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Augmented Production of Tumor Necrosis Factor-α in Obese Mice

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Non-insulin-dependent diabetes mellitus develops in obesity. The insulin resistance of this disease may be mediated by tumor necrosis factor-α (TNF-α). In particular, the TNF-α derived from adipose tissues might be involved in the induction of peripheral insulin resistance in rodent models of obesity. In general, monocytes/macrophages have been considered as the major source of TNF-α. This study was designed to examine the potential production of TNF-α from monocyte/macrophages in obese mice. In obese (*ob/ob*) and obese diabetic (*db/db*) mice, both of which are known to have severe insulin resistance, unstimulated serum bioactivity of TNF-α was significantly higher than that in lean control mice. Spontaneous TNF-α mRNA expression in splenic macrophages was also enhanced in obese mice, but not in monosodium-L-glutamate (MSG)-induced obese mice which have no insulin resistance. In addition, both *ob/ob* and *db/db* mice produce more TNF-α than lean mice upon *in vivo* lipopolysaccharide (LPS) stimulation. The LPS-induced increase in serum TNF-α activity was not observed in MSG-induced obese mice. Taken together, it is postulated that TNF-α produced by monocytes/macrophages may also play an important role in the genesis of insulin resistance in obesity. Further study is needed to reveal the mechanism of enhanced TNF-α production in obese states and its possible etiologic relevance to obesity.

INTRODUCTION

Non-insulin-dependent diabetes mellitus (NIDDM) is the most common serious metabolic disorder. Obesity and NIDDM are usually both associated with pronounced insulin resistance (1, 2). This insulin resistance may also have an important role in the development of hypertension and cardiovascular disease (3). However, the cellular and molecular mechanisms by which insulin resistance develops in obesity are not well established.

Tumor necrosis factor-α (TNF-α) is recognized as a multifunctional cytokine (2, 4, 5), which suppresses lipoprotein lipase activity (6–8), activates of neutrophils (9), mediates endotoxin-induced shock (10, 11), and is a central regulator of inflammation and immunity (5, 10, 12). The recent finding that chronic administration of TNF-α to rodents induces systemic insulin resistance which can be blocked by specific TNF-α antibodies, indicates that TNF-α could be relevant to the pathogenesis of insulin resistance. It has been shown that adipose tissue expresses TNF-α mRNA. It is possible that TNF-α derived from adipose tissues might be involved in the induction of peripheral insulin resistance in rodent models of obesity (13, 14). Although TNF-α has recently received considerable attention, the cellular source for TNF-α production relevant to insulin resistance is not clear. In general, monocyte/macrophage lineages have been considered as the major source of TNF-α (15, 16). In order to test the hypothesis that monocytes/macrophages may be involved in the development of insulin resistance, we examined circulatory levels of TNF-α, *in vivo* TNF-α production induced by LPS, and TNF-α mRNA expression in splenic macrophages in obese (*ob/ob*) and obese diabetic (*db/db*) mice.

MATERIALS AND METHODS

Mice

C57BL/6 (*ob/ob*) and lean control (+/?) mice were originally donated by Professor M. Nishimura (Hamamastu University), and C57BL/KS (*db/db*) and lean (+/?) mice were kindly provided by Professor M. Fujiwara (University of Tokyo). All mice were maintained at the Laboratory Animal Facility, Yokohama City University. Male mice were used in this study, and all were 10–12 weeks old. They were given free access to water and standard chow pellets and kept under uniform conditions.

Assay of TNF-α Activity

TNF-α levels were assayed using a bioassay of LM cells and a subline of TNF-sensitive mouse fibroblast (L929) and by recombinant human TNF-α (kindly provided by Asahi Chemical Industry, Shizuoka, Japan) as a standard (10). Briefly, 2×10^5 LM cells suspended

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in 0.1 ml of RPMI 1640, supplemented with 5% heat-inactivated fetal bovine serum, were cultured in a 96-well microculture plate. After confluent cell growth, the same volume of mouse serum containing Actinomycin D (Sigma, St. Louis, MO), final concentration 2 μg/ml, was added. Twenty-four hours later, the plates were stained with 0.2% crystal violet and optical density (OD) at 540 nm was measured with an autoreader (SJeia autoreader, Sanko Junyaku, Tokyo, Japan). The detection limit of our bioassay was about 50 pg/ml. To study the influence of TNF-β on the bioassay, the serum samples were preincubated with polyclonal rabbit anti-human TNF-β (Genzyme, Cambridge, MA) or anti-mouse TNF-α (Genzyme, Cambridge, MA) and assayed. In addition, immunoreactive TNF-α concentrations were determined using commercial ELISA kit (Genzyme, Cambridge, MA).

Treatment with LPS

ob/ob and *db/db* mice were injected intravenously with 10 μg of LPS (LPS W *Escherichia coli* 055:B5, Difco Laboratories, Detroit, MI) via the tail vein. Blood samples were obtained by cardiac puncture under ether anesthesia. Previously we investigated the time course of *in vivo* TNF-α production after LPS injection and found that TNF-α activity reached a maximum level at 90 min after injection in rats (17). Similarly, in both *ob/ob* and *db/db* mice, 30 min after LPS injection, TNF-α activity appeared in the serum, reached maximum level at 90 min, and then gradually decreased to less than 1 unit/ml at 180 min in all strains of mice examined. We therefore obtained blood samples for TNF-α assay 90 min after LPS injection throughout the study.

Treatment of MSG

C57BL/6 mice were injected subcutaneously with monosodium-L-glutamate (MSG) (Wako Pure Chemicals Industries, Tokyo, Japan) at a dosage of 2 mg/kg body weight daily for 5 days after birth (18, 19). Control mice received an equal volume of physiological saline.

RNA Isolation and Northern Blot Analysis

Spleens were isolated before and 60 min after LPS administration. Splenic macrophages were separated by their adhesion to plastic as reported (20). The adherent cells were >95% stained with α-naphthyl acetate esterase. Total RNAs were extracted from the adherent cells by guanidinium thiocyanate and purified by ultracentrifugation (21). For Northern blot analysis, 20 μg of total RNA was applied per lane. RNA was size-separated by electrophoresis on 1% agarose/formaldehyde gels and then transferred to Hybond-N membranes (Amersham, Japan, Tokyo). The RNA was immobilized on nylon filters by vacuum-baking at 80°C for 2 hr. The blots were prehybridized for 6 hr at 42°C in a mixture of 50% Formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS, and 100 μg/ml denatured salmon sperm DNA. They were subsequently hybridized for another 24 hr at 42°C in the same solution containing 0.2 μg of a nick-translated ^{32}P-labeled mouse TNF-α cDNA probe (4×10^8 cpm/μg DNA). After hybridization, the nylon filters were washed three times for 10 min at room temperature in 2× SSC and 0.05% SDS and then for 1 hr in 1× SSC and 01% SDS at 65°C. The nylon filters were exposed at −70°C to Kodak XAR film with intensifying screens. Resulting autoradiographs were scanned by densitometry to quantitate the relative levels of TNF-α mRNA with a Fujix BAS 2000 Bio-image Analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan). The mouse TNF-α cDNA probe was kindly provided by Asahi Chemical Industry (Shizuoka, Japan).

Statistical Analysis

Differences in each parameter between the lean and obese mice were evaluated by unpaired t tests after ensuring that the data were normally distributed and that they met criteria for equality of variance. χ^2 test was performed at comparison with serum TNF-α activity without LPS stimulation in *ob/ob* and lean mice.

RESULTS

Body Weight in Obese Mice

The body weights of *ob/ob* were significantly higher than those of their lean (*ob*/+ or +/+) counterparts (45.8 ± 7.0 vs. 24.3 ± 1.6, $P < 0.001$) and body weights in *db/db* mice were significantly higher than that in *db*/+ or +/+ mice (46.9 ± 5.8 vs. 26.7 ± 3.5, $P < 0.001$). MSG-treated mice also showed significantly higher body weights than MSG-free controls (39.7 ± 4.1 vs. 24.2 ± 3.8, $P < 0.001$).

Serum Concentration of TNF-α

Serum concentrations of TNF-α were measured using the bioassay (Fig. 1). In *ob/ob* mice, TNF-α was detectable in 13 of 18 (72%), with concentrations ranging from 55 to 225 pg/ml (mean ± SE = 89 ± 24). Only 2 of 13 (15%) lean mice had detectable amounts of TNF-α activity (60 and 124 pg/ml). The difference in the fraction of lean and obese mice having detectable TNF-α was statistically significant ($P < 0.01$, χ^2 test). In *db/db* mice, serum TNF-α levels were also increased compared with lean (data not shown), indicating that circulating TNF-α is elevated in both obese animals.

Northern Blotting Analysis

To elucidate the TNF-α production at the transcriptional level, we examined TNF-α-specific mRNA ex-

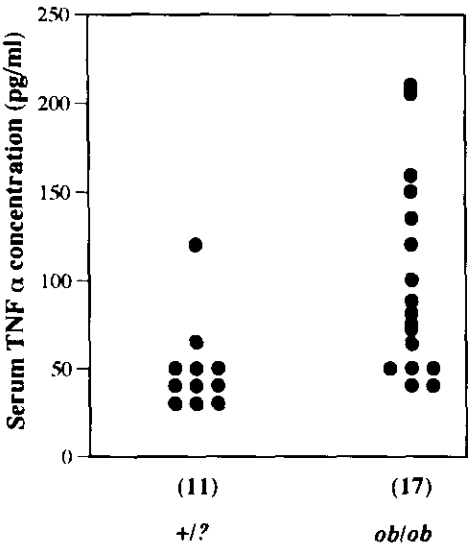

FIG. 1. Serum TNF-α cytotoxic activity in *ob/ob* and *ob/+* or +/+ mice. Blood samples were collected and TNF-α levels were examined by bioassay as described under Materials and Methods.

pression of splenic macrophages. The amount of spontaneous TNF-α mRNA expression is significantly increased in *ob/ob* and *db/db* mice compared with their lean controls (Fig. 2). We also examined the MSG model of chemically induced obesity. In this model the obesity is less severe and develops without hyperphagia, hyperinsulinemia, or insulin resistance. TNF-α mRNA expression was not enhanced in MSG-treated obese mice. The LPS stimulated TNF-α mRNA expression was at a level similar to that seen in control mice pattern. These results indicated that TNF-α mRNA expression might be closely relevant to insulin resistance.

LPS-Stimulated in Vivo TNF-α Production

We next examined the maximum *in vivo* TNF-α production induced by LPS. As shown in Fig. 3, serum cytotoxic activity of TNF-α in *ob/ob* mice was significantly increased compared with that in lean controls (121.5 ± 19.5 vs 22.5 ± 18.0, $P < 0.001$). In order to investigate whether enhanced TNF-α production is also shown in aged mice, we examined LPS-stimulated TNF-α production at 35 weeks of age in *ob/ob* and lean control mice. The results showed that bioactive TNF-α levels of *ob/ob* mice at 35 weeks of age were also significantly enhanced compared with that of lean control (114.5 ± 23.6 vs 12.4 ± 7.5, $P < 0.001$). *db/db* mice also showed an enhanced TNF-α production when stimulated by LPS compared with leans (52.5 ± 10 vs 2.5 ± 0.7, $P < 0.01$). However, MSG-induced obese mice did not show a significant difference in TNF-α production compared with their lean controls (7.8 ± 2.9 vs 5.3 ± 3.5). To study whether the serum bioactivity induced in LM cells was caused by TNF-α or TNF-β, the serum

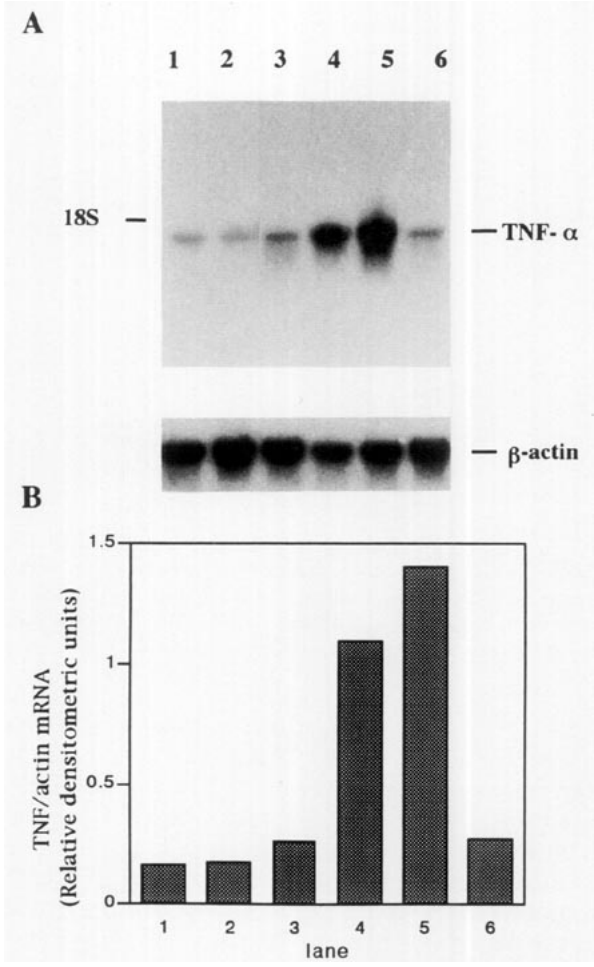

FIG. 2. Expression of TNF-α mRNA of splenic macrophages in various models of obesity. (A) The mRNA from mouse spleen (20 μg/lane) were electroded, blotted, and hybridized with radiolabeled TNF-α, as described under Materials and Methods. β-Actin mRNA is shown as a control for loading and integrity of the RNA. (B) Levels of mRNA were determined by densitometer and expressed as relative densitometric units of TNF-α/β-actin. Lane 1, *db/db* lean (+/?) mice; lane 2, *ob/ob* lean (+/?) mice; lane 3, C57BL/6 MSG-nontreated mice; lane 4, *db/db* obese mice; lane 5, *ob/ob* obese mice; lane 6, MSG-treated C57BL/6 mice.

samples were preincubated with polyclonal rabbit anti-human TNF-β or rabbit anti-mouse TNF-α and subjected to assay. Serum cytotoxic activity for LM cells was significantly decreased by the anti-mouse TNF-α, but not by the anti-hTNFβ, demonstrating the specificity to TNF-α (data not shown).

In addition, immunoreactive TNF-α concentrations were determined by ELISA. Immunoreactive TNF-α concentrations were similar to those by the LM cell bioassay (Fig. 4).

DISCUSSION

The pathogenesis of insulin resistance in obesity has not yet been identified. Until recently, there were no reports of associations between insulin resistance and TNF-α. Now, some reports showed that there is a direct

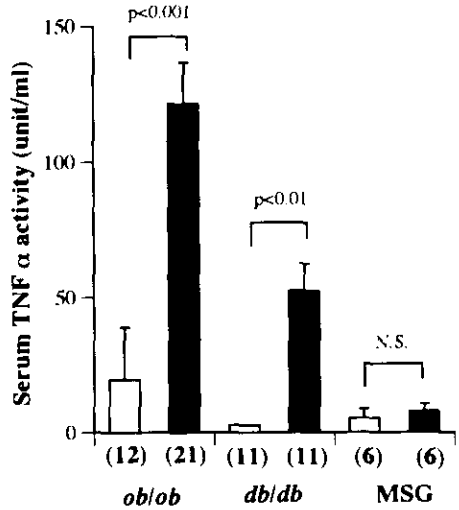

FIG. 3. Serum cytotoxic activity of TNF-α induced by LPS in *ob/ob* mice, *db/db* mice, MSG-treated C57BL/6 mice, and each lean control mice. Serum TNF activity is measured by LM cell using bioassy. Each bar represents mean ± SEM. Open bars, lean control mice; closed bars, obese mice.

role for TNF-α in causing insulin resistance. Chronic, low-level administration of TNF-α to rodents induces systemic insulin resistance (13). Hotamisligil *et al.* showed that the induction of TNF-α mRNA expression was observed in adipose tissue from four different rodent models of obesity and diabetes (14). Neutralization of TNF-α in obese *fa/fa* rats caused a significant increase in the peripheral uptake of glucose in response to insulin. These results indicate a role for TNF-α in obesity and particularly in the insulin resistance that often accompanies obesity and NIDDM.

Although these results are very provocative and interesting, the question of the source of TNF-α remains. Monocytes and macrophages have been considered the major source of TNF-α (15, 16, 22, 23). Our question is whether TNF-α production from monocytes and macrophages is increased in obesity. In order to investigate this, we examined *in vivo* TNF-α production with or without stimulation by LPS in obese mice and controls. In the present study, the results show that serum levels of TNF-α are significantly elevated in both *ob/ob* and *db/db* mice. Northern blotting analysis showed that spontaneous TNF-α mRNA expression in splenic macrophages was significantly increased in both *ob/ob* and *db/db* mice, but not in MSG-treated mice. In addition, *in vivo* TNF-α production induced by LPS was also increased in obesity. Thus, it is likely that the different levels of TNF-α production between obese and lean strains may be an enhancement of TNF-α production from macrophages which is controlled at the transcriptional level. These results also suggest that this TNF-α production may be relevant to insulin resistance. Although we examined TNF-α mRNA expression in splenic macrophages in this study, it seems to be important to elucidate the major sources of TNF-α among macrophages in various tissues. Since it is well known that Kupffer cells are one major source of TNF-α in a variety of experimental models (24), it might be necessary to study in the future whether TNF-α production from Kupffer cells is enhanced in these insulin-resistant obese mice.

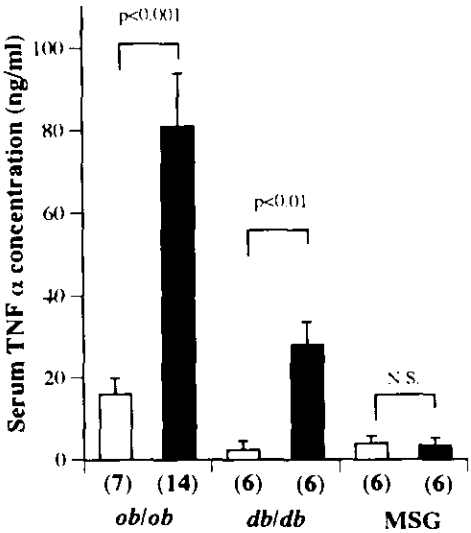

FIG. 4. Serum immunoreactive TNF-α concentration in *ob/ob* mice, *db/db* mice, MSG-treated C57BL/6 mice, and lean control mice. Immunoreactive TNF-α is measured by ELISA. Each bar represents mean ± SEM. Open bars, lean control mice; closed bars, obese mice.

In obesity and non-insulin-dependent diabetes, the effect of sex steroids, including DHEA, on insulin resistance has been already examined. DHEA treatment has been shown to reduce the hyperglycemia of diabetic (*db/db*) mice (25, 26), streptozotocin diabetic mice (27), obese Zucker rats (28), and obese (*ob/ob*) mice (29). Glucose tolerance and/or the glucose to insulin ratios were improved by DHEA treatment in these models and the improvement was attributed to an increased sensitivity of adipocytes to insulin. This improvement in sensitivity was likely to be due to the decrease in fat cell mass (30, 31). Recently, Leiter *et al.* showed that impairment of the balance of androgen and estrogen sulfotransferase in hepatocytes of *db/db* mice indicates that sex steroids are important for the induction of insulin resistance in these mice (32). Although many studies reported a relationship between DHEA and insulin resistance, the role of sex steroids including DHEA on TNF-α production from monocytes/macrophages has not yet been clearly identified. Thus, it seems to be important to elucidate whether the improvement of insulin sensitivity by DHEA may be mediated by suppressing the TNF-α production.

Besides insulin resistance, other metabolic abnormalities are found in obesity. Plasma lipid levels are elevated in obese subjects and those with non-insulin-dependent diabetes mellitus. The importance of hyperlipidemia is related to its increased risk of atheroscle-

rosis. It has been shown that TNF-α stimulates hepatic triglyceride production and increases serum triglycerides (33, 34, 5). In addition, TNF-α secreted from macrophage can enhance the expression of vascular cell-adherence molecules on endothelial cells (35) and can induce smooth muscle cell proliferation. TNF-α may play some part in the progression of atherosclerotic process through these processes (17). Thus, enhancement of TNF-α production in obese syndromes might also be related to the pathogenesis of hyperlipidemia and atherosclerosis in obesity and non-insulin-dependent diabetes.

In conclusion, it is clear that serum TNF-α levels are elevated with or without LPS stimulation in models of obesity and are pretranscriptionally regulated and that macrophages play a major part in the enhancement of TNF-α production (although TNF-α derived from adipose tissues was not examined in our study). The present study, and previous reports, lead us to believe that the central mechanism of insulin resistance in obese syndromes may be caused by TNF-α derived from macrophages and/or adipose tissues. However, the pathogenesis and significance of enhancement of TNF-α production in obesity and related metabolic abnormalities remains to be described.

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REFERENCES

1. Kolterman, O., Gray, R. S., Griffin, J., Burstein, P., Insel, J., Scarlett, J. A., and Olefsky, J. M., Receptor and postreceptor defects contribute to the insulin resistance in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* 68, 957–969, 1981.
2. Defronzo, R. A., Simonson, D., and Ferrannini, E., Hepatic and peripheral insulin resistance: A common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23, 313–319, 1982.
3. Bjorntorp, P., Obesity, insulin resistance and diabetes. *Diabetes Annu.* 6, 347–370, 1991.
4. Beutler, B., and Cerami, A., Tumor necrosis, cachexia, shock, and inflammation: A common mediator. *Annu. Rev. Biochem.* 57, 505–518, 1988.
5. Old, L. J., Tumor necrosis factor. *Sci. Am.* 258, 41–59, 1988.
6. Kawakami, M., Cerami, A., Studies of endotoxin-induced decrease in lipoprotein lipase activity. *J. Exp. Med.* 154, 631–639, 1981.
7. Kawakami, M., Pekala, P. H., Lane, M. D., and Cerami, A., Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. *Proc. Natl. Acad. Sci. USA* 79, 912–916, 1982.
8. Beutler, B., Mahoney, J., Pekala, P., Le Trang, N., and Cerami, A., The major endotoxin-inducible secretory product of cultured macrophages ia a novel lipolytic hormone. *Blood* 64, 65a, 1984.
9. Shalaby, M. R., Aggarwal, B. B., Rinderknecht, E., Svedersky, L. P., Finkle, B. S., and Palladino, Jr. M. A., Activation of polymorphonuclear neutrophil functions by gamma-interferon and tumor necrosis factors. *J. Immunol.* 135, 2069–2073, 1985.
10. Beutler, B., and Cerami, A., The biology of cachectin/TNF-α primary mediator of the host response. *Annu. Rev. Immunol.* 7, 625–655, 1989.
11. Beutler, B., Milsark, I. W., Cerami, A. C., Passive immunization against Cachectin/Tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* 229, 869–871, 1985.
12. Paul, N. L., and Ruddle, N. H., Lymphotoxin. *Annu. Rev. Immunol.* 6, 407–438, 1988.
13. Lang, C. H., Dobrescu, C., and Bagby, G. J., Tumor necrosis factor impairs insulin action on peripheral glucose proposal and hepatic glucose output. *Endocrinology* 130, 43–52, 1992.
14. Hotamisligil, G. S., Shagill, N. S., and Spiegelman, B. M., Adipose expression of TNF-α: Direct role in obesity-linked insulin resistance. *Science* 259, 87–91, 1993.
15. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B., An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA* 72, 3666–3670, 1975.
16. Higuchi, M., Higashi, N., Taki, H., and Osawa, T., Cytolytic mechanism of activated macrophages. Tumor necrosis factor and L-arginine-dependent mechanisms act synergistically as the major cytolic mechanisms of activated macrophages. *J. Immunol.* 144, 1425–1431, 1990.
17. Lopes-Virella, M. F., and Virella, G., Immune mechanisms of atherosclerosis in diabetes mellitus. *Diabetes 41* (Suppl.2), 86–91, 1992.
18. Olney, J. W., Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 164, 719–721, 1969.
19. Cameron, D. P., Catbush, L., and Opat, F. Effects of monosodium glutamate-induced obesity in mice on carbohydrate metabolism in inulin secretion. *Clin. Exp. Pharmacol. Physiol.* 5, 41–51, 1978.
20. Newman, S. L., Mussen, R. A., and Henson, P. M., Development of functional complement receptors during in vitro maturation of human monocytes into macrophages. *J. Immunol.* 125, 2236–2244, 1980.
21. Sambrook, J., Fritch, E. F., and Maniatis, T., "Molecular Cloning: A Laboratory Manual," p. 7.19, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.
22. Mannel, D. N., Moore, R. N., and Mergenhager, S. E., Macrophages as a major source of tumoricidal activity (tumor necrotizing factor). *Infect. Immunol.* 30, 523–530, 1980.
23. Matthews, N., Production of an anti-tumor cytotoxin by human monocytes. *Immunology* 44, 135–142, 1981.
24. Magilavy, D. B., and Pothstein, J. L., Spontaneous production of tumor necrosis factor α by Kupffer cells of MRL/lpr mice. *J. Exp. Med.* 168, 789–794, 1988.
25. Coleman, D. L., Antiobesity effects of etiocholanolone in diabetes (db), viable yellow (A^{vy}) and normal mice. *Endocrinology* 117, 2279–2283, 1985.
26. Coleman, D. L., Leiter, E. H., and Applezweig, N., Therapeutic effects of dehydroepiandrosterone metabolites in diabetes mutant mice (C57BL/KsJ-db/db) *Endocrinology* 115, 239–243, 1984.
27. Coleman, D. L., Leiter, E. H., and Schwizer, R. W., Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. *Diabetes* 31, 830–833, 1982.
28. Muller, S., and Cleary, M. P., Glucose metabolism in isolated adipocytes from lean and obese Zucker rats following treatment with dehydroepiandrosterone. *Metabolism* 34, 278–284, 1985.
29. Coleman, D. L., Schwizer, R. W., and Leiter, E. H., Effect of genetic background on the therapeutic effects of dehydroepiandros-

terone (DHEA) in diabetes-obesity mutants and in aged normal mice. *Diabetes* 33, 26–32, 1984.

30. Tagliaferro, A. R., Davis, J. R., Truchon, S., and Van Hamont, N., Effects of DHEA acetate on metabolism, body weight and composition in male and female rats. *J. Nutr.* 116, 1977–1983, 1986.
31. Muller, S., Cleary, M. P., Glucose metabolism in isolated adipocytes from lean and obese Zucker rats following treatment with dehydroepiandrosterone. *Metabolism* 34, 278–284, 1985.
32. Leiter, E. H., Chapman, H. D., Obesity-induced diabetes (diabesity) in C57BL/KsJ mice produces aberrant trans-regulation of sex steroid sulfotransferase genes. *J. Clin. Invest.* 93, 2007–2013, 1994.

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33. Chajek-Shaul, T., Friedman, G., Stein, O., Shiloni, E., Etiene, J., and Stein, Y., Mechanism of the hyperlipidemia induced by tumor necrosis factor administration to rats. *Biochim. Biophys. Acta* 1001, 316–324, 1989.
34. Feingold, K. R., Serio, M. J., Adi, S., Moser, A. H., and Grunfeld, C., Tumor necrosis factor simulates hepatic lipid synthesis and secretion. *Endocrinology* 124, 2336–2342, 1989.
35. Luxninskas, F. W., Brock, A. F., Arnaout, M. A., and Gimbrone, M. A., Jr., Endothelial-leukocyte adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. *J. Immunol.* 142, 2257–2263, 1989.