

Subcutaneous lipectomy causes a metabolic syndrome in hamsters

R. V. WEBER,¹ M. C. BUCKLEY,¹ S. K. FRIED,² AND J. G. KRAL¹

¹Department of Surgery, State University of New York, Health Science Center at Brooklyn, Brooklyn, New York 11203; and ²Department of Nutritional Sciences, Rutgers University, New Brunswick, New Jersey 08901

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Weber R. V., M. C. Buckley, S. K. Fried, and J. G. Kral.

Subcutaneous lipectomy causes a metabolic syndrome in hamsters. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R936–R943, 2000.—The insulin resistance syndrome X is related to excess intra-abdominal adipose tissue. With lipectomy of >50% of subcutaneous adipose tissue (SQAT) in nonhibernating, adult female Syrian hamsters on high-fat (HF; 50 calorie%) diet and measurements of oral glucose tolerance, oral [¹⁴C]oleic acid disposal, serum triglycerides, serum leptin, liver fat, perirenal (PR) adipose tissue cellularity, and body composition, we studied the role of SQAT. Sham-operated (S) animals on HF or low-fat (LF; 12.5 calorie%) diets served as controls. After 3 mo there was no visible regrowth of SQAT but HF diet led to similar levels of body weight and body fat in lipectomized and sham-operated animals. Lipectomized (L) animals had more intra-abdominal fat as a percentage of total body fat, higher insulinemic index, a strong trend toward increased liver fat content, and markedly elevated serum triglycerides compared with S-HF and S-LF. Liver and PR adipose tissue uptake of fatty acid were similar in L-HF and S-HF but reduced vs. S-LF, and were inversely correlated with liver fat content and insulin sums during the oral glucose tolerance test. In summary, lipectomy of SQAT led to compensatory fat accumulation implying regulation of total body fat mass. In conjunction with HF diet these lipectomized hamsters developed a metabolic syndrome with significant hypertriglyceridemia, relative increase in intra-abdominal fat, and insulin resistance. We propose that SQAT, via disposal and storage of excess ingested energy, acts as a metabolic sink and protects against the metabolic syndrome of obesity.

syndrome X; fatty liver; high-fat diet; insulin resistance; triglycerides; leptin

CONSIDERABLE EVIDENCE demonstrates the adverse health effects of a central distribution of adipose tissue with a preponderance of intra-abdominal or visceral accumulation of fat. The factors causing such distribution and the pathogenetic mechanisms are not known, but excess visceral fat is believed to have a key role in the etiology of the metabolic “syndrome X” of obesity (17).

This experiment investigates the role of subcutaneous adipose tissue (SQAT) in the metabolic syndrome

of obesity by surgically creating a relative “deficiency” of SQAT. An analogy is proposed with various lipodystrophies, demonstrating metabolic abnormalities such as insulin resistance (45) and dyslipidemia (22, 34), considered to be “paradoxically” similar to abnormalities found in obesity or adipose tissue excess.

Earlier we noted that some of our patients undergoing extensive surgical reduction of SQAT postoperatively had increased serum insulin and triglyceride levels (21). Other patients in our experience who had had prior lipectomy subsequently developed severe or “morbid” obesity with non-insulin-dependent diabetes and dyslipidemia (19), implying a detrimental effect of removal of subcutaneous fat. Indeed, it actually has been suggested that thigh fat might be “protective” against lipoprotein abnormalities associated with cardiovascular disease (48), and one animal study demonstrates dysfunction of adipose tissue lipoprotein lipase as a determinant of hypertriglyceridemia (15).

Herein we extend our previous animal work using excision of adipose tissue, adipectomy (10, 18), to reduce the amount of SQAT in an animal model of dietary obesity to test the hypothesis that SQAT deficiency causes metabolic abnormalities. We also explored the impact of subcutaneous lipectomy on serum leptin, the adipose tissue-derived ob protein.

METHODS AND MATERIALS

Animals, Acclimatization, and Handling

Twenty-eight adult (125–135 g) female Syrian hamsters (Harlan Sprague Dawley) were housed in individual Plexiglas cages with cedar chip bedding. Room conditions were controlled for temperature (75°F), humidity (40%), and 16:8-h light-dark cycle. Animals were handled on a daily basis during daily gavage with tap water, as conditioning for future oral glucose tolerance tests (OGTT) and [¹⁴C]oleic acid uptake. Animals were weighed biweekly and were acclimatized to housing and handling for 2 wk preoperatively.

Diets

Animals had ad libitum access to tap water and isocaloric high-fat (HF) or low-fat (LF) control diets that met require-

Address for reprint requests and other correspondence: J. G. Kral, Dept. of Surgery, Box 40, SUNY Downstate Medical Center, 450 Clarkson Ave., Brooklyn, New York 11203.

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ments for protein, minerals, and vitamins (Research Diets, New Brunswick, NJ). The HF diet contained 50 calorie% fat, 27.5% carbohydrate, and 22.5% protein. The LF diet contained 12.5 calorie% fat, 65% carbohydrate, and 22.5% protein. The fat sources were one-half each of soybean and hydrogenated coconut oils.

Groups

Animals were randomly assigned to one of three groups: lipectomy on an HF diet (L-HF, $n = 9$), sham lipectomy on an HF diet (S-HF, $n = 9$), or sham lipectomy on an LF diet (S-LF, $n = 9$).

Procedures

Surgical. After fasting for 16 h with free access to water, the animals were anesthetized intraperitoneally with 100 mg/kg ketamine plus 10 mg/kg xylazine. The abdomen was shaved and scrubbed with Betadine. A 3-cm transverse incision was made across the inguinal region. The superior cutaneous flap was dissected to the level of the costal arch; the inferior flap was dissected around the perineum on the inside of the thighs down to the knees. Both flaps were extended posterolaterally to the vertebral column ("belt lipectomy"). The subcutaneous fat pad was then sharply dissected from the fascia, excised, and weighed. There was no visible evidence of any brown adipose tissue in the surgical specimen. This procedure removes more than 50% of all subcutaneous dissectable adipose tissue in 130-g female hamsters corresponding to ~15–20% of all adipose tissue.

The incision was closed with interrupted 3-0 nylon sutures. Animals were returned to their cages after recovery from anesthesia. Sham procedures were identical to lipectomy with elevation of flaps but without fat-pad excision. All animals were followed daily for 2 wk to monitor postoperative wound healing and weight gain.

Fasting OGTTs. After 11 wk, 1 wk before death, all animals underwent an OGTT. After a 16-h fast with free access to water, 1.5 g/kg body wt of dextrose was mixed in 0.5 ml sterile water and given via gavage. Blood samples (0.5–0.75 ml) were taken from the retro-orbital sinus under CO₂ sedation, at baseline, after 30 min, and after 120 min for serum glucose and serum insulin determination.

Nonfasting [¹⁴C]oleic acid uptake. All animals were gavaged with 0.9 ml sesame oil containing 1 μCi [¹⁴C]oleic acid (Dupont NEN Products, Boston, MA) 16 h before they were killed. This time interval was chosen based on the data of Li et al. (25) in rats demonstrating maximum uptake in adipose tissue after 16 h.

Death

At 12 wk, without fasting, at midday the animals were sedated with CO₂, and 4–6 ml of blood were collected from the retro-orbital sinus into nonheparinized tubes for further serum analyses. The animals were then killed by exsanguination.

Carcasses were inspected for any regrowth at lipectomy sites. Subcutaneous (SQ), parametrial (PM), and perirenal (PR) fat pads were dissected, excised, weighed, and stored at –20°C until they were analyzed.

The liver was removed and weighed after discarding the gallbladder. Portions of liver were stored at –20°C for protein and lipid extractions and ¹⁴C analysis. One aliquot was stored in liquid nitrogen for determination of glutathione as a marker of lipid peroxidation (41). A small sample was fixed in Formalin for histological examination.

The gastrointestinal tract from esophagus to rectum was removed. The remaining carcass was homogenized in water, and aliquots were analyzed for lipid gravimetrically after lipid extraction using Dole's solution. Carcass dry weight was measured while fat-free dry weight and water content were calculated.

Chemical Analyses

Blood. Serum was stored at –20°C until spectrophotometric determination of alanine aminotransferase, alkaline phosphatase, and triglycerides (Sigma, St. Louis, MO). A radioimmunoassay (RIA) kit was used for leptin determination. Samples collected during OGTT were measured for glucose levels using a Beckman Glucose Analyzer 2. Insulin was determined using ¹²⁵I-RIA with rat insulin standard (IncStar, Stillwater, MN).

Liver. Liver protein determination was done spectrophotometrically from homogenized wet tissue samples (Sigma, modified Lowry method). Lipid extraction was performed according to Folch and measured gravimetrically. Liver glutathione, a sensitive marker of hepatocellular injury, was determined with the method described by Salazar et al. (41).

Histological hematoxylin and eosin stain of liver tissue was prepared according to the routines of the Department of Pathology. Specimens were examined by the pathologist without knowledge of the experimental groups, and graded on a semiquantitative scale for amount of lipid within hepatocytes.

Adipose tissue. Samples from the PR fat depot were placed in 0.9% NaCl. They were blotted and weighed and used for Folch extraction and osmium fixation for cell sizing and calculation of cell number (14). This depot was selected for fat cell sizing because it has been demonstrated consistently to be most sensitive to manipulations of body fat (e.g., Ref. 23). [¹⁴C]oleic acid counts in fat and liver tissues were determined before lipid extraction using a Packard Tricarb Liquid Scintillation Spectrometer.

Statistics

SPSS and Statview II, and Microsoft Excel 97 programs were used to calculate *t*-tests, ANOVAs, and Scheffé post hoc tests as appropriate. All results are presented as means ± SE. Pearson correlations were calculated for selected continuous variables.

The protocol was approved by the Institutional Animal Care and Use Committee in accordance with Association for Accreditation and Assessment of Laboratory Animal Care guidelines.

RESULTS

General

HF diet resulted in equal body weights in the two HF groups, which were higher than in S-LF animals (Table 1; Fig. 1; $P < 0.05$). Close inspection of the lipectomy sites in the L-HF groups did not reveal any signs of adipose tissue regrowth.

Adipose Tissue Depots

Fat pad wet weights (SQ, PM, PR) were significantly greater in the HF groups than in S-LF group ($P < 0.001$; Table 1). The absolute weights of "visceral" or intra-abdominal depots (PR and PM) were not statistically different in lipectomized and S-HF groups but

Table 1. Body weight, adipose tissue weights, and carcass lipid content in lipectomized or sham-operated female Syrian hamsters after 12 wk on HF or LF diets

	L-HF	S-HF	S-LF
Operation wt, g	128.7 ± 2.4	128.9 ± 2.2	129.3 ± 1.8
Term wt, g	145.8 ± 3.6	147.0 ± 3.6	138.4 ± 1.2 ^a
SQAT wt, g		4.25 ± 0.17	3.12 ± 0.16 ^c
Adipose tissue wt			
PR, g	1.23 ± 0.08 ^f	1.12 ± 0.08 ^f	0.61 ± 0.06 ^{c,f}
PM, g	1.64 ± 0.10 ^f	1.46 ± 0.13 ^f	0.80 ± 0.09 ^{c,f}
IA, g	2.87 ± 0.16 ^f	2.59 ± 0.19 ^f	1.41 ± 0.15 ^{c,f}
Carcass lipid, g	9.6 ± 1.2 ^e	7.9 ± 0.8 ^e	4.7 ± 0.5 ^{b,e}
Depot lipid, g	2.3 ± 0.1 ^{c,f}	5.5 ± 0.2 ^f	3.7 ± 0.2 ^{c,f}
Total body lipid, g	11.9 ± 1.3 ^e	13.4 ± 0.8 ^e	8.4 ± 0.6 ^{c,e}
IA wt/term wt, %	1.96 ± 0.08 ^d	1.75 ± 0.10	1.02 ± 0.11 ^c
IA lipid/total lipid, %	21.6 ± 2.9 ^d	15.8 ± 1.3	13.6 ± 1.2
PR fat cell wt, μg	0.24 ± 0.01	0.24 ± 0.02	0.14 ± 0.01 ^c
Fat cell number	4.22 ± 0.18 ^f	4.41 ± 0.31 ^f	7.55 ± 0.57 ^{e,f}

Values are means ± SE; *n* = 9 hamsters/group. HF, high-fat diet; LF, low-fat diet; SQAT, subcutaneous adipose tissue; PR, perirenal; PM, parametrial; IA, intra-abdominal (PR + PM); L-HF, lipectomized HF group, S-HF and S-LF, sham-operated HF and LF groups. ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001, ^d*P* = 0.10 for S-HF vs. L-HF or S-LF; ^e*P* < 0.01, ^f*P* < 0.001 by ANOVA.

were greater than in the S-LF group. L-HF animals showed a strong trend to greater visceral fat vs. S-HF animals when expressed as %body weight (1.96 ± 0.08 vs. 1.75 ± 0.10%; *P* = 0.10) and %body fat (21.6 ± 2.9% vs. 15.8 ± 1.3%; *P* = 0.10). PR fat cell sizes were larger in the HF groups (*P* < 0.001), but there was no difference between L-HF and S-HF. There were no statistically significant differences in fat cell numbers in the PR depots between groups.

Carcass Analyses

The absolute amount of residual carcass lipid (after removal of major fat pads) was not statistically significantly different between the two HF groups [L-HF = 9.6 ± 1.2 g vs. S-HF = 7.9 ± 0.8 g; *P* = not significant (NS)], although expressed as %total body lipid, carcass lipid was 17% greater in the lipectomized group (62.7 ± 2.3 vs. 46.3 ± 2.9% in S-HF; *P* < 0.001), indicating

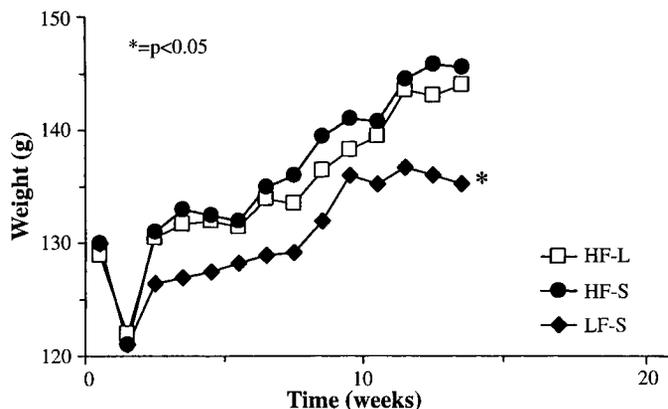


Fig. 1. Weights in lipectomized (L) and sham-operated (S) adult female hamsters on high-fat (HF) or control, low-fat (LF) diets followed for 12 wk.

Table 2. Liver tissue in lipectomized or sham-operated female Syrian hamsters on HF or LF diets

	L-HF	S-HF	S-LF
Liver wet wt, g	5.2 ± 0.2	5.2 ± 0.3	5.6 ± 0.2
Liver protein, mg/g wet wt	2.87 ± 0.14	3.03 ± 0.15	2.77 ± 0.11
Liver fat, mg/g protein	7.7 ± 0.7*	6.6 ± 0.7	5.2 ± 0.8
Liver glutathione, μmol/g protein	0.66 ± .04	0.60 ± .03	0.62 ± .04

Values are means ± SE; *n* = 9 hamsters/group. **P* = 0.07 L-HF vs. S-LF.

increased fat deposition in the carcass after lipectomy. Both HF groups had greater carcass lipid content than the S-LF group. Total body lipid content (carcass lipid plus dissected depot lipid) in L-HF and S-HF hamsters was similar and higher than in S-LF hamsters.

Body composition was similar in the HF groups (L-HF: lipid = 10.5%, water = 68.8%, fat-free dry weight = 20.7%; S-HF: lipid = 11.8%, water = 68.4%, fat-free dry weight = 19.8%). The S-LF group had less lipid (8.0%; *P* < 0.001) and more water (72.3%; *P* < 0.001) than the two HF groups.

Liver analyses

Neither wet weight nor protein content per gram wet weight of liver tissue differed significantly between groups (Table 2). However, there was a 48% increase in chemically determined liver fat in the lipectomized group compared with the S-LF group (*P* = 0.07; Table 2), which was not evident on histological examination (data not shown). Differences in liver glutathione per gram protein between L-HF and S-HF groups did not reach statistical significance (Table 2). Serum alanine aminotranspeptidase and serum alkaline phosphatase also were not statistically significant (Table 3).

Glucose and Lipid Metabolism

When killed the S-HF animals had significantly higher baseline serum glucose than the other two groups (*P* < 0.05) and higher baseline serum insulin than S-LF (*P* < 0.01) and L-HF groups (*P* < 0.05; Table 3). However, the baseline insulinemic index (serum

Table 3. Blood chemistry and oral glucose tolerance tests in female Syrian hamsters

Serum	L-HF	S-HF	S-LF
Insulin, μU/ml	23.7 ± 1.8 ^{a,e}	24.8 ± 3.3 ^e	20.1 ± 1.1 ^{b,e}
Insulin sums	88.4 ± 4.4 ^{a,g}	108.1 ± 5.8 ^g	72.3 ± 2.6 ^{c,g}
Glucose, mg/dl	75.6 ± 2.5 ^{a,e}	86.6 ± 3.7 ^e	75.4 ± 1.9 ^{a,e}
Glucose sums	316.7 ± 12.2	327.4 ± 12.7	324.8 ± 15
Triglycerides, mg/dl	208.8 ± 18.8 ^{d,e}	161.0 ± 19.7 ^e	145.4 ± 12.2 ^e
ALAT, μ/l	25.7 ± 5.4	23.0 ± 1.7	22.2 ± 2.0
AP, μ/l	70.6 ± 3.5	73.6 ± 4.7	78.0 ± 3.6
Leptin, ng/ml	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.2

Values are means ± SE; *n* = 9 hamsters/group. ALAT, alanine aminotranspeptidase; AP, alkaline phosphatase. ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001, ^d*P* = 0.10 for S-HF vs. L-HF or S-LF; ^e*P* < 0.05, ^g*P* < 0.001 by ANOVA.

Table 4. [^{14}C]oleate uptake in SQ, PM, and PR adipose tissue and liver in female Syrian hamsters

	L-HF	S-HF	S-LF
Adipose tissue			
SQ, $\text{cpm} \times 10^{-3}/\text{g}$			
wet wt		4.2 ± 0.4	8.4 ± 1.7^a
PM, $\text{cpm} \times 10^{-3}/\text{g}$			
wet wt	7.4 ± 1.1^e	7.0 ± 0.6^e	$17.0 \pm 2.8^{b,e}$
PR, $\text{cpm} \times 10^{-3}/\text{g}$			
wet wt	4.9 ± 0.7^e	4.5 ± 0.4^e	$14.0 \pm 2.3^{b,e}$
PR, $\text{cpm} \times 10^{-3}/10^6$ cells	15.6 ± 0.2^e	15.3 ± 2.1^d	$26.5 \pm 3.9^{a,d}$
Liver, cpm/g protein	235 ± 17^c	223 ± 22^c	$306 \pm 27^{a,c}$

Values are means \pm SE; $n = 9$ hamsters/group. cpm, counts/min. ^a $P < 0.05$, ^b $P < 0.01$ for S-HF vs. L-HF or S-LF; ^c $P < 0.05$, ^d $P < 0.01$, ^e $P < 0.001$ by ANOVA.

insulin /serum glucose) was higher in the L-HF group than in either sham-operated group (0.31 ± 0.01 vs. 0.29 ± 0.02 in S-HF and 0.27 ± 0.01 in S-LF; $P < 0.05$ by ANOVA). Both HF groups had elevated serum insulin sums during OGTT ($P < 0.001$) with the S-HF group being highest ($P < 0.001$ by ANOVA). There were no statistically significant differences in serum glucose sums between groups.

Serum triglycerides were markedly elevated in the L-HF group compared with the S-HF and S-LF groups ($P < 0.02$ by ANOVA).

[^{14}C]oleic acid uptake in adipose tissue was significantly greater in the S-LF group than in either HF group with no difference between lipectomized and sham-operated animals (Table 4). In the S-LF animals uptake in the intra-abdominal depots (PM and PR) was greater than in SQAT [PM, 17.0 ± 2.8 counts/min ($\text{cpm}) \times 10^{-3}/\text{g}$; PR, 14.0 ± 2.3 $\text{cpm} \times 10^{-3}/\text{g}$; SQ, 8.4 ± 1.7 $\text{cpm} \times 10^{-3}/\text{g}$; $P < 0.05$ by ANOVA]. In the PR adipose tissue depot there was an inverse relationship between fat cell size and [^{14}C]oleic acid uptake ($r = -0.61$; $P < 0.001$; Fig. 2). Furthermore, PR lipid uptake was inversely related to insulin sums during the

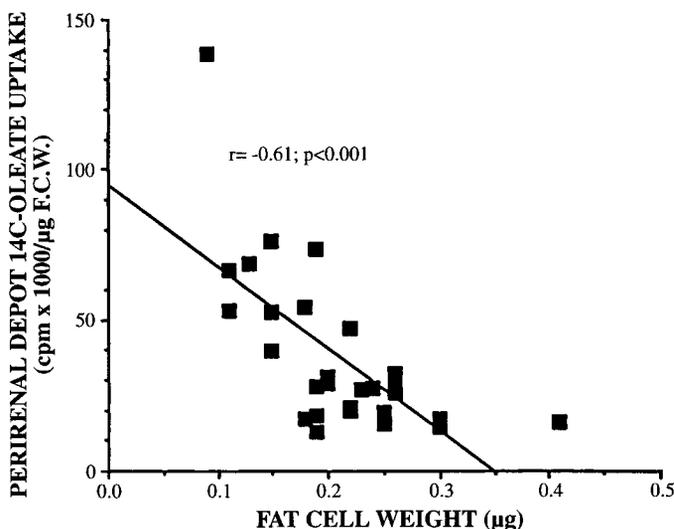


Fig. 2. Relationship between fat cell weight and gavaged [^{14}C]oleate uptake in perirenal adipose tissue. FCW, fat cell weight.

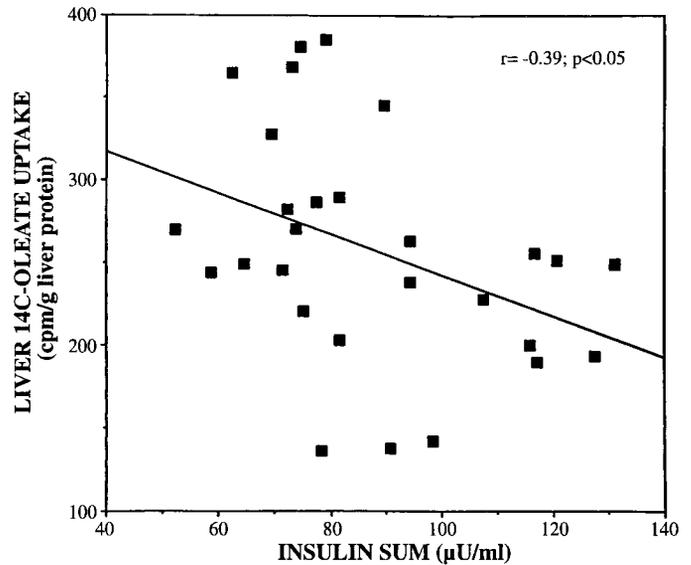


Fig. 3. Insulin sum during oral glucose tolerance tests and [^{14}C]oleate uptake in liver tissue.

OGTT ($r = -0.48$; $P < 0.05$). Liver uptake of [^{14}C]oleic acid was also greater in the S-LF group than in both HF groups ($P = 0.004$; Table 4).

There were inverse relationships between liver uptake and insulin sums ($r = -0.39$; $P < 0.05$; Fig. 3) and liver fat ($r = -0.40$; $P < 0.05$), with a trend toward a positive correlation between insulin sums and liver fat content ($r = 0.34$; $P = 0.075$). However, there were no statistically significant relationships between serum insulin and serum triglycerides nor between liver fat and serum triglycerides.

Leptin Analysis

Mean serum leptin values were similar in all three groups of hamsters (Table 3). There were statistically significant positive correlations between serum leptin values and dissectable adipose tissue depot weights with similar slopes in each group (L-HF, $r = 0.75$; S-HF, $r = 0.85$; S-LF, $r = 0.81$; $P < 0.05$). Serum leptin did not correlate with body weight, total lipid, or serum insulin.

DISCUSSION

HF diet in these adult female hamsters was associated with relative hyperinsulinemia, increased body fat and body weight, larger PR fat cell size, and increased weights of dissectable adipose tissue depots compared with sham-operated hamsters on a control, LF diet (S-LF). Lipectomy in conjunction with the HF diet resulted in compensatory deposition of lipid in the carcass and remaining fat depots leading to similar levels of body fat and body weight as in the sham-operated group (S-HF), thus clearly demonstrating regulation of body fat in hamsters as previously determined by Hamilton and Wade (12) in adult female Syrian hamsters having more extensive lipectomy. Furthermore, in the present study lipectomy was associated with significant hypertriglyceridemia, a 40%

increase in liver fat content, a relative increase in intra-abdominal adipose tissue, and an elevated basal insulinemic index, components of the metabolic, insulin resistance syndrome X of obesity described in people (1, 11, 17).

There are three potential sites of peripheral insulin resistance in this model: 1) adipose tissue, 2) liver, and 3) muscle. We studied *in vivo* oleic acid uptake to detect differences in fatty acid uptake into tissues and as a marker of insulin resistance. In the absence of tissue studies of insulin action, however, we are unable to determine the extent or relative contributions of the various tissues to overall insulin resistance. Both of our HF-fed groups exhibited decreased uptake of oral oleic acid compared with the group on control diet in all dissected white adipose tissue, implying insulin resistance. On the control diet oleic acid uptake was greater in the metabolically more active intra-abdominal depots than in subcutaneous fat, in agreement with findings by Li et al. (25) in intact fed rats.

The substantial increase in serum triglycerides in the lipectomized hamsters implies a quantitative role for SQAT as a "metabolic sink" for dietary fat. The lack of triglyceride elevation in the sham-operated hamsters on an HF diet is consistent with the findings of others of an "absence of changes in circulating triglyceride levels" during fat feeding (39). Similar elevations of triglycerides as those in the lipectomized animals have been described during seasonal obesity in female Syrian hamsters (4), in lipectomized female ground squirrels (9), and in lipectomized male rats with dexamethasone-induced insulin resistance (31). A recent study of lipectomy of inguinal and interscapular adipose tissue in female obese and lean Zucker rats on a LF diet demonstrated great variability in serum insulin and glucose levels as well as serum triglycerides in both groups at different times postoperatively (26). Unfortunately, there were no sham-operated controls, and the sampling circumstances were not described. Furthermore, the interscapular depot of rats and other rodents contains substantial amounts of brown adipose tissue (which were not described in the paper), further contributing to difficulties in interpreting their data.

We did not measure adipose tissue lipoprotein lipase (LPL) levels in our animals and cannot determine whether quantitative or qualitative (15) changes in LPL could account for impaired clearance of circulating lipids. However, female ground squirrels undergoing extensive visceral and subcutaneous lipectomy exhibited elevated plasma triglyceride levels in the face of markedly elevated SQAT LPL activity (9). On the other hand, Mauer and Bartness (28), in a study of long-day-housed male Siberian hamsters with lipectomy of the epididymal white adipose tissue depot (a relatively small depot), did not detect any changes in SQAT LPL.

The reduction of a significant amount of SQAT in these hamsters on an HF diet was associated with a trend toward increased fatty infiltration of the liver. There was a reduction in the uptake of oleic acid by the liver, which might reflect hepatic insulin resistance similar to that found in adipose tissue. Earlier we

showed that elevated plasma insulin levels and serum free fatty acids are correlated with liver fat and triglyceride synthesis (20), and we demonstrated recently that insulin resistance is associated with fatty infiltration of the liver and increased intra-abdominal fat in humans (1, 11) without being able to determine the mechanism(s). One mechanism of insulin resistance is thought to be mediated by free fatty acids delivered to liver tissue via the portal vein, interfering with insulin binding and degradation (13, 47) contributing to the Randle effect (35).

A different mechanism of hepatic insulin resistance with decreased hepatic insulin clearance (6) might be due to toxic effects of lipid peroxidation in the fatty liver (5), reflected in the slight elevation of serum alanine aminotransferase in the lipectomized animals. However, in the absence of elevations of serum alkaline phosphatase or decreases in liver glutathione levels we are unable to conclusively determine the validity of this proposed mechanism. Nevertheless, in our lipectomy model with reduced SQAT acting as a metabolic sink and with a relative increase in intra-abdominal fat, it is likely that the fatty infiltration of the liver is a cause rather than a consequence of insulin resistance.

It has been speculated recently that leptin may contribute to hepatic steatosis via effects on insulin secretion (16). In the present experiment using an HF diet known to decrease leptin, we found no differences between leptin levels among the groups in the face of increased fatty infiltration and varying levels of plasma insulin. Our finding in conjunction with findings of fatty liver in the leptin-deficient models described below (30, 43) lead us to believe that increased leptin likely does not contribute to fatty liver in this context, although decreased levels might be of pathogenetic importance.

Lipectomy did not cause any change in serum leptin levels compared with the sham-operated controls on HF diets (S-HF). This is not surprising in the face of similar levels of total body lipid and the leptin-lowering effects of the HF diet. We are unable, however, to explain the lack of a difference in serum leptin level in the S-LF group, with 37% less body lipid, particularly in view of the robust correlations between serum leptin and dissectable adipose tissue depot weights in all groups.

The third peripheral site of insulin resistance may be muscle tissue. Deposition of lipid within or between muscle fibers in these lipectomized hamsters on an HF diet would be consistent with the findings of Perseghin et al. (33) in people. Insulin resistance is well documented in skeletal muscle tissue of rats fed an HF diet (46). Although not measured, we have no reason to expect significant differences in physical activity, which hypothetically also could contribute to differences in insulin action between the groups.

The metabolic manifestations of surgical reduction of SQAT in our study are similar to those described in lipodystrophy (22, 34, 45) characterized by an absence of subcutaneous but presence of visceral adipose tissue.

Another “natural model” for reduced SQAT mass might be fetal undernutrition, inhibiting the development and growth of adipose tissue. Fetal undernutrition has been described recently as a risk factor for subsequent development of components of the metabolic syndrome X (2, 36). Interestingly, in utero exposure to famine during the fetal period when adipose tissue normally develops, resulted in significantly higher obesity rates in young men (38). Recent follow-up studies of women and men at 50 yr of age have demonstrated an abdominal distribution of adipose tissue in the women (37), which might explain a higher prevalence of metabolic abnormalities later in life.

Based on the present experiments and our earlier clinical observations, we believe that surgical reduction of SQAT can be used as an experimental model for studying the importance of this adipose tissue depot in the pathogenesis of the metabolic syndrome of obesity. Earlier studies of antiserum with cytotoxic effects to rat adipocyte plasma membranes *in vivo* affected both visceral and SQAT cell numbers with a predominant effect on the internal depots (8). In a follow-up study these authors noted “grossly affected liver morphology” but no effects of the antiserum on lipid or glucose metabolism (32). Wright and Hausman (51) demonstrated cytotoxicity of monoclonal antibodies in rats *in vivo*, with greater effects on inguinal than PR depot weights. They did not address metabolic effects of the intervention.

Initial studies on various transgenic mice with knock-out of white adipose tissue led to lethal phenotypes characterized by enlarged, fatty livers and metabolic disturbances (40, 50). More recent studies on transgenic mice with alterations of transcription factors successfully created phenotypes with absence of white fat (30, 43). The metabolic consequences in those experiments, expressed after life-long or delayed exposure to generalized adipose tissue deficiency were similar to our findings after the more acute intervention of surgically reducing SQAT in adult animals. The conclusions of those authors are similar to ours, postulating a role for adipose tissue deficiency in the pathogenesis of diabetes and hyperlipidemia. Interestingly a recent sophisticated study of hepatic insulin resistance in male rats with lipectomy of epididymal and PR depots (3) is complementary to ours. Although their study neither reduced SQAT nor visceral depots draining into the portal vein (omental and mesenteric) they found a significant increase in hepatic insulin sensitivity in their lipectomized rats as well as altered gene expression in SQAT. Although performed in different species, with different sex and different designs with respect to diet and duration, taken together these two studies seem to imply the importance of optimal amounts of subcutaneous vs. visceral adipose tissue, as has been proposed in clinical studies (27, 44).

In conclusion, the present results confirm earlier findings by others and by us of regulation of body fat and imply that a lack of SQAT may have detrimental consequences. These findings in hamsters are in line with preliminary data from severely obese patients

with extensive subcutaneous lipectomy (19). Further studies will be necessary to determine whether there is a critical relative or absolute amount of subcutaneous fat necessary to “protect” against the metabolic abnormalities of the insulin resistance syndrome X. Such studies might require greater numbers of animals and longer periods of observation.

Perspectives

With the recognition of the seriousness of the worldwide epidemic of obesity and its significant comorbidities, all interest has focused on detrimental aspects of excess adipose tissue without considering the possibility of necessary functions of the tissue beyond simple storage of substrate. Separate from concerns over health consequences of obesity, the prevailing cosmetic ideals have driven the wide-spread performance of suction-assisted lipectomy among the overweight and obese and even among normal weight individuals exclusively targeting SQAT (27). This study demonstrates that lipectomy, by reducing the number of subcutaneous adipocytes, causes lipid deposition in visceral adipose tissue and the liver with metabolic consequences similar to the insulin resistance syndrome X.

The results also have implications for the controversy over the etiology and earliest events of syndrome X. Candidates range from genes, fetal stress, undernutrition, and dietary excess (mainly lipid and simple carbohydrates) to sedentary lifestyle and include hypothalamic neuroendocrine activity, pancreatic β -cell responsiveness, and neoglucogenetic enzyme abnormalities. Most of these factors influence adipogenesis, either through inhibition (e.g., via undernutrition) or stimulation (e.g., via fatty diet or sedentariness) of fat cell growth causing adipose tissue deficiency or adipocyte hypertrophy. Syndrome X is prevalent in people born in areas with perinatal and infant undernutrition when they are exposed to “Western” diets and lifestyles (29). Their decreased numbers of fat cells in SQAT, developmentally the earliest fat compartment, places them at risk for lipid deposition in tissues poorly equipped to handle excess lipid [e.g., pancreas (23), liver, artery, skeletal muscle, or heart (52)].

Further support for this metabolic sink hypothesis can be found in clinical lipodystrophy, a naturally occurring state of adipose tissue deficiency. All lipodystrophies associated with components of syndrome X described to date involve dystrophy exclusively of subcutaneous fat. In this context, mutations affecting lamin have just been discovered in people with partial lipodystrophy (42), in turn contributing to the development of type II diabetes (7).

The broad general implication of this study of lipectomized hamsters is recognition of the fact that SQAT may protect against lipotoxicity in nonadipose tissue and thus be of primary pathogenetic importance for avoiding the development of metabolic abnormalities. It raises concerns over long-term adverse effects of large-volume lipectomy.

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