has the potential to intensify the process resulting in an earlier onset of CHD.

Our normal weight group data appears to be at the low end of the normal limits for leukocyte counts[22,29]; whereas the overweight group appears to be slightly above normal. These differences were not related to gender representation between the groups. Bao *et al*.[22] have suggested that girls may have slightly higher total counts than boys. Other studies [16,29] using larger samples sizes than Bao *et al*.[22] have noted no significant differences between genders. A potential factor contributing to leukocyte counts not accounted for in this study was pubertal status, that may exacerbate gender differences.[22] However, the NW and OW groups in our study had the same proportion of pre-, early- and late-pubertal children, based on gender-adjusted mean age of developmental stages, rendering it unlikely that major pubertal status differences were present and interfered with our reported results. It would therefore appear that in peripubertal children, obesity can influence leukocyte status independent of gender.

Nieman *et al*.[5,30] has reported that in obese and nonobese adults and elderly women, aerobic fitness (VO_2max) was a 'potent factor' predicting lymphocyte proliferation and NK cell function. Thus, we wondered if aerobic power (VO_{2peak}) was related to circulating leukocyte concentrations in children. The results of our correlations (Tables 3 and 4) do not support a strong relationship between VO_{2peak} and leukocytes or NK cells. There was a weak, but significant, relationship between NK cells and VO_{2peak} expressed in units of lean body mass, but the relationship only accounted for 11% of the total variance, suggestive that some there may be some small effect of fitness independent of obesity. There are several possible reasons for the differing results. First, we used children with a relatively narrow range of VO_{2peak} and no highly fit individuals (our range: 14–41 ml/kg/min), whereas Nieman *et al*.[22] used adults with a larger range of responses ($\sim$14–50 ml/kg/min). Second, we had a relatively small sample size that could have limited our ability to find a significant correlation. Interestingly, Nieman *et al*.[22] reported significant correlations in the range of 0.21–0.28; similar to our results of an $r = 0.213$ (Table 4). Third, the methodology used by Nieman *et al*.[22] to determine VO_2max includes weight (ml/kg/min), and since their obese subjects weighed substantially more with greater fat mass than the normal weight subjects, their ml/kg/min would compute to be low, yet their cell count high. We chose to eliminate the fat mass (ml/kgLBM/min) and when doing so, we found only a marginal relationship. Thus, our data would suggest that in children, weight status mediates leukocytes more so than fitness, but there is a small measurable obesity independent effect as well.

Table 3 shows that the relationships between total leukocyte count, granulocytes, or neutrophils with percent body fat were very strong, indicating that fat mass is likely the critical component that influences leukocyte counts. The fact that for the direct measurement of body fat we utilized DEXA, the emerging new gold standard technique for this type of measurements, guaranteed accurate assessment independent of the wide range of body composition (9–53% body fat), that would have limited accuracy with most other available techniques. BMI and BMI % in our study also correlated reasonably well with white blood cells (WBC) counts, although slightly less than for fat mass, confirming that these may be useful indices in field studies, where more sophisticated equipment may not be available. In fact, our data confirm those by Visser *et al*.,[16] who also reported increased total WBC in children with obese, that was assessed by BMI only.

If obesity is considered an inflammatory disease,[12,16] then one might expect that NK (CD16/CD56) cell number, or activity, should be increased. In contrast, the adult literature suggests that NK cell activity may not be changed by obesity.[5,30,31] Thus, our results would suggest that the response of children is similar to adults. It is interesting to note that the helper T cells (CD4) were elevated in our overweight children, suggesting an inflammatory state. Similar results have been published in adults,[5] but the reason appears obscure.

In summary, our data suggest that even with a relatively small sample size, obese children have an elevation in circulating leukocyte counts, in particular neutrophils, monocytes, and lymphocytes. Although the mechanisms of these increases are not clear, obesity in children, like in adults, is related to increased circulating cytokines, like IL-6 and TNF-α,[14] which may contribute to the increased numbers of circulating leukocytes.[32] In addition, it appears that aerobic fitness, independent of its effect on body composition, only minimally influences circulating leukocyte levels in children and adolescents. Since the literature in adults has concluded that elevation of leukocytes contributes to several disease states, significant efforts must be implemented to keep the weight of children within normal limits or reducing weight if the child is overweight in order to reduce the potential harmful effects of the obesity-induced inflammatory state.

Acknowledgements

We would like to acknowledge the outstanding support provided by the nursing and support staff of the UCI General

Clinical Research Center. This research was supported by NIH Grants MO1 RR00827 and RO1-HL080947.

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