Middle-aged C57BL/6 mice have impaired responses to leptin that are not improved by calorie restriction

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Jacobson, Lauren. Middle-aged C57BL/6 mice have impaired responses to leptin that are not improved by calorie restriction. Am J Physiol Endocrinol Metab 282: E786–E793, 2002; 10.1152/ajpendo.00495.2001.—Midlife weight gain occurs in many species, suggesting that leptin signaling is impaired at middle age. To test this hypothesis, we measured changes in food intake and body composition in young (Y) and middle-aged (MA) C57BL/6 male mice infused subcutaneously with phosphate-buffered saline or leptin. Leptin-induced decreases in food intake and body fat were delayed in MA mice and associated with catabolism after longer treatment periods. Endogenous plasma leptin levels did not correlate with body fat in MA mice. Calorie restriction (CR) reduced body fat, plasma leptin, and insulin in MA mice to levels in Y mice but did not upregulate leptin sensitivity. CR mice did not respond to leptin doses that inhibited food intake in MA mice and reduced food intake and body fat in Y mice significantly below levels in CR mice. Plasma corticosterone was significantly higher in leptin-treated CR vs. MA mice. We conclude that MA C57BL/6 mice exhibit impaired leptin signaling and that CR, possibly by elevating glucocorticoids, impairs appetite control without improving the metabolic actions of leptin.

weight gain; food intake; glucocorticoids; insulin; longevity

WEIGHT GAIN AT MIDDLE AGE occurs in at least 50% of the population and has been linked to diabetes, cardiovascular disease, stroke, and reduced lifespan. Conversely, avoiding midlife weight gain, whether by virtue of genes, diet, or exercise, increases longevity (4, 10, 24). Understanding the etiology of this increase in body weight will aid in improving the length and quality of life.

Weight gain in adulthood is almost exclusively in the form of fat (4). Body fat levels are signaled by the adipocyte hormone leptin, which is normally produced in proportion to fat mass and acts on the brain to decrease appetite and increase metabolic rate (11). Greater adiposity in middle age could be caused by decreased leptin sensitivity, such that slower or smaller leptin-induced changes in food intake and metabolism fail to prevent further fat deposition. Indeed, leptin has been shown to inhibit food intake and stimulate energy expenditure less effectively in aged (24-

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METHODS

Animals

The Institutional Animal Care and Use Committee of Albany Medical College approved all animal procedures for these experiments. All mice were purchased through the Office of Biological Resources and Resource Development of the National Institute on Aging. Mice were housed individually in an American Association for Accreditation of Laboratory Animal Care-approved, viral antibody-free facility and allowed to acclimatize for 10–14 days before experiments were begun. Lights were on a 12:12-h cycle (on at 7 AM). As described for experiment 2 below, mice were fed either a standard or a vitamin-fortified pelleted diet (NIH-31), which has been characterized for aging research by Tururtro et al. (27). Diets were shipped with mice from National Institute of Aging contract facilities and were used throughout acclimatization and experiments. No mice died before the end of experiments. Gross inspection at death did not reveal overt pathology in middle-aged mice.

Experiment 1

Y (2 mo old) and MA (11–12 mo old) male C57BL/6 mice were implanted with subcutaneous osmotic minipumps (Du- rect, Cupertino, CA) under isoﬂurane anesthesia. Minipumps delivered either phosphate-buffered saline vehicle (PBS) or 0.15, 0.5, or 1.5 µg/h murine leptin (donated by Amgen, Thousand Oaks, CA). All mice were implanted with pumps on the same day. Body weight and ad libitum (ad lib) intake of standard NIH-31 diet were measured daily within 2 h of the same time each day throughout the experiment. Body weight was corrected for the weight of the minipump. Mice were killed without fasting in the morning after 3 or 10 days of infusion to assess early and sustained responses to leptin, respectively. Mice were bled for plasma hormones by retroorbital puncture and immediately killed by decapitation for tissue collection.

Experiment 2

Experiment 2 included an additional group of MA mice from the National Institute on Aging colony that had been calorie restricted (CR) from 16 wk of age to 60% of normal intake. This restriction paradigm has been shown to increase longevity and is used as a standardized procedure at the National Institute on Aging Aged Rodent Colonies to provide longevity and is used as a standardized procedure at the National Institute on Aging. Mice were housed in an American Association for Accreditation of Laboratory Animal Care-approved, viral antibody-free facility and allowed in an American Association for Accreditation of Laboratory Animal Care-approved, viral antibody-free facility and allowed to acclimatize for 10–14 days before experiments were begun. Lights were on a 12:12-h cycle (on at 7 AM). As described for experiment 2 below, mice were fed either a standard or a vitamin-fortified pelleted diet (NIH-31), which has been characterized for aging research by Tururtro et al. (27). Diets were shipped with mice from National Institute of Aging contract facilities and were used throughout acclimatization and experiments. No mice died before the end of experiments. Gross inspection at death did not reveal overt pathology in middle-aged mice.

Assays

Plasma glucose, corticosterone, leptin, and insulin were measured using commercial kits as previously described (26). Body composition was analyzed using previously described modifications of published methods (26).

Statistics

ANOVA and post hoc analyses were performed using Statview version 5.0 (SAS Institute, Cary, NC). Significance was defined as P < 0.05. Three-way ANOVA for age, treatment, and time (experiment 1) or two-way ANOVA for age and treatment (experiment 2) revealed significant effects of age or interaction of age and treatment for all variables examined except postinfusion leptin levels in experiment 1. Post hoc comparisons between each of three leptin infusions and PBS to assess leptin responsiveness were performed in Y and MA mice in experiment 1 by Dunnett’s test. Post hoc comparisons among Y, MA, and CR mice receiving either leptin or PBS in experiment 2 were performed by t-test with Bonferroni correction for three comparisons: 1) and 2) to the other age groups receiving the same treatment and 3) between leptin and PBS in the same age group. Data are presented throughout as means ± SE; the lack of error bars indicates that the scale of the symbol or graph exceeded that of the error. Regressions of plasma leptin against body fat levels (Fig. 7) for Y and MA mice were evaluated by analysis of covariance (Statview).

RESULTS

Experiment 1

Basal food intake and body weight. Before the experimental treatments, MA and Y mice differed significantly in absolute levels of body weight and food intake. MA mice weighed 31.09 ± 0.35 g (n = 39), whereas Y mice weighed 22.81 ± 0.4 g (n = 42; P < 0.05). Food intake in MA mice in this experiment was slightly but significantly lower than that in Y mice (Y, 3.88 ± 0.1 g/mouse; MA, 3.60 ± 0.08 g/mouse; P < .05); this difference was accentuated when food intake was normalized to body weight (not shown).

Leptin effects on food intake and body weight in Y and MA mice. After 3 or 10 days of subcutaneous infusion of 0.15, 0.5, or 1.5 µg/h murine leptin, plasma leptin levels were similar both between Y and MA mice and over time (Table 1). Y mice exhibited leptin-depen-

<table>
<thead>
<tr>
<th>Age</th>
<th>Infusion</th>
<th>PBS</th>
<th>3 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>PBS</td>
<td>4.0 ± 1.0</td>
<td>4.2 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 µg/h</td>
<td>4.6 ± 0.4</td>
<td>4.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 µg/h</td>
<td>10.9 ± 3.4</td>
<td>13.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 µg/h</td>
<td>31.9 ± 6.4</td>
<td>39.7 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>PBS</td>
<td>3.0 ± 0.3</td>
<td>3.7 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 µg/h</td>
<td>6.7 ± 2.4</td>
<td>7.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 µg/h</td>
<td>12.0 ± 1.5</td>
<td>14.4 ± 2.2</td>
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<tr>
<td></td>
<td>1.5 µg/h</td>
<td>42.4 ± 4.4</td>
<td>31.5 ± 4.9</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4–6/group.

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dent decreases in food intake within 1 day of treatment (Fig. 1, A1). However, MA mice showed a delayed response to leptin, with no change in food intake at any leptin dose by day 1 and reduced food intake evident only by day 3 at the two higher doses (Fig. 1, A2). As has been reported previously for prolonged leptin infusion (11, 12), daily food intake returned to initial levels in leptin-treated Y and MA mice by 10 days.

To determine whether slower feeding responses to leptin affected total calorie consumption in MA mice, we calculated cumulative food intake over 3 or 10 days (Fig. 1, B1). Each leptin dose significantly reduced cumulative food intake in Y but not MA mice by 3 days (Fig. 1, B1). By day 10, leptin-treated Y and MA mice showed similar decreases in total food intake (Fig. 1, B2).

As with food intake, Y mice exhibited rapid, significant weight loss at all leptin doses within 3 days (Fig. 2, A1 and B1). MA mice showed no change in body weight at 1 day and exhibited significant weight loss only at the two higher doses of leptin by 3 days (Fig. 2, A2 and B1). By 10 days, leptin-treated Y and MA mice exhibited significant weight loss at all leptin doses (Fig. 2, B2).

Leptin effects on body composition in Y and MA mice. We analyzed carcass fat and protein content to determine whether changes in total food intake and body weight affected body composition. PBS-treated MA mice had almost twice the body fat of the Y PBS group (Fig. 3, A and B). However, only Y mice exhibited dose-related decreases in carcass fat content by 3 days of leptin treatment; these decreases were significant at the two higher leptin doses (Fig. 3A). Although carcass fat content tended to be lower at 3 days in MA mice infused with 0.15 μg/h leptin vs. PBS, body fat did not decrease progressively with increasing leptin levels, and there were no significant effects of any leptin dose on body fat at this time. By 10 days of infusion, as with food intake and weight loss, both MA and Y mice showed similar significant, leptin-induced fat loss (Fig. 3B).

The higher body weight of MA mice correlated with significantly higher levels of body protein as well as body fat (Fig. 3, C and D). Body protein was not significantly affected by 3 days of leptin infusion in either age group (Fig. 3C), although it was significantly reduced in both age groups by 10 days of infusion of 1.5 μg/h leptin (Fig. 3D). However, MA mice also exhibited

Fig. 1. A: daily food intake, expressed as grams per mouse, in young (1) and middle-aged mice (2) infused subcutaneously with PBS (•) or 0.15 (○), 0.5 (□), or 1.5 (●) μg/h leptin in experiment 1. Mice were killed after either 3 or 10 days of infusion, indicated by the arrows. B: cumulative food intake (g/mouse) in Y (gray bars) and MA mice (black bars) after 3 (●) or 10 (□) days of infusion with either PBS or the indicated doses of murine leptin (in μg/h) in experiment 1. Differences in daily food intake mentioned in the text were significant by post hoc testing. However, for clarity of data presentation, significance symbols are shown only for cumulative data in B. Data through day 3 are pooled from mice in both the 3-day and 10-day infusion groups (n = 9–12 (days 1–3) and 4–6 mice per group (days 4–10)). *P < 0.05 vs. PBS-infused mice in the same age group.

Fig. 2. A: daily change in body weight in Y (1) and MA (2) mice from experiment 1, expressed in grams, relative to body weight at minipump implantation on day 0. B: net change in body weight after 3 (●) or 10 (□) days of infusion. Symbols and analysis are as in Fig. 1. *P < 0.05 vs. PBS-infused mice in the same age group.
significant loss of body protein after 10 days of infusion of 0.5 μg/h leptin, which did not significantly affect body protein in Y mice (Fig. 3D).

Experiment 2

Basal food intake, body weight, and hormone levels in Y, MA, and CR mice. We next tested whether calorie restriction, which minimizes weight gain and improves insulin sensitivity in aging (3), would improve leptin responsiveness in middle-aged mice. As in experiment 1, middle-aged mice fed ad libitum (MA) were heavier than Y mice (Table 2). Middle-aged mice that had been calorie-restricted (CR) according to National Institute on Aging protocols (27) weighed significantly less than MA but significantly more than Y mice (Table 2). In this experiment, initial daily food intake was slightly but significantly higher in MA mice than in either Y or CR mice (Table 2). However, normalized food intake in MA mice was still significantly lower than that in Y mice and did not differ from that in CR mice (Table 2).

Corroborating studies of age-related adiposity and hyperinsulinemia in other species (4, 10, 19), postprandial plasma leptin and insulin levels 3–4 h into the light (inactive) period were significantly higher in MA mice but were comparable between Y and CR mice (Table 2).

Leptin effects on body protein and food intake in Y, MA, and CR mice. We infused leptin at an intermediate dose and time (0.25 μg/h) in experiment 2 to increase plasma leptin measurably but minimize protein loss in MA mice. Data in Table 3 confirm that leptin infusion caused comparable, significant elevations in plasma leptin in all three age groups without affecting body protein (Table 3). This dose of leptin caused similar, significant decreases in 4-day food intake in MA as well as Y mice (Fig. 4B). In contrast,

### Table 2. Preinfusion values of body weight, food intake (expressed either as total grams per mouse or as grams of food per gram of body weight), plasma leptin, and plasma insulin in Y, MA, and CR mice from experiment 2

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Body Weight, g</th>
<th>24-h Food Intake, g/mouse⁻¹\·24 h⁻¹</th>
<th>Normalized Food Intake, g/g body wt⁻¹\·24 h⁻¹</th>
<th>Initial Plasma Leptin, ng/ml</th>
<th>Initial Plasma Insulin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>22.34 ± 0.29†‡</td>
<td>3.36 ± 0.10†‡</td>
<td>0.153 ± 0.005†‡</td>
<td>2.2 ± 0.2</td>
<td>0.6 ± 0.06</td>
</tr>
<tr>
<td>MA</td>
<td>32.27 ± 1.36†‡</td>
<td>3.81 ± 0.17†‡</td>
<td>0.115 ± 0.005†‡</td>
<td>7.9 ± 1.7†‡</td>
<td>1.1 ± 0.2†‡</td>
</tr>
<tr>
<td>CR</td>
<td>25.43 ± 0.44†‡</td>
<td>2.95 ± 0.02†‡</td>
<td>0.120 ± 0.002†‡</td>
<td>1.8 ± 0.1†‡</td>
<td>0.6 ± 0.06†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12–15/group. CR, middle-aged and calorie-restricted mice. †P < 0.05 vs. young mice; ‡P < 0.05 vs. MA mice.

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Leptin treatment did not reduce food intake in CR mice (Fig. 4B). Y mice actually ate significantly less, and MA mice ate marginally less, during leptin infusion (P = 0.095 after Bonferroni correction) than did leptin-treated CR mice (Fig. 4B). Leptin effects were similar whether absolute or normalized food intake was analyzed (not shown).

Experiment 2: leptin effects on body fat in Y, MA, and CR mice. Leptin infusion also had divergent effects on body fat. In the PBS control groups, carcass fat content was similar between Y and CR mice and significantly lower than that in MA mice (Fig. 4A). However, only Y mice exhibited significant fat loss after leptin treatment (Fig. 4). Carcass fat in Y mice was also significantly lower than that in MA and CR mice after leptin treatment (Fig. 4A).

Leptin effects on hormones and glucose in Y, MA, and CR mice. Young mice were also the only group to show lower levels of glucose and insulin in association with leptin-induced decreases in food intake and body fat (Fig. 5). Morning plasma glucose and insulin levels (Fig. 5) were marginally lower in leptin- vs. PBS-treated Y mice (P = 0.0504 and 0.098, respectively, after Bonferroni correction). Despite similar leptin-induced decreases in food intake in MA mice, changes in plasma glucose and insulin did not even approach significance in this group (P > 0.5 before correction). Morning plasma insulin in leptin-treated Y mice was also marginally lower than that in leptin-treated CR and MA mice (P = 0.07 after correction).

### Table 3. Levels of plasma leptin and carcass protein in Y, MA, and CR mice after 4 days of PBS or 0.25 μg/h leptin infusion in experiment 2

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Infusion</th>
<th>Plasma Leptin, ng/ml</th>
<th>Carcass Protein, g/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>PBS</td>
<td>3.0 ± 0.6</td>
<td>2.51 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>6.8 ± 0.8*</td>
<td>2.42 ± 0.14</td>
</tr>
<tr>
<td>MA</td>
<td>PBS</td>
<td>4.8 ± 1.2</td>
<td>4.53 ± 0.15†</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>9.8 ± 2.4*</td>
<td>4.51 ± 0.46†</td>
</tr>
<tr>
<td>CR</td>
<td>PBS</td>
<td>2.1 ± 0.1‡</td>
<td>3.15 ± 0.15‡</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>8.5 ± 0.7*</td>
<td>3.11 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6–8/group. *P < 0.05 vs. PBS-infused mice in the same age group; †P < 0.05 vs. Y mice in the same infusion group; ‡P < 0.05 vs. MA mice in the same infusion group.

Fig. 4. Carcass fat (A) and cumulative food intake (B) after 4 days of infusion with PBS (gray bars) or leptin (black bars) in Y, MA, or CR mice in experiment 2 (n = 6–8 per group). *P < 0.05 vs. PBS in same age group. †P < 0.05 vs. Y mice in the same infusion group. ‡P < 0.05 vs. MA mice in the same infusion group.

Fig. 5. Morning plasma glucose (A) and insulin (B) after 4 days of either PBS (gray bars) or 0.25 μg/h leptin infusion (black bars) in Y, MA, and CR mice from experiment 2 (n = 6–8 per group). Samples were obtained when mice were killed, within 2.5 h of lights-on without prior fasting. P values from post hoc testing after correction for multiple comparisons were >0.05; therefore, no significance symbols are shown.
Because glucocorticoids affect responsiveness to leptin (26), we also measured circadian nadir (morning; AM) and peak (early evening; PM) plasma corticosterone levels. At the times examined, leptin infusion did not alter plasma corticosterone in either Y or MA mice (Fig. 6). In PBS-treated mice, AM plasma corticosterone levels were significantly higher in CR vs. Y mice, whereas PM levels were significantly higher in CR than in either Y or MA mice (Fig. 6, A and B, respectively). Leptin-treated CR mice tended to have marginally lower PM corticosterone levels than their PBS-infused counterparts ($P = 0.054$ after correction). However, even after leptin treatment, PM corticosterone was still significantly higher in CR than in MA mice (Fig. 6B).

Experiments 1 and 2: relationship of endogenous plasma leptin to body fat in Y and MA mice

To determine whether the endogenous leptin signal might also be altered in middle age, we performed linear regression of plasma leptin against carcass fat by using values from each PBS-treated Y and MA mouse in experiments 1 and 2. There was a significant linear correlation between plasma leptin and body fat levels in Y mice (Fig. 7). However, plasma leptin tended to be lower and was not significantly correlated with body fat in middle-aged mice (Fig. 7).

DISCUSSION

In support of our first hypothesis and extending evidence for age-related leptin resistance (22, 25) to middle age, we have shown that leptin signaling is impaired during the period of midlife weight gain in male C57BL/6 mice (27). We found several deficits in MA mice, including slower and less selective metabolic responses to leptin, as well as a lack of correlation between body fat and endogenous plasma leptin levels. Contrary to our second hypothesis, we found that although calorie restriction reduces body fat, leptin, and insulin levels, it actually increases resistance to the appetite-suppressing effects of leptin and does not improve metabolic responses in middle-aged mice.

Slower Responses to Leptin in MA Mice

Results from experiment 1 indicated that, although leptin rapidly reduced food intake and body fat in Y mice, leptin had no consistent effect on these endpoints in MA mice for up to 3 days. Delayed leptin effects are unlikely to be due to slower equilibration of leptin levels in the larger MA mice, because we have found that these infusion rates significantly increase plasma leptin within 2 h in even bigger (>40 g) mice (not shown). Experiment 2 confirmed impaired fat mobilization in MA mice, even after comparable, leptin-induced decreases in food intake (Fig. 4). A variety of hypothalamic and adipocyte responses to leptin have been shown to be impaired in 24-mo-old rats (20, 22, 23, 25). However, because body weight declines after this age in rats (22), it was unclear whether leptin resistance was relevant to the midlife weight gain that decreases longevity (4, 7, 14, 24). The delayed and reduced leptin responsiveness we have found at an earlier life stage could account for weight gain at middle age by allowing calorie intake and storage to outpace compensatory responses to increasing fat mass.

Fig. 7. Relationship between plasma leptin and body fat in PBS-treated young (Y, ▲) and ad libitum-fed MA mice (○) in experiments 1 and 2. Linear regression of plasma leptin ($y$) against carcass fat ($x$) in each mouse revealed the following relationships: Y mice ($n = 17$): $y = 1.833x + 0.538$, $r^2 = 0.4246$, $P = 0.0046$; MA mice ($n = 15$): $y = 0.545x + 1.689$, $r^2 = 0.1381$, $P = 0.1727$. 
Less Selective Metabolic Responses to Leptin in MA Mice

Although leptin resistance of MA mice appeared to be overcome by longer treatment times, leptin-induced decreases in food intake, body weight, and body fat occurred by 10 days in MA mice at the price of greater protein catabolism. Although Y and MA mice lost body protein at the highest leptin dose, this infusion increased plasma leptin almost 10-fold and was probably supraphysiological. However, MA mice also lost significant amounts of body protein after 10 days of infusion with 0.5 μg/h leptin, a dose that, in agreement with previous literature (12), did not affect body protein in Y mice. Thus MA mice not only respond to leptin more slowly but are also less able to mobilize fat preferentially after sustained leptin exposure. The etiology of this leptin resistance remains to be elucidated but could include loss (6) or impaired intracellular signaling (23) of leptin-responsive hypothalamic neurons, as well as adipocyte resistance to leptin-induced increases in sympathetic stimulation (20).

No Correlation Between Body Fat and Endogenous Plasma Leptin in MA Mice

The correlation between plasma leptin and body fat is lost in older humans, suggesting that increasing age-related adiposity represents a compensatory response to disproportionately low leptin levels (16). In agreement with the human data (16), we found that body fat was not a significant predictor of plasma leptin levels in MA mice. Deficits in maintaining plasma leptin levels are clearly not the only cause of altered weight regulation in MA mice, since MA responses were impaired even after plasma leptin was increased by infusion. The finding that transgenic mice overproducing leptin still get fat with age (18) also suggests that leptin resistance is a key factor in midlife weight gain.

CR Mice Do Not Respond to Leptin Infusions Inhibiting Food Intake in MA Mice

We have further shown that calorie restriction, which minimizes weight gain and improves insulin sensitivity in aging (4), actually increases resistance to the appetite-suppressing effects of leptin. Infusion of a moderate leptin dose (0.25 μg/h) that reduced 4-day food intake in MA mice had no effect in CR mice. Suppression of MA food intake was not inconsistent with experiment 1, since 4 days of treatment probably augmented leptin-related differences emerging by 3 days; calculation of 4-day cumulative food intake for experiment 1 did show significant decreases in the 0.5 and 1.5 μg/h MA groups (not shown). The failure of leptin to suppress food intake in CR mice was not due to restricted food availability during the infusion, since leptin-treated Y mice ate significantly less than did leptin-treated CR mice (Fig. 4). Initial levels of food intake in CR mice were also similar to those in MA mice when expressed relative to body weight (Table 2) or lean body mass (calculated as carcass weight minus fat weight; not shown). Therefore, CR food rations during the experiment were neither so absolutely nor disproportionately low that further decreases were impossible.

Metabolic Responses to Leptin Are Not Improved in CR Mice

Like their MA counterparts, CR mice did not decrease body fat or plasma insulin as efficiently as Y mice after leptin treatment. CR mice were not so lean that further reductions were impossible, since Y mice were as lean and yet underwent further, leptin-induced loss of body fat. MA and CR mice not only failed to exhibit leptin-dependent decreases but also had significantly higher levels of body fat and marginally higher levels of plasma insulin than Y mice after leptin treatment. Significant leptin-induced loss of body fat might have been expected not only in MA mice, which decreased food intake, but also in CR mice, because leptin can stimulate fat mobilization independently of food intake (11). These results suggest that leptin-induced fat mobilization in MA mice is not improved by calorie restriction. Leptin effects on glucose homeostasis in Y mice, which were probably underrepresented because mice were not fasted, could have been due to greater loss of body fat as well as leptin inhibition of food intake. However, because leptin can enhance insulin action independently of changes in food intake and fat deposition (5, 9), the lack of leptin effects on insulin in CR as well as MA mice also suggests that calorie restriction may not fully reverse age-related insulin resistance.

Incomplete Leptin Suppression of CR Plasma Glucocorticoid Levels

As expected from previous literature (2, 13, 17), CR mice had markedly higher circadian peak corticosterone levels that tended to be reduced by leptin infusion. However, even after leptin treatment, plasma corticosterone was still significantly higher in CR vs. MA mice. Because we have previously shown that corticosterone levels in this range can block leptin effects on food intake and body fat (26), higher corticosterone levels in CR mice may contribute to their relative refractoriness to leptin. Notably, leptin treatment did not reduce trough or peak corticosterone levels in Y or MA mice. We suspect that the reported inhibition of circadian glucocorticoid secretion by leptin infusion (1) was confounded by stress effects on adrenocortical activity.

We have shown that MA mice exhibit impaired responses to leptin that are not improved or, in the case of food intake, are further disrupted by calorie restriction. The failure of leptin action in MA mice is consistent with the likelihood that excess body weight reflects leptin resistance rather than leptin deficiency (11) and affirms the anticipated difficulty of achieving significant public adherence to lifelong calorie restriction. Identifying the factors causing leptin resistance
during aging and calorie restriction will be necessary to make dietary restraint a more tolerable, if not avoidable, strategy for healthy aging.

I am grateful to Brian D. Mooney and Rebecca Rokow-Kittell for technical assistance. This study was supported in part by National Institute on Aging Grant AG-18241 to the author.

REFERENCES