Removal of Visceral Fat Prevents Insulin Resistance and Glucose Intolerance of Aging

An Adipokine-Mediated Process?

Ilan Gabriely,[1,2] Xiao Hui Ma,[1,2] Xiao Man Yang,[1,2] Gil Atzmon,[1,2] Michael W. Rajala,[3] Anders H. Berg,[3] Phillip Scherer,[3] Luciano Rossetti,[1] and Nir Barzilai[1,2]

Age-dependent changes in insulin action and body fat distribution are risk factors for the development of type 2 diabetes. To examine whether the accumulation of visceral fat (VF) could play a direct role in the pathophysiology of insulin resistance and type 2 diabetes, we monitored insulin action, glucose tolerance, and the expression of adipo-derived peptides after surgical removal of VF in aging (20-month-old) F344/Brown Norway (FBN) and in Zucker Diabetic Fatty (ZDF) rats. As expected, peripheral and hepatic insulin action were markedly impaired in aging FBN rats, and extraction of VF (accounting for ~18% of their total body fat) was sufficient to restore peripheral and hepatic insulin action to the levels of young rats. When examined at the mechanistic level, removal of VF in ZDF rats prevented the progressive decrease in insulin action and delayed the onset of diabetes, but VF extraction did not alter plasma free fatty acid levels. However, the expression of tumor necrosis factor-α and leptin in subcutaneous (SC) adipose tissue were markedly decreased after VF removal (by approximately three- and twofold, respectively). Finally, extracted VF retained ~15-fold higher resistin mRNA compared with SC fat. Our data suggest that insulin resistance and the development of diabetes can be significantly reduced in aging rats by preventing the age-dependent accumulation of VF. This study documents a cause-and-effect relationship between VF and major components of the metabolic syndrome. *Diabetes* 51:2951–2958, 2002

A progressive increase in visceral adiposity is a common feature of aging, and epidemiological evidence supports its role as a prominent risk factor for insulin resistance, diabetes, and mortality from atherosclerotic cardiovascular disease (1–5). Among various body fat depots, the amount of visceral fat (VF) best correlates with insulin sensitivity in animal models and in humans. Insulin action is markedly impaired in individuals with visceral obesity (6,7), and epidemiological studies have shown that VF can account for most of the variability in insulin sensitivity in heterogeneous populations (2,4,6,7). However, these studies are associational in nature, and VF may be simply a "marker" of more complex endocrine and metabolic changes rather than playing a "causative" role in the pathogenesis of insulin resistance and its metabolic consequences. Putative mechanisms responsible for the modulation of insulin action by VF include increased portal release of free fatty acids (FFAs) (8,9) and/or abnormal expression and secretion of fat-derived peptides, such as resistin (10), leptin, ACRP30, and tumor necrosis factor-α (TNF-α) (11).

A consistent observation in the biology of aging is that chronic restriction of caloric intake in rodents markedly improves survival and prevents the onset of insulin resistance. We and others have hypothesized that the beneficial effects of caloric restriction (CR) on the metabolic alterations of aging are largely accounted for by its prevention of VF accumulation (12,13). To directly examine the contribution of VF to the insulin resistance of aging, it is important to separate the potential effects of a decrease in VF per se from other nutritional, anthropometric, and metabolic consequences of CR. Having demonstrated that surgical removal of VF rapidly improves hepatic insulin action in young rats (14), we made use of this novel animal model to investigate the following two main questions: *1*) Does VF play a causative role in the peripheral and hepatic insulin resistance of aging? and *2*) Can VF removal alter the natural history of developing diabetes in a rodent model of obesity and diabetes ?

RESEARCH DESIGN AND METHODS

Animals. A total of 34 F1 hybrids of F344/Brown Norway rats obtained from the National Institutes of Aging were housed in individual cages and subjected to a standard light (6:00 A.M. to 6:00 P.M.)/dark (6:00 P.M. to 6:00 A.M.) cycle. Rats were assigned into five experimental groups: *1*) VF^- (n = 6): 15-month-old ad libitum–fed rats that were anesthetized (pentobarbital 50 mg/kg body wt i.p.), and their epididymal and the perinephric fat pads were removed, weighed, and immediately frozen in liquid nitrogen; *2*) SC^- (n = 8): 15-month-old ad libitum–fed rats in which subcutaneous (SC) fat was removed from both inguinal areas to match the amount of fat removed in VF^-; *3*) SO (n = 6): 15-month-old ad libitum–fed rats that underwent a sham operation (the abdominal cavity was incised, and fat was mobilized, but not removed); *4*) CR (n = 8): 15-month-old caloric-restricted rats (fed since 3 months of age 60% of the calories consumed by the ad libitum–fed rats) that received a similar sham

From the [1]Diabetes Research and Training Center and Division of Endocrinology, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York; the [2]Institute for Aging Research, Albert Einstein College of Medicine, Bronx, New York; and the [3]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York.

Address correspondence and reprint requests to Nir Barzilai, Institute for Aging Research, Belfer Bldg. no. 701, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. E-mail: barzilai@aecom.yu.edu.

Received for publication 15 April 2002 and accepted in revised form 2 July 2002.

CR, caloric restriction; dsDNA, double-stranded DNA; EGP, endogenous glucose production; FFA, free fatty acid; SC, subcutaneous; TNF-α, tumor necrosis factor-α; VF, visceral fat.

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operation; and *5*) young ($n = 6$): 2-month-old (post-puberty) rats that were ad libitum–fed and also received a sham operation.

At 20 months of age, all rats were anesthetized again, and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery (15,16). Recovery was continued until body weight and daily food intake was within 5% of their preoperative levels. Clamp studies were performed in 6-h fasted, awake, unstressed, chronically catheterized rats.

To study whether VF removal prevents diabetes, another set of Zucker (+/+) rats ($n = 24$; Charles River Laboratories, Wilmington, MA) were used as a model of "diabesity" (diabetes associated with marked obesity). At 2 months of age (before the development of glucose intolerance), these rats were assigned into two groups: *1*) ZDVF$^-$ ($n = 12$): rats in which the epididymal epididymal and the perinephric fat pads were removed (using the same technique described above); and *2*) ZDVF$^+$ ($n = 12$): rats that received a sham operation. At 8 weeks after the procedure, six rats from each group received indwelling catheters and were allowed to recover as previously described. The other remaining (control) rats (six in each group) were followed at 1-month intervals and monitored for diabetes (fasting plasma glucose >12 mmol/l).

Body composition. Lean body mass and fat mass were calculated as described elsewhere (15,16). Briefly, rats received an intra-arterial bolus injection of 20 μCi tritiated-labeled water (^3H_2O; New England Nuclear, Boston, MA), and plasma samples were obtained at 30-min intervals. Steady-state conditions for plasma ^3H_2O specific activity were achieved within 45 min in all studies. Five plasma samples obtained between 1 and 3 h were used to calculate the total body distribution of water. At the completion of each experiment, epididymal, perinephric, and mesenteric fat (or their remnants) were dissected and weighed.

Hyperinsulinemic-euglycemic clamp. All rats received a primed-continuous (15–40 μCi bolus, 0.4 μCi/min) infusion of high-performance liquid chromatography–purified [^{3}H-3]glucose (New England Nuclear) throughout the study. After 120 min (to allow for the measurement of basal endogenous glucose production [EGP]), a primed-continuous infusion of insulin (6 mU · kg^{-1} · min^{-1}) and a variable infusion of a 25% glucose solution were started (and periodically adjusted) to clamp the plasma glucose concentration at the basal level for an additional 120 min of the clamp. Somatostatin (1.5 μg · kg^{-1} · min^{-1}) was infused to suppress endogenous insulin secretion (12).

Plasma samples for determination of ^{3}H-glucose specific activity were obtained at 10-min intervals throughout the insulin infusion. Samples were also obtained for determination of plasma insulin, leptin, and FFA concentrations at 30-min intervals throughout the study. The total volume of blood withdrawn was ~3.0 ml/study. To prevent volume depletion and anemia, a solution (1:1 vol/vol) of ~3.0 ml fresh blood (obtained by heart puncture from a littermate of the tested animal) and heparinized saline (10 units/ml) was infused at a constant rate throughout the study. Epididymal, mesenteric, and perinephric fat pads were dissected and weighed at the end of each experiment (12). The study protocol was reviewed and approved by the animal care and use committee of the Albert Einstein College of Medicine.

Whole-body glycolysis and glycogen synthesis. The rate of glycolysis was estimated from the rate of conversion of [^{3}H-3]glucose to ^3H_2O as previously described (17,18). Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present in either ^{3}H water or glucose. Plasma-tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness.

Expression of adipo-derived peptides. Total RNA from fat depots was prepared following Clontech's protocol with some modifications as previously described (19). Before use, the total RNA was analyzed by 1% agarose gel containing 2.2 mol/l formaldehyde. First-strand cDNA was synthesized with random primers from 5 μg total RNA in 40 μl final incubation volume by using the SuperScript preamplification system for first-strand cDNA (Gibco). Initially and for visualization, leptin and TNF-α were assessed by RT-PCR, whereas resistin was measured by Northern blot analysis (described in detail in 14). Real-time PCR was used for quantification. Real-time PCR was performed by a light cycle (Roche, Mannheim, Germany) using the one-step RT-PCR system. Primers for resistin, leptin, and TNF-α were chosen with the assistance of the computer program Oligo 4.0 (National Biosciences, Plymouth, MN). For resistin, the sequence for the upper primer was 5′-GAT GAAGCCA TCAGCAAGAAGATC-3′, and the lower primer was 5′-CACATTGTAT CCTCACGGAC GTC-3′. For leptin, the sequence of upstream primer was TCC TAT CTG TCC TAT GTT CAA GCT GTG, and the downstream primer was CAA CTG TTG AAG AAT GTC CTG CAG AGA. For TNF-α, the sequence of the upstream primer was CTC CAC CAA GGA AGT TTT CC, and the downstream primer was CAC CCC GAA GTT CAG TAG AC.

The reactions were performed using the Light Cycler–Fast Start DNA Master SYBR green kit. The components for PCR in a final volume of 20 l included 2 l of a commercial ready-to-use reaction mix for PCR (Light Cycler–DNA master SYBR Green–I) of *Taq* DNA polymerase, reaction buffer, DNTP MIX, SYBR Green–I dye, and 10 mmol/l $MgCl_2$. SYBR Green-I dye binds to the minor groove of double-stranded DNA (dsDNA), and the fluorescence is greatly enhanced by binding. The $MgCl_2$ final concentration was 4 mmol/l, the primer's final concentration was 1.0 mol/l, and 2 l of 1:10 diluted template DNA was added. The reactions were cycled 50 times with a 95°C denaturation for 0 s, a 62°C annealing for 7 s, and 72°C for 12 s, with slopes of 20°C/s, 20°C/s, and 20°C/s, respectively. Fluorescence was acquired after heating at 20°C/s to a temperature 2°C below the product melting temperature (T_m) and holding for 60 s. During the various stages of PCR, different intensities of fluorescence signals can be detected depending on the amount of dsDNA present. The fluorescence is recorded at the end of each cycle and monitored from cycle to cycle. Serial dilutions of the peptide's plasmid DNA were used to create the standard curve. The crossing points are identified by the intersection of the best-fit line with the log linear portion of the standards amplification curve. The standard curve is the plot of the crossing point versus the log of copy number. The concentration of the products in the sample was calculated by extrapolation to the standard curve by using the Light Cycler analysis software. mRNA obtained from all rats and real-time PCR were repeated three times on each sample.

Acrp30 was quantified by Western blot analysis. After SDS-PAGE, proteins were transferred to BA83 nitrocellulose (Schleicher & Schuell). A rabbit antibody raised against full-length murine Acrp30 that was derivatized with 125-I was used to decorate the blots. Blots were analyzed with a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA) and quantified with ImageQuant 1.2 software. Each gel contained four standards of purified mouse Acrp30 at four different concentrations to ensure linearity and reproducibility of the signal. Intra-assay variability was measured by assaying the same serum sample 10 times on one blot with standards and quantifying the SE of the 10 measurements; the SE was 3%). Interassay variability based on the measurement of serum samples from the same experiment assayed at different times gave an SE of 10%, indicating a high level of reproducibility of the assay. The lower limit of detection was 1–5 ng of Acrp30.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA), and plasma insulin and glucagon were measured by radioimmunoassay (Linco Research, St. Charles, MO). Plasma ^{3}H-glucose specific activity 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 (Waco Pure Chemical Industries, Osaka, Japan) concentrations were determined by enzymatic methods according to the manufacturer's specifications.

Statistical analysis. The significance of group differences was evaluated using repeated-measures ANOVA for multiple comparisons. The Pearson correlation coefficient was calculated to estimate the linear relationship between variables. All values are presented as means ± SE. A P value <0.05 was considered significant.

RESULTS

VF removal and the prevention of insulin resistance in aging rats

Body composition and fat distribution in aging rats. Similar amounts of fat were extracted in VF$^-$ and SC$^-$ rats (Table 1). After ~8 weeks, these rats had similar food intake (not shown), body weight, and lean body mass. The amounts of epididymal and perinephric fat removed from the VF$^-$ group at the abdominal surgery was similar to that removed at the end of the study from the SC$^-$ and SO groups; similarly, the weight of mesenteric fat was not different in these three groups. The young and CR groups were much thinner and had less fat in all locations than VF$^-$ and SC$^-$ rats, but they were similar to each other.

Insulin sensitivity in aging rats. Although postabsorptive plasma glucose and FFA levels were similar in all groups, plasma insulin levels were increased by ~50% in the SC$^-$ and SO rats compared with other groups (Table 2, Figs. 1 and 2). During the pancreatic clamps (insulin and somatostatin infusions), plasma glucose levels were maintained at baseline, and plasma FFA and insulin levels were similar in all groups. Plasma glucagon concentrations

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TABLE 1
Body composition and fat distribution of aging rats

	young	SO	SC^-	VF^-	CR
n	6	6	8	6	8
VF/SC removed (g)	0	0	14.9 ± 1.7	15.7 ± 1.3	0
Body weight (g)	291 ± 13	478 ± 19*	451 ± 14*	452 ± 20*	303 ± 9
Lean body mass (g)	264 ± 14	373 ± 14*	364 ± 14*	372 ± 20*	266 ± 8
Non-VF fat mass (g)	21 ± 5	82 ± 10*	65 ± 11*	75 ± 10*	32 ± 6
Total VF after study (g)	5.8 ± 0.9	23.4 ± 4.3*	21.3 ± 1.6*	4.6 ± 0.7†	6.1 ± 1.0
Epididymal (g)	2.9 ± 0.3	9.2 ± 1.6*	8.2 ± 0.7*	0.1 ± 0.1†	2.6 ± 0.5
Perinephric (g)	1.4 ± 0.3	8.3 ± 0.7*	8.1 ± 0.6*	0.4 ± 0.4†	1.9 ± 0.5
Mesenteric (g)	1.5 ± 0.3	5.9 ± 0.4*	4.8 ± 0.6*	4.1 ± 0.4*	1.4 ± 0.4

young (2-month-old), and old (20-month-old) F1 hybrids of F344 and Brown Norway rats were used in these experiments. The old rats underwent VF removal (VF^-), SC fat removal (SC^-), sham operation (SO), or CR (see RESEARCH DESIGN AND METHODS). The table depicts the amounts of VF or SC removed at the surgery, body weight, lean body mass, non-VF fat mass, total VF, and epididymal, perinephric, and mesenteric fat, which were determined at killing after the experiments. *$P < 0.001$ vs. young and CR; †$P < 0.01$ vs. young, CR, and SC^-. All data are presented as means ± SE.

were 98 ± 15 and 109 ± 13 pg/ml in VF^- and SC^- rats, respectively ($P = 0.496$), and plasma corticosterone concentrations were 243 ± 27 and 263 ± 35 ng/ml in VF^- and SC^- rats, respectively ($P = 0.348$). However, VF extraction resulted in an ~80% increase in the rates of glucose infusion compared with the SC^- and SO rats ($P < 0.001$), and glucose uptake was significantly higher in VF^- rats than in SC^- and SO rats ($R_d = 23.3 \pm 2.5$ mg · kg^{-1} · min^{-1} in VF^- compared with 15.5 ± 1.6 and 16.1 ± 1.3 mg · kg^{-1} · min^{-1} in SC^- and SO rats, respectively, $P < 0.001$) (Fig. 1). Furthermore, these rates were similar to those observed in young and CR rats (25.7 ± 1.6 and 22.1 ± 1.6 mg · kg^{-1} · min^{-1}, respectively).

The action of insulin to suppress EGP was also significantly affected by removal of VF (Fig. 2). VF^- rats demonstrated a ~50% increase in the ability of insulin to suppress EGP (5.4 ± 0.8 mg · kg^{-1} · min^{-1}), compared with SC^- (8.1 ± 1.0 mg · kg^{-1} · min^{-1}) and SO rats (7.8 ± 1.2 mg · kg^{-1} · min^{-1}, $P < 0.001$ vs. both). Furthermore, these rates were similar to those observed in young (4.3 ± 0.8 mg · kg^{-1} · min^{-1}) and CR rats (6.3 ± 0.9 mg · kg^{-1} · min^{-1}).

VF removal prevents progression to frank diabetes in ZDF rats

Body composition and fat distribution in ZDF rats. ZDF rats had surgery when they weighed ~280 g. At 8 weeks after surgery, their body weight was similar to the equivalent FBN rats (i.e.,. the SC^-, SO, and VF^- rats in Table 1) (Table 3). Interestingly, ~10 g of epididymal and perinephric fat were removed during surgery; however, these fat pads increased in parallel with weight gain and reached ~16 g when the rats weighed ~480 g. Thus, these 8 weeks were associated with rapid fat growth. Moreover, this period allowed for significant regeneration, so that up

TABLE 2
Metabolic characteristics of aging rats

	young	SO	SC^-	VF^-	CR
n	6	6	8	6	8
Glucose (mmol/l)					
Basal	7.2 ± 0.2	7.6 ± 0.5	7.4 ± 0.7	7.7 ± 0.3	7.2 ± 0.7
Clamp	7.3 ± 0.3	7.5 ± 0.3	7.5 ± 0.4	7.6 ± 0.3	7.3 ± 0.7
Insulin (μU/ml)					
Basal	14 ± 2	29.5 ± 3.5*	25 ± 2*	18 ± 2	15 ± 2
Clamp	98 ± 13	96 ± 10	102 ± 11	102 ± 22	92 ± 18
FFA (mmol/l)					
Basal	0.99 ± 0.15	1.1 ± 0.24	0.81 ± 0.14	1.06 ± 0.14	1.11 ± 0.11
Clamp	0.21 ± 0.08	0.35 ± 0.1	0.23 ± 0.1	0.27 ± 0.04	0.32 ± 0.03
R_a (mg · kg^{-1} · min^{-1})					
Basal	12.1 ± 0.4	9.1 ± 0.3*	8.7 ± 0.2*	10.7 ± 0.3	11.0 ± 0.8
GIR (mg · kg^{-1} · min^{-1})					
Clamp	21.3 ± 3.0	8.4 ± 4.2*	10.4 ± 3.2*	18.0 ± 2.0	21.8 ± 4.6
Gly (mg · kg^{-1} · min^{-1})					
Basal	7.1 ± 0.6	6.0 ± 0.2	6.4 ± 0.5	6.2 ± 0.3	7.4 ± 0.7
Clamp	14.4 ± 1.5	6.9 ± 0.4*	8.6 ± 1.4*	14.7 ± 2.1	13.7 ± 2.0
GS (mg · kg^{-1} · min^{-1})					
Basal	5.0 ± 0.7	3.1 ± 0.1*	2.3 ± 0.5*	4.4 ± 0.3	3.6 ± 0.3
Clamp	12.6 ± 1.4	5.1 ± 0.7*	6.5 ± 0.6*	9.3 ± 0.9	10.4 ± 2.4

Plasma glucose, insulin, FFA concentrations, basal rate of EGP (R_a), glucose infusion rate (GIR), glycolysis (Gly), and glycogen synthesis (GS) during the basal period and during the pancreatic (euglycemic) clamp are shown. These parameters were measured at basal conditions and during the insulin clamps (see group description in RESEARCH DESIGN AND METHODS). *$P < 0.001$ vs. young, VF^-, and CR. All data are presented as means ± SE.

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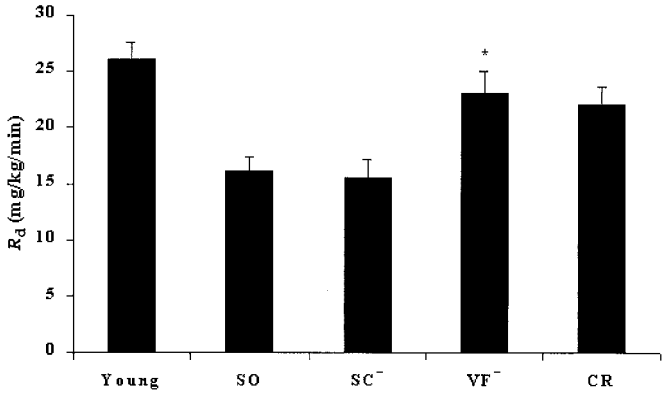

FIG. 1. Peripheral insulin sensitivity in aging rats. Shown are results for young (2-month-old) and old (20-month-old) F1 hybrids of F344 and Brown Norway rats. The old rats underwent VF removal (VF$^-$), SC fat removal (SC$^-$), or sham operation (SO), or they were calorically restricted (see RESEARCH DESIGN AND METHODS). Glucose uptake (R_d) during hyperinsulinemic (6 mU · kg^{-1} · min^{-1}) pancreatic clamps is shown. *P < 0.001 vs. SC$^-$ and SO rats.

to one-third of the VF observed in ZDVF$^+$ was weighed in the ZDVF$^-$ group. This regeneration continued during the following 2 months (observed in the control set [n = 6] of ZDVF$^-$ rats and compared with the control set [n = 6] of ZDVF$^+$ rats), attaining >80% of the amount of fat observed in the ZDVF$^+$ rats (Fig. 3).

Insulin sensitivity in ZDF rats. Postabsorptive plasma glucose and FFA levels were similar in both groups (Table 4). However, the fasting plasma insulin concentration decreased by ~50% in the ZDVF$^-$ rats compared with the ZDVF$^+$ rats. During the pancreatic clamps (insulin and somatostatin infusion), plasma insulin concentrations were similar in both groups, although glucose infusion was needed only in the ZDVF$^-$ group to maintain plasma glucose at basal levels. In fact, ZDVF$^+$ rats became hyperglycemic (10.1 ± 0.7 mmol/l) during this period. This was accompanied by a significant increase in the plasma FFA concentration, suggesting resistance to the antilipolytic effect of insulin as well. Although plasma insulin levels were higher than during the basal period, it should be pointed out that insulin was infused peripherally during the pancreatic clamp procedure. In the presence of similar peripheral insulin concentrations, insulin induced a significant suppression of EGP in ZDVF$^-$ rats, whereas there

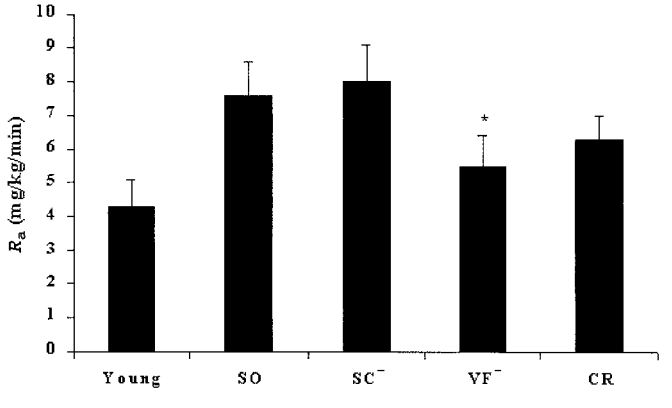

FIG. 2. Hepatic insulin sensitivity in aging rats. The ability of insulin to suppress EGP was studied using glucose tracer methodology (see RESEARCH DESIGN AND METHODS). *P < 0.001 vs. SC$^-$ and SO rats. R_a, rate of EGP.

TABLE 3
Body composition and fat distribution of ZDF rats

	ZDVF$^+$	ZDVF$^-$
n	6	8
VF removed (g)	0	10.5 ± 0.3*
Body weight (g)	488 ± 17	468 ± 16
Lean body mass (g)	327 ± 19	313 ± 18
Non-VF fat mass (g)	121 ± 15	139 ± 11
Total VF after study(g)	40.0 ± 3.8	15.8 ± 1.1*
Epididymal (g)	15.0 ± 2.0	2.0 ± 0.4*
Perinephric (g)	15.8 ± 1.7	5.1 ± 0.3*
Mesenteric (g)	9.2 ± 0.8	8.8 ± 1.2

At 8 weeks prior to the study, epididymal and perinephric pads were removed in ZDF (*fa/fa*) rats (ZDVF$^-$), whereas sham operation was performed in control rats (ZDVF$^+$). The table depicts the amounts of VF removed at surgery, body weight, lean body mass, non-VF fat mass, and total VF, epididymal, perinephric, and mesenteric fat that were detected after the study. *P < 0.001 vs. ZDF$^+$. All data are presented as means ± SE.

was no detectable increase in the rate of glucose uptake. Indeed, resistance to the inhibitory action of insulin on EGP was also present in the face of hyperglycemia, which may have an independent effect on decreasing EGP.

In a follow-up for the appearance of diabetes, measuring the fasting plasma glucose concentration in the control ZDF rats demonstrated a significant delay in the development of diabetes in the ZDFV$^-$ rats compared with the ZDFV$^+$ rats. However, all animals became diabetic when most of the VF regenerated (Fig. 3).

Adipo-derived peptides in aging FBN rats. The expression of leptin and TNF-α were estimated in VF and SC fat. Whereas SC$^-$ rats had similar expression of these peptides in all tissues, leptin expression was decreased by ~75%, and TNF-α was decreased by ~65% in SC compared with

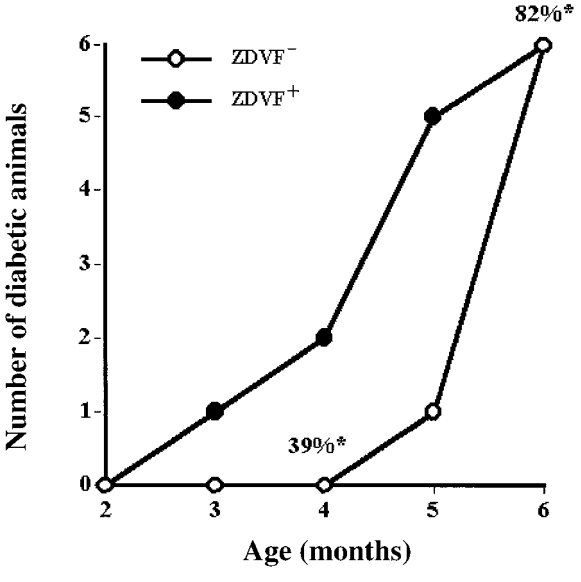

FIG. 3. Development of diabetes and the regrowth of VF in ZDF rats. At 2 months of age, rats were assigned to either have their epididymal and perirenal fat removed (ZDVF$^-$, n = 12) or undergo a sham operation (ZDVF$^+$, n = 12). Six rats from each group were studied using a pancreatic clamp, and the rest were monitored for 4 months until they developed diabetes. The six ZDVF$^-$ rats studied at 4 months had 38% of the fat observed in ZDVF$^+$ rats. When diabetes (defined as glucose >12 mmol/l) appeared, ZDVF$^-$ VF accounted for 82% of that observed in ZDVF$^+$ rats. *Percent VF in ZDVF$^-$ versus ZDVF$^+$ rats.

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TABLE 4
Metabolic characteristics of ZDF rats

	BASAL		CLAMP	
	ZDVF+	ZDVF−	ZDVF+	ZDVF−
n	6	6	6	6
Glucose (mmol/l)	8.9 ± 0.4	8.5 ± 0.5	10.1 ± 0.7*	8.4 ± 0.3
Insulin (pmol/l)	76 ± 9*	44 ± 2	112 ± 10	101 ± 5
FFA (mmol/l)	1.99 ± 0.22	1.67 ± 0.13	2.53 ± 0.11*	1.51 ± 0.16
GIR (mg · kg^{-1} · min^{-1})	0	0	0	2.1 ± 0.6
EGP (mg · kg^{-1} · min^{-1})	13.8 ± 0.8	13.1 ± 1.6	13.3 ± 0.5*	10.7 ± 0.6†
R_d (mg · kg^{-1} · min^{-1})	13.8 ± 0.8	13.1 ± 1.6	13.7 ± 0.8	14.2 ± 0.8

Plasma glucose, insulin, FFA levels, glucose infusion rate (GIR), EGP, and glucose uptake (R_d) at basal conditions and during the pancreatic clamps in ZDVF$^+$ (no VF removed) and ZDVF$^-$ (VF removed) rats. *$P < 0.001$ vs. ZDVF−, †$P < 0.001$ vs. basal. All data are presented as means ± SE.

mesenteric fat of VF$^-$ rats (Fig. 4), demonstrating differential regulation at these two fat depots. Epididymal and perinephric fat displayed a ~15-fold increase in resistin mRNA compared with SC fat (Fig. 5*A*) and a 3.5-fold increase in ACRP30 gene expression compared with SC fat (Fig. 5*B*).

In concert with leptin gene expression, plasma leptin concentrations were significantly lower in the VF$^-$ compared with SC$^-$ and SO rats (2.37 ± 0.4, 6.60 ± 0.9, and 9.12 ± 0.6 ng/ml, respectively; $P < 0.001$ for both). CR rats had plasma leptin levels (3.10 ± 0.2 ng/ml) similar to the VF$^-$ rats, and the young rats had the lowest (1.40 ± 0.2 ng/ml) plasma leptin concentration. Interestingly, although the ZDF rats have alterations in the leptin receptor and subsequently have extremely high plasma leptin levels, removal of VF in these rats induced a significant decrease in the plasma leptin concentration (41 + 3 ng/ml in ZDVF$^-$ vs. 71 + 9 ng/ml in ZDVF$^+$ rats, $P < 0.001$). Finally, plasma levels of ACRP30 were decreased by ~50% in VF$^-$ compared with SC$^-$ rats (7.21 vs. 14.5 arbitrary units, respectively).

DISCUSSION

Prevention treatment strategies have been successfully applied to type 2 diabetes. Lifestyle modifications largely designed to alter fat mass and distribution are the cornerstone of these interventions and have been shown to delay the onset of hyperglycemia in individuals at high risk for the disease (20,21). Similarly, chronic CR has dramatic beneficial effects on glucose tolerance and insulin action in rodents (12) and in primates (22). Importantly, reduction in visceral adiposity is a common feature of all these interventions. However, it has been difficult to discern whether a reduction in the size of intra-abdominal fat depots plays a causative role or is simply a covariant tightly associated with one or more causative factors. Here, we show that surgical removal of two intra-abdominal fat depots prevents the onset of age-dependent insulin resistance and markedly delays the onset of glucose intolerance and diabetes in a rodent model of obesity and diabetes.

Our findings indicate that a modest decrease in total fat mass per se does not have a significant impact on insulin action. In fact, the surgical removal of SC adipose tissue accounting for ~18% of the overall fat mass had no appreciable impact on any of the measured metabolic parameters. However, not all fat depots have equal metabolic import. Adipose tissue from different anatomical sites has markedly different effects on metabolic outcomes. In fact, removal of a similar amount of adipose tissue from the peri-renal and peri-epididymal sites (VF) led to dramatic improvements in peripheral and hepatic insulin sensitivity and glucose tolerance. Of interest, unlike the old F1 hybrid of F344 and Brown Norway rats, the ZDVF$^-$ rats demonstrated rapid VF regeneration due to their young age at the time of the VF extraction operation, and possibly due to their relative resistance to leptin, which plays a key role in body fat distribution. The onset of diabetes in the ZDF rats tracked very closely with the regeneration of VF several weeks after its surgical removal.

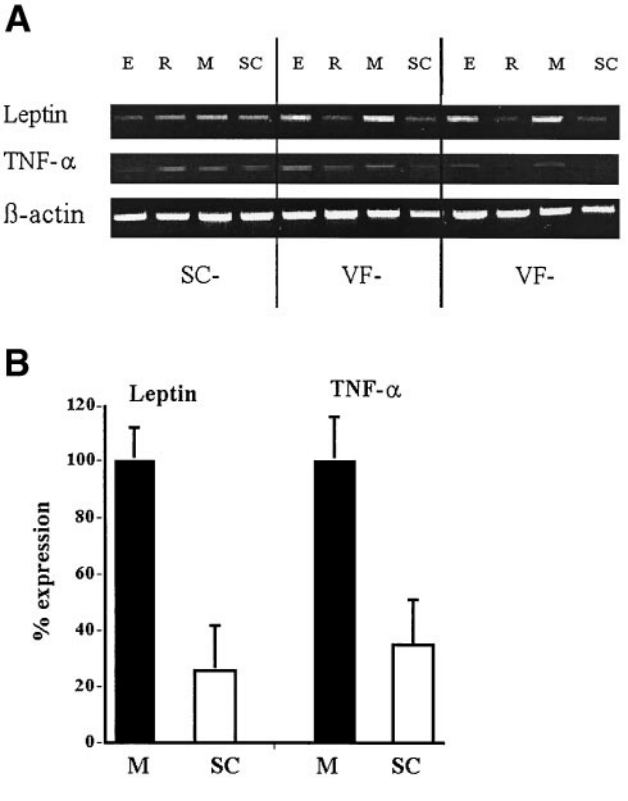

FIG. 4. Gene expression of TNF-α and leptin. Measurements were performed in epididymal (E), perinephric (R), mesenteric (M), and SC fat after extraction of VF (VF$^-$) or SC fat (SC$^-$). RT and real-time PCR analysis for TNF-α, leptin, and β-actin are described in RESEARCH DESIGN AND METHODS. *A*: Example of agarose gel analysis of RT-PCR products from different fat depots in SC$^-$ and VF$^-$ rats. *B*: Analysis of all real-time PCR data obtained in all rats, corrected for intensity of β-actin and presented in arbitrary units. $P < 0.001$ vs. mesenteric fat.

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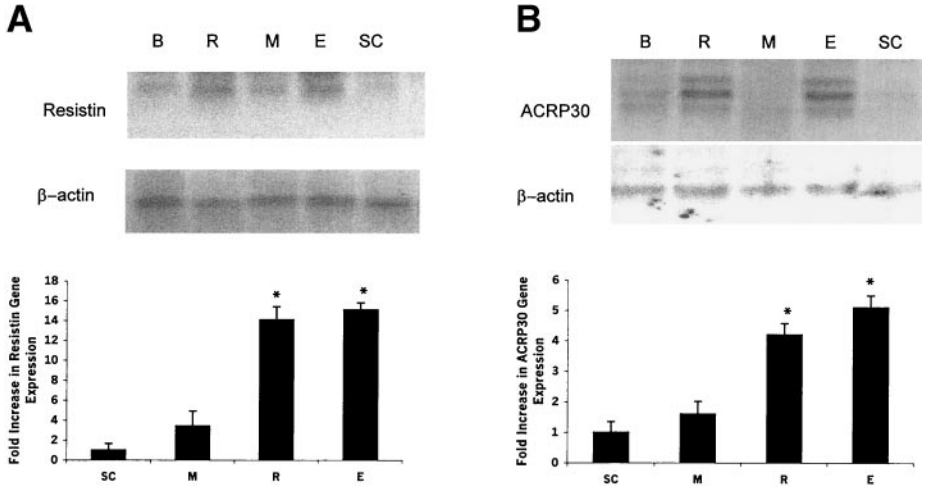

FIG. 5. Gene expression of resistin (*A*) and ACRP30 (*B*). A: Northern blot analysis of brown (B), epididymal (E), perinephric (R), mesenteric (M), and SC fat obtained from SC$^-$ rats. *B*: Real-time PCR data obtained from all rats corrected for the intensity of β-actin and presented in arbitrary units. *$P < 0.01$ vs. M and SC.

Our results may provide useful information in the contentious debate regarding the mechanism(s) by which CR improves metabolic parameters and extends life in rodent models (23). Until recently, the effects of CR on life extension (24,25) were related directly to the decreased intake of nutrients rather than to secondary effects on body weight, fat mass, or distribution of body fat. This study documents that insulin action can be improved in the absence of CR, challenging the notion that nutrient excess per se is the prominent cause of insulin resistance in this aging model. Overall, the dramatic improvement in peripheral and hepatic insulin action after removal of visceral but not SC adipose tissue resembles the effects of prolonged CR in aging rodents (12). However, a closer look at other associated variables in the two intervention models also reveals striking differences. CR results in marked reductions in body weight, total fat mass, and lean body mass. Conversely, the surgical removal of VF markedly improves metabolic parameters in the absence of any detectable changes in body weight, fat mass, and lean body mass. Furthermore, the reduction in VF is the result of a proportional decrease in all VF depots with CR, whereas it is entirely due to decreased epididymal and perinephric fat in our surgical model. Thus, decreased VF could largely account for the beneficial metabolic effects of chronic CR. However, our results cannot quantify the relative contribution of mesenteric, epididymal, and perinepric fat depots in mediating these effects.

Insulin resistance is a major risk factor for the development of diabetes in humans and animals (26). Here, we used the Zucker Fatty rat model, which resembles common features of obese patients developing type 1 diabetes (27). This model is characterized by marked hyperinsulinemia and insulin resistance (28). Despite peripheral hyperinsulinemia (insulin clamp procedure), plasma glucose levels increased during the study in ZDVF$^+$ rats. By contrast, glucose infusion was required to maintain the plasma glucose concentration at the basal levels in ZDVF$^-$. Thus, removal of VF dramatically improved insulin sensitivity in Zucker Fatty rats. Furthermore, all ZDVF$^+$ rats developed diabetes during the 3 weeks after sham operation. However, only one ZDVF$^-$ rat developed diabetes during the same period. Finally, further follow-up of a subgroup (control) of ZDVF$^-$ rats shows that regrowth of VF closely tracked with the late onset of hyperglycemia.

Thus, selective intra-abdominal fat depots play a major role in modulating insulin action and glucose tolerance in these two animal models. Can we speculate on potential mechanism(s) by which these depots regulate insulin action in distant sites? One possibility is that increased plasma levels of FFAs and glycerol impair insulin action in both the liver and muscle (29–31). In fact, it has been suggested that VF is resistant to the antilipolytic effects of insulin, and its removal may have decreased the flux of FFAs and glycerol to these target organs (32). However, the concentrations of FFAs and glycerol were unchanged in these experimental models at both basal conditions and during the studies. It should also be pointed out that the venous drainage of the mesenteric fat is portal, whereas that of the epididymal and perinephric fat is caval. Because the mesenteric fat was intact in VF$^-$ rats, an increased "portal" delivery of FFA to the liver is not a likely explanation for the observed changes in hepatic glucose fluxes (33).

Recent evidence indicates that adipose cells are also capable of biosynthesis and secretion of several metabolically active factors (34). Some of these factors circulate in plasma and are active at distant targets (19,35). Thus, the surgical removal of selective fat pads may have removed an important factor implicated in the pathophysiology of insulin resistance and/or may have disrupted a cross-talk between fat depots and distant sites (36). In this regard, it is of interest that the mRNA encoding for the novel circulating protein resistin was much higher in epididymal and perinephric adipose tissue than in SC adipose tissue. Although the differential expression of resistin in these fat depots was striking and reproducible, it is difficult to evaluate whether its removal plays a significant role in the improved insulin action in the VF$^-$ models. The extraction of VF also resulted in marked changes in the expression of the fat-derived peptide TNF-α in the SC adipose tissue. TNF-α may be directly involved in the development of insulin resistance in obesity through its effects on insulin signaling (37). It may be postulated that a factor that

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modulates TNF-α gene expression is selectively expressed in VF and has been removed in the VF$^-$ group. Alternatively, a decrease in TNF-α gene expression may be secondary to the altered metabolic and hormonal milieu in the VF$^-$ rats. It is of interest that marked (45%) decreases in TNF-α gene expression were also demonstrated in fat obtained from obese humans who lost weight (38) and after dietary manipulations in obese mice (39). VF removal was also associated with decreased plasma concentrations of both insulin and leptin. Leptin mRNA in SC adipose tissue was also decreased. The decline in circulating levels of these hormones may simply reflect their improved biological action. However, it is also likely that a decrease in plasma insulin concentrations and perhaps decreased carbon flux into the hexosamine pathway may account for the decreased expression of leptin in SC adipose tissue after VF removal (19). Finally, removal of VF resulted in a significant decrease in plasma Acrp30 levels. Thus, because Acrp30 normally induces a potentiation of insulin action, the improvements in systemic insulin sensitivity seen with VF removal was probably mediated through mechanisms distinct from an increase in circulating Acrp30 levels.

Our results indicate that the surgical removal of selective intra-abdominal fat depots prevents the age-related decrease in peripheral and hepatic insulin action and may regulate gene expression in SC adipose tissue. Furthermore, removal of VF delays the onset of diabetes in the ZDF model of obesity and diabetes. Thus, we propose that specific interventions designed to reduce intra-abdominal adiposity will greatly improve insulin action. Further studies will be necessary to identify the specific fat-derived signals by which selective depots of adipose tissue regulates glucose fluxes and gene expression at distant sites.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (Paul Beeson Physician Faculty Scholar in Aging Award and RO1-AG18381 to N.B. and R01-DK 45024 and ROI-DK 48321 to L.R.), the American Diabetes Association, and the Core Laboratories of the Albert Einstein Diabetes Research and Training Center (DK 20541).

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