

Surgical Removal of Visceral Fat Reverses Hepatic Insulin Resistance

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We directly examined whether visceral fat (VF) modulates hepatic insulin action by randomizing moderately obese (body wt ~400 g) Sprague-Dawley rats to either surgical removal of epididymal and perinephric fat pads (VF⁻; *n* = 9) or a sham operation (VF⁺; *n* = 11). Three weeks later, total VF was fourfold increased (8.5 ± 1.2 vs. 2.1 ± 0.3 g, *P* < 0.001) in the VF⁺ compared with the VF⁻ group, but whole-body fat mass (determined using ³H₂O) was not significantly different. The rates of insulin infusion required to maintain plasma glucose levels and basal hepatic glucose production in the presence of hepatic-pancreatic clamp were markedly decreased in VF⁻ compared with VF⁺ rats (0.57 ± 0.02 vs. 1.22 ± 0.19 mU · kg⁻¹ · min⁻¹, *P* < 0.001). Similarly, plasma insulin levels were more than twofold higher in the VF⁺ group (*P* < 0.001). The heightened hepatic insulin sensitivity is supported by the decrease in gene expression of both glucose-6-phosphatase and PEPCK and by physiological hyperinsulinemia in VF⁻ but not VF⁺ rats. The improvement in hepatic insulin sensitivity in VF⁻ rats was also supported by a ~70% decrease in the plasma levels of insulin-like growth factor binding protein-1, a marker of insulin's transcription regulation in the liver. The removal of VF pads also resulted in marked decreases in the gene expression of tumor necrosis factor- α (by 72%) and leptin (by 60%) in subcutaneous fat. We conclude that visceral fat is a potent modulator of insulin action on hepatic glucose production and gene expression. *Diabetes* 48:94–98, 1999

Increased visceral adiposity is a risk factor for insulin resistance, diabetes, and mortality from arteriosclerotic disease and has been shown to largely account for the variability in insulin sensitivity in heterogeneous populations (1–4). Although postabsorptive hyperinsulinemia has been shown to maintain glucose production (GP) within normal limits, its suppression by insulin is signifi-

cantly impaired in individuals with visceral obesity (5). Bjorntorp (6) hypothesized that visceral fat (VF) results in hepatic insulin resistance via a "portal" effect of free fatty acids (FFAs) and glycerol released by increased omental fat, as previously demonstrated (7). Alternative hypotheses have also been proposed to explain the link between VF and peripheral and hepatic insulin resistance. For example, the novel evidence for "endocrine functions" of adipose tissue allows one to speculate that alterations in the expression of fat-derived proteins, such as tumor necrosis factor (TNF)- α , may also play a role (8).

Insulin regulates hepatic GP and the gene expression of several proteins, including glucose-6-phosphatase (Glc-6-Pase), PEPCK, and insulin-like growth factor binding protein (IGFBP)-1 (9–11). A common motif has been characterized for the insulin response element of PEPCK, Glc-6-Pase, and IGFBP-1 genes (9–11). In the presence of insulin resistance, insulin may also fail to regulate the transcription of those genes. In that case, the expression of the proteins may be normal or even increased, despite markedly elevated circulating insulin levels.

While IGFBP-1 is not directly involved in the regulation of GP, the marked similarities between the IGFBP-1 and PEPCK promoters, including regions conferring insulin, glucocorticoid, and cAMP responses, have spurred interest in the use of this circulating protein as a noninvasive index of the liver-specific transcriptional action of insulin in vivo (12,13). In fact, recent data suggest that serum IGFBP-1 is the most direct and accurate indicator of insulin sensitivity in humans and serves as a marker of diseases associated with insulin resistance (14–22).

We previously reported that the ability of insulin to inhibit GP is markedly impaired in an obese rodent model of aging (23). Recently, we demonstrated that lowering VF by caloric restriction (24) or leptin administration (25) dramatically improved hepatic insulin sensitivity independent of whole-body fat mass. In this study, we provide direct evidence in support of a cause-effect relationship between VF and hepatic insulin action by examining the impact of the surgical removal of intra-abdominal fat pads on hepatic insulin sensitivity, Glc-6-Pase and PEPCK mRNA levels, circulating IGFBP-1 levels, and adipose tissue expression of TNF- α .

RESEARCH DESIGN AND METHODS

Animals. Four-month-old male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) (*n* = 20) were anesthetized (pentobarbital 50 mg/kg body wt intraperitoneally) and randomly assigned to one of two surgical procedures: 1) VF⁻ (*n* = 9), epididymal (Epi) and perinephric (Peri) fat pads removed, weighed, and immediately frozen in liquid nitrogen; 2) VF⁺ (*n* = 11), sham operation. Three weeks later, rats were anesthetized, and indwelling catheters were inserted in the right internal jugular vein and the left carotid artery (12–14). Recovery continued until body weight and daily food intake were within 5% of preoperative levels. Studies were performed in 6-h fasted, awake, unstressed, chronically catheterized rats. **Body composition.** Lean body mass (LBM) and fat mass (FM) were calculated

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ECL, enhanced chemiluminescence; Epi, epididymal; FFA, free fatty acid; FM, fat mass; Glc-6-Pase, glucose-6-phosphatase; GP, glucose production; IGFBP, insulin-like growth factor binding protein; LBM, lean body mass; PCR, polymerase chain reaction; Peri, perinephric; RT, reverse transcriptase; TBS, Tris-buffered saline; TNF, tumor necrosis factor; VF, visceral fat.

from the whole-body volume of distribution of water, estimated by tritiated water bolus (20 μCi) injection in each experimental rat as previously described (23–25). At the completion of each experiment, Epi, Peri, and mesenteric fat (or their remnant) were dissected and weighed.

Hepatic-pancreatic clamp studies. All rats received a primed-continuous infusion of high-performance liquid chromatography-purified [^3H]glucose (New England Nuclear, Boston, MA) (15–40 μCi bolus, 0.4 $\mu\text{Ci}/\text{min}$). Somatostatin (1.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused to suppress endogenous insulin secretion (23,24), and during the 1st hour, insulin was infused peripherally at variable rates designed to clamp the plasma glucose levels at their fasting levels ($n = 14$). That rate of insulin infusion was then maintained for an additional hour. Plasma samples for determination of [^3H]glucose specific activity were obtained at 10-min intervals throughout the saline and insulin infusions. Plasma samples for determination of plasma insulin, lactate, FFA, glycerol, and glucagon concentrations were obtained at 30-min intervals. The total volume of blood drawn was ~ 4.0 ml/study; to prevent volume depletion and anemia, a solution (1:1 vol/vol) of ~ 4.0 ml fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml) was infused. At the end of the insulin infusion, rats were anesthetized (pentobarbital 60 mg/kg body wt intravenously), the abdomen was quickly opened, and all fat was removed, frozen in liquid nitrogen, and weighed. All tissue samples were stored at -80°C for subsequent analysis. These study protocols were reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

Preparation of total RNA from liver and fat depots and reverse transcriptase-polymerase chain reaction. Total hepatic RNA was prepared from frozen tissues using TRIzol reagent (GIBCO BRL, Gaithersburg, MD) as previously described (25). Total RNA from fat depots was prepared following Clontech's protocol with some modifications, as previously described (26). Total RNA was analyzed by 1% agarose gel containing 2.2 mol/l formaldehyde. First-strand cDNA was synthesized from 3 μg total RNA in 20 μl final incubation volume by using SuperScript Preamplification System for First Strand cDNA Synthesis (GIBCO BRL) with random primer. Polymerase chain reaction (PCR) was carried out in a 50- μl reaction mixture containing 4 μl of the above first-strand cDNA, 5 μl of $10\times$ PCR buffer (Mg $^{2+}$ plus, Boehringer, Indianapolis, IN), 1 μl of 10 mmol/l dNTP mix, 4 pmol of each primer, and 2.5 U Taq DNA polymerase (GIBCO BRL). For Glc-6-Pase, the sequence of upstream primer is AGG TGA GCC GCA AGG TAG ATC C; downstream primer, TGT CTT GGT GTC TGT GAT CGC TG; and the expected reverse transcription (RT)-PCR product, 441 bp. For PEPCK, the sequence of upstream primer is TGG TCT GGA CTT CTC TGC CAA G; downstream primer, ACC GTC TTG CTT TCG ATC CTG G; and expected RT-PCR product, 258 bp. For leptin, the sequence of upstream primer is TCC TAT CTG TCC TAT GTT CAA GCT GTG; downstream primer, CAA CTG TTG AAG AAT GTC CTG CAG AGA; and expected RT-PCR product, 454 bp. For TNF- α , the sequence of upstream primer is CTC CAC CAA GGA AGT TTT CC; downstream primer, CAC CCC GAA GTT CAG TAG AC; and expected RT-PCR product, 418 bp. The conditions for PCR were, for leptin, 94°C for 45 s, 69°C for 2 min (42 cycles), and for TNF- α , 95°C for 30 s, 72°C for 90 s (34 cycles), using GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT).

IGFBP-1 immunoblots. Rat serum (4 μl) was electrophoresed through nonreducing 10% SDS-PAGE and electroblotted onto nitrocellulose. The nitrocellulose membranes were immersed in blocking solution (5% fat free milk in Tris-buffered saline [TBS]) for 45 min, washed with 0.1% Tween in TBS, and incubated with primary antibodies (anti-IGFBP-1 [UBI, St. Louis, MO]) (1:2,000) for 2.5 h. Nitrocellulose membranes were washed and incubated with secondary goat anti-rabbit antibodies (1:10,000) for 1 h. After washing excessive unbound antibodies, we used the peroxidase-linked enhanced chemiluminescence (ECL) detection system from Amersham (Arlington Heights, IL) for visualization. Each lane of the autoradiographs was scanned and analyzed densitometrically (27).

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA) and plasma insulin and glucagon by radioimmunoassay (Linco Research, St. Charles, MO). Plasma [^3H]glucose radioactivity was measured in duplicate on the supernatants of Ba(OH) $_2$ and ZnSO $_4$ precipitates of plasma samples after evaporation to dryness to eliminate tritiated water. Plasma FFA (Wako Pure Chemical Industries, Osaka, Japan) and glycerol (Sigma Diagnostics, St. Louis, MO) concentrations were determined by enzymatic methods according to the manufacturers' specifications. All values are presented as means \pm SE.

RESULTS

Body composition and fat distribution. Body weight, food intake, and rate of weight gain were similar in VF $^-$ and VF $^+$ rats (Table 1). The amounts of Epi and Peri fat removed from the VF $^-$ group at the abdominal surgery were similar to that removed at the end of the study from the VF $^+$ group (Table 1).

Furthermore, FM (57 ± 7 and 63 ± 9 g in VF $^-$ and VF $^+$; respectively), LBM, and the weight of mesenteric fat (Fig. 1A and Table 1) were not significantly different in the two groups.

Hepatic insulin sensitivity. While postabsorptive GP (Fig. 1C), plasma glucose, FFA, glycerol, and glucagon were similar (Table 2), plasma insulin levels were decreased by 49% in VF $^-$ compared with VF $^+$ rats. The rate of insulin infusion needed to maintain the plasma glucose levels at baseline during the hepatic-pancreatic clamp was 1.22 ± 0.19 mU \cdot kg $^{-1} \cdot$ min $^{-1}$ in VF $^+$ and was dramatically decreased after VF removal (0.57 ± 0.02 mU \cdot kg $^{-1} \cdot$ min $^{-1}$, $P < 0.001$, Fig. 1D). During that period, plasma glucose concentrations were kept constant throughout the study, while plasma glucagon levels and GP fell slightly during the somatostatin infusion (Table 2 and Fig. 1C). Plasma FFA, glycerol (Table 2), and lactate levels were similar in both groups during the insulin infusion.

Glc-6-Pase and PEPCK gene expression. Multiple densitometric scanning of PCR products (Fig. 2A) shows that Glc-6-Pase and PEPCK gene expression were similar between VF $^-$ and VF $^+$ rats. This result has to be interpreted in reference to the twofold difference in the chronic portal exposure to insulin between these groups. Thus, the gene expression of Glc-6-Pase and PEPCK seems resistant to the suppressive effect of insulin in VF $^+$ rats, and hepatic insulin sensitivity was improved in VF $^-$ rats. To confirm the heightened sensitivity of Glc-6-Pase and PEPCK gene expression to insulin in VF $^+$ ($n = 3$) and VF $^-$ ($n = 3$), rats were studied after 2 h of somatostatin (1.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and physiological hyperinsulinemia (3 mU \cdot kg $^{-1} \cdot \text{min}^{-1}$). Indeed, this acute study demonstrated a marked reduction in the expression of these hepatic enzymes in VF $^-$ (VF $^-$ /INS) but not VF $^+$ (VF $^+$ /INS) rats.

IGFBP-1 levels in serum. Serum IGFBP-1 levels were estimated by immunoblotting with anti-IGFBP-1 antibodies. As depicted in Fig. 3A, IGFBP-1 was present in both the VF $^+$ samples and (to a lesser degree) in the VF $^-$ samples and migrated at 29 kDa. Figure 2B shows the results of densitometric analyses of IGFBP-1 immunoblots in sera, indicating a significant reduction (to 32% of VF $^+$ levels) in the IGFBP-1 concentration in the VF $^-$ rats. Other IGFBPs were also assessed by Western ligand blotting, confirming the change in IGFBP-1 and indicating no changes in other IGFBPs (data not shown).

TNF- α and leptin gene expression in VF and subcutaneous fat. There were similar levels of TNF- α and leptin gene expression among different fat pads in VF $^+$ (Fig. 4A) rats. Most impressively, the gene expression of TNF- α in the subcutaneous fat was approximately four- to fivefold

TABLE 1
Body composition and fat distribution

	At surgery		At time of study	
	VF $^-$	VF $^+$	VF $^-$	VF $^+$
<i>n</i>	6	8	6	8
Body weight (g)	391 \pm 32	409 \pm 21	410 \pm 16	414 \pm 18
LBM (g)	ND	ND	353 \pm 15	348 \pm 18
Epi fat (g)	3.44 \pm 0.23	ND	U	3.41 \pm 0.65
Peri fat (g)	3.08 \pm 0.6	ND	U	2.47 \pm 0.40
Mesenteric fat (g)	ND	ND	2.10 \pm 0.26	2.11 \pm 0.49

Data are means \pm SD. ND, not determined; U, undetected.

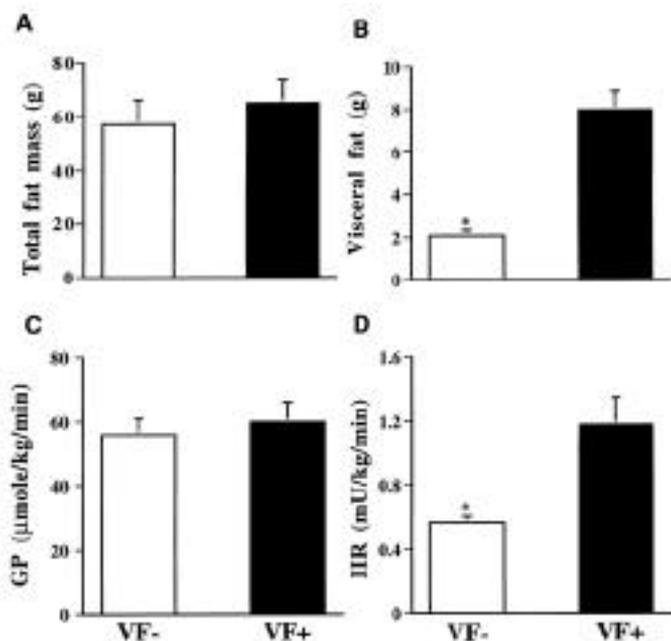


FIG. 1. Total fat mass, total visceral fat (epididymal, perinephric, and mesenteric fat), hepatic GP, and insulin infusion rate (IIR) during the hepatic-pancreatic clamp. Studies were performed in rats from which visceral fat was removed (VF⁻) and from sham-operated control rats (VF⁺). Fat mass was calculated from the whole-body volume of distribution of water, estimated by ³H₂O bolus injection in each experimental rat (A). Total visceral fat was removed and weighed at the end of the study (B). After the basal turnover period of a primed-continuous infusion of [³H-3]glucose to determine HGP (C), somatostatin was infused (1.5 µg · kg⁻¹ · min⁻¹) to suppress endogenous insulin secretion. Insulin was then infused peripherally at variable rates in the 1st hour to determine the rate required to clamp the plasma glucose levels at fasting levels and maintained at that rate for an additional hour, at which time IIR was determined (D). *P < 0.001 vs. VF⁺.

higher and that of leptin approximately two- to threefold higher in rats with intact VF (VF⁺) than in the rats from which the Epi and Peri fat were extracted (VF⁻). Conversely, TNF-α and leptin gene expression was unchanged in the mesenteric fat, demonstrating differential regulation at these two fat depots (Fig. 4B).

DISCUSSION

Hepatic insulin sensitivity. Both plasma insulin level and the rate of insulin infusion needed to maintain the plasma glucose levels were decreased in VF⁺ compared with VF⁻ rats.

These results demonstrate that the hepatic responses to either endogenous (basal) or exogenous (pancreatic clamp) insulin were markedly enhanced in VF⁻ compared with VF⁺ rats. Thus, surgical removal of VF dramatically improved hepatic insulin action. To the best of our knowledge, this is the first report to directly implicate VF in the regulation of insulin action. While the mechanisms whereby VF determines insulin sensitivity remain to be delineated, the unique metabolic characteristics of the intra-abdominal fat depots with regard to the turnover of glycerol, FFA, and lactate may play a role through a portal effect (6,7); that is, the hepatic load of FFA, lactate, and glycerol can modulate liver glucose metabolism (28–30). The concentrations of these substrates were unchanged in this experimental model, however. Furthermore, while the venous drainage of the mesenteric fat is portal, that of Epi and Peri is caval. Because the mesenteric fat was intact in VF⁻ rats, our data do not support a role of such a portal effect on the observed changes in hepatic glucose homeostasis.

IGFBP-1 levels in serum. Serum IGFBP-1 is known to be secreted almost exclusively by the liver and to be negatively regulated by insulin (11). The serum levels of IGFBP-1 serve as a sensitive short-term indicator of hepatic insulin sensitivity (31), and subjects with visceral obesity manifest increased levels of IGFBP-1 despite hyperinsulinemia (14,15), reflecting hepatic insulin resistance. The markedly decreased concentration of IGFBP-1 in serum from VF⁻ rats, in face of a ~50% decrease in circulating insulin, indicates that the surgical removal of specific visceral fat pads leads to increased hepatic insulin sensitivity.

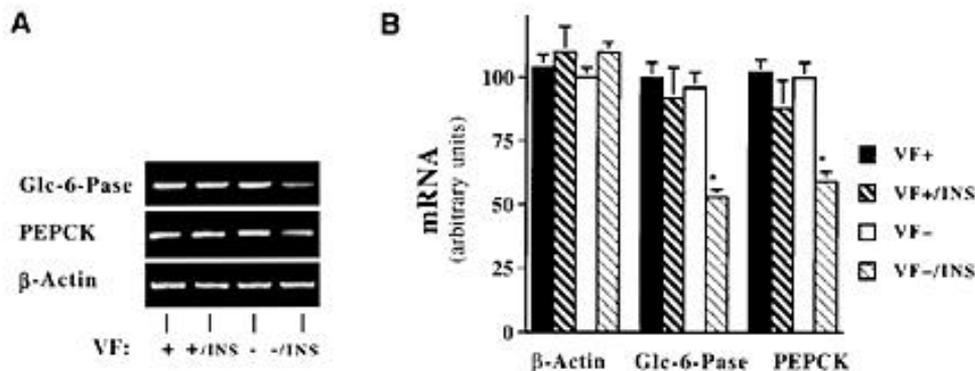
TNF-α and leptin gene expression in VF and subcutaneous fat. It should be pointed out that here we studied exclusively male rats. While differences in leptin expression have been reported between omental and subcutaneous fat in humans, the latter differences were mostly seen in women (32). Marked decreases (45%) in TNF-α gene expression have been demonstrated in fat obtained from obese humans who lost weight (33) and after dietary manipulations in obese mice (34). This fat-derived protein has been suggested to be directly involved in the development of insulin resistance in obesity through its effects on insulin signaling (8). Several hypotheses may be advanced regarding the modalities by which the removal of specific intra-abdominal fat depots (Epi and Peri) leads to such a striking decrease in TNF-α gene expression in subcutaneous adipose tissue. It may be hypothesized that a factor that modulates TNF-α gene expression is selectively

TABLE 2
Metabolic characteristics at baseline and during hepatic-pancreatic clamp

	Baseline		During clamp	
	VF ⁻	VF ⁺	VF ⁻	VF ⁺
n	6	8	6	8
Glucose (mmol/l)	7.6 ± 0.2	7.4 ± 0.7	7.7 ± 0.3	7.5 ± 0.7
Insulin (pmol/l)	110 ± 21	214 ± 32*	136 ± 49	258 ± 38*
FFA (mmol/l)	1.47 ± 0.25	1.37 ± 0.18	1.36 ± 0.18	1.34 ± 0.11
Glycerol (µmol/l)	178 ± 24	174 ± 23	174 ± 22	167 ± 17
Glucagon (pg/ml)	72 ± 9	70 ± 4	60 ± 7	60 ± 8

Data are means ± SE. ND, not determined; U, undetected. *P < 0.001 vs. VF⁻.

FIG. 2. Gene expression of hepatic Glc-6-Pase and PEPCK after sham operation (VF⁺) or extraction of visceral fat (VF⁻) in basal state and after exposure to physiological hyperinsulinemia (VF⁺/INS and VF⁻/INS). Livers from each of the rats were rapidly obtained, clamp-frozen with liquid nitrogen, and stored at -80°C for subsequent analysis. **A:** Example of RT-PCR analysis from VF⁺, VF⁺/INS, VF⁻, and VF⁻/INS (as described in RESULTS). **B:** Analysis of all RT-PCR data obtained from all rats (VF⁺, $n = 8$; VF⁺/INS, $n = 3$; VF⁻, $n = 6$; and VF⁻/INS, $n = 3$), corrected for intensity of β -actin and presented in arbitrary units. * $P < 0.005$ vs. all others.



expressed in VF and has been removed in the VF⁻ group. The latter effect may in turn contribute to the improvement in hepatic insulin action. While we cannot point to a specific peptide, a growing number of fat-secreted peptides have been described (35–37) and may have a direct or indirect role in modulating insulin action. Alternatively, the decrease in TNF- α gene expression may be secondary to the markedly decreased circulating insulin levels in the VF⁻ rats. The second mechanism may also account for the decreased expression of leptin. In fact, insulin has been shown to stimulate leptin gene expression (38). We have recently shown that the flux of glucose into the glucosamine biosynthetic pathway is also a potent stimulus for leptin gene expression (26). Thus, both decreased insulin concentrations and decreased carbon flux into the glucosamine pathways may explain the decreased leptin gene expression in fat following VF removal. Conversely, a decrease in leptin gene expression is not expected to account for the improvement in hepatic insulin action observed in our study (25).

Our results indicate that the surgical removal of selective intra-abdominal fat depots leads to a marked increase in hepatic insulin action and may regulate gene expression in subcutaneous adipose tissue. Thus, we propose that specific interventions designed to reduce VF might greatly improve hepatic insulin action. Further studies will be necessary to

identify the specific hormonal and metabolic signals by which VF regulates hepatic glucose fluxes in the hope to ultimately devise alternative therapeutic interventions.

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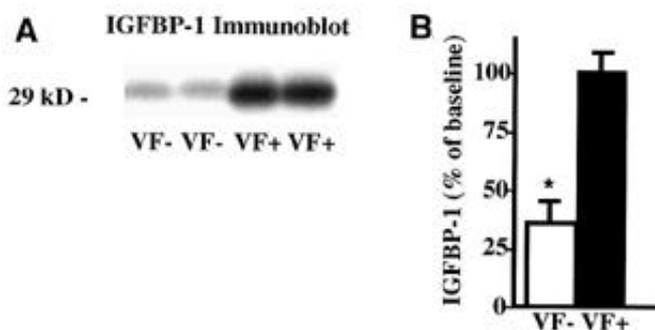


FIG. 3. IGFBP-1 in sera. **A:** Western immunoblot analysis of sera from VF⁺ and VF⁻ rats. Serum (4 μl) was electrophoresed on 10% SDS-PAGE, probed with an IGFBP-1 antibody, and visualized by ECL. **B:** Densitometrically analyzed values of IGFBP-1 peptide levels in sera of VF⁻ and VF⁺. Data are means \pm SE. * $P < 0.001$.

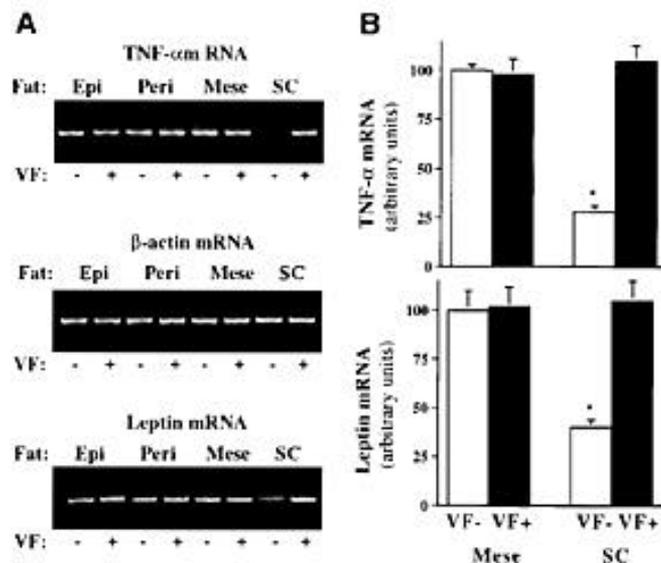


FIG. 4. Gene expression of TNF- α and leptin in Epi, Peri, mesenteric (Mese), and subcutaneous (SC) fat after extraction of visceral fat (VF⁻) or sham operation (VF⁺). Individual fat depots from each of the rats were rapidly obtained, clamp-frozen with liquid nitrogen, and stored at -80°C for subsequent analysis. Epi and Peri fats were obtained from VF⁻ during the surgery (~ 3 weeks previously); all other fat depots were obtained after the study. RT-PCR analysis for TNF- α , leptin, and β -actin is described in METHODS. **A:** Example of RT-PCR analysis from different fat depots from one VF⁻ and one VF⁺ rat. **B:** Analysis of all RT-PCR data obtained from all rats (VF⁻, $n = 6$; VF⁺, $n = 8$), corrected for intensity of β -actin and presented in arbitrary units. * $P < 0.001$ vs. VF⁺.

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Author Queries (please see Q in margin and underlined text)

Q1: Please specify the affiliations of each author.

Q2: Bjornatorp is an author of Ref. 6 but not 7; please clarify why 7 is cited here.

Q3: Should this be cAMP?

Q4: Please supply location of Gibco.

Q5: Is "Mg⁺² plus" the name of the buffer?

Q6: Please supply location of Boehringer.

Q7: Please supply location of Perkin-Elmer.

Q8: Please supply location of UBI.

Q9: Please supply location of Amersham.

Q10: According to *Diabetes* publication style, the Results and Discussion sections have been separated. Please read them carefully to ensure that text has been put in the proper section.

Q11: Please note that your original Table 1A and B are now Tables 1 and 2.

Q12: Please check sentence beginning "Both plasma insulin levels" carefully for meaning; it is a paraphrase of the information in Results.

Q13: By "caval," do you mean "vena caval"? Please clarify.

Q14: Author, please explain significance of *(asterisk) in 3B.