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CLINICAL RESEARCH

Bariatric Surgery Reverses Natural Killer (NK) Cell Activity and NK-Related Cytokine Synthesis Impairment Induced by Morbid Obesity

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Abstract
Background Obesity is related to a higher rate of infections and some types of cancer. Here we analyzed the impact of obesity and weight loss induced by Roux-en-Y gastric bypass (RYGB) on immunological parameters, i.e., cytokine productions and natural killer cell function.
Methods We analyzed 28 morbidly obese patients before and 6 months after RYGB. Biochemical parameters were analyzed in plasma. The percent of natural killer (NK) cells, their cytotoxicity, and the production of cytokines by peripheral blood mononuclear cells were analyzed. The percent of NK cells was determined by flow cytometry and cytokine production determined by enzyme-linked immunosorbent assay. NK cytotoxicity was determined by the lactate dehydrogenase release assay.
Results The weight loss 6 months following surgery was 35.3±4.5 kg. RYGB also improves biochemical parameters. No significant difference was found in the percent of NK cells after surgery. We found an increase in the production of interferon-γ, interleukin (IL)-12 and IL-18, but not in IL-2, 6 months after RYGB. Cytotoxic activity of NK cells was significantly enhanced 6 months after RYGB [17.1±14.7% before RYGB vs 51.8±11.3% at 6 months after, at 40:1 effector to target cell ratio; $p<0.001$]. We observed significant post-surgical improvement in the cytotoxic activity curve in 22 out of 28 patients (78.6%), irrespective of the target to effector cell ratio.
Conclusions The weight loss induced by RYGB modifies the production of cytokines related with NK cell function and improves its activity.

Keywords Morbid obesity · Bariatric surgery · Immunity · Natural killer cells · Weight reduction

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Introduction

Obesity is a worldwide public health problem associated with several comorbidities including cardiovascular diseases, reproductive disorders, osteoarthritis, gallstones, and respiratory disease [1]. Furthermore, obesity has also been linked to decreased immunocompetence [2]. Epidemiological data indicate that the incidence and severity of infections are higher in obese individuals [3]. Results from meta-analysis show the association between high body mass index (BMI) and an increased incidence of many types of cancer [4]. The

biological mechanism that links obesity to cancer seems to be multifactorial and to involve a group of metabolic and immunological factors.

Bariatric surgery is currently the only effective therapy for morbid obesity and it can improve most obesity-related conditions [5]. The observational Swedish Obese Subjects (SOS) study found that those treated with surgery had significantly better weight loss than controls in more than a 15-year follow-up [6]. A meta-analysis of effectiveness associated with surgical treatment of obesity confirmed substantial improvement or resolution of diabetes, hypertension, dyslipidemia, and sleep apnea [7]. A recent report from the SOS study showed a reduced cancer incidence in obese women submitted to surgery [8]. Bariatric surgery in obese subjects was also associated with a reduction in overall mortality [6, 9]. Adams et al. [9] observed that long-term total mortality after gastric bypass surgery was significantly reduced, particularly deaths from diabetes, heart disease, and cancer.

Natural killer (NK) cells are innate immunity cells critically involved in the control of cancer and infections. Human NK cells target various cancer cells of different origins [10]. NK cells also kill cells infected with a variety of pathogens such as viruses, bacteria, and fungi [11]. NK cell cytotoxic activity is regulated by interleukin (IL)-2, IL-12, IL-18, and interferons [12]. In an 11-year prospective study, lower NK cytotoxicity was associated with increased cancer incidence [13]. Patients with Chediak–Higashi syndrome, a genetic disorder characterized by abnormal NK cytotoxic function, have a 200-fold increased risk of developing malignancy [14]. NK cytotoxicity has been shown to be inversely associated with risk and severity of some infections [15]. Aside from its effector functions, NK cells also play a fundamental role in the regulation of the immune response and their presence or absence will influence the pathway adaptive immunity will take, mostly under infectious conditions [16].

Although obese individuals have a higher rate of infections and are more prone to develop cancer, studies on NK cell function in these subjects and the effect of weight loss in this parameter of immunity are scarce. In this study, we analyzed NK cell numbers and function in a morbidly obese population to address the effect of weight loss induced by Roux-en-Y gastric bypass (RYGB) in this parameter of immunity.

Methods

Subjects and Research Design

We evaluated 28 patients with BMI≥40 kg/m^2, who underwent RYGB, and with ages ranging between 23 and 58 years. We excluded patients with fasting glucose≥ 126 mg/dL, autoimmune diseases, cancer, chronic infections, or under treatment with immunosuppressive drugs. Written informed consent was obtained from all patients. The study was approved by the Ethics Committee of the University of São Paulo Medical School.

Medical evaluation and blood collection were conducted in all subjects before and 6 months after RYGB. After surgery, all patients took polyvitamin replacement as part of the nutritional follow-up supervised by an attending endocrinologist and a nutritionist.

Body Composition and Metabolic Parameters

Weight was measured with a balance beam scale (Health-o-meter, Sunbeam, Boca Raton, FL, USA) with subjects barefoot and wearing light clothing. Height was measured to the nearest 1 cm using a Harpenden stadiometer.

Plasma glucose was determined by the glucose oxidase method (Hitachi Modular P, Roche Diagnóstica Brasil Ltda). Serum total cholesterol (C), low-density lipoprotein (LDL)-C, and triglycerides (TG) were also measured enzymatically (Hitachi Modular P, Roche Diagnóstica Brasil Ltda). High-density lipoprotein (HDL)-C was measured with a homogenous method. Plasma leptin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) kit (Linco Research, Inc). The minimum detectable leptin concentration in this assay is 0.5 ng/mL. Samples from individual subjects were analyzed in the same assay.

Evaluation of Immunologic Parameters

Blood samples were obtained after a 12-h overnight fast and were processed immediately for immunological examinations. Peripheral blood mononuclear cells (PBMCs) from heparinized blood were isolated by density gradient centrifugation with Isolymph (Gallard-Schlesinger Industries, Inc, Norway) followed by washing with phosphate-buffered saline (PBS), pH 7.2. Cells were resuspended in 1 mL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% human AB$^+$ serum (Cambrex) and counted.

Flow Cytometry

Ten million cells per milliliter were diluted in supplemented DMEM and incubated with antigen-specific monoclonal antibodies against CD3, CD4, CD8, CD19, CD25, CD45RA and R0, CD56, CD69, and CD95 (Pharmingen, San Diego, CA, USA) for 30 min at room temperature in the dark. The cells were subsequently washed with PBS and then washed twice with PBS with 3% human AB serum and read on a flow cytometer (FACSCalibur—Becton Dickinson, Sunnyvale, CA, USA). A minimum of 10,000 cells per sample were acquired. Data were evaluated

using CellQuest analytical software (Tree Star Inc., Palo Alto, CA, USA). CD56 was considered a specific marker of NK cell. Nonspecific staining was evaluated using isotype-control antibodies. Nonspecific staining was below detectable levels in all experiments.

Cytokine Measurement

Cytokines were measured by ELISA using paired antibody (PharMigen, San Diego, CA, USA) as described elsewhere [17]. Briefly, PBMCs were diluted to 10^6 cells/mL and added to 96-well flat-bottom microtiter plates (Falcon, Oxnard, CA, USA) in medium alone or stimulated with 5 μg/mL phytohemagglutinin (PHA). Cultures were assayed after 24 h for IL-2, IL-12, and IL-18 synthesis and after 48 h for IFN-γ.

Cytotoxicity Assay

NK activity was measured by a 4-h enzyme release cytotoxicity assay. Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) activity released in the supernatant by lysed target cells according to the method of Korzeniewski and Callewaert [18]. The results obtained with the LDH release method have been shown to correlate with those of 51Cr release [18]. K562 (target) cells were mixed with PBMCs (effector) to give different effector to target cell ratios (40:1, 20:1, 10:1, 5:1, and 1:1). LDH activity was measured by colorimetric procedure, performed by an automatic PC-aided microtiter plate reader (UltraMax 180 with SoftMax software, Molecular Devices, Menlo Park, USA). Data on the NK activity of PBM cells incubated with or without modifiers were expressed as lytic units (LU)/10^7 cells. The results are presented as percentage of specific lysis at the median of E/T 40:1. Specific lysis was calculated using the mean counts per minute (cpm) of triplicate values for each effector to target ratio by the formula: % cytotoxicity=[1−(cpm with effector cells/cpm without effector cells)]×100. In all experiments, spontaneous release (target cells alone) was less than 20% of maximal release (effector plus target cells).

The method of serial dilutions is used because different ratios of effector to target cells allow the evaluation of the cytotoxic effects that are dependent on cell contact, as well as those that are cytokine-dependent. The establishment of a dose–response curve also helps to unveil discrete results that would be otherwise hard to appreciate and are revealed using a curve because of the tendencies shown by the curve slope.

Statistical Analysis

Shapiro–Wilk's test was used to verify the normal distribution of data. Student's *t* test was performed to compare anthropometric and biochemical data before and after surgery and Wilcoxon test to compare the immunological parameters, except NK cell cytotoxicity. Results were considered statistically significant when a 95% confidence level was achieved. Fisher test was used to evaluate cell cytotoxicity assessed by LDH release.

Results

RYGB Improves Anthropometric and Biochemical Parameters in Obese Patients

Anthropometric and biochemical parameters were evaluated before and 6 months after RYGB. We analyzed 28 subjects (20 females and 8 males), nonsmokers and sedentary, with mean age of 39.9±10.9 years (range 23–58) and mean preoperative BMI of 49.5±7.1 kg/m^2 (range 40.1–62.4). As corroborated by the literature [5–7], patients demonstrated a better BMI associated with a significant weight loss after the surgery (Table 1). The weight loss 6 months following surgery was 35.3±4.5 kg, an average loss of 26% of the initial weight. We observed better and stable blood glucose level, associated with a decrease in total C, TG, and LDL-C. We also observed a decreased in plasma leptin (Table 1), consistent with previous findings by other groups [19].

RYGB Induces Changes in Cytokines Involved in NK Cell Function

To evaluate changes in the immune response of patients after RYGB, we assayed the capacity of PBMCs stimulated in vitro to secrete proinflammatory and regulatory cytokines. Cytokine synthesis was evaluated in each patient individually prior to surgery and 6 months after surgery. Our data showed increased secretion of innate immunity-

Table 1 Anthropometric and biochemical parameters of patients (n=28) before and 6 months after RYGB

Parameter	Before	6 months
Weight (kg)	136.2±24.4	100.9±19.9*
BMI (kg/m^2)	49.5±7.1	36.6±6.2*
Glucose (mg/dL)	90.8±12.8	80.2±6.5*
Total cholesterol (mg/dL)	211.8±34.8	173.8±34.7*
Triglycerides (mg/dL)	163.7±107.1	105.7±39.5*
HDL (mg/dL)	50.2±11.5	52.9±9.9
LDL (mg/dL)	132.5±31.7	99.7±28.6*
Leptin (ng/mL)	105.6±87.2	29.0±19.8*

Blood was taken at both time points and plasma was evaluated for the mentioned biochemical parameters

*p<0.01, Student's *t* test

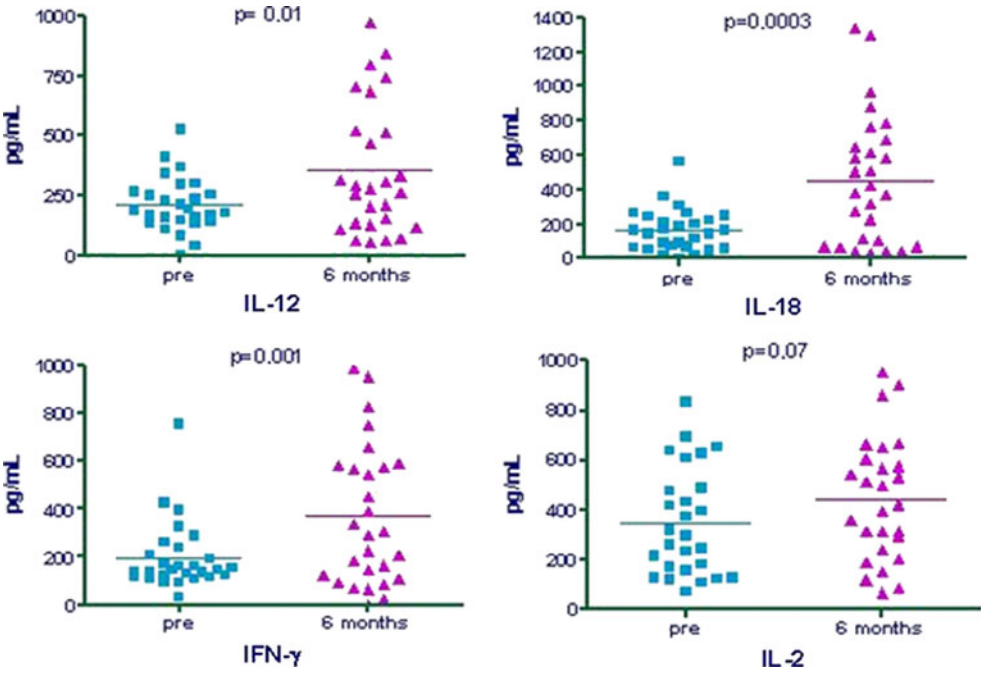

Fig. 1 Cytokine secretion by PBMCs from patients (n=28) before (pre) and 6 months after RYGB. PBMCs were isolated and plated with 5 μg/mL of PHA. Supernatants were harvested and cytokines were measured by ELISA. Wilcoxon test was applied to compare the synthesis in the two time points

associated cytokines 6 months after RYGB, such as IL-12 (210.6±113.6 vs 355.8±272.9 pg/mL; p=0.01) and IL-18 (163.0±122.7 vs 455.1±374.6 pg/mL; p=0.0003). We also found an increase in IFN-γ synthesis (196.7±142.7 vs 369.9±291.8 pg/mL; p=0.01). We did not observe any changes in the production of IL-2 (Fig. 1).

Lymphocyte populations were evaluated by flow cytometry. Surface markers CD3, CD4, CD8, and CD19 were evaluated in combination with the activation and differentiation markers CD45RA and CD45R0, CD95, CD25, and CD69. We could not detect any changes in these cellular populations (data not shown).

Improved Cytotoxic Activity of NK Cells After Surgery

We first analyzed the percentage of NK cells in the patient's PBMCs and found no differences between the results before and 6 months after surgery.

Interestingly, our data showed an increased cytotoxic activity of NK cells over K562 target cells (MHC class I negative). Cytotoxic activity of NK cells was enhanced 6 months after RYGB [17.1±14.7% before RYGB vs 51.8±11.3% at 6 months after, at effector to target cell (NK cell/K562 cell) ratio 40:1; p<0.001] (Fig. 2a). We observed in Fig. 2b that severely obese patients have a significant decrease in the activity of NK cells in comparison to normal individuals matched for age and gender (the red curve represent data obtained from controls with normal BMI). We found significant post-surgical improvement in the cytotoxic activity curve and the NK cells of patients recovered their activity to values similar to the controls. We observed significant post-surgical improvement in the cytotoxic

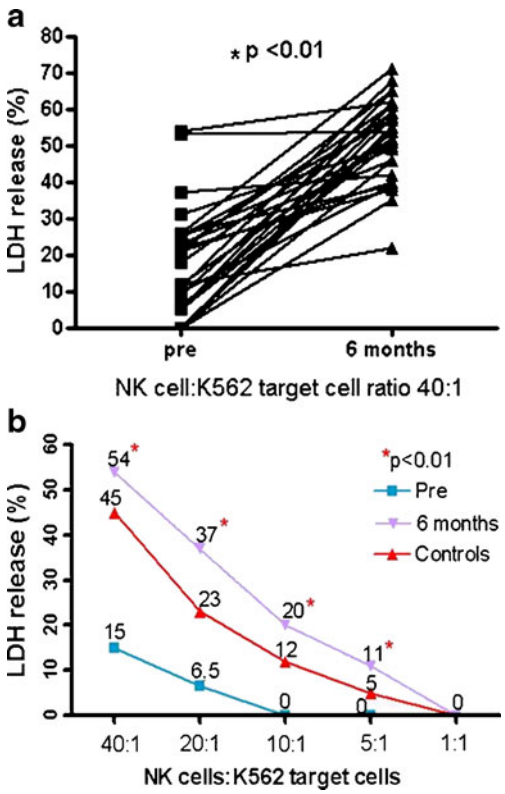

Fig. 2 Cytotoxic activity of NK cells from patients (n=28) before (pre) and 6 months after RYGB. PBMCs were plated at different ratios with K562 target cells, and cytotoxicity was evaluated through LDH release according to the "Methods" section. Pre and 6 months later, data for 40:1 NK/target ratio only (**a**) or at different ratios from 40:1 to 1:1 (**b**). The curve (**b**) represents the data obtained from controls with normal BMI matched for age and gender. Variations in effector to target ratios are essential in this assay to reveal discrete differences, as described in the "Methods" section of this paper. *Asterisk*, Fisher test pre vs 6 months

activity curve in 22 out of 28 patients (78.6%), irrespective of the target to effector cell ratio.

Discussion

Several groups have described a strong relationship between obesity and certain types of tumors, as well as recurrent infections. Changes in immune function have been described in obese experimental animals and human. Taking into consideration the role of NK cells in immune surveillance and response to infections, we decided to address the status of this cell population before and 6 months after RYGB. To our knowledge, this is the first study evaluating the impact of weight loss induced by RYGB in the activity of NK cells.

Diet-induced obese animals present lower cytotoxic activity of NK cells, which was restored by energy restriction [20]. In elderly women, a negative correlation was shown between the activity of NK cells and percentage of body fat [21]. In contrast, other reports showed no difference in this parameter between obese and nonobese subjects [22, 23]. The varied degree of obesity in the patients enrolled in these studies confounded its interpretation. In our study, only patients with a BMI above 40 were included. We have shown that severely obese patients have a significant decrease in the activity of NK cells compared to normal individuals matched for age and gender (Fig. 2). This decrease could not be subscribed to a decrease in the numbers of circulating NK cells since the individual numbers as well as the averages were similar, prior to and 6 months after surgery (data not shown). Nevertheless, we can associate the diminished function to impaired production of cytokines known to regulate NK function (IL-12, IL-18, and IFN-γ) that were decreased prior to surgery (Fig. 1).

The effect of weight reduction strategies on NK cell function has been evaluated in a few studies in humans. The difference in assays, characteristics of patients studied, type, and duration of the weight loss treatment makes it difficult to compare the available data. One study has reported that NK cells' cytolytic activity was enhanced by fasting in obese individuals [24]. On the other hand, suppression of the activity of NK cells, without change in NK cell proportion, was described in obese women after an 8-week weight loss program with a diet of 950 kcal per day [25]. Most studies that evaluated the effects of bariatric surgery on immune function have focused on inflammation markers but have not assessed any aspect of the immune response effector mechanisms. We observed a post-surgical increase in NK cell activity (Fig. 2) although NK cell proportion, defined as CD56+ cells, remained unchanged after surgery, as corroborated by Cottam et al. [26]. We can suggest that the increase in the production of IL-12 and IL-18 6 months after surgery is one important factor in the recovery of NK cell activity (Fig. 1). Because cytotoxicity was assessed using whole PBMCs and not sorted CD56+ cells, one could argue that our measurements included cell death induced by other cytotoxic cells, mainly CD3+CD8+ cells. However, the fact that the K562 cells are MHC class I negative makes them a preferential target for NK cells, and therefore, the impact of lysis induced by other cells is negligible [27].

Adipose tissue has been recognized to be a multifunctional organ, playing an important role not only as energy storage organ, but also exerting important immune functions through the released of leptin, resistin, adiponectin, IL-6, and tumor necrosis factor (TNF)-α [28]. All of those molecules may act on immune cells leading to local and systemic inflammation. Leptin is the most studied adipocyte-derived hormone and BMI is the main determinant of circulating leptin in the body [29, 30]. Its functional receptor is expressed not only in the hypothalamus where it regulates energy homeostasis and neuroendocrine function, but also in several cell types of innate and adaptive immunity [31]. In vitro studies using both animal and human cells with complete leptin deficiency have demonstrated the role of leptin in modulating immune function [32, 33]. In vivo studies in fasting, diet-induced obese mice showed that leptin administration prevents pre- and post-starvation reduction in spleen weight compared with lean controls, but it showed no effect on IL-2, IL-10, and IFN-γ production, suggesting that under these circumstances the effect of leptin on immune cells in the obese state may be minimal [34]. It is possible that there is leptin resistance in obese individuals' immune cells, rendering them refractory to leptin stimulation despite its high concentration in the serum. As expected, we observed a decreased in serum leptin levels 6 months after RYGB. Nevertheless, we could not relate individual drop out in leptin levels with any specific parameter of the immune response analyzed.

Although we could not clarify the mechanism by which weight loss controls NK cell function, it is conceivable that it is associated with the synthesis of cytokines involved in NK cell activation. We observed a significant post-surgical increase in the production of IL-12, IL-18, and IFN-γ. IL-12 and IL-18 have been implicated in the functions of NK cells. IL-12 is crucial for the induction of IFN-γ released by NK cells and also for the enhancement of NK cell cytotoxicity [35]. IL-18 operates synergistically with IL-12 in the induction of IFN-γ production by NK expression, and consequently, Fas-FasL-mediated cytotoxicity is enhanced by IL-18 [36]. IL-18-deficient mice have reduced NK cell cytotoxic activity that can be restored by exogenous IL-18 [37]. The production of IFN-γ can inhibit viral replication and also has a direct toxic effect on tumor cells [38]. Moreover, IFN-γ is a

cytokine capable of activating macrophages and CD8 T cells and, by these means, stimulating cell-mediated immunity [38]. As the NK cell proportion was unchanged after surgery, the increase in these cytokines can explain the improved cytotoxic activity observed.

Diet-induced obese animals have increased mortality and altered immune responses when infected with influenza virus. These mice have reduced expression of antiviral cytokines IFN-α and IFN-β and reduced NK cytotoxicity [39]. Albanes [40] showed that tumor incidence in mice is proportional to the level of caloric intake and resulting body weight, and body weight accounted may be a more sensitive indicator for cancer risk. Epidemiological data suggest that obesity is associated with increased risk of certain types of cancer. Several mechanisms by which obesity induces or promotes tumorigenesis have been proposed, among them a deregulation of immune response effected through leptin, TNF-α, and or IL-6 [4, 41]. Although we have not studied the clinical outcomes but rather the effect of obesity on the functional status of NK cells, is important to notice the correlation between low NK cytotoxic activity, found by us, and a higher incidence of tumors and infections in obese subjects. Our work offers a cellular explanation for the epidemiological data available. Furthermore, our data also show that the functional status of NK cells can be restored upon weight loss, demonstrating a previously unappreciated effect of weight loss in immunity.

In conclusion, the weight loss induced by RYGB increases the production of cytokines related with NK cell cytotoxic function and improves the killing capacity of these cells. The impairment in NK cells' cytotoxic activity and cytokines observed in patients with severe obesity can be at least one of the possible explanations for developing infections and cancer. Our data show that weight loss induced by bariatric surgery can positively impact these factors in a significant way. Nevertheless, long-term studies should be performed to address whether these changes positively affect the ability of these subjects to control viral infections and tumor development.

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References

1. WHO Consultation on Obesity. Obesity: preventing and managing the global epidemic. WHO Tech Rep Series no. 894. Geneva, Switzerland: World Health Organization, 2000.
2. Martí A, Marcos A, Martínez JA. Obesity and immune function relationships. Obes Rev. 2001;2:131–40.
3. Falagas ME, Kompoti M. Obesity and infection. Lancet Infect Dis. 2006;6:438–46.
4. Renehan AG, Tyson M, Egger M, et al. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008;371:569–78.
5. Choban PS, Jackson B, Poplawski S, et al. Bariatric surgery for morbid obesity: why, who, when, how, where, and then what? Cleve Clin J Med. 2002;69:897–903.
6. Sjostrom L, Narbro K, Sjostrom CD, et al. Effects of bariatric surgery on mortality in Swedish obese subjects. N Engl J Med. 2007;357:741–52.
7. Maggard MA, Shugarman LS, Suttorp M, et al. Meta-analysis: surgical treatment of obesity. Ann Intern Med. 2005;142:547–59.
8. Sjöstrom L, Gummesson A, Sjöström CD, et al. Effects of bariatric surgery on cancer incidence in obese patients in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. Lancet Oncol. 2009;10:653–62.
9. Adams TD, Gress RE, Smith SC, et al. Long-term mortality after gastric bypass surgery. N Engl J Med. 2007;357:753–61.
10. Nagatomi R. The implication of alterations in leukocyte subset counts on immune function. Exerc Immunol Rev. 2006;12:54–71.
11. Orange JS, Ballas ZK. Natural killer cells in human health and disease. Clin Immunol. 2006;118:1–10.
12. Hallett WHD, Murphy WJ. Positive and negative regulation of natural killer cells: therapeutic implications. Sem Cancer Biol. 2006;16:367–82.
13. Imai K, Matsuyama S, Miyake S, et al. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;356:1795–9.
14. Ghiringhelli F, Ménard C, Martin F, et al. The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. Immunol Rev. 2006;214:229–38.
15. Whiteside TL, Herberman RB. Role of human natural killer cells in health and disease. Clin Diagn Lab Immunol. 1994;1:125–33.
16. Eissmann P, Davis DM. Inhibitory and regulatory immune synapses. Curr Top Microbiol Immunol. 2010;340:63–79.
17. Correa-Sales C, Tosta CE, Rizzo LV. The effects of anesthesia with thiopental on T lymphocyte responses to antigen and mitogens in vivo and in vitro. Int J Immunopharmacol. 1997;19:117–28.
18. Korzeniewski C, Callewaert DM. An enzyme-release assay for natural cytotoxicity. J Immunol Methods. 1983;64:313–20.
19. Kotidis EV, Koliakos GG, Baltzopoulos VG, et al. Serum ghrelin, leptin and adiponectin levels before and after weight loss: comparison of three methods of treatment—a prospective study. Obes Surg. 2006;16:1425–32.
20. Lamas O, Martinez JA, Marti A. Energy restriction restores the impaired immune response in overweight (cafeteria) rats. J Nutr Biochem. 2004;15:418–25.
21. Nieman DC, Henson DA, Gusewich G, et al. Physical activity and immune function in elderly women. Med Sci Sports Exerc. 1993;25:823–31.
22. Nieman DC, Henson DA, Nehlsen-Cannarella SL, et al. Influence of obesity on immune function. J Am Diet Assoc. 1999;99:294–9.
23. Dovio A, Caramello V, Masera RG, et al. Natural killer cell activity and sensitivity to positive and negative modulation in uncomplicated obese subjects: relationships to leptin and diet composition. Int J Obes. 2004;28:894–901.

24. Wing EJ, Stanko RT, Winkelstein A, et al. Fasting-enhanced immune effector mechanisms in obese subjects. Am J Med. 1983;75:91–6.
25. Scanga CB, Verde TJ, Paolone AM, et al. Effects of weight loss and exercise training on natural killer cell activity in obese women. Med Sci Sports Exerc. 1998;30:1666–71.
26. Cottam DR, Schaefer PA, Shaftan GW, et al. Dysfunctional immune-privilege in morbid obesity: implications and effect of gastric bypass surgery. Obes Surg. 2003;13:49–57.
27. Nagatake H, Watanabe N, Kobayashi Y. Granule exocytosis in a cultured human natural killer cell-rich population induced by K562 target cells and by PMA/A23187. Cell Immunol. 1995;161:295–8.
28. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006;6: 772–83.
29. Geloneze B, Tambascia MA, Pareja JC, et al. Serum leptin levels after bariatric surgery across a range of glucose tolerance from normal to diabetes. Obes Surg. 2001;11:693–8.
30. Zabrocka L, Raczynska S, Goyke E, et al. BMI is the main determinant of the circulating leptin in women after vertical banded gastroplasty. Obes Res. 2004;12:505–12.
31. Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. J Immunol. 2005;173:3137–42.
32. Loffreda S, Yang SQ, Lin HZ, et al. Leptin regulates proinflammatory immune responses. FASEB J. 1998;12:57–65.
33. Lord GM, Matarese G, Howard JK, et al. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature. 1998;394:897–901.
34. Mito N, Yoshino H, Hosoda T, et al. Analysis of the effect of leptin on immune function in vivo using diet-induced obese mice. J Endocrinol. 2003;180:167–73.
35. Watford WT, Moriguchi M, Morinobu A, et al. The biology of IL-12: coordinating innate and adaptive immune responses. Cytokine Growth Factor Rev. 2003;14:361–8.
36. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. J Leukoc Biol. 2003;73:213–24.
37. Takeda K, Tsutsui H, Yoshimoto T, et al. Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity. 1998;8:383–90.
38. Schroder K, Hertzog PJ, Ravasi T, et al. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004;75:163–89.
39. Smith AG, Sheridan PA, Harp JB, et al. Diet-induced obese mice have increased mortality and altered immune responses when infected with influenza virus. J Nutr. 2007;137:1236–43.
40. Albanes D. Total calories, body weight and tumor incidence in mice. Cancer Res. 1987;47:1987–92.
41. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4: 579–91.