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Reduced tetanus antibody titers in overweight children

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Abstract

Under-nutrition impairs immune responses, but far less is known about the impact of over-nutrition, such as obesity, on the response to vaccines. We measured the effect of childhood overweight status on inflammatory mediators, circulating immunoglobulins and tetanus antibodies in fifteen overweight children (BMI $>$ 85 age-adjusted percentile) and 15 age-matched normal weight controls. Fitness was measured by a progressive ramp type exercise test. Lean body mass (LBM) and fat mass were determined by DXA. Tumor necrosis factor-a (TNF-a), interleukin-6 (IL-6), interleukin-1 β (IL-1β) and interleukin-1 receptor antagonist (IL-1ra) were used to assess the inflammatory status; and circulating immunoglobulins (IgM, IgA, IgG and IgG subclasses) and specific IgG titer to tetanus were used to assess humoral immunity. Overweight children had higher LBM and percent fat mass, and lower peak VO_2 normalized to body weight. IL-6 was significantly higher in the obese children (2.6 ± 0.3 vs. 1.3 ± 0.3 pg/ml, in overweight and normal weight children, respectively; $p < 0.05$). No significant differences were found in TNF-a, IL-1β and IL-1ra between the groups. No significant differences were found in immunoglobulin levels (IgM, IgA, IgG and IgG subclasses) between the groups. Anti-tetanus IgG antibodies were significantly lower in the overweight children compared to normal weight controls (2.4 ± 0.6 vs. 4.2 ± 0.5 IU/ml, in overweight and normal weight children, respectively; $p < 0.05$). The reduced specific antibody response to tetanus in obese children and adolescent might be due to mechanical factors such as lower relative vaccination dose, or reduced absorption from the injection site due to increased adipose tissue, or related to reduce immune response due to the chronic low grade inflammation expressed by the higher levels of IL-6.

Keywords: *Childhood, obesity, tetanus, immunoglobulin, humoral immunity*

Introduction

Altered energy balance can impair immune function and the response to vaccines in children [1]. In particular, the adverse impact of under-nutrition on immunity and infectious diseases in children is substantial and well-studied. But far less is known about the immune effects of other abnormal conditions of energy balance such as obesity, now a virtual epidemic in children in many areas of the world [2]. There are emerging data to suggest that in otherwise healthy children, obesity and levels of physical activity can, indeed, alter immune and inflammatory function [3–5]. For example, in adults, Weber and coworkers [6] and in children, Simo-Minana and coworkers [7] found reduced antibody responsiveness to hepatitis B vaccine in obese individuals.

The mechanisms for this impaired responsiveness to hepatitis B are not known, nor is it known whether antibody titers to other vaccines are also influenced by obesity. Mechanical factors, such as differences in concentration and volume of distribution of the vaccine in obese compared with normal weight individuals could play a role [8]. Alternatively, the impaired response to vaccines might be related to the chronic, low-level inflammatory state (characterized by elevated cytokines such as IL-6 and TNF-a) now known to be associated with obesity in adults and children [9].

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Indeed, in adults with a history of cytomegalovirus infection, those with higher levels of circulating IL-6 (indicating chronic inflammation) were significantly less likely to respond to subsequent influenza vaccine [10]. Consequently, we hypothesized that the altered vaccine responsiveness found in obesity reflected systemic immune alteration and would, therefore, also be found in the antibody titers for other common immunizations, specifically, to tetanus.

To test this hypothesis, we evaluated fitness, levels of circulating inflammatory mediators, and the humoral immune system in a group of obese and normal-weight children and adolescents. The inflammatory mediators tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and the anti-inflammatory mediator interleukin-1 receptor antagonist (IL1ra) were used to assess the inflammatory status, and circulating immunoglobulins (IgM, IgA, IgG and IgG subclasses) and specific IgG antibody titer to tetanus were used to assess humoral immunity.

Methods

Subjects

Thirty subjects (age range 8–17) participated in this study (Table I). Fifteen subjects were overweight (BMI > 85%) and 15 subjects had normal body weight. The participants were matched by age. Individuals with a history of any chronic medical conditions or use of any medications were excluded from participation. The Institutional Review Board at the University of California, Irvine approved the study, and written informed assent and consent was obtained from all participants and their parents upon enrollment.

Immunization history

We determined immunization history using an interview with the participants and their parents. All subjects had received childhood immunizations at the recommended intervals. None of the participants reported having received additional anti-tetanus boosters.

Table I. Anthropometric characteristics of the study participants.

	Normal weight (n=15)	Overweight (n=15)
Age (years)	13.2 ± 0.7	12.9 ± 0.6
Male/female (#)	8/7	8/7
Height (cm)	153.6 ± 4.4	154.9 ± 3.3
Weight (kg)	44.8 ± 3.6	71.5 ± 5.7⋆
BMI (kg/m^2)	18.4 ± 0.7	29.1 ± 1.6⋆
BMI percentile (%)	42.1 ± 6.6	95.3 ± 1.0⋆
Percent fat (%)	18.9 ± 1.4	36.2 ± 2.0⋆
Lean body mass (kg)	35.7 ± 3.1	42.1 ± 2.5⋆
VO_2 peak (ml/min/kg)	32.3 ± 1.8	25.0 ± 1.3⋆
VO_2 peak (ml/min/kg$_{LBM}$)	40.2 ± 1.8	40.3 ± 1.4

⋆Statistical significance compared to normal weight, $p < 0.01$.

Anthropometric measurements

Standard, calibrated scales and stadiometers were used to determine height, body mass, and body mass index (BMI = wt/ht^2). Since BMI changes with age, we also calculated BMI percentile for each child using the recently published standards from the Centers for Disease Control, National Center for Health Statistics [11].

Body composition assessment by DEXA

Fat mass and muscle mass were measured by DEXA using the hologic QDR 4500 densitometer (Hologic Inc., Bedford, MA, USA), a well-established technique for assessing body composition in children. Subjects were scanned in light clothing while lying flat on their backs. DEXA scans were performed and analyzed using pediatric software, and the DEXA was calibrated routinely.

Measurement of fitness

Each subject performed a ramp-type progressive cycle-ergometer exercise test to the limit of his/her tolerance [12]. Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath-by-breath and the peak VO_2 was calculated using the Sensor Medics metabolic system.

Blood sampling and analysis

Morning fasting blood samples were drawn using standard phlebotomy. Blood samples were spun at 3000 rpm, at 4°C for 20 min. The serum was separated and stored at −80°C. All samples were analyzed in the same batch by an experienced technician who was blinded to the individual's group (overweight or normal weight).

Tumor necrosis factor-alpha (TNF-α). TNF-α serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system; Minneapolis, MN). Intra-assay CV was 8.7–14.8%, inter-assay CV was 16.1–22.6%, and the sensitivity was 0.18 pg/ml.

Interleukin-6 (IL-6). IL-6 serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system).

Intra-assay CV was 3.8–11.1%, inter-assay CV was 7.1–29.5%, and the sensitivity was 0.0094 pg/ml.

Interleukin-1 beta (IL-1β). IL-1β serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system). Intra-assay CV was 1.6–4.0%, inter-assay CV was 5.3–9.0%, and the sensitivity was 0.059 pg/ml.

Interleukin-1 receptor antigen (IL-1ra). IL-1ra serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system). Intra-assay CV was 3.1–6.2%, inter-assay CV was 4.4–6.7%, and the sensitivity was 22 pg/ml.

Immunoglobulins. Levels of IgA, IgM, IgG and IgG subclasses 1 through 4 were measured using commercially available kits. Total IgA, IgM and IgG were detected using Beadlyte Human kit (Upstate technologies, Lake Placid, NY). IgA sensitivity was >0.7 ng/ml. IgM and IgG sensitivity was >6.0 ng/ml. IgG subclasses levels were determined using the Beadlyte Human IgG subclass isotyping kit (Upstate, Lake Placid, NY). IgG1 sensitivity was 3 ng/ml, IgG2 sensitivity was 16 ng/ml, IgG3 sensitivity was 0.2 ng/ml, and IgG4 sensitivity was 0.1 ng/ml.

Tetanus titer. Tetanus titers were determined using ELISA (SCIMEDX, Denville, NJ), Intra-assay CV was 4.6–8.8%, inter-assay CV was 6.6–10.2%, and the sensitivity was 0.1 IU/ml. Tetanus titer ranges are: <0.01 IU/ml indicate no protection, 0.01–0.1 IU/ml indicate uncertain protection, >0.1–0.5 IU/ml indicate short-term immunization, and >5 IU/ml indicate long-term protection. With the cut-off of 0.1 IU/ml, 92% of blood donors show immunization protection.

Statistical analysis

Unpaired *t*-test was used to assess differences in anthropometric measurements, inflammatory cytokines, immunoglobulins and tetanus titer levels between obese and normal weight children. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SEM.

Results

Subject characteristics

Anthropometric characteristics of the study participants are summarized in Table I. By design, the group of children classified as overweight had significantly higher body weight, BMI, BMI percentile, percent body fat and lean body mass (LBM). Peak VO_2 was significantly lower in obese children when normalized to body weight, but not when normalized to LBM.

Table II. Circulating levels of inflammatory cytokines in normal and overweight children.

	Normal weight (n=15)	Overweight (n=15)
IL-6 (pg/ml)	1.3 ± 0.3	2.6 ± 0.3*
TNF-α (pg/ml)	2.2 ± 0.6	2.3 ± 0.3
IL1-β (pg/ml)	0.20 ± 0.07	0.17 ± 0.02
IL1ra (pg/ml)	347.2 ± 65.8	456.1 ± 70.0

*Statistical significance, $p < 0.05$.

Inflammatory cytokines

Circulating levels of inflammatory cytokines in the study participants are summarized in Table II. Circulating IL-6 was significantly higher in overweight compared to normal weight children. IL-6 was significantly correlated BMI percentile ($r = 0.53$, $p < 0.01$), and negatively correlated with VO_2 peak/kg ($r = -0.51$, $p < 0.01$). There were no significant differences in TNF-α, IL-1β and IL1ra levels between the groups.

Immunoglobulins and anti-tetanus titer

Circulating levels of immunoglobulins are summarized in Table III. There were no significant differences in IgM, IgA, IgG, and IgG subclasses 1–4 between the groups. IgG anti-tetanus titer was significantly reduced in the obese subjects compared to normal weight controls.

Discussion

This study demonstrates that in obese children with a history of standard immunization to tetanus, anti-tetanus titers were significantly lower than in normal-weight controls with a similar history of tetanus immunization. The lower levels could not be

Table III. Circulating immunoglobulin levels and anti-tetanus IgG levels in normal and overweight children.

	Normal weight (n=15)	Overweight (n=15)
IgM (ng/ml)	504.5 ± 106.4	583.0 ± 47.9
IgA (ng/ml)	67.5 ± 10.3	77.3 ± 4.4
IgG (ng/ml)	1209.9 ± 199.1	1389.7 ± 76.5
IgG 1 (ng/ml)	686.6 ± 41.2	622.3 ± 90.0
IgG 2 (ng/ml)	18.6 ± 4.2	15.2 ± 1.6
IgG 3 (ng/ml)	39.9 ± 5.0	41.8 ± 10.9
IgG 4 (ng/ml)	93.2 ± 20.9	93.9 ± 20.2
Anti-tetanus IgG (IU/ml)	4.2 ± 0.5	2.6 ± 0.6*

*Statistical significance, $p < 0.05$.

explained by a global impairment in immunoglobulin levels since these values did not differ between the two groups. Taken together with previous data, these results suggest that under-nutrition is not the only energy-balance alteration that can influence immune status in children. The lower tetanus antibody levels that we found in obese children, along with previous observations of an impaired antibody response to hepatitis B vaccine in obese individuals, suggests that an excessively positive energy balance leading to increased body fat can alter immune responses in otherwise healthy children.

There are several possible mechanisms that individually or in combination might explain these results. Obesity could attenuate either the initial or sustained immune response to a given vaccine. An attenuated response could occur either because of mechanical factors such as an insufficient dose relative to body size, or suboptimal absorption and distribution of the injected vaccine in obese individuals. Alternatively, obesity related changes in inflammatory state, e.g. the elevated IL-6, could attenuate the initial humoral immune response or limit the duration of immune effectiveness following administration of the vaccine.

Clearly dose and mechanical factors might have played a role. The obese children that we studied were above the 85th percentile of BMI. Although the exact age that our study participants developed overweight is not available, recent data suggest that there exists a high likelihood (greater than 50% [13]) that a child who is obese at age 13 years-old (the mean age of the children studied) was obese at the time of his/her most recent booster (most likely 11–12 years-old [14]). It is currently recommended that tetanus immunizations be administered intramuscularly because absorption from the intramuscular site is known to be substantially better than from adipose depots [15,16]. Interestingly, in the case of hepatitis B vaccine, buttock injection was associated with a reduced antibody response, perhaps because of inadvertent injection of the vaccine into the fat rather than into the muscle [16]. In obese individuals, an increased subcutaneous fat layer in the deltoid muscle, the recommended site for tetanus toxoid injections, might also have led to inadvertent adipose injection and to reduced vaccine effectiveness.

An alternative or complementary mechanism for the impaired immune response might be related to the low-grade chronic inflammatory state that is now known to be associated with obesity in both adults and children [3]. In particular, the pleiotropic inflammatory cytokine IL-6 is consistently found to be elevated in obese individuals and has been demonstrated to be a direct link between obesity and inflammation in children [17]. Adipocytes produce IL-6 which induces synthesis of acute-phase proteins, such as C-reactive protein, leading to the chronic inflammatory state. The mechanism of the effect of IL-6 on the response to vaccination is not completely clear, however, it was shown that chronically elevated levels of IL-6 interfere with the humoral immune system, leading to a decrease in antibody (IgG) production [18]. In addition, it was suggested that inflammatory cytokines also play a role in the reduced cellular response in obesity, demonstrated by decreased lymphocytic blastogenic responsiveness to phytohemaglutinin and concanavalin A [19,20], and by a preferential decrease in the T cell subset CD + CD45RO + , indicating a possible impairment of memory T cells [21]. Indeed, it has been shown, recently, that non-responders to the influenza vaccine have exaggerated pro-inflammatory activity with the presence of chronically elevated IL-6 [10].

In the present study, a possible effect of low-grade inflammatory state on generalized immunoglobulin production can be ruled out, since no differences were found in immunoglobulin levels (i.e. total IgM, IgA, IgG and IgG subclasses) between the overweight and normal weight children. This is consistent with other previous reports in children [22] and adults [23,24]. In contrast, other investigators [19] found increased levels of circulating IgA and decreased levels of IgG in a group of obese children and adolescents compared with normal weight controls. In addition, there is one report that nutritional deprivation in obese subjects resulted in increases in serum concentrations of IgA, IgM and IgG [25].

Our study adds to the growing body of data indicating that energy balance can alter immune function. Energy balance is determined by the relationship between caloric intake and energy expenditure. Therefore, levels of physical activity may also affect the immune response. Interestingly, intense exercise training in elite athletes, in particular during the training season when there are many competitions, was associated with reduced specific antibody titers to diphtheria and staphylococcus [26]. In our study, although the peak VO_2 normalized to LBM did not differ between the two groups, overweight participants had significantly lower peak VO_2 per kg body weight indicating that reduced fitness was associated with increased adiposity in children. Ultimately, only by examining immune responses in weight-matched children with varying fitness levels can we determine independent effects of body composition and fitness on immune responses.

Although the anti-tetanus titers were significantly reduced in the obese subjects, their levels were high above the recommended threshold for anti-tetanus titer level (>0.1 IU/ml, [14]). In addition, to the best of our knowledge, there are no reports on increased prevalence of tetanus infection in the obese population. However, since tetanus infections are very rare, this study suggests that the specific antibody response to other, more common, pathogens should be investigated relative to overall energy balance and

body composition in children. Moreover, while abnormalities of the immune system in under-nutrition are well described, efforts should be made to better define the association between immune functions over-nutrition, overweight and obesity.

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