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 versus young. However, it is unclear to what extent the portally-drained mesenteric fat depot influences this susceptibility.

**Research Design and Methods:** Young (Y) high-fat-fed and old (O) obese rats were subjected to 0, 2, 4, or 6 weeks of caloric restriction (CR). Insulin sensitivity (SI) was assessed by hyperinsulinemic clamp and lean body mass (LBM) and total body fat by $^{18}$O-water administration.

**Results:** Six weeks of CR caused a similar reduction in body weight in Y and O animals (p=0.748) which 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 Y and O rats after 6 weeks of CR (~↓53%; p=0.537). Despite similar visceral fat loss, SI improved less in O rats (↑32.76±9.80% compared to Y (↑82.91±12.66%) versus week 0. Also, there was significantly more reversal of fat accumulation in the liver in Y (% Reduction in Y: 89±2 vs. O: 64±5; p<0.0001). Furthermore, in Y, SI 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 SI during CR correlated with a preferential abdominal fat loss. This improvement was refractory in older animals, likely due to slower liberation of hepatic lipid. Furthermore, mesenteric fat was a better predictor of SI than other abdominal depots in young rats, but not old. These results suggest a singular role for mesenteric fat to determine insulin resistance. This role might be related to delivery of lipid to liver, and associated accumulation of liver fat.
Increased abdominal adipose mass has been of particular interest in elucidating the mechanisms of insulin resistance. Visceral fat has been implicated due to its distinct anatomical characteristics, since it has a circulation draining into the portal vein and hence the liver. Compared to subcutaneous, visceral adipocytes have a higher secretion rate of some adipokines and metabolites linked to insulin resistance, including free fatty acids (FFA). 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 man. Rodents present 3 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 non-rodent species. It is therefore unclear what role the mesenteric fat depot 

**RESEARCH DESIGN AND METHODS**

**Animals:** 2 groups of male Fischer Brown Norway rats (F344XBN F1; National Institute on Aging, Bethesda, MD) were used in this study: [1] young rats (Y: 5-6 months; n=34) fed a high fat diet (HFD) for 3 weeks and [2] old rats (M: 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 (CR). The HFD obtained from Harlan Teklad (Madison, WI) consisted of 5.0 kcal/g, 66.5% of which were from fat (lard), 21% from protein, and 12.5% carbohydrate. Ad libitum-chow-fed rats were given a standard diet provided by NIH (Diet NIH-31; 4.02 kcal/g). Once subjected to CR, animals were fed 60% of their typical chow ad libitum calorie per day using a NIH-31 fortified diet (3.95 kcal/g) based upon feeding instructions provided by the 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 (C). 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 (12:12 light: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 hours), any remaining food was removed from the cage and catheters were placed. Two tail vein catheters for infusion and 1 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 U/ml heparin (1.0 ml/hr). Each animal underwent a euglycemic hyperinsulinemic clamp at ~1200 hours (6 hour fast).

**Hyperinsulinemic Euglycemic Clamp:** D-[3-3H]-glucose (0.2 µCi/min; “tracer”) began at t = -240 min. Four samples to measure basal (B) glucose turnover were taken at t = -60, -45, -30, and -15 minutes. At t=0, infusion of insulin (5 mU/min per kg) was started. Samples were taken at 10-minute 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 minutes of the clamp (t = 120-150 min).

**Body Composition:** A bolus of water labeled with the stable isotope 18O 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 18O-water bolus at t = -120 min (0.5g/kg BW). Animals equilibrated for 2 hours and a sample was taken at t = -1 min to assess steady state 18O 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 euthanasia.

**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 FFA and glycerol were collected in tubes containing diethyl p-nitrophenyl phosphate (Paraoxan) to inhibit lipoprotein lipase within samples (9), centrifuged immediately, and plasma 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 Wako Chemicals (Neuss, Germany). Plasma nonesterified FFA were measured using the FFA Assay Kit from Wako Chemicals (Neuss, Germany), glycerol using Triglyceride Reagent from Sigma Diagnostics (St. Louis, MO) and triglyceride using a commercially available kit from Stanbio (Boerne, Texas). Triglyceride values were normalized per gram of liver.

To determine D-[3-3H]-glucose, plasma samples were deproteinized with BaOH2 and Zn2SO4 as described by Somogyi (11).

Samples for 18O water measurement were assayed by Metabolic Solutions in Nashua, New Hampshire using the Europa 20/20 Automated Breath Carbon Analyzer.
Isotope Ratio Mass Spectrometer with an intra-assay CV of 0.2%.

**Data Analysis:** Glucose turnover and insulin sensitivity (SI) were calculated as previously described (8), utilizing 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 (TBW) was determined as described previously (13-15) 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 TBW/0.72 (16) and total body fat as the difference between BW and LBM.

**Statistics:** All data are represented as means±SEMs. Within-group comparisons were made using Analysis of Variance (ANOVA) with Tukey’s multiple comparisons test for individual variances. Student’s T tests or paired T, when appropriate, were performed for individual comparisons. Multiple linear regression analysis was employed 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≤0.05.

**RESULTS**

**Food Intake:** Using recommendations from the NIA, young and old animals were fed a diet consisting of approximately 41 and 47 kcal/day, respectively during the CR 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:** While 3wks of HFD in Y rats did not cause a significant increase in BW, LBM, or SubQ fat (Y$_0$ vs. C: p=0.054, p=0.634, p=0.367, respectively; Table 1) it did promote abdominal fat accretion (Total AB Fat; ~90%), and the 3 depots comprising total AB fat (Epi Fat, Peri Fat, and V Fat) were markedly larger (64±7%, 111±10%, 121±9%, respectively).

As expected, BW was ~1.5-fold greater in M rats versus C and Y$_0$ rats (M$_0$ vs C, Y$_0$: p<0.001) and while LBM was unaltered (C: 219±24g vs. M$_0$: 199±6g, p=0.445), SubQ fat was markedly augmented with age (C: 132±26g vs. M$_0$: 307±12g; p<0.001; data not shown). However, %LBM was reduced in older animals (↓39%), while %SubQ fat was increased by 61%. Abdominal fat depots were significantly increased with age (Table 1).

Caloric restriction in Y and M rats caused a modest BW reduction, reaching significance by 4wks (Y: ↓6.8%, p<0.05; M: ↓11.4%, p<0.05) and arriving at its nadir by 6wks (Y: ↓12.8%, p<0.05; M: ↓13.7%, p<0.05; Table 1, Figure 1). These changes could not be explained by reductions in either %LBM (Y: p=0.584; M: p=0.399) or %SubQ fat (Y: p=0.624; M: p=0.355 by ANOVA; Table 1). However, CR did effectively reduce abdominal fat by 4wks in both Y and M rats, with no further reductions at 6wks (Y: ~45%↓; M: ~30%↓; Figure 1). Interestingly, obesity was reversed slightly more in Y versus M rats by 6wks for both epididymal and perirenal fat pads; in contrast, strikingly similar reductions in the visceral fat depot were observed over the period of 6wks of CR for both Y and M rats (Y$_6$: ↓44±3% vs. M$_6$: ↓47±4, p=0.537; Figure 1).

**Basal Plasma Chemistry:** Plasma glucose was unaltered by either HFD, age, or CR (Table 2). In contrast, 3wks of HFD in Y rats induced significant basal hyperinsulinemia (↑71±20% from C, 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, 6wks of CR was not sufficient to correct hyperinsulinemia in either group (Y
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As expected, CR improved $S_I$ for both Y and M rats ($Y$ vs. Time: $p=0.001$; $M$ vs. Time: $p=0.032$; Figure 3a). However $Y$ animals responded more rapidly to fat loss than did $M$; $Y$ rats exhibited significant improvement in $S_I$ by 4wks of CR ($\uparrow 47\%$ from $Y_0$; $p<0.05$) versus $M$ rats responding only after 6wks ($\uparrow 25\%$ from $M_0$; $p<0.05$). Despite the fact that $S_{IRd}$ was not significantly impaired in either $Y$ or $M$ obese animals, there was a significant effect of time on $S_{IRd}$ ($S_{IRd}$ vs. Time: $p=0.002$) as well as age ($S_{IRd}$ vs. Age: $p=0.008$) with CR (Figure 3b). Similarly, $S_{IEGP}$ was also influenced by both time ($S_{IEGP}$ vs. Time: $p=0.034$) and age ($S_{IRd}$ vs. Age: $p=0.005$; Figure 3c). Most notably, the changes in $S_{IEGP}$ in $Y$ rats during CR exactly mirrored those changes in $S_{IEGP}$ in $M$ rats ($Y$ vs. $M$: $p\geq 0.128$).

Although this study was not designed to measure lipid turnover as low-dose heparin was used to maintain catheter patency, we did observe that while control animals exhibited ~90% suppression during clamps, FFA 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 FFA in both obese models, although this recovery, like $S_{IEGP}$, 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 ($1/S_I$; Figure 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, was not different for young and old rats (effect of age Epi Fat: $p=0.227$, Peri Fat: 0.100). Additionally, there

As suggested by $G_{INF}$, we observed ~50% reduction in whole body insulin sensitivity ($S_I$) in obese $Y$ and $M$ groups compared to $C$ rats (Figure 3a). However, we did not find any notable resistance in the periphery in either group; obese rats had similar $S_{IRd}$ when compared to $C$ rats ($Y_0$ vs $C$: $p=0.105$; $M_0$ vs $C$: $p=0.196$; Figure 3b). Rather, both groups appeared to manifest liver resistance solely as evidenced by 67% and 105% reductions in $S_{IEGP}$ in $Y_0$ and $M_0$ rats respectively versus control (Figure 3c).

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was no age \times fat interaction for either depot (Epi Fat: p=0.499, Peri 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 Y: 0.053, M: 0.17 kg•min/mg per % fat; effect of age: p=0.001; age \times 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 evidence 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 (Figure 5a-c). Changes in neither adiponectin nor resistin appeared to be responsible for the differential response to CR in M rats. As might be expected, leptin levels followed the changes in absolute adiposity observed in both obese groups, and the persistently high levels after 6wks of CR in old rats are likely due to an appreciable degree of remaining adiposity in these animals.

Since the primary defect in insulin sensitivity resided in the liver in both obese models, we measured hepatic lipid content (Figure 5d). Liver triglyceride reached similar levels in both young and old rats (Y: 608±140 vs. M: 653±95 mg/mg tissue, p>0.05). Astonishingly, reduction of liver triglyceride with CR 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 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, 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 2-fold 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 to a defect in hepatic lipid turnover. The corollary is that adiposity, per se, can not completely account for the insulin resistance of the aging rat.

Short-term CR has been shown to decrease both weight and adiposity in other models (17;18). Interestingly, our data suggests a preferential loss in abdominal depots (30-45%) rather than subcutaneous in both young and old rats with 6wks of restriction. Studies of lifetime CR have been less apt to clarify this issue since 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;19;20). These latter studies have examined the effects of gonadal and perirenal fat removal on $S_I$ depots which 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 to other adipose (21). Data from our laboratory in dogs has 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 [(22); with further support from (5;23)]. Greater loss of visceral versus subcutaneous depots, as observed in the current study, might be expected due to the inherently increased lipolytic activity and insulin resistance of visceral fat (24-27). And while 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 $S_I R_d$, are expressed per LBM, the apparent resistance observed in many studies, especially those examining animals of varying sizes (i.e. different ages), disappears (28;29). While 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. 4wks).

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 action improved dramatically in both groups when exposed to CR, 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 (see Figure 4) at least in young rats. Thorne and colleagues achieve similar results with surgical removal of visceral fat in obese patients undergoing gastric banding surgery (30), a result also observed in the non-obese canine model (31). Meanwhile studies of the removal of subcutaneous fat in humans have been less clear (32;33). 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. While 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 $S_I$ (Figure 4c, $p<0.001$) suggesting that “old visceral fat” is different from “young visceral fat”. While it’s possible that inflammatory cytokines, which are elevated with age (34;35), 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. Since 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, especially at 2wks. However, because this study was not designed to study lipid turnover directly, it is impossible to discern the tissue source of these circulating FFA.

It has been suggested that HFD, like that used in this study, induces insulin resistance not through fat depot accretion, but rather ectopic fat storage (e.g. liver and/or muscle) (36;37). 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 CR 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.

While CR reduces adipose mass, it also has profound effects on eating patterns and insulin-independent energy balance (38). 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 CR 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 2-fold higher influence on the improvements in S1 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. While 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.

ACKNOWLEDGMENTS
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REFERENCES


**FIGURE LEGENDS**

**Figure 1.** Effect of CR on body composition. A) Total abdominal fat, B) Epididymal fat, C) Perirenal Fat, and D) Mesenteric fat pad weights over 6 weeks of CR in Young (○) and Old (□) rats in grams represented as a percent of Week 0. Data are means ± SEMs. Significant differences determined using two-way ANOVAs for effect of time and age, with post-hoc Tukey’s tests for individual comparisons. * p<0.05 for Y vs. Wk and O vs. Wk 0; § p<0.05 Y vs. O.

**Figure 2.** Glucose infusion rates necessary to maintain euglycemia. Glucose infusion rates in mg/min per kg of body weight for Young (○) and Old (□) rats exposed to A) 0, B) 2, C) 4, or D) 6 weeks of CR. Infusion rates for Control rats (*) fed an 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’s t tests. * p<0.05 vs. control.

**Figure 3.** Effect of CR on insulin sensitivity. A) Whole body, B) Peripheral, and C) Hepatic insulin sensitivity in dl/min per kg body weight (lean body mass for Peripheral) per pM insulin x 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 CR. 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’s tests for individual comparisons. * p<0.05 for Y vs. Wk 0 and O vs. Wk 0; § p<0.05 Y vs. O.

**Figure 4.** Dependency of insulin sensitivity on abdominal fat depot size. The correlation between A) Epididymal, B) Perirenal, and C) Mesenteric fat normalized to body weight versus Insulin Resistance (1/whole body S_i) in all Young (○) and Old (□) rats used in the study. Correlations are determined for all Young (solid lines) and all Old (dashed lines) 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.

**Figure 5.** Circulating adipokine and liver triglyceride content. Plasma levels of A) Adiponectin, B) Resistin, and C) Leptin in Young (solid bars) and Old (open bars) rats exposed to 0, 2, 4, or 6 weeks of CR. Data are means ± SEMs. Statistical significance was determined using two-way ANOVAs for the effect of time and age with post hoc Tukey’s tests for individual comparisons. * p<0.05 for Y vs. Wk 0 and O vs. Wk 0; § p<0.05 Y vs. O.
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<td>496 ± 13 *§</td>
<td>43 ± 4 *</td>
<td>52 ± 4 *</td>
<td>8.81 ± 0.67 *</td>
<td>7.99 ± 0.57 *§</td>
<td>6.59 ± 0.34 *§</td>
<td>23.39 ± 1.19 *§</td>
</tr>
<tr>
<td>4 wks (n=10)</td>
<td>472 ± 14 *§</td>
<td>41 ± 2 *</td>
<td>56 ± 2 *</td>
<td>7.12 ± 0.67 *§</td>
<td>6.44 ± 0.76 *§</td>
<td>4.02 ± 0.31 *§</td>
<td>17.58 ± 1.54 *§</td>
</tr>
<tr>
<td>6 wks (n=8)</td>
<td>460 ± 11 *§</td>
<td>39 ± 0 *</td>
<td>57 ± 0 *</td>
<td>7.05 ± 0.48 *§</td>
<td>5.47 ± 0.47 §</td>
<td>3.99 ± 0.34 *§</td>
<td>16.51 ± 1.24 *§</td>
</tr>
</tbody>
</table>

Data are means ± SEMs. BW (g), lean body mass (LBM, % BW), subcutaneous fat (SubQ Fat, % BW), epididymal fat (Epi Fat, g), perirenal fat (Peri Fat, g), mesenteric/visceral fat (V Fat, g) and the sum of the three abdominal depots (Total AB Fat, g) in control rats as well as young fat fed and old rats exposed to 0, 2, 4, and 6 weeks of caloric restriction. * indicates a significant difference between control vs. a single experimental group as determined by Student’s T Tests, where p < 0.05. § indicates value significantly differs from young rats at the same time exposure to caloric restriction using 2-way ANOVA with post-hoc tests where p<0.05.
### Table 2. Basal and Steady State plasma characteristics for all animals used in study.

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

Data are means ± SEMs. Fasting and steady state (SS) plasma glucose (mM), insulin (pM), free fatty acids (FFA; mM), and glycerol (mM) in control rats as well as young fat fed and old rats exposed to 0, 2, 4, and 6 weeks of caloric restriction. * indicates a significant difference between control vs. a single experimental group as determined by Student’s T Tests, where p < 0.05. § indicates value significantly differs from young rats at the same time exposure to caloric restriction using 2-way ANOVA with post-hoc tests where p<0.05.
Figure 1

A. Total Abdominal Fat

B. Epididymal Fat

C. Perirenal Fat

D. Mesenteric Fat

Weeks on CR

Body Fat (% Week 0)
Figure 2

A. Week 0 of CR

B. Week 2 of CR

C. Week 4 of CR

D. Week 6 of CR

G_{inf} (mg/min per kg)

Time (min)
Figure 3

A. Whole Body Sensitivity

B. Peripheral Sensitivity

C. Hepatic Sensitivity

Weeks on CR

Whole Body Sensitivity

Peripheral Sensitivity

Hepatic Sensitivity

dl/min * kg⁻¹ per pM * 10⁻⁴

dl/min * kg⁻¹ per pM * 10⁻⁴

dl/min * kg⁻¹ per pM * 10⁻⁴
Figure 4

**Epididymal Fat**
- Y: m = 0.21
- O: m = 0.17
- $R^2 = 0.92$

**Perirenal Fat**
- Y: m = 0.27
- O: m = 0.18
- $R^2 = 0.91$

**Mesenteric Fat**
- Y: m = 0.53
- O: m = 0.17
- $R^2 = 0.92$
Figure 5

A

Adiponectin (µg/ml)

Young
Old

0 5 10 15 20

0 Wks 2 Wks 4 Wks 6 Wks

B

Resistin (ng/ml)

0 5 10 15

Control 0 Wks 2 Wks 4 Wks 6 Wks

C

Leptin (pg/ml)

0 500 1000 1500 2000

Control 0 Wks 2 Wks 4 Wks 6 Wks

D

Triglyceride (mg/mg tissue)

0 250 500 750

Control 0 Wks 2 Wks 4 Wks 6 Wks

* §