Critical Role of the Mesenteric Depot Versus Other Intra-abdominal Adipose Depots in the Development of Insulin Resistance in Young Rats

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OBJECTIVE—Age-associated insulin resistance may be caused by increased visceral adiposity and older animals appear to be more susceptible to obesity-related resistance than young animals. However, it is unclear to what extent the portally drained mesenteric fat depot influences this susceptibility.

RESEARCH DESIGN AND METHODS—Young high-fat–fed and old obese rats were subjected to 0, 2, 4, or 6 weeks of caloric restriction. Insulin sensitivity (S_I) was assessed by hyperinsulinemic clamp and lean body mass (LBM) and total body fat were assessed by ^{18}O-water administration.

RESULTS—Six weeks of caloric restriction caused a similar reduction in body weight in young and old animals ($P = 0.748$) that was not due to reduced subcutaneous fat or LBM, but rather preferential loss of abdominal fat ($P < 0.05$). Most notably, mesenteric fat was reduced equivalently in young and old rats after 6 weeks of caloric restriction ($\sim \downarrow 53\%$; $P = 0.537$). Despite similar visceral fat loss, S_I improved less in old ($\uparrow 32.76 \pm 9.80\%$) than in young ($\uparrow 82.91 \pm 12.66\%$) rats versus week 0. In addition, there was significantly more reversal of fat accumulation in the liver in young (% reduction: 89 ± 2) versus old (64 ± 5) rats ($P < 0.0001$). Furthermore, in young rats, S_I changed much more rapidly for a given change in mesenteric fat versus other abdominal depots (slope = 0.53 vs. ≤0.27 kg/min/mg per % fat).

CONCLUSIONS—Improved S_I during caloric restriction correlated with a preferential abdominal fat loss. This improvement was refractory in older animals, likely because of slower liberation of hepatic lipid. Furthermore, mesenteric fat was a better predictor of S_I than other abdominal depots in young but not old rats. These results suggest a singular role for mesenteric fat to determine insulin resistance. This role may be related to delivery of lipid to liver, and associated accumulation of liver fat.
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Increased abdominal adipose mass has been of particular interest in elucidating the mechanisms of insulin resistance. Visceral fat has been implicated because of its distinct anatomic characteristics, because it has a circulation draining into the portal vein and hence the liver. Compared with subcutaneous, visceral adipocytes have a higher secretion rate of some adipokines and metabolites linked to insulin resistance, including free fatty acids (FFAs). In addition, visceral fat cells are resistant to insulin-mediated suppression of lipolysis, leading to elevated FFA delivery to the liver (1–3). Increased release of FFA and/or adipokines from the visceral depot may disrupt insulin action, most likely at the liver, a primary site of insulin resistance in diet-induced obesity (4,5).

Many rodent studies suggesting a linkage between visceral fat and insulin resistance have focused on the removal of specific intra-abdominal fat depots: epididymal and perirenal fat (6,7). However these depots do not have the same circulation as most intra-abdominal fat depots in larger mammals, or primates, including humans. Rodents present three morphologically distinct fat depots: subcutaneous, intra-abdominal (epididymal and perirenal pads), and portally draining "true" visceral fat (mesenteric depot). Thus, removal of abdominal fat shown to influence insulin sensitivity in rats does not necessarily mirror visceral fat depletion in nonrodent species. It is therefore unclear what role the mesenteric fat depot per se may play in the development of insulin resistance in rodent models of obesity. Furthermore, because mesenteric fat in the rodent closely resembles visceral fat in the human, greater experimental attention to this depot and its role in resistance is required.

Although increased adiposity per se may contribute to insulin resistance associated with aging, it is possible that the susceptibility to obesity-linked resistance is altered by age. In this regard, our laboratory has demonstrated that for a given degree of intra-abdominal adiposity, old rats have a greater decrement in insulin sensitivity compared with young (8). It was therefore one goal of the present study to examine the vulnerability of old versus young rats to fat-induced insulin resistance. We tested the hypothesis that fat reduction due to short-term caloric restriction is less effective in enhancing insulin sensitivity in naturally obese old animals compared with a similarly obese group of young rats. Furthermore, we hypothesized that the mesenteric fat depot, in particular, distinct from other intra-abdominal fat pads, plays a pivotal role in this altered susceptibility to resistance with age.

RESEARCH DESIGN AND METHODS

Animals. Two groups of male Fischer Brown Norway rats (F344XBN F1; National Institute on Aging, Bethesda, MD) were used in this study: *1*) young rats (5–6 months; $n = 34$) fed a high-fat diet (HFD) for 3 weeks and *2*) old rats (23–4 months; $n = 35$) fed a standard chow diet ad libitum. Rats from each group were subjected to 0, 2, 4, or 6 weeks of short-term caloric restriction. The HFD obtained from Harlan Teklad (Madison, WI) consisted of 5.0 kcal/g, 66.5% of which was from fat (lard); 21%, from protein; and 12.5%, carbohydrate. Ad libitum chow–fed rats were given a standard diet provided by the National Institutes of Health (NIH) (Diet NIH-31; 4.02 kcal/g). Once subjected to caloric restriction, animals were fed 60% of their typical chow ad libitum calorie per day using a NIH-31 fortified diet (3.95 kcal/g) based on feeding

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TABLE 1
Body composition for all animals used in study

	Body wt (g)	LBM (% body wt)	Subcutaneous fat (% body wt)	Epididymal fat (g)	Perirenal fat (g)	Mesenteric/ visceral fat (g)	Total abdominal fat (g)
Control	360 ± 10	61 ± 7	36 ± 7	4.40 ± 0.26	3.06 ± 0.22	1.58 ± 0.08	9.04 ± 0.51
Young							
0 weeks (n = 8)	385 ± 4	52 ± 7	44 ± 7	7.20 ± 0.33*	6.45 ± 0.30*	3.49 ± 0.14*	17.15 ± 0.60*
2 weeks (n = 8)	389 ± 6	44 ± 3	52 ± 3	7.16 ± 0.31*	5.10 ± 0.39*	2.56 ± 0.17*	14.82 ± 0.78*
4 weeks (n = 8)	359 ± 9	52 ± 5	45 ± 5	5.00 ± 0.48	3.44 ± 0.65	1.47 ± 0.17	9.90 ± 1.27
6 weeks (n = 10)	335 ± 5	52 ± 4	46 ± 4	3.98 ± 0.11	2.54 ± 0.16	1.55 ± 0.09	8.07 ± 0.30
Old							
0 weeks (n = 9)	533 ± 19*§	37 ± 0*§	58 ± 0*§	9.96 ± 0.74*§	8.47 ± 0.58*§	8.43 ± 0.79*§	26.86 ± 1.96*§
2 weeks (n = 8)	496 ± 13*§	43 ± 4*	52 ± 4*	8.81 ± 0.67*	7.99 ± 0.57*§	6.59 ± 0.34*§	23.39 ± 1.19*§
4 weeks (n = 10)	472 ± 14*§	41 ± 2*	56 ± 2*	7.12 ± 0.67*§	6.44 ± 0.76*§	4.02 ± 0.31*§	17.58 ± 1.54*§
6 weeks (n = 8)	460 ± 11*§	39 ± 0*	57 ± 0*	7.05 ± 0.48*§	5.47 ± 0.47 §	3.99 ± 0.34*§	16.51 ± 1.24*§

Data are means ± SEMs. Body weight, LBM, subcutaneous fat, epididymal fat, perirenal fat, mesenteric/visceral fat, and the sum of the three abdominal depots (total abdominal fat) in control rats as well as young fat-fed and old rats exposed to 0, 2, 4, and 6 weeks of caloric restriction.*Significant difference between control and a single experimental group as determined by Student t test, where $P < 0.05$. §Value significantly differs from young rats at the same time exposure to caloric restriction using two-way ANOVA with post hoc tests where $P < 0.05$.

instructions provided by the National Institute on Aging (NIA) for their age group. A separate group of young rats (5–6 months, n = 8) fed the standard diet ad libitum were used as a control group. All animals had free access to water and were housed in the University of Southern California vivarium in separate cages under controlled temperature and lighting (12-h light/12-h dark cycle). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California.

Surgeries. Animals were prepared for the euglycemic clamp protocol as described previously (8). Forty-eight hours prior to the day of the experiment, those animals on restricted diet were returned to a standard chow ad libitum feeding regimen to prevent any acute effects of restriction on fasting state during experiments. On the morning of the experiment (0600 h), any remaining food was removed from the cage and catheters were placed. Two tail vein catheters for infusion and one tail artery catheter for sampling were inserted under local anesthesia (2% lidocaine; Phoenix Pharmaceuticals, St. Joseph, MO). Sampling catheter patency was maintained by infusion of saline with 10 units/ml heparin (1.0 ml/h). Each animal underwent a hyperinsulinemic-euglycemic clamp at ~1200 h (6-h fast).

Hyperinsulinemic-euglycemic clamp. D-[3-^{3}H]-glucose (0.2 μCi/min; "tracer") began at $t = -240$ min. Four samples to measure basal glucose turnover were taken at $t = -60$, -45, -30, and -15 min. At $t = 0$, infusion of insulin (5 mU/min/kg) was started. Samples were taken at 10-min intervals and plasma glucose was monitored. Dextrose (20% unlabeled) was infused via the remaining venous catheter at a variable rate to maintain euglycemia. Euglycemia was defined as the average basal glucose for each rat. Steady state (SS) was defined as the last 30 min of the clamp (t = 120–150 min).

Body composition. A bolus of water labeled with the stable isotope ^{18}O was given to assess total body water, and subsequently total body fat, during the euglycemic clamp. One basal plasma sample was taken at $t = -121$ min, followed by an ^{18}O-water bolus at $t = -120$ min (0.5 g/kg body wt). Animals were equilibrated for 2 h and a sample was taken at $t = -1$ min to assess steady-state ^{18}O levels. To determine the contribution of abdominal fat to total body fat, three abdominal fat pads (epididymal, perirenal, and mesenteric) were excised and weighed upon sacrifice.

Blood sampling. Blood samples for glucose, insulin, adiponectin, resistin, leptin, and tracer determination were collected in tubes coated with heparin and lithium fluoride and centrifuged immediately for separation of plasma. Samples collected for FFAs and glycerol were collected in tubes containing diethyl p-nitrophenyl phosphate (Paraoxan) to inhibit lipoprotein lipase within samples (9) and centrifuged immediately, and plasma was stored at −20°C until assay.

Liver triglycerides. To determine hepatic lipid content, lipid was extracted from frozen liver samples by chloroform:methanol using an adaptation of the Folch method (10).

Assays. Plasma glucose was measured using the automated glucose analyzer YSI 2300 STAT Plus (Yellow Springs, OH). Insulin was assessed using an Ultrasensitive Rat Insulin ELISA Kit from Alpco (Salem, NH). Plasma adiponectin, resistin, and leptin were assayed using commercially available kits from Alpco. Plasma nonesterified FFAs were measured using the FFA Assay Kit from Wako Chemicals (Neuss, Germany), glycerol was measured using Triglyceride Reagent from Sigma Diagnostics (St. Louis, MO), and triglyceride was measured using a commercially available kit from Stanbio (Boerne, TX). Triglyceride values were normalized per gram of liver.

To determine D-[3-^{3}H]-glucose, plasma samples were deproteinized with $BaOH_2$ and Zn_2SO_4 as described by Somogyi (11).

Samples for ^{18}O water measurement were assayed by Metabolic Solutions (Nashua, NH) using the Europa 20/20 Automated Breath Carbon Analyzer Isotope Ratio Mass Spectrometer with an intra-assay coefficient of variation of 0.2%.

Data analysis. Glucose turnover and insulin sensitivity (S_I) were calculated as previously described (8), using classic tracer dilution methodology (12), to estimate glucose disappearance (Rd) expressed per lean body mass and endogenous glucose production (EGP) expressed per body weight.

Total body water was determined as described previously (13,14) and measures were corrected for a known 1% overestimation of this parameter with the $H_2{}^{18}O$ technique (13). Lean body mass (LBM) was calculated as the TOTAL body water/0.72 (15) and total body fat, as the difference between body weight and LBM.

Statistics. All data are represented as means ± SEMs. Within-group comparisons were made using ANOVA with Tukey multiple comparisons test for individual variances. Student t or paired t tests, when appropriate, were performed for individual comparisons. Multiple linear regression analysis was used to assess the relationship between sensitivity and adiposity with a t test for slope comparison between age groups. ANOVAs and regressions were performed using MINITAB Statistical Software (State College, PA) and t tests, using Excel 2000, with statistical significance set at $P \leq 0.05$.

RESULTS

Food intake. Using recommendations from the NIA, young and old animals were fed a diet consisting of ~41 and 47 kcal/day, respectively, during the caloric restriction phase of the study versus 66 and 68 kcal/day in control and fat-fed young rats, respectively (data not shown).

Body weight and composition. Although 3 weeks of HFD in young rats (Y_0) did not cause a significant increase in body weight, LBM, or subcutaneous fat (Y_0 vs. control: $P = 0.054$, $P = 0.634$, $P = 0.367$, respectively; Table 1), it did promote abdominal fat accretion (total abdominal fat; ~90%), and the three depots comprising total abdominal fat (epididymal, perirenal, and visceral fat) were markedly larger (64 ± 7%, 111 ± 10%, 121 ± 9%, respectively).

As expected, body weight was ~1.5-fold greater in old rats (M_0) versus control and Y_0 rats (M_0 vs. control, Y_0: $P < 0.001$) and although LBM was unaltered (control: 219 ± 24 g vs. M_0: 199 ± 6 g, $P = 0.445$), subcutaneous fat was markedly augmented with age (control: 132 ± 26 g vs. M_0: 307 ± 12 g; $P < 0.001$; data not shown). However, percentage of LBM was reduced in older animals (↓ 39%), whereas percentage of subcutaneous fat was increased by 61%. Abdominal fat depots were significantly increased with age (Table 1).

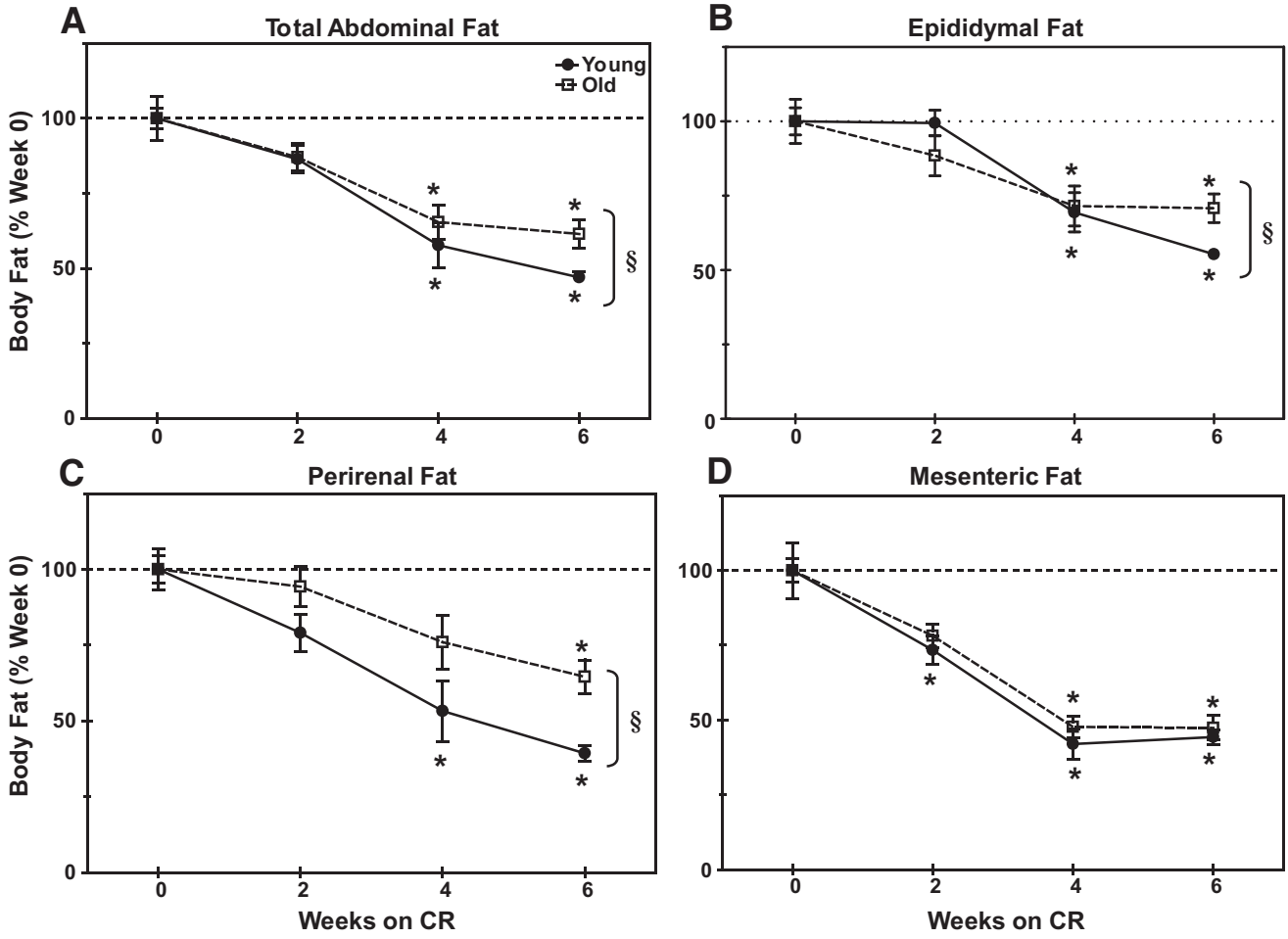

FIG. 1. Effect of caloric restriction on body composition. Total abdominal fat (*A*), epididymal fat (*B*), perirenal fat (*C*), and mesenteric fat (*D*) pad weights during 6 weeks of caloric restriction in young and old rats in grams represented as a percentage of week 0. Data are means ± SEMs. Significant differences were determined using two-way ANOVAs for effect of time and age, with post hoc Tukey tests for individual comparisons. *$P < 0.05$ for young vs. week 0 and old vs. week 0; §$P < 0.05$ young vs. old. CR, caloric restriction.

Caloric restriction in young and old rats caused a modest body weight reduction, reaching significance by 4 weeks (young: ↓6.8%, $P < 0.05$; old: ↓11.4%, $P < 0.05$) and arriving at its nadir by 6 weeks (young: ↓12.8%, $P < 0.05$; old: ↓13.7%, $P < 0.05$; Table 1, Fig. 1). These changes could not be explained by reductions in either percentage of LBM (young: $P = 0.584$; old: $P = 0.399$) or percentage of subcutaneous fat (young: $P = 0.624$; old: $P = 0.355$ by ANOVA; Table 1). However, caloric restriction did effectively reduce abdominal fat by 4 weeks in both young and old rats, with no further reductions at 6 weeks (young: ~45%↓; old: ~30%↓; Fig. 1). Interestingly, obesity was reversed slightly more in young (Y_6) versus old (M_6) rats by 6 weeks for both epididymal and perirenal fat pads; in contrast, strikingly similar reductions in the visceral fat depot were observed over the period of 6 weeks of caloric restriction for both young and old rats (Y_6: ↓44 ± 3% vs. M_6: ↓47 ± 4, $P = 0.537$; Fig. 1).

Basal plasma chemistry. Plasma glucose was unaltered by either HFD, age, or caloric restriction (Table 2). In contrast, 3 weeks of HFD in young rats induced significant basal hyperinsulinemia (↑71 ± 20% from control, $P = 0.012$; Table 2). To a greater extent, age also provoked basal hyperinsulinemia (M_0: 2.5-fold vs. Y_0, $P < 0.001$) as observed previously (8). Surprisingly, 6 weeks of caloric restriction was not sufficient to correct hyperinsulinemia in either group (young vs. time: $P = 0.120$; old vs. time: $P = 0.247$), although Y_6 insulin was no longer different from control ($P = 0.072$; Table 2).

To examine indirectly the effect of caloric restriction on the adipocyte, basal plasma FFA and glycerol were measured. No significant trends were observed for plasma glycerol due to either diet or age (Table 2). However, FFAs were noticeably elevated in both young and old obese models (Y_0 vs. control: ↑43 ± 7%, $P = 0.036$; M_0 vs. control: ↑59 ± 8%, $P = 0.008$). In contrast to insulin, this elevation was completely attenuated by 6 weeks of caloric restriction in young and old rats (Y_6 vs. control: $P = 0.202$; M_6 vs. control: $P = 0.509$; Table 2).

Insulin sensitivity. Glucose in all animals was successfully clamped to fasting levels (basal vs. SS: $P \geq 0.10$; data not shown). When exposed to hyperinsulinemia, control animals required a steady-state glucose infusion rate (G_{INF}) of 24.26 ± 1.15 mg/min/kg to maintain euglycemia (Fig. 2*A*). Both HFD and age caused a significant and similar decrease in G_{INF} (30–40%; SS_{Ginf}; Y_0 vs. M_0, $P = 0.121$). After only 2 weeks of caloric restriction, G_{INF} increased by 43% in young rats (Y_0: 14.62 ± 0.56 vs. Y_2: 20.88 ± 0.55 mg/min/kg; $P < 0.001$) and continued to improve after 4 weeks reaching levels comparable with control (Y_4: 24.34 ± 1.26 mg/min/kg vs. control; $P = 0.961$; Fig. 2*B* and *C*). In stark contrast, 2-week G_{INF} for old rats

TABLE 2
Basal and steady-state plasma characteristics for all animals used in study

	Glucose (mM)	Insulin (pM)	Basal FFA (mM)	SS FFA (mM)	Basal glycerol (mM)	SS glycerol (mM)
Control	6.15 ± 0.13	137 ± 17	0.30 ± 0.05	0.02 ± 0.01	0.14 ± 0.02	0.11 ± 0.01
Young						
0 weeks (n = 8)	6.44 ± 0.11	234 ± 27*	0.42 ± 0.02*	0.14 ± 0.03*	0.17 ± 0.01	0.17 ± 0.02*
2 weeks (n = 8)	6.08 ± 0.19	143 ± 27	0.41 ± 0.02	0.07 ± 0.01*	0.16 ± 0.01	0.12 ± 0.01
4 weeks (n = 8)	5.95 ± 0.28	196 ± 27	0.38 ± 0.04	0.05 ± 0.01*	0.15 ± 0.01	0.14 ± 0.01*
6 weeks (n = 10)	6.27 ± 0.16	190 ± 21	0.37 ± 0.02	0.06 ± 0.01*	0.11 ± 0.01	0.07 ± 0.00*
Old						
0 weeks (n = 9)	6.10 ± 0.13	349 ± 25*	0.47 ± 0.02*	0.14 ± 0.02*	0.14 ± 0.01§	0.16 ± 0.01*
2 weeks (n = 8)	6.01 ± 0.14	384 ± 53*§	0.43 ± 0.03*	0.17 ± 0.02*§	0.14 ± 0.01	0.17 ± 0.01*§
4 weeks (n = 10)	6.77 ± 0.13*§	421 ± 58*§	0.36 ± 0.02	0.09 ± 0.01*	0.15 ± 0.01	0.14 ± 0.01*
6 weeks (n = 8)	6.47 ± 0.09	298 ± 11*	0.33 ± 0.03	0.06 ± 0.01*	0.13 ± 0.01	0.13 ± 0.01*§

Data are means ± SEMs. Fasting and steady-state (SS) plasma glucose, insulin, FFAs, and glycerol in control rats as well as young fat-fed and old rats exposed to 0, 2, 4, and 6 weeks of caloric restriction. *Significant difference between control and a single experimental group as determined by Student t test, where $P < 0.05$. §Value significantly differs from young rats at the same time exposure to caloric restriction using two-way ANOVA with post hoc tests where $P < 0.05$.

was unchanged (M_0: 17.12 ± 1.37 vs. M_2: 17.00 ± 1.07 mg/min/kg; P = 0.945), and only after 6 weeks of caloric restriction was significant improvement observed in old rats where G_{INF} was not different from control (M_6: 21.00 ± 1.28 mg/min/kg vs. control; P = 0.08).

As suggested by G_{INF}, we observed ~50% reduction in whole body insulin sensitivity (S_I) in obese young and old groups compared with control rats (Fig. 3*A*). However, we did not find any notable resistance in the periphery in either group; obese rats had similar $S_{I\ Rd}$ compared with control rats (Y_0 vs. control: P = 0.105; M_0 vs. control: P = 0.196; Fig. 3*B*). Rather, both groups appeared to manifest liver resistance solely as evidenced by 67 and 105% reductions in $S_{I\ EGP}$ in Y_0 and M_0 rats, respectively, versus control (Fig. 3*C*).

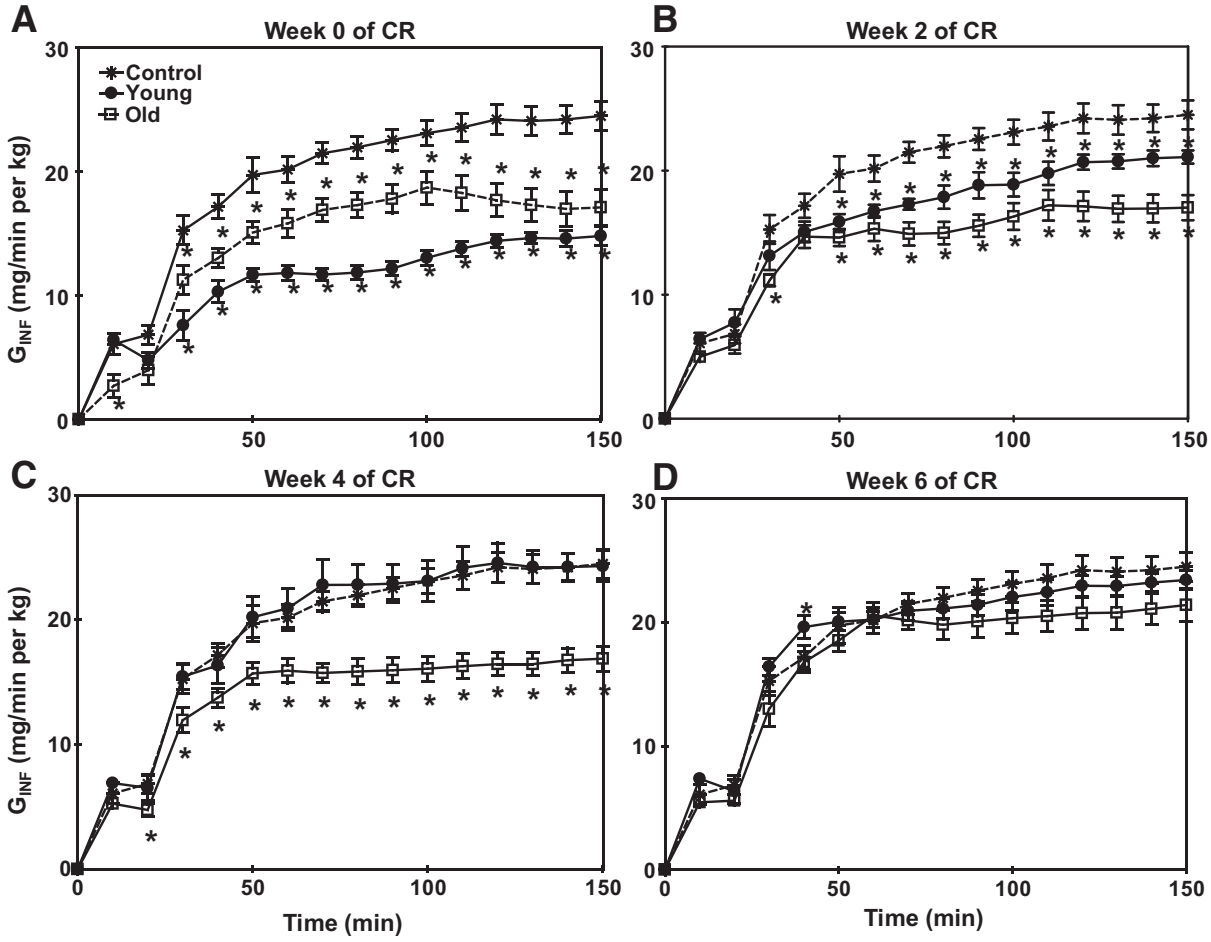

FIG. 2. Glucose infusion rates necessary to maintain euglycemia. Glucose infusion rates in mg/min/kg of body wt for young and old rats exposed to 0 (*A*), 2 (*B*), 4 (*C*), or 6 weeks (*D*) of caloric restriction. Infusion rates for control rats fed ad libitum are recapitulated in each panel for comparison. Data are represented as means ± SEMs. Statistical significance was determined using ANOVAs for the effect of group with post hoc paired Student t tests. *$P < 0.05$ vs. control.

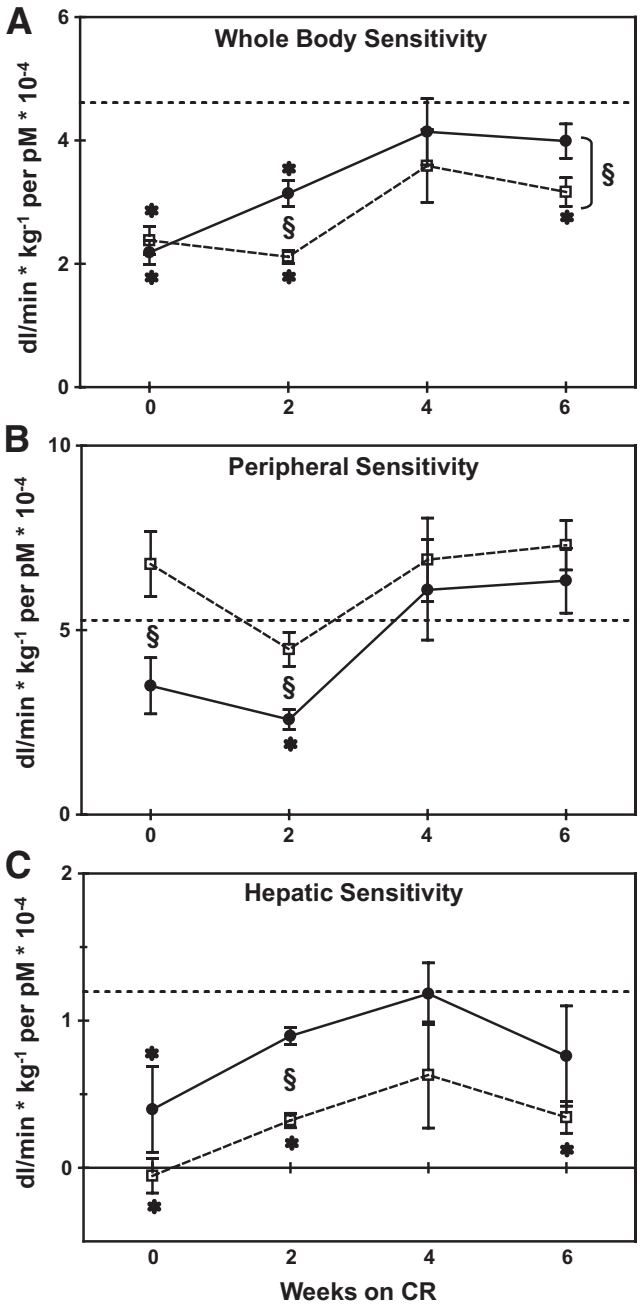

FIG. 3. Effect of caloric restriction on insulin sensitivity. Whole body (*A*), peripheral (*B*), and hepatic insulin sensitivity (*C*) in dl/min/kg body wt (lean body mass for peripheral) per picomolar of insulin × 10^{-4} as measured by tracer dilution technique during hyperinsulinemic-euglycemic clamps in young (○) and old (□) rats exposed to 0, 2, 4, or 6 weeks of caloric restriction. Dashed lines in each panel represent the values from control rats for each parameter for comparison. Data are means ± SEMs. Statistical significance was determined using two-way ANOVAs for the effect of time and age with post hoc Tukey tests for individual comparisons. *$P < 0.05$ for young vs. week 0 and old vs. week 0; §$P < 0.05$ young vs. old.

As expected, caloric restriction improved S_I for both young and old rats (young vs. time: $P = 0.001$; old vs. time: $P = 0.032$; Fig. 3*A*). However young animals responded more rapidly to fat loss than did old; young rats exhibited significant improvement in S_I by 4 weeks of caloric restriction (↑47% from Y_0; $P < 0.05$) versus old rats responding only after 6 weeks (↑25% from M_0; $P < 0.05$). Although $S_{I\ Rd}$ was not significantly impaired in either young or old obese animals, there was a significant effect of time on $S_{I\ Rd}$ ($S_{I\ Rd}$ vs. time: $P = 0.002$) as well as age ($S_{I\ Rd}$ vs. age: $P = 0.008$) with caloric restriction (Fig. 3*B*). Similarly, $S_{I\ EGP}$ was also influenced by both time ($S_{I\ EGP}$ vs. time: $P = 0.034$) and age ($S_{I\ Rd}$ vs. age: $P = 0.005$; Fig. 3*C*). Most notably, the changes in $S_{I\ EGP}$ in young rats during caloric restriction exactly mirrored those changes in $S_{I\ EGP}$ in old rats (young vs. old: $P \geq 0.128$).

Although this study was not designed to measure lipid turnover as low-dose heparin was used to maintain catheter patency, we did observe that whereas control animals exhibited ~90% suppression during clamps, FFAs in obese animals were suppressed by only ~70%, suggestive of adipocyte resistance (Table 2; $P \leq 0.004$). Diet restriction appeared to recover insulin's ability to suppress FFAs in both obese models, although this recovery, like $S_{I\ EGP}$, was somewhat slower in old animals. Similar results were obtained for glycerol.

Dependency of S_I on adiposity. To determine the dependency of S_I on abdominal adiposity, we performed multiple linear regression analysis to correlate changes in abdominal fat pads versus whole-body insulin resistance ($1/S_I$; Fig. 4). Abdominal fat correlated positively with insulin resistance for young and old rats ($P < 0.001$). The impact of fat depots classically called visceral fat, epididymal and perirenal fat was not different for young and old rats (effect of age epididymal fat: $P = 0.227$, perirenal fat: $P = 0.100$). In addition, there was no age × fat interaction for either depot (epididymal fat: $P = 0.499$, perirenal fat: 0.139). In striking contrast, true visceral fat loss, as measured by the mesenteric depot, exerted a much stronger effect on insulin sensitivity in young versus old rats (slope: young: 0.053, old: 0.17 kg/min/mg per % fat; effect of age: $P = 0.001$; age × fat interaction: $P < 0.001$). Moreover, mesenteric fat appeared to play a far greater role in determining S_I than did other depots in young rats as evidenced by more than a doubling of slope, whereas this increased dependence was not observed with old rats (slope = 0.17). These data suggest not only a stronger effect of true (i.e., mesenteric) visceral fat in insulin resistance versus other abdominal depots, but also a sharply reduced interaction in older animals.

Circulating adipokines and hepatic lipid accumulation. To determine potential mechanisms of this differential relationship, we measured several circulating adipokines: adiponectin, resistin, and leptin (Fig. 5*A*–*C*). Changes in neither adiponectin nor resistin appeared to be responsible for the differential response to caloric restriction in old rats. As might be expected, leptin levels followed the changes in absolute adiposity observed in both obese groups, and the persistently high levels after 6 weeks of caloric restriction in old rats are likely due to an appreciable degree of remaining adiposity in these animals.

Because the primary defect in insulin sensitivity resided in the liver in both obese models, we measured hepatic lipid content (Fig. 5*D*). Liver triglyceride reached similar levels in both young and old rats (young: 608 ± 140 vs. old: 653 ± 95 mg/mg tissue, $P > 0.05$). Astonishingly, reduction of liver triglyceride with caloric restriction completely mirrored the delayed recovery in hepatic insulin sensitivity in old rats, suggesting a defect in lipid turnover in livers of old animals.

DISCUSSION

Recently, our laboratory presented data demonstrating an increased susceptibility of old rodents to obesity-related

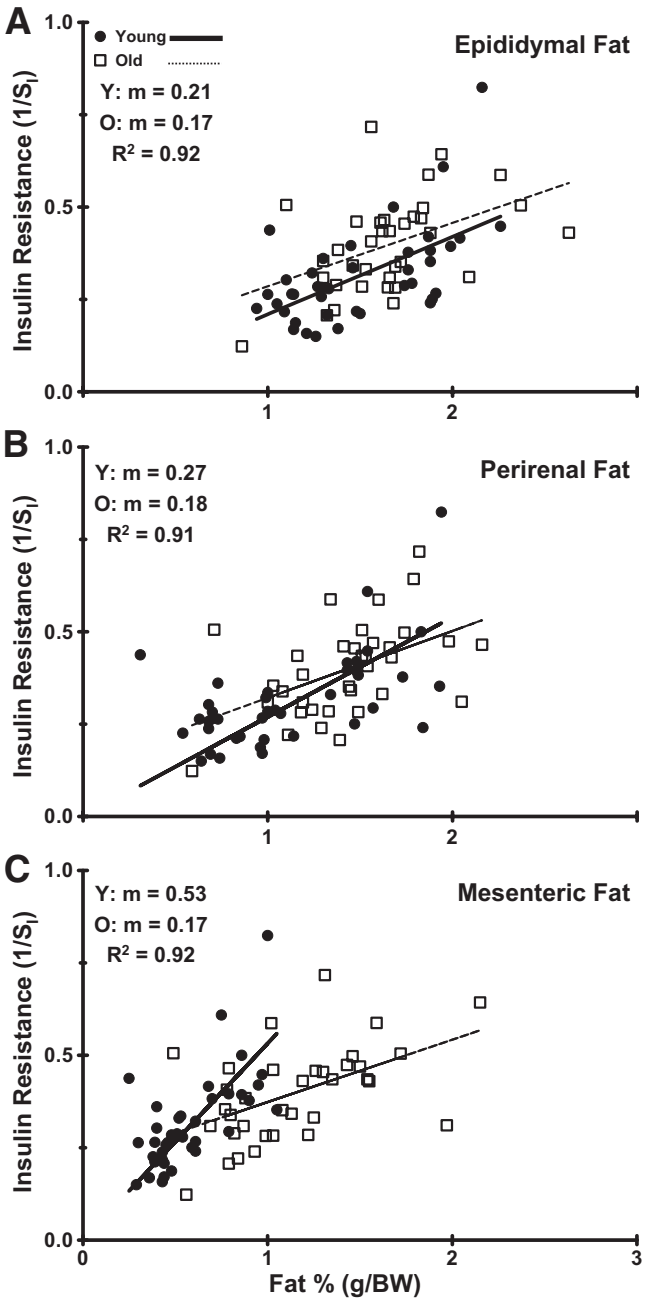

FIG. 4. Dependency of insulin sensitivity on abdominal fat depot size. The correlation among epididymal (*A*), perirenal (*B*), and mesenteric fat (*C*) normalized to body weight vs. insulin resistance (1/whole-body S_I) in all young and old rats used in the study. Correlations are determined for all young and all old rats using the general linear model. A significant difference between young and old slopes was only found for the mesenteric fat depot, and thus visceral fat. Y, young; O, old; m, slope.

insulin resistance (8). However, we did not address the importance of mesenteric fat, true visceral fat in the rodent, in the manifestation of insulin resistance. Here, we test the susceptibility hypothesis in more depth by examining the response to short-term weight loss in young fat-fed versus old rats. Six weeks of restriction reduced body weight and abdominal adiposity in both young and old groups. Remarkably, reductions in mesenteric fat in old rats exactly mirrored those in young rats, whereas this similarity did not exist for other abdominal fat depots. Whole-body insulin sensitivity was markedly improved in both groups, which significantly coincided with a preferential loss of abdominal fat in both young and old rats. However, improvement in young rats exhibited a more than twofold greater dependence on the mesenteric fat depot versus other abdominal depots. Furthermore, this association was altered by age such that a similar degree of mesenteric fat loss led to much lesser amelioration of insulin resistance in older rats, and slower recovery of insulin sensitivity in older rats was accompanied by a remarkably subdued reduction of hepatic triglyceride. These data support the hypothesis that old animals are at higher risk for development of insulin resistance in the face of visceral obesity, which may be coupled with a defect in hepatic lipid turnover. The corollary is that adiposity, per se, cannot completely account for the insulin resistance of the aging rat.

Short-term caloric restriction has been shown to decrease both weight and adiposity in other models (16,17). Interestingly, our data suggest a preferential loss in abdominal depots (30–45%) rather than subcutaneous fat in both young and old rats with 6 weeks of restriction. Studies of lifetime caloric restriction have been less apt to clarify this issue because these animals typically exhibit marked reduction in all fat depots. Studies looking at specific adipose depots have resorted to surgical removal, such as that reported by Barzilai and colleagues (7,18,19). These latter studies have examined the effects of gonadal and perirenal fat removal on S_I, depots that do not fit the specific criteria of visceral fat, namely circulatory drainage into the portal vein. To our knowledge, our present study is the first to examine reductions in true visceral (i.e., mesenteric) fat as they relate to recovery of S_I.

Other studies examining fat distribution during weight loss have demonstrated a similar preferential diminution of the visceral depot compared with other adipose depots (2). Data from our laboratory in dogs have suggested that the visceral depot may serve as a favored site of fat accumulation in the early stages of diet-induced obesity with subsequent fat gain appearing in the periphery once the visceral depot's capacity is exhausted (21) (with further support from [5,22]). Greater loss of visceral versus subcutaneous depots, as observed in the current study, might be expected because of the inherently increased lipolytic activity and insulin resistance of visceral fat (23–26). And although this characteristic of visceral fat has been used to suggest its importance in the etiology of insulin resistance, it can equally be exploited to help explain a potential preferential loss of the tissue in times of energy deficit.

In contrast to our previous observations, here we find that resistance in young and old obese rats exists primarily at the liver, rather than the periphery (8). It has been demonstrated that once R_d and therefore $S_{I\ Rd}$ are expressed per LBM, the apparent resistance observed in many studies, especially those examining animals of various sizes (i.e., different ages), disappears (27,28). Although in our previous study LBM was only estimated, here we use a more precise measure of LBM using a stable isotope. Furthermore, that fat-fed animals did not exhibit any substantial peripheral resistance is likely explained by the fact that these animals were on HFD for less time (3 vs. 4 weeks).

Despite these differences, we found a similar degree of whole-body insulin resistance as observed previously in both fat-fed young animals and ad libitum–fed old rats (~50%). More importantly, in concert with fat loss, insulin

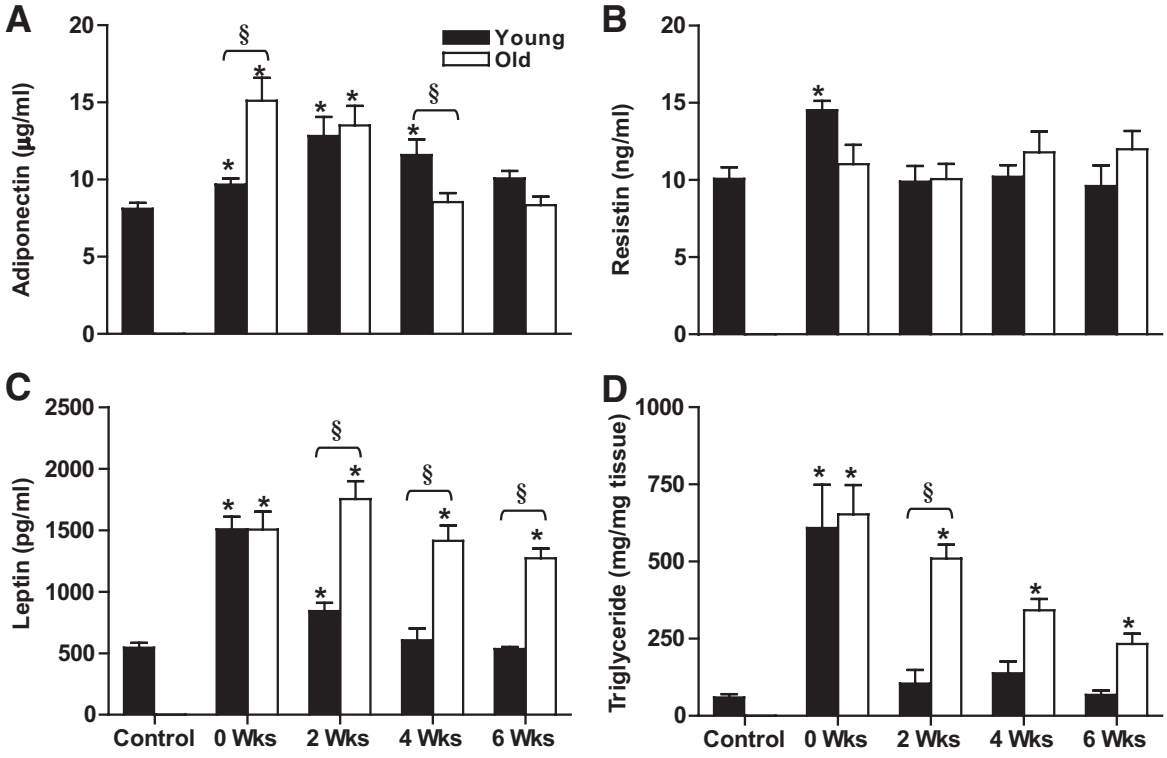

FIG. 5. Circulating adipokine and liver triglyceride content. Plasma levels of adiponectin (*A*), resistin (*B*), leptin (*C*), and lipid content (*D*) in liver in young and old rats exposed to 0, 2, 4, or 6 weeks of caloric restriction. Data are means ± SEMs. Statistical significance was determined using two-way ANOVAs for the effect of time and age with post hoc Tukey tests for individual comparisons. *$P < 0.05$ for young vs. week 0 and old vs. week 0; §$P < 0.05$ young vs. old.

action improved dramatically in both groups when exposed to caloric restriction, albeit refractory in old animals. Our data also demonstrate a highly significant dependence of S_I on abdominal fat of all types in both ages. However reductions in true visceral fat appear to have a much stronger influence on attenuating resistance (Fig. 4) at least in young rats. Thörne et al. achieve similar results with surgical removal of visceral fat in obese patients undergoing gastric banding surgery (29), a result also observed in the nonobese canine model (30). However, studies of the removal of subcutaneous fat in humans have been less clear (31,32). Thus, our data suggest that visceral fat "removal" reverses insulin resistance whether induced by diet or age, further suggesting its causative role in the impairment of insulin action with obesity.

Our data demonstrate a blunted association between visceral fat loss and the recovery of insulin sensitivity in old rats, and although this study was not designed to look at the potential mechanisms for this disparity, several explanations might exist. Although it is possible that there is an adipose-independent effect at work in old rats, regression analysis revealed a significant interaction between age and visceral adiposity for S_I (Fig. 4*C*, $P < 0.001$) suggesting that "old visceral fat" is different from "young visceral fat." Although it is possible that inflammatory cytokines, which are elevated with age (33,34), could explain this difference, the refractory recovery of S_I in old animals could not be explained by altered regulation of either adiponectin or resistin. Because the primary site of resistance in this study was the liver, we must ask what role the "portal hypothesis" might play in the differences observed between young and old rats. Old animals appeared to have greater resistance to FFA suppression during clamp conditions versus young animals, especially at 2 weeks. However, because this study was not designed to study lipid turnover directly, it is impossible to discern the tissue source of these circulating FFAs.

It has been suggested that HFD, such as that used in this study, induces insulin resistance not through fat depot accretion, but rather ectopic fat storage (e.g., liver and/or muscle) (35,36). Here we demonstrate that young and old obese animals did in fact have similar degrees of fat accumulation in the liver. Most remarkable was the finding that liver triglyceride in old animals, like liver insulin resistance, reduced more slowly with caloric restriction versus young fat-fed rats. Our data suggest that old rats do not mobilize liver triglyceride stores as readily as young, contributing to their persistent insulin resistance. To test this, further studies examining hepatic lipid turnover will be required.

Although caloric restriction reduces adipose mass, it also has profound effects on eating patterns and insulin-independent energy balance (37). Furthermore, although not measured in this study, it cannot be discounted that there are inherent differences in activity levels between young and old animals, and the effect that caloric restriction has on activity in each of these age groups may not be equivalent. Further investigation would be necessary to clarify these potential differences.

In conclusion, our data demonstrate that short-term diet restriction is an effective way to reverse insulin resistance in two obese rat models and this reversal is highly correlated with abdominal fat and hepatic triglyceride loss. In particular, we have shown that a commonly neglected fat depot representing true visceral fat in the rodent, the mesenteric fat pad, has a more than twofold higher

influence on the improvements in S_I observed with restriction. Yet, old animals appear to have a refractory response to visceral fat loss, suggesting an altered association between obesity and resistance in old versus young animals. Although the potential mechanism(s) of this altered association in young and old animals is not known, our data suggest that differences in lipid handling by the liver may be a candidate. Moreover, further investigation into the potential role of differences in local delivery of adipokines from mesenteric fat will be required.

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