Decline in Delayed-Type Hypersensitivity Response in Obese Women following Weight Reduction

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The effect of weight loss on immune function was evaluated in 14 middle-aged obese women. Cutaneous delayed-type hypersensitivity to five recall antigens was measured before a weight loss of 21 kg and again after weight had been stabilized. Weight loss was associated with a decrease in both number and magnitude of delayed-type hypersensitivity responses. The number of positive skin tests decreased from 3.1 ± 0.2 to 2.4 ± 0.3 per subject, and the mean cutaneous induration size decreased by 38%. The decrease in induration size was positively correlated with the decrease in body mass index (r = 0.63), weight (r = 0.59), and fat mass (r = 0.52). These preliminary findings suggest that large weight losses may result in decreased expression of immune function.

Over two million overweight Americans enter weight reduction programs every year. Although they do not suffer from chronic protein-calorie malnutrition (3, 9, 26, 34), impairment of cell-mediated immunity has been found in morbidly obese persons (23), moderately obese persons (21), obese children and adolescents (8), and in genetically obese mice (6). Weight reduction may further depress their immune function: we report here an apparent impairment of cell-mediated immunity following a weight loss of 21 kg achieved by dietary means.

MATERIALS AND METHODS

The subjects were 14 obese women who ranged in age from 28 to 54 years (mean, 43.8 ± 1.8 years; Table 1). Subjects completed a 26-week weight loss program, described previously (14), that included 12 weeks of a very-low-calorie protein-sparing diet (420 to 800 kcal/day [ca. 1,800 to 3,000 kJ/day]; 70 g of protein, at least 30 g of carbohydrate, less than 14 g of fat, and 100% of the U.S. recommended daily allowance of vitamins and minerals). It was followed by 6 weeks of a refeeding diet and 8 weeks of a 1,200- to 1,500-kcal/day (ca. 5,000- to 6,300-kJ/day) balanced deficit diet, each of which contained 30% fat divided approximately equally among polyunsaturated, monounsaturated, and saturated forms. Behavioral therapy was delivered in weekly group sessions. Reasons for exclusion from the study included the following: a history of immunological, endocrine, kidney, or liver disease; insulin-dependent diabetes; cancer; any other disease with immunologic sequelae; medication known to affect immune responses; adverse reaction to previous tetanus toxoid; and any clinically significant psychiatric illness by the Diagnostic and Statistical Manual of the American Psychiatric Association (vol. III, revised) criteria.

Body composition. Body composition was determined before the weight loss program began and in the last 2 weeks of the balanced deficit diet (weeks 25 to 26 of the weight loss program). Body fat and fat-free mass were determined by hydrostatic weighing (16). Residual lung volume was measured by the oxygen dilution technique (36). Body density was calculated from the formula of Brozek et al. (5), and percent body fat was calculated from the formula of Siri (33). Fat-free mass was defined as body weight minus body fat. Weight was measured weekly with a balance-beam scale with the subjects barefoot and wearing light clothing. Height was measured by using a stadiometer. Body mass index (BMI), a standard index of the degree of adiposity, was calculated as weight in kilograms divided by height in meters squared.

Cellular immunity. Cellular immunity was evaluated in 14 subjects by delayed-type hypersensitivity (DTH) skin testing. Testing was conducted at the Allergy Clinic of the Hospital of the University of Pennsylvania by two nurses who were highly experienced in the technique, with excellent interrater reliability. Tests were performed 1 to 2 weeks before treatment and again during weeks 24 to 25 of the weight loss program, at a time when subjects had been receiving the 1,200- to 1,500-kcal (ca. 5,000- to 6,300-kJ/day) balanced deficit diet for 6 to 7 weeks. Five common recall antigens were used: mumps (Connaught; 40 complement-fixing units per ml), tetanus toxoid (Wyeth; 10 Loeffler units/ml), Candida albicans (Greer; 500 protein nitrogen units per ml), purified protein derivative (Connaught; 5 TU), and Trichophyton sp. (Hollister; 1:30). In quality control studies using freshly diluted reagents, which were used well within the expiration period of the stock solutions, we have found consistent patterns of responses over a period of years. Repeat testing in healthy volunteers induces similar responses.

Antigens (0.1 ml) were injected intradermally with a 26-gauge needle on a tuberculin syringe. Forty-eight hours later, the mean diameter of each induration was measured and recorded in millimeters. An induration response of at least 5 mm was considered positive.

A contrast group that had been studied previously was composed of 15 nonobese volunteers of the same age as that of the obese subjects (32).

All procedures were approved by the Committee on Research Involving Human Beings of the University of Pennsylvania.

Data analysis. Average induration sizes, including positive
and negative responses, were calculated. Changes in weight, body fat, and cutaneous DTH were assessed by paired t tests. Relationships between changes in cutaneous DTH and changes in weight, body fat, BMI, and fat-free mass were assessed by using Pearson product-moment correlations. Data were analyzed by using the Statistical Analysis System (31).

RESULTS

The weight loss program produced large reductions in body weight (21.0 ± 1.8 kg) and fat (17.1 ± 1.3 kg; Table 1). Most of the weight loss occurred in the first 12 weeks of the program while patients were receiving the very-low-calorie diet. In the 6 weeks preceding the second skin tests, the average weight loss was 1.03 ± 0.7 kg. Serum levels of three markers of nutritional status, total protein, albumin, and globulin, were within the normal range both prior to and following weight reduction (Table 1).

Skin tests. All 14 subjects showed positive responses to at least two antigens prior to weight loss; all responded to mumps, 13 responded to C. albicans, 10 responded to tetanus toxoid, 5 responded to Trichophyton sp., and 1 responded to purified protein derivative. Following weight loss, 11 of the 14 subjects (79%) showed decreases in skin test reactivity. The average number of positive tests decreased from 3.1 ± 0.2 to 2.4 ± 0.3 per subject (P ≤ 0.02), and reactivity to at least one antigen changed from positive to negative in five subjects. One subject who responded to three antigens before weight loss was completely unreactive after a weight loss of 26 kg. The average induration size decreased 38% overall, 56% for mumps, 47% for tetanus toxoid, and 25% for C. albicans (Fig. 1). The responses of the 15 members of the nondieting non-obese contrast group were quite stable over a period of 15 months, as measured at 3-month intervals, in sharp contrast to the decline in the obese subjects (32).

Correlations. The magnitude of change in the DTH response, in millimeters of induration, was related to the degree of weight loss. The change in the average skin test response to all five antigens was correlated with the change in BMI (r = 0.63, P ≤ 0.02; Fig. 2), body weight (r = 0.59, P ≤ 0.03), fat mass (r = 0.52, P ≤ 0.05), and waist circumference (r = 0.65, P ≤ 0.01). The change in DTH did not correlate with change in fat-free mass (r = 0.40, P = 0.20) or age (r = 0.20, P = 0.50).

Follow-up. Seven of the 11 subjects who showed decreased cutaneous DTH following weight loss were retested 8 months after the end-of-treatment skin tests (the other four subjects declined to participate). In the five subjects who had regained less than 41% of their weight loss, cutaneous DTH remained as depressed or became more depressed. In the two subjects who had regained a greater percentage of their weight loss (39 and 84%), DTH responses returned towards prediet levels (Table 2).

DISCUSSION

In the present study, diet-induced weight loss was associated with a decline in the DTH responsiveness of obese women, a decline that was strongly correlated with the extent of weight loss and differed markedly from the stable response of the normal-weight contrast group. Although interpretation of these preliminary findings is limited by the small number of subjects and the lack of an appropriate control group of nondieting obese subjects, they raise troubling uncertainties for the more than two million overweight Americans who enter weight reduction programs each year.

Cutaneous DTH is the in vivo immune response most commonly used to assess cellular immunodeficiency. The usual criterion for normal DTH responsiveness is a minimum of two positive tests (induration of ≥5 mm) out of a battery of five tests with common skin test antigens (10, 11, 27). All of our subjects manifested such normal DTH responsiveness prior to weight loss.

DTH skin tests carried out sequentially in subjects in a steady state are generally consistent in size (30). In both healthy and diseased subjects, cutaneous DTH correlates well with a number of in vitro tests that measure specific functions involved in cell-mediated immunity, including mitogen-stimu-

![FIG. 1. DTH skin test results, in millimeters of induration, before and after weight loss, for the antigens C. albicans, mumps, and tetanus toxoid. Baseline responses to Trichophyton sp. and purified protein derivative were limited, and data on responses to these antigens are not shown. AVG, average combined response to C. albicans, mumps, and tetanus toxoid. Error bars indicate standard error of the mean.](http://cvi.asm.org/)
related blast transformation, lymphocyte cytotoxicity, and macrophage and leukocyte migration inhibition assays (1, 4, 28, 29).

The inability to mount a positive response to at least two of a battery of five or more recall antigens correlates highly with postoperative morbidity, cancer prognosis, and clinical disease state (10, 18, 22, 24, 25, 29). In addition, changes in DTH responses over time are predictive of clinical outcome. Sequential skin testing of surgery or trauma patients has shown that patients whose responses remain normal or improve from abnormal to normal have far lower rates of infection than patients whose responses change from normal to abnormal or remain abnormal (10, 11, 27).

In addition to the categorical classification of normality based on the number of positive tests, the magnitude of DTH response, in millimeters of induration, is also related to clinical outcome. Studies in laboratory animals and surgery patients have shown an inverse and exponential correlation between average induration size and subsequent bacterial infection (10, 35). In addition to a decline in DTH responsiveness, we also found that tetanus antibody titer rose less in six subjects following weight loss than it had in six subjects before weight loss, although the difference did not reach statistical significance.

Neither protein-energy malnutrition nor caloric restriction appears to be responsible for the decreased expression of cellular immunity following weight loss. Subjects were prescribed a diet that provided 100% of the U.S. recommended daily allowance of protein, vitamins, and minerals throughout the study, and their weights were stable at the time of repeat testing, suggesting that they had not been severely restricting their food intake. Further, serum levels of protein, albumin, and globulin were unchanged from baseline values. Finally, immune responses remained suppressed 8 months after the end of treatment in subjects who had regained less than 41% of their weight loss. Thus, the decrease in cutaneous DTH appears to be related to the weight loss, and more specifically to the decrease in adiposity, rather than to a decrease in fat-free mass or to caloric restriction.

No other study, to our knowledge, has assessed the effects of voluntary weight reduction on immune function in obese persons after the diet period has ended. Two studies assessed these effects during dieting or fasting. Field et al. found no decrease in responsiveness during the fifth week of a very-low-calorie diet (13), while Wing et al. reported enhanced DTH responsiveness at the end of a 14-day fast (37).

A number of endocrine factors modulate immune function, including adrenocorticotropic, growth hormone, prolactin, endogenous opiates, sex hormones, and the catecholamines (2, 12, 17, 20). Since obesity, dieting, and weight loss are each associated with altered endocrine status (15), endocrine factors may contribute to the effects we observed. In addition, although subjects were not suffering from malnutrition, nutritional factors such as the type and quantity of dietary fat and micronutrients known to affect DTH could conceivably have had an effect (7, 19).

Although based on a small number of subjects and without a non-dieting obese control group, the results of this study suggest that dieting and weight loss may depress the immune function of obese persons. Further study of this troubling finding is indicated.

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