Transplantation of wild-type white adipose tissue normalizes metabolic, immune and inflammatory alterations in leptin-deficient *ob/ob* mice

Joseph A. Sennello, **Raja Fayad**, **Maria Pini**, **Melissa E. Gove**, and **Giamila Fantuzzi**
Department of Human Nutrition, University of Illinois at Chicago, Chicago, IL

Abstract

Leptin-deficient *ob/ob* mice exhibit several metabolic and immune abnormalities, including thymus atrophy and markedly reduced inflammatory responses. We evaluated whether transplantation of wild type (WT) white adipose tissue (WAT) into *ob/ob* mice could mimic the effect of recombinant leptin administration in normalizing metabolic, immune and inflammatory abnormalities. Female *ob/ob* mice received a subcutaneous transplantation of WAT obtained from WT littermates. A separate group of *ob/ob* mice was sham-operated. Despite raising leptin levels to only 15% of those observed in WT mice, WAT transplantation normalized metabolic abnormalities (glycemia, ALT, liver weight) in *ob/ob* mice and prevented further body weight gain. The transplanted group demonstrated normalization of thymus and spleen cellularity, thymocyte subpopulations and rates of thymocyte apoptosis. In the model of dextran sulfate sodium-induced colitis, WAT transplantation restored inflammation to levels equivalent to those of WT mice. Colonic production of IL-6 and MIP-2 was markedly reduced in the non-transplanted *ob/ob* group compared to transplanted *ob/ob* and WT mice. Our data indicate that WAT transplantation is an effective way to normalize metabolic as well as immune and inflammatory parameters in *ob/ob* mice. The threshold of leptin sufficient to normalize metabolic, immune and inflammatory function is significantly lower than levels present in lean WT mice. Finally, leptin derived exclusively from WAT is sufficient to normalize metabolic, immune and inflammatory parameters in *ob/ob* mice.

Introduction

Leptin, a 16 kDa protein encoded by the *ob* gene, is a pleiotropic hormone which regulates appetite and metabolism as well as immunity and inflammation [1]. In addition to being massively obese and infertile, leptin-deficient *ob/ob* mice exhibit several metabolic alterations, including hepatomegaly with steatosis and increased transaminase levels as well as hyperglycemia and insulin resistance [2]. Leptin deficiency is also associated with immune and inflammatory abnormalities. In fact, decreased thymus cellularity accompanied by increased apoptosis of $CD4^+$ $CD8^+$ immature thymocytes, resistance to several experimental models of inflammation/autoimmunity and altered production of pro-inflammatory cytokines are observed in *ob/ob* mice [3–6]. In particular, *ob/ob* mice exhibit markedly reduced disease severity in the experimental model of intestinal inflammation induced by administration of the

Corresponding author: Giamila Fantuzzi, PhD, Department of Human Nutrition, University of Illinois at Chicago, 1919 W. Taylor Street M/C 517, Chicago, IL 60612. Phone 312-413-5398. Fax 312-413-0319. e-mail: giamila@uic.edu

Disclosure statement: The authors have no conflicts to disclose.

irritant dextran sodium sulfate (DSS), whereas reconstitution with exogenous leptin restores their response [6].

Recently, transplantation of white adipose tissue (WAT) has been reported to effectively and protractedly reduce body weight and food intake, reduce insulin, glucose and corticosterone levels and restore fertility in *ob/ob* mice, despite the fact that circulating leptin levels reached at best 20% of WT levels in transplanted mice [7], thus supporting the concept of a threshold level for leptin in regulating neuroendocrine and metabolic function. However, it is currently unclear if such a threshold effect is also important for regulation of immune responses by leptin.

Quantitatively, adipocytes are the most important source of leptin [2]. However, leptin is also produced - albeit at much reduced levels compared to adipocytes - by other tissues and cell types, including gastric fundic mucosa, inflamed colonic epithelium as well as activated T lymphocytes [8–11]. The function of non adipocyte-derived leptin in regulating metabolic and inflammatory response has not been completely characterized, although cell transfer experiments indicate lack of a significant role for T cell-derived leptin in hepatic and intestinal inflammation [12].

In the present study, we evaluated the effect of WT WAT transplantation on body weight, metabolic, immune and inflammatory parameters in *ob/ob* mice. Our results confirm previous data indicating that transplantation of a small amount ($\sim$ 1g) of WT WAT is sufficient to stop body weight gain and normalize glucose levels in *ob/ob* mice [7]. We also demonstrate for the first time that WAT transplantation restores thymus and spleen cellularity and the intestinal inflammatory response in *ob/ob* mice.

Materials and Methods

WAT transplantation

Animal protocols were approved by the Animal Studies Committee of the University of Illinois at Chicago. Six-week-old female leptin-deficient (B6.V-Lep^{ob}/J, referred to as *ob/ob*) mice and their lean littermates were obtained from The Jackson Laboratory. A group of *ob/ob* mice received WAT transplantation as described in [13]. Briefly, gonadal WAT from WT littermates euthanized by cervical dislocation was placed in sterile PBS and cut into small pieces. The grafts were implanted subcutaneously through small incisions in the shaved skin of the back of recipient *ob/ob* mice anesthetized with isoflurane. Approximately 1 g of tissue was transplanted per mouse. Incisions were closed using wound clips and mice observed for wound closure. A second group of *ob/ob* mice was sham-operated. Mice were weighed twice weekly, beginning one day before transplant. A group of WT mice was also weighed and observed for comparison. Mice were observed for 6 weeks after transplant, at which point they were sacrificed by cervical dislocation under isoflurane anesthesia immediately after obtaining blood from the retroorbital plexus. Liver, thymus and spleen were obtained and processed immediately after euthanasia.

DSS-induced colitis

DSS colitis was induced and evaluated as previously described [6], with minor modifications. Beginning on day 47 after WAT transplant, mice were fed 2% DSS (mol wt, 40 kD; ICN, Aurora, OH) dissolved in sterile water ad libitum for 5 days followed by 5 days of normal drinking water. Control mice were fed tap water (without DSS). Mice were weighed daily and monitored for appearance of diarrhea and blood in the stools. A previously described clinical score which takes into account body weight loss, diarrhea and blood in the stools was used to quantify disease [14]. At the end of the experiment, colon cultures were performed as previously described [6]. Culture supernatants were used to evaluated production of IL-6 and

macrophage inflammatory protein-2 (MIP-2) by specific ELISA (R&D Systems, Minneapolis, MN).

Thymocyte and splenocyte isolation and flow cytometry

Thymocytes and splenocytes were freshly isolated using a 100μm cell strainer (Fisher Scientific, Pittsburgh, PA). Cells were suspended in complete cell culture medium (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin; Invitrogen Life Technologies, Carlsbad, CA), washed once and counted. For CD4 and CD8 T cell staining, thymocytes (10^6) were incubated with 1 μg of FITC-conjugated anti-CD4 and 1 μg of PE-conjugated anti-CD8 (BD Pharmingen, San Diego, CA) for 20 minutes at 4° C and washed with cold PBS three times. Rat IgG conjugated with FITC and PE served as negative control. Cells were then analyzed using a FACS Calibur (Becton Dickinson, San Diego, CA).

Miscellaneous measurements

Leptin, adiponectin, resistin, IL-6 and MIP-2 levels were measured using specific ELISA kits (R&D Systems). ALT levels were measured using a kit from Teco Diagnostics (Anaheim, CA). Glucose levels were measured using a glucometer (Bayer, Leverkusen Germany).

Statistical analysis

Data are expressed as mean $+/-$ SEM. Statistical significance of differences between treatment and control groups were determined by factorial ANOVA. Statistical analyses were performed using the XLStat software (Addinsoft, Brooklyn, NY).

Results and Discussion

Effect of WAT transplantation on body weight, metabolic parameters and adipokine levels

Transplantation of WT WAT effectively reduced adiposity (Fig. 1) and normalized the pronounced hepatomegaly, increased serum ALT and glucose levels characteristically observed in *ob/ob* mice (Fig. 2), in agreement with a previous report [7]. Interestingly, reduced adiposity and normalization of metabolic alterations were observed even in the presence of sharply reduced leptin levels in transplanted *ob/ob* compared to WT mice (Table 1). In fact, serum leptin in WAT transplanted *ob/ob* mice reached only 15% of the levels observed in WT mice, a result in agreement with data published by Klebanov et al [7]. The observation that very low circulating levels of leptin were sufficient to significantly ameliorate the phenotype of *ob/ob* mice has two possible explanations, not necessarily mutually exclusive: 1) *ob/ob* mice are exquisitely sensitive to leptin, as also suggested by their response to administration of low levels of recombinant leptin [15]. This increased sensitivity is likely due to overexpression of leptin receptors [16]; 2) increasing leptin levels above a low, critical threshold is not necessary for this adipokine to exert its full biological activity and higher levels may actually reduce sensitivity and lead to development of leptin resistance, as suggested by studies evaluating the effect of short-term fasting on hormonal and immune parameters in humans [17].

In contrast to leptin, serum levels of adiponectin and resistin in *ob/ob* mice were not significantly altered by WAT transplantation (Table 2). Serum adiponectin and resistin levels were slightly but significantly higher in both sham and transplanted *ob/ob* compared to WT mice, in agreement with some [18–20] though not other [21,22] previously published data. The reasons for the above-mentioned discrepancies in circulating adiponectin and resistin levels in *ob/ob* mice are currently unclear and may be secondary to experimental differences such as age, gender and genetic background of the mice studied by different investigators.

Effect of WAT transplantation on thymus and spleen cellularity

Several alterations of the immune system are present in *ob/ob* mice and are normalized by leptin administration, including thymus atrophy and reduced cellularity of the spleen [3,23]. Although leptin is mostly produced by white adipocytes, activated T lymphocytes - in addition to other cell types - also produce this adipokine [10]. Since *ob/ob* mice lack production of leptin from every cell type and administration of recombinant leptin unspecifically raises systemic leptin levels, it has until now not been possible to discriminate the cellular source of leptin necessary for modulation of the immune system.

Transplantation of WT WAT completely reversed thymus atrophy as well as reduced spleen cellularity in *ob/ob* mice (Table 2) and normalized thymocyte subpopulations, increasing the number of immature CD4$^+$ CD8$^+$ and reducing CD4$^-$ CD8$^+$ thymocytes (Fig. 3), an effect comparable to that achieved by administration of recombinant leptin [3]. Since in WAT-transplanted *ob/ob* mice leptin is exclusively produced by the transplanted tissue, these data clearly demonstrate that WAT-derived leptin is sufficient to maintain normal thymus and spleen cellularity and that lymphocyte-derived leptin is not necessary for this process, although we cannot exclude that leptin produced by lymphocytes might play a role under normal physiological conditions. Furthermore, the same remarks mentioned above concerning leptin threshold effects as well leptin sensitivity in *ob/ob* mice apply not only to the regulation of appetite and metabolism by leptin, but also to its effects on immunity.

Effect of WAT transplantation on DSS-induced intestinal inflammation

In addition to reduced cellularity of immune organs, *ob/ob* mice also develop significantly reduced inflammatory responses in a variety of experimental models [3–6]. In particular, leptin deficiency is associated with a markedly impaired colonic inflammatory response following administration of the irritant DSS [6]. Using cell transfer experiments, we have previously demonstrated that leptin produced by activated T lymphocytes does not play a critical role in mediating colonic inflammation in the model of colitis induced by transfer of CD4$^+$ CD45RB^{high} cells into *scid* mice [12]. However, activated T lymphocytes are not the only cells able to produce leptin in the inflamed intestine, since colonic epithelial cells activated by inflammatory stimuli can express and release leptin [9]. The contribution of WAT- *versus* colon-derived leptin in modulating intestinal inflammation is currently unclear.

As shown in Fig. 4, transplantation of WT WAT into *ob/ob* mice completely reversed the impaired inflammatory response to DSS, as evaluated by a clinical score that include measurements of body weight loss, diarrhea and presence of blood in the stools. Whereas sham-operated *ob/ob* mice developed only a very mild disease, the clinical scores of WT and transplanted *ob/ob* mice were not significantly different from each other at any of the time points examined. Colon shortening is a typical manifestation of inflammation in response to DSS [24]. Whereas sham-operated *ob/ob* mice did not demonstrate any significant shortening in the colon after DSS administration, marked shortening occurred in both WT and transplanted *ob/ob* mice (Table 3), further confirming the effectiveness of WAT transplantation in restoring colonic inflammation in *ob/ob* mice. Finally, production of the pro-inflammatory cytokines IL-6 and MIP-2 was significantly lower in colon cultures obtained from sham-operated *ob/ob* mice as compared to cultures obtained from WT or transplanted *ob/ob* mice (Fig. 5). Taken together, these data indicate that WAT-derived leptin is sufficient to restore colonic inflammation in *ob/ob* mice, whereas colon-derived leptin is not necessary.

Conclusions

The present report demonstrates that leptin derived exclusively from white adipose tissue is sufficient to reverse the metabolic, immune and inflammatory alterations of *ob/ob* mice. Although our data do not exclude the possibility that local production of leptin in immune

organs and in the inflammatory microenvironment might further modulate immunity and inflammation, the results clearly indicate that local leptin production is not necessary to maintain normal thymus and spleen cellularity and to allow for a full development of colonic inflammation.

References

1. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911–919. [PubMed: 15867843]

2. Friedman JM. The function of leptin in nutrition, weight, and physiology. Nutr Rev 2002;60:S1–S14. [PubMed: 12403078]

3. Faggioni R, Jones-Carson J, Reed DA, Dinarello CA, Feingold KR, Grunfeld C, et al. Leptin-deficient (*ob/ob*) mice are protected from T cell-mediated hepatotoxicity: role of tumor necrosis factor-a and IL-18. Proc Natl Acad Sci USA 2000;97:2367–2372. [PubMed: 10681432]

4. Matarese G, Di Giacomo A, Sanna V, Lord GM, Howard JK, Di Tuoro A, et al. Requirement for leptin in the induction of autoimmune encephalomyelitis. J Immunol 2001;166:5909–5916. [PubMed: 11342605]

5. Busso N, So A, Chobaz-Peclat V, Morard C, Martinez-Soria E, Talabot-Ayer D, et al. Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis. J Immunol 2002;168:875–882. [PubMed: 11777985]

6. Siegmund B, Lehr HA, Fantuzzi G. Leptin: a pivotal mediator of intestinal inflammation in mice. Gastroenterology 2002;122:2011–2025. [PubMed: 12055606]

7. Klebanov S, Astle CM, DeSimone O, Ablamunitis V, Harrison DE. Adipose tissue transplantation protects *ob/ob* mice from obesity, restores insulin sensitivity and restores fertility. J Endocrinol 2005;186:203–211. [PubMed: 16002549]

8. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, et al. The stomach is a source of leptin. Nature 1998;394:790–793. [PubMed: 9723619]

9. Sitaraman S, Liu X, Charrier L, Gu LH, Ziegler TR, Gewirtz A, et al. Colonic leptin: source of a novel proinflammatory cytokine involved in IBD. Faseb J 2004;18:696–698. [PubMed: 14977884]

10. Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest 2003;111:241–250. [PubMed: 12531880]

11. Siegmund B, Sennello JA, Lehr HA, Batra A, Fedke I, Zeitz M, et al. Development of intestinal inflammation in double IL-10- and leptin-deficient mice. J Leukoc Biol 2004;76:782–786. [PubMed: 15240754]

12. Fantuzzi G, Sennello JA, Batra A, Fedke I, Lehr AH, Zeitz M, et al. Defining the role of T cell-derived leptin in the modulation of hepatic or intestinal inflammation in mice. Clin Exp Immunol 2005;142:31–38. [PubMed: 16178853]

13. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest 2000;105:271–278. [PubMed: 10675352]

14. Siegmund B, Sennello JA, Jones-Carson J, Gamboni-Robertson F, Lehr HA, Batra A, et al. Leptin receptor expression on T lymphocytes modulates chronic intestinal inflammation in mice. Gut 2004;53:965–969. [PubMed: 15194645]

15. Montez JM, Soukas AA, Asilmaz E, Fayzikhodjaeva G, Fantuzzi G, Friedman JE. Acute leptin deficiency, leptin resistance and the physiologic response to leptin withdrawal. Proc Nat Acad Sci USA. 2005In press

16. Baskin DG, Seeley RJ, Kuijper JL, Lok S, Weigle DS, Erickson JC, et al. Increased expression of mRNA for the long form of the leptin receptor in the hypothalamus is associated with leptin hypresensitivity and fasting. Diabetes 1998;47:538–543. [PubMed: 9568684]

17. Chan JL, Matarese G, Shetty GK, Raciti P, Kelesidis I, Aufiero D, et al. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. Proc Nat Acad Sci USA 2006;103:8481–8486. [PubMed: 16714386]

18. Haluzik M, Dietz KR, Kim JK, Marcus-Samuels B, Shulman GI, Gavrilova O, et al. Adrenalectomy improves diabetes in A-ZIP/F-1 lipoatrophic mice by increasing both liver and muscle insulin sensitivity. Diabetes 2002;51:2113–2118. [PubMed: 12086940]

19. Delporte ML, El Mkadem SA, Quisquater M, Brichard SM. Leptin treatment markedly increased plasma adiponectin but barely decreased plasma resistin of ob/ob mice. Am J Physiol Endocrinol Metab 2004;287:E446–E453. [PubMed: 15126241]

20. Sennello JA, Fayad R, Morris AM, Eckel RH, Asilmaz E, Montez JM, et al. Regulation of T cell-mediated hepatic inflammation by adiponectin and leptin. Endocrinology 2005;146:2157–2164. [PubMed: 15677756]

21. Fujinami A, Ohta K, Matsui H, Kitada N, Kitaura Y, Kawahara Y, et al. Resistin concentrations in murine adipose tissue and serum measured y a new enzyme immunoassay. Obesity (Silver Spring) 2006;14:199–205. [PubMed: 16571844]

22. Makimura H, Mizuno TM, Bergen H, Mobbs CV. Adiponectin is stimulated by adrenalectomy in ob/ob mice and is highly correlated with resistin mRNA. Am J Physiol Endocrinol Metab 2002;283:E1266–E1271. [PubMed: 12388167]

23. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 1998;394:897–901. [PubMed: 9732873]

24. Okayasu I, Hatakeyama S, Yamada M, Okhusa T, Inagaki Y, Nakaya R. A novel method for the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 1990;98:694–702. [PubMed: 1688816]
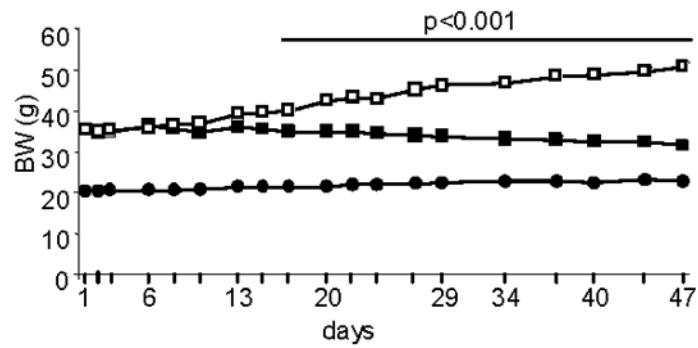
Figure 1. Effect of WAT transplantation on body weight
Body weight was measured in *ob/ob* mice transplanted with WAT (closed squares), sham-operated *ob/ob* mice (open squares) and WT mice (closed circles) starting on the day of WAT transplant and continuing until day 47. Data are mean +/− SEM of 10 mice per group. BW of *ob/ob* sham was significantly different from that *ob/ob* transplant starting on day 17 and continuing until day 47.
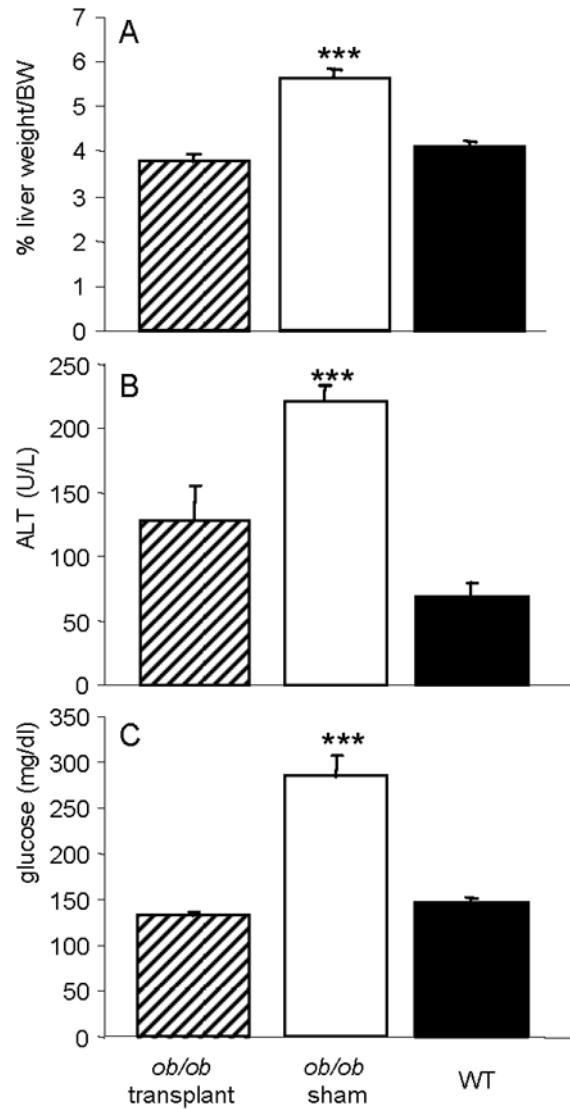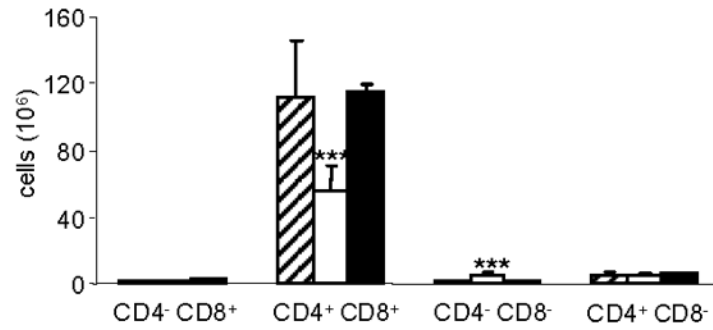
Figure 2. Effect of WAT transplantation on liver weight, ALT and glucose levels
Liver and serum were obtained from *ob/ob* mice transplanted with WAT (hatched columns),
sham-operated *ob/ob* mice (open columns) and WT mice (closed columns) on day 47. Panel
A: liver weight as percent of BW; Panel B: Serum ALT levels; Panel C: blood glucose levels.
Data are mean +/− SEM of 10 mice per group. *** p<0.001 *versus ob/ob* transplant and WT.
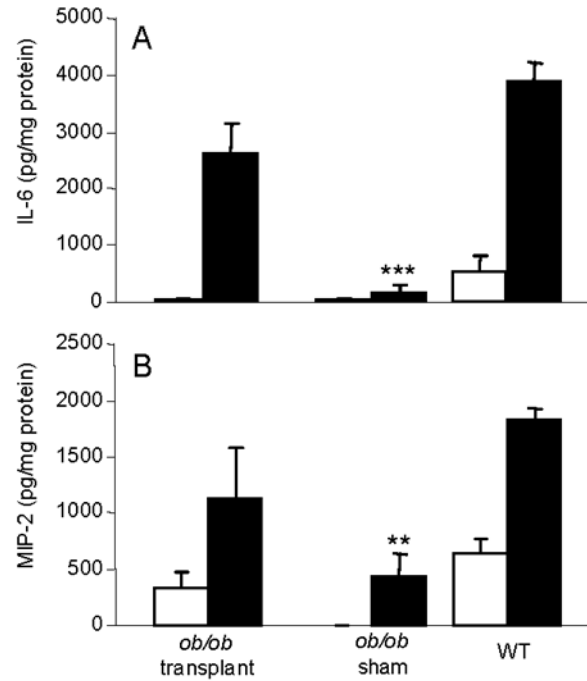
Figure 3. Effect of WAT transplantation on thymocyte subpopulations
Thymocytes were isolated from the thymus of *ob/ob* mice transplanted with WAT (hatched columns), sham-operated *ob/ob* mice (open columns) and WT mice (closed columns) and analyzed by flow cytometry as described in the Methods section. Data are mean +/− SEM of 5 mice per group. *** p<0.001 *versus ob/ob* transplant and WT.
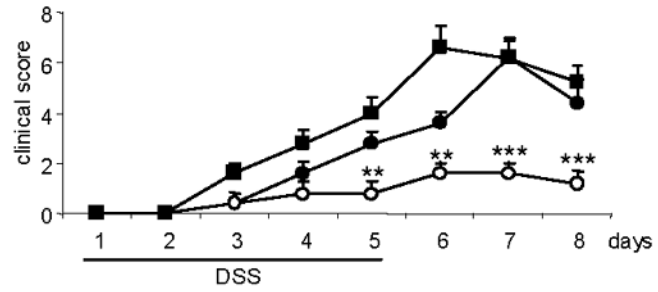
Figure 4. DSS-induced intestinal inflammation: effect of WAT transplantation
ob/ob mice transplanted with WAT (closed squares), sham-operated *ob/ob* mice (open circles) and WT mice (closed circles) received DSS for 5 days following the schedule described in the Methods section. Clinical score was evaluated daily. Data are mean +/− SEM of 5 mice per group. ** p<0.01, *** p<0.001 *versus ob/ob* transplant and WT.

Figure 5. Effect of WAT transplantation on colonic production of IL-6 and MIP-2
On day 8 of administration of either regular drinking water (open columns) or DSS (closed colums), colon cultures were performed and levels of IL-6 (panel A) and MIP-2 (panel B) evaluated in the supernatants. Data are mean +/− SEM of 5 mice per group. ** p<0.01, *** p<0.001 *versus ob/ob* transplant and WT treated with DSS.

Table 1

Effect of WAT transplantation on circulating adipokine levels

	Leptin (ng/ml)	Adiponectin (µg/ml)	Resistin (ng/ml)
ob/ob transplant	0.60 +/− 0.10 ***	8.11 +/− 0.72*	2.51 +/− 0.09*
ob/ob sham	<0.02	7.58 +/− 0.51*	2.63 +/− 0.07*
WT	4.10 +/− 0.99	6.95 +/− 0.63	2.11 +/− 0.14

Blood was obtained on day 47 from *ob/ob* transplant, *ob/ob* sham and WT mice and serum prepared for measurement of leptin, adiponectin and resistin by ELISA. Data are mean +/− SEM of 10 mice per group. *p<0.05, *** p<0.001 *versus* WT.

Table 2

Effect of WAT transplantation on thymus and spleen cellularity

	Thymocytes (10^6)	Splenocytes (10^6)
ob/ob transplant	118.67 +/− 32.47	189.33 +/− 11.02
ob/ob sham	66.75 +/− 14.52 **	138.50 +/− 18.45 *
WT	124.40 +/− 5.08	186.00 +/− 19.32

Thymi and spleens were obtained on day 47 from *ob/ob* transplant, *ob/ob* sham and WT mice and cellularity evaluated. Data are mean +/− SEM of 5 mice per group. * p<0.05, ** p<0.01 *versus* WT and *ob/ob* transplant.

Table 3

Effect of WAT transplantation on DSS-induced colon shortening

	Water	DSS
ob/ob transplant	7.75 +/− 0.10	5.54 +/− 0.31**
ob/ob sham	7.88 +/− 0.27	7.14 +/− 0.26
WT	7.18 +/− 0.11	4.96 +/− 0.19**

Mice received either regular drinking water or DSS as detailed in the Methods sections. On day 10, colons were removed and their length measured using a ruler. Data are expressed as cm of colon length. Data are mean +/− SEM of 5 mice per group. ** $p < 0.01$ *versus* respective water.