Aging does not contribute to the decline in insulin action on storage of muscle glycogen in rats

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Gupta, Gaurav, Li She, Xiao-Hui Ma, Xiao-Man Yang, Meizhu Hu, Jane A. Cases, Patricia Vuguin, Luciano Rossetti, and Nir Barzilai. Aging does not contribute to the decline in insulin action on storage of muscle glycogen in rats. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R111–R117, 2000.—Increase in fat mass (FM) and changes in body composition may account for much of the age-associated impairment in insulin action on muscle glycogen storage. To examine whether preventing the increase in FM abolishes this defect seen with aging. We studied the novel aging model of F1 hybrids of BN/F344 NIA rats fed ad libitum (AL) at 2 (weighing 259 ± 17 g), 8 (459 ± 17 g), and 20 (492 ± 10 g) mo old. To prevent the age-dependent growth in FM, rats were caloric restricted (CR) at 2 mo by decreasing their daily caloric intake by 45% (weighing 292 ± 5 g at 8 mo, 294 ± 6 g at 20 mo). As designed, the lean body mass (LBM) and %FM remained unchanged through aging (8 and 20 mo old) in the CR rats and was similar to that of 2-mo-old AL rats. However, 8- and 20-mo-old AL-fed rats had three- to fourfold higher FM than both CR groups. Peripheral insulin action at physiological hyperinsulinemia was determined (by 3 mU·kg−1·min−1 insulin clamp) and similar plasma insulin levels (during hyperinsulinemic clamp), the pathway of muscle glycogen synthesis (GS) was also studied at 30% (8 mo old) and 70% (20 mo old), respectively. These levels were significantly increased (P < 0.001) compared with AL rats with higher %FM (Rd, 22 ± 1 and 22 ± 2 and GS, 7 ± 1 and 8 ± 2 mg·kg−1·LBM−1·min−1) at youthful levels (2 mo AL) in 8- and 20-mo-old CR rats, respectively. The increase in whole body GS in age-matched CR rats was accompanied by ~40% increased accumulation of [3H]glucose into glycogen and a similar increase in insulin-induced muscle glycogen content. Furthermore, the activation of glycogen synthase increased, i.e., ~50% decrease in the Michaelis constant, in both CR groups (P < 0.01). We conclude that chronic CR designed to prevent an increase in storage of energy in fat maintained peripheral insulin action at youthful levels, and aging per se does not result in a defect on the pathway of glycogen storage in skeletal muscle.

fatty mass; caloric restriction; lean body mass; insulin-mediated glycogen synthesis

INCREASED FAT MASS (FM) and/or decreased exercise capacity (4, 16, 22) may account for much of the decrease in peripheral insulin action observed with aging. Human and animal studies have shown that, for similar plasma insulin levels (during hyperinsulinemic clamp), the pathway of muscle glycogen synthesis (GS) is often found to be impaired with increased fat and obesity (9, 24), effecting peripheral glucose uptake (Rd). Furthermore, insulin action in humans may be maximally impaired when FM is more than a certain percent of body weight (6). In another rat model, we previously demonstrated that insulin responsiveness is maximally decreased when FM is >14% of body weight (1, 2, 4).

Body weight and exercise capacity may also determine lean body mass (LBM; see Ref. 14). In particular, human aging is characterized by an increase in LBM that plateaus in the third decade and decreases after the sixth decade of life (12, 21). Thus decreased insulin action with aging may be related to a decrease in LBM or to the combined effect of an increased ratio of FM to LBM. Alternatively, this impaired ability of the muscle to respond to insulin may be, at least partly, due to an age-related decrease in the muscle metabolic pathways, such as glycogenesis or GS (8, 29), or to decreased enzymatic function (20), independent of the effects of body composition.

This study was designed to delineate whether the pathway of insulin-mediated GS is impaired, in vivo and in vitro, with aging. Because human and animal studies have shown that FM has an overwhelming negative effect on insulin action, we used the tool of chronic caloric restriction (CR) to keep percent FM at youthful (2 mo old) levels. A novel aging model of rats (F1 hybrid of Brown Norway × Fischer 344 crosses) was also studied at 30% (8 mo old) and 70% (20 mo old) of their average life span to compare postdevelopmental and old ages. In addition, we matched young and old rats for LBM to exclude this as a potentially confounding variable. We hypothesized that, if aging is characterized by a defect in the pathway of GS in skeletal muscle, it will be revealed when percent FM is kept at youthful levels throughout aging.

MATERIALS AND METHODS

Animals. Male F1 hybrids of Brown Norway × Fischer 344 rats [obtained from National Institute of Aging (NIA)] were used for this study. These hybrids were selected as an alternative model to the Fischer 344, based on a study by NIA of several F1 hybrids resulting from various crosses that showed Brown Norway × Fischer 344 crosses produce prog-
nergy with the fewest detrimental pathologies and at later age of onset than other crosses studied (NIA animal catalog). Thus this model combines the advantages of the Sprague-Dawley model, which, like humans, gains substantial amounts of body weight and FM, and the previously popular thin Fischer 344 aging model, which lives longer. To maintain FM below 14%, young rats (2 mo old) were caloric restricted (CR, n = 12), with 55% of the calories consumed by ad libitum feeding (AL). The chow contained 64% carbohydrates, 30% proteins, and 6% fats with a physiological fuel value of 3.3 kcal/g chow, and the CR rats were given vitamin supplements. Rats were housed in individual cages and were subjected to a standard light (6:00 AM to 6:00 PM)-dark (6:00 PM to 6:00 AM) cycle. A second group of control rats were fed AL with subgroups studied when they were young (2 mo old, n = 6) and after they attained 30% (8 mo old, n = 8) and 70% (20 mo old, n = 6) of their average life span (obtained by mortality curves provided by NIA). Similarly, the CR rats were studied at 8 mo (n = 6) and 20 mo (n = 6) of age. One week before the in vivo study, rats were anesthetized by inhalation of methoxyflurane, and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery. This method of anesthesia allows for fast recovery and normal food consumption at 24 h. The venous catheter was extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch (4, 25, 26). Recovery was then continued until body weight was within 3% of the preoperative weight. Studies were performed in awake, unstressed, chronically catheterized rats (4, 25, 26).

Body composition. LBM and FM were calculated from the whole body volume of distribution of water, estimated by $^{3}$H$_2$O bolus injection in each experimental rat (4). On the morning of the study, 20 µCi of $^{3}$H$_2$O (New England Nuclear, Boston, MA) were injected intra-arterially. Steady state for $^{3}$H$_2$O specific activity in rats is generally achieved within 30–45 min, and eight samples were collected between 1 and 1.5 h after injection. The distribution space of water was obtained by dividing the total radioactivity injected by the steady-state specific activity of plasma water, which was assumed to be 93% of the total plasma volume. LBM was calculated from the whole body water distribution space divided by 0.73 (the %water content of LBM). FM was calculated as the difference between total body weight and LBM. The fat-derived peptide leptin is another index of adiposity, and its plasma leptin levels were determined to reflect FM and insulin action (31). Plasma leptin (Leptin RIA kit; Linco Research, St. Charles, MO) concentrations were measured by RIA. Skeletal muscle triglyceride content, which was suggested as an index of insulin sensitivity, was measured from frozen muscle homogenate extracted by chloroform-methanol and was measured by a triglyceride (GPO-Trinder) kit (Sigma diagnostics, St. Louis, MO).

Hyperinsulinenic euglycemic clamp. All rats received a primed-continuous (15–40 µCi bolus, 0.4 µCi/min) infusion of HPLC-purified $[3^{-3}$H]glucose (New England Nuclear) throughout the study. A primed continuous infusion of insulin (3 mU·kg$^{-1}$·min$^{-1}$) and a variable infusion of a 25% glucose solution was started and periodically adjusted to damp the plasma glucose concentration at the basal level for the 120 min of the clamp. Somatostatin (1.5 µg·kg$^{-1}$·min$^{-1}$) was infused to suppress endogenous insulin secretion. Plasma samples for determination of $^{3}$H glucose specific activity were obtained at 10-min intervals throughout the insulin infusion. Samples were also obtained for determination of plasma insulin, leptin, and free fatty acid (FFA) concentrations at 30-min intervals throughout the study. The total volume of blood withdrawn was ~3.0 ml/study; to prevent volume depletion and anemia, a solution (1:1 vol/vol) of ~3.0 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml) was infused at a constant rate throughout the study. At the end of the insulin infusion, rats were anesthetized (60 mg pentobarbital sodium/kg body wt iv), the abdomen was quickly opened, and the rectus abdominal muscle was freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen (4, 25, 26). The time from the injection of the anesthetic until freeze clamping of the muscle was <1 min. Epididymal, mesenteric, and perinephric fat pads were dissected and weighed at the end of each experiment. All tissue samples were stored at −80°C for subsequent analysis.

The study protocol was reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

Whole body glycolysis and GS. The rate of glycolysis was estimated from the rate of conversion of $[3^{-3}$H]glucose to $^3$H$_2$O as previously described (4, 25, 26). Because $^3$H on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma $^3$H is present either in $^3$H$_2$O or glucose. Plasma $^3$H$_2$O specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Whole body GS was estimated by subtracting whole body glycolysis from whole body $^{3}$H$_2$O. Glycogen formation. Indexes for muscle GS were obtained by two additional independent methods. First, it was quantified by measuring the incorporation of $[^3$H]glucose into glycogen. Second, muscle glycogen after insulin infusion was determined after digestion with amyloglucosidase, as previously described (4, 25, 26), and subtracted from basal muscle glycogen determined in age-matched control rats that were infused with saline (n = 3). The intra-assay and the interassay coefficients of variation were <10% (at 0.25 g/100 g tissue wt) when a muscle homogenate was assayed as multiple aliquots. Glycogen was precipitated by washing in 10 volumes of absolute ethanol and by incubation for 1 h at −20°C. The procedure was repeated three times, and then the precipitate was collected, dried down, and dissolved in water before assay. Muscle glycogen was freeze-clamped in situ, and the glycogen phosphorylase assay was performed to determine glycogen phosphorylase activity. Muscle glycogen synthase activity was measured by a modification (4, 25, 26) of the method of Thomas et al. (28) and was based on the measurement of the incorporation of radioactivity in glycogen from UDP-[U-$^{14}$C]glucose at 30°C. Tissue samples (20–30 mg) were homogenized in 2.0 ml of Tris-HCl buffer, pH 7.8, containing 10 mmol/l EDTA, 5 mmol/l dithiothreitol, 50 mmol/l NaF, and 2.5 g/l type III rabbit liver glycogen. To approximate the in vivo conditions, synthase activity was measured in the presence of physiological 0.11 mmol/l glucose 6-phosphate. Total glycogen synthase activity was measured in the presence of 7.2 mmol/l glucose 6-phosphate. For the kinetic analysis, the assay was conducted at final concentrations of 0.003, 0.017, 0.033, 0.09, 0.33, and 1.4 mM UDPG; the data were linearized as Eadie-Hofstee plots and were fit using linear regression. The Michaelis constant ($K_m$) for UDPG is the reciprocal of the slope, whereas maximal velocity ($V_{max}$) is calculated as the y-intercept divided by the slope.

Glycogen phosphorylase activity. Muscle glycogen phosphorylase activity was measured as previously described (4, 25, 26). This assay is based on the measurement of the incorporation of $^{14}$C into glycogen from labeled glucose 1-phosphate in the absence of AMP. The supernatant was used for glycogen phosphorylase assay by measuring the incorporation of $^{14}$C into glycogen at 30°C in a
mixture containing 33 mM MES, 200 mM KF, 0.45% mercaptopetoanol, 15 mM glucose 1-phosphate (50 µCi/mmol), and 3.4 mg/ml glycogen.

Analytic procedures. Plasma glucose was measured by the glucose oxidase method (GlucoseAnalyzer II; Beckman Instruments, Palo Alto, CA), and plasma insulin was measured by RIA using rat and porcine insulin standards. Plasma [3H]glucose radioactivity was measured in duplicate on the supernatants of Ba(OH)2 and ZnSO4 precipitates of plasma samples before and after evaporation to dryness to eliminate [3H]2O. Plasma nonesterified fatty acid concentrations were determined by an enzymatic method with an automated kit according to the manufacturer’s specifications (Waco Pure Chemical Industries, Osaka, Japan).

Calculations. Rates of whole body glycolysis were estimated from the increment per unit time in [3H]2O (dpm·ml⁻¹·min⁻¹) times body water (ml) divided by [3H]glucose specific activity (dpm/mg). All values are presented as the means ± SE. Comparisons between groups were made using repeated-measures ANOVA where appropriate. When F ratios were significant, further comparisons were made using Student’s t-tests.

RESULTS

Body composition and biochemical characteristics. In the AL rats, the percent of FM increased with age from 9% in 2-, to 20% in 8-, and to 25% in 20-mo-old rats (P < 0.01 between all ages), although the LBM between 8- and 20-mo-old rats remained unchanged (Table 1). By subjecting the rats to CR beginning at 2 mo of age, this increase in FM was prevented. The total FM was attenuated to approximately threefold less than that of old AL rats, and the percent FM was maintained below 14% of total body weight, a level similar to young (2 mo old) rats (Table 1 and Fig. 1). LBM was similar in 8- and 20 mo old CR rats. Muscle triglyceride content was three- to fourfold higher in 8- and 20-mo-old AL rats than in young or CR aging rats.

Interestingly, the plasma leptin levels doubled between 8- and 20-mo-old AL rats, although FM was increased only by ~30% between these ages. Although plasma leptin levels were approximately fivefold lower in CR rats, they were doubled between 2-mo-old AL to 8-mo-old CR (not significantly) and between 8- and 20-mo-old CR (P < 0.01).

All groups had similar basal glucose levels (Table 2). However, the basal plasma insulin levels were ~60% decreased in CR rats compared with AL-fed rats. The basal plasma FFA levels were increased with age in AL compared with CR rats.

Insulin-mediated Rₐ and GS. During the insulin clamp studies, the steady-state plasma insulin levels were increased similarly in all groups to physiological postprandial levels (Table 2). Steady-state plasma glucose levels were also similar in all groups. Hyperinsulinemia was unable to suppress plasma FFA levels in 8- and 20-mo-old AL rats. However, FFA levels were similarly decreased by ~30% in the CR rats.

The insulin-mediated Rₐ was significantly increased (P < 0.01) in CR rats (29 ± 2 and 31 ± 4 mg·kg⁻¹·min⁻¹ in 8- and 20-mo-old rats, respectively; Fig. 1) compared with AL rats (22 ± 1 and 22 ± 2 mg·kg⁻¹·min⁻¹ in 8- and 20-mo-old rats, respectively). Indeed, it was similar to the Rₐ of young (2 mo old) controls (28.8 ± 1.9 mg·kg⁻¹·min⁻¹). This improvement was mostly due to the ~60% increase in GS with CR (12 ± 1 and 14 ± 2 mg·kg⁻¹·min⁻¹ in 8- and 20-mo-old CR rats vs. 7 ± 1 and 8 ± 2 mg·kg⁻¹·min⁻¹ in 8- and 20-mo-old AL rats, respectively). Additionally, there was marginal improvement in glycolysis (17 ± 1 mg·kg⁻¹·min⁻¹ in both 8- and 20-mo-old CR; 15 ± 2 and 14 ± 2 mg·kg⁻¹·min⁻¹ in 8- and 20-mo-old AL, respectively). Indeed, both GS and glycolysis in the CR rats were restored to the

Table 1. Body composition

<table>
<thead>
<tr>
<th></th>
<th>2 mo old AL</th>
<th>8 mo old AL</th>
<th>20 mo old AL</th>
<th>20 mo old CR</th>
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<tr>
<td>Body weight, g</td>
<td>259 ± 17</td>
<td>459 ± 17*</td>
<td>292 ± 5</td>
<td>492 ± 10*</td>
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<tr>
<td>LBM, g</td>
<td>240 ± 15</td>
<td>359 ± 6*</td>
<td>259 ± 9</td>
<td>369 ± 12*</td>
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<td>FM, g</td>
<td>19 ± 4</td>
<td>93 ± 7*</td>
<td>28 ± 6</td>
<td>126 ± 2*†</td>
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<tr>
<td>FM, %</td>
<td>9 ± 1</td>
<td>20 ± 1*</td>
<td>9 ± 1</td>
<td>25 ± 1†</td>
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<tr>
<td>TG, mg/g muscle</td>
<td>2.8 ± 1.3</td>
<td>9.2 ± 3.6*</td>
<td>4.4 ± 2.1</td>
<td>17.0 ± 5.1*</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>1.2 ± 0.3</td>
<td>11.1 ± 1*</td>
<td>2.1 ± 1.3</td>
<td>21.1 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Body weight, lean body mass (LBM), fat mass (FM), percent fat mass, muscle triglyceride (TG) content, and leptin levels in 2-mo-old ad libitum (AL), 8-mo-old AL and caloric restricted (CR), and 20-mo-old AL and CR rats. *P < 0.001 vs. young controls and CR rats. †P < 0.01 vs. 8-mo-old AL, ‡P < 0.01 vs. 8-mo-old CR.

Fig. 1. Insulin-mediated glucose uptake (Rₐ). Rₐ measured during physiological hyperinsulinemia levels (insulin clamp − 3 mU·kg⁻¹·min⁻¹) in 2-mo-old ad libitum-fed (AL), 8-mo-old AL and caloric-restricted (CR) rats, and 20-mo-old AL and CR rats. LBM, lean body mass. Percent fat mass (%FM) shown above bars. *P < 0.001 vs. young controls and CR rats.
youthful levels seen in 2-mo-old AL rats (GS, 12 ± 1 mg·kg LBM⁻¹·min⁻¹ and glycolysis, 17 ± 2 mg·kg LBM⁻¹·min⁻¹; Fig. 2). This increase in GS was confirmed by a 50% increase in insulin-stimulated accumulation of [3H]glucose into glycogen in CR rats compared with AL rats (743 ± 119 and 693 ± 116 dpm·10⁻⁷ ·g wet wt⁻¹ in 8- and 20-mo-old CR rats vs. 490 ± 91 and 337 ± 35 in 8- and 20-mo-old AL rats, P < 0.01; Fig. 3). In addition, after 2 h of hyperinsulinemia, the CR had more accumulation of glycogen than AL rats. Because this was measured over basal glycogen levels obtained from AL and CR rats, which were infused with saline (other rats), significance was noted only in the 20-mo-old rats.

Muscle glycogen synthase and glycogen phosphorylase activities. The K_m of glycogen synthase decreased by 50% in both CR groups (0.16 ± 0.03 and 0.12 ± 0.03 mM in 8- and 20-mo-old CR rats vs. 0.26 ± 0.04 and 0.26 ± 0.11 mM in 8- and 20-mo-old AL rats, P < 0.01 vs. young controls and CR rats.

### Table 2. Biochemical characteristics

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<tr>
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<th>2 mo old AL</th>
<th>AL</th>
<th>CR</th>
<th>8 mo old</th>
<th>AL</th>
<th>CR</th>
<th>20 mo old</th>
<th>AL</th>
<th>CR</th>
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<tr>
<td>Glucose, mM</td>
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<tr>
<td>Basal</td>
<td>8.0 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>7.8 ± 0.1</td>
<td>8.0 ± 0.1</td>
<td>7.5 ± 0.3</td>
<td>7.7 ± 0.3</td>
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<tr>
<td>Clamp</td>
<td>7.8 ± 0.1</td>
<td>7.8 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>7.7 ± 0.5</td>
<td>7.6 ± 0.2</td>
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<td>Insulin, µU/ml Basal</td>
<td>16 ± 3</td>
<td>33 ± 4*</td>
<td>13 ± 2</td>
<td>35 ± 3*</td>
<td>14 ± 4</td>
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<tr>
<td>Clamp</td>
<td>68 ± 3†</td>
<td>60 ± 6†</td>
<td>56 ± 12†</td>
<td>63 ± 9†</td>
<td>68 ± 6†</td>
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<td>FFA, mM Basal Clamp</td>
<td>0.81 ± 0.12</td>
<td>1.14 ± 0.14</td>
<td>0.90 ± 0.04</td>
<td>1.30 ± 0.11</td>
<td>0.99 ± 0.19</td>
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<td></td>
<td>0.57 ± 0.09†</td>
<td>1.06 ± 0.11*</td>
<td>0.70 ± 0.08†</td>
<td>1.36 ± 0.04*</td>
<td>0.69 ± 0.15†</td>
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Values expressed as means ± SE. Plasma glucose, insulin, and free fatty acids (FFA) during baseline (basal) and physiological hyperinsulinemia (clamp) in 2-mo-old AL, 8-mo-old AL and CR, and 20-mo-old AL and CR rats. *P < 0.001 vs. young controls and CR rats. †P < 0.001 vs. basal.

Fig. 2. Incorporation of [3H]glucose into glycogen and Michaelis constant (K_m) of glycogen synthase (GSy). Muscle was obtained in situ at the end of insulin infusion in all rats. Incorporation of [3H]glucose into glycogen was done as described in MATERIALS AND METHODS. K_m of glycogen synthase was determined in the presence of physiological 0.11 mM glucose 6-phosphate concentration in 2-mo-old AL, 8-mo-old AL and CR, and 20-mo-old AL and CR rats. *P < 0.01 vs. CR rats.
greatly determined by FM. Although increased FM in Table 3. had similar V
The AMP-independent form of glycogen phosphorylase
8- and 20-mo-old CR, although CR was
difference between young and old rats (19). LBM was
part of their life span such that there can be a twofold
more stringent CR was implemented to attain lower
FM was no more than 14% (1, 3). In this study design, a
CR animals have demonstrated that insulin responsive-
insulin responsiveness is fully decreased when ex-
1 to 35%, when FM is over 14% of the body weight, that
examining this question. First, we have previously
composition (Table 1) overcomes previous obstacles in
insulin is intact with aging.

0.01; Fig. 3 and Table 3), whereas V_max was unchanged.
The AMP-independent form of glycogen phosphorylase
had similar V_max values in both AL and CR rats.

DISCUSSION
This study confirms the notion that an increase in
FM determines the decrease in insulin action and GS in
an animal model of aging. To unveil a defect specific to
aging per se rather than to FM, rats were chronically
caloric restricted from young adulthood to maintain
FM at youthful levels. This experimental manipulation
provided evidence in support of the hypothesis that the
capacity of the muscle to store glycogen in response to
insulin is intact with aging.

Our ability to achieve the desired alterations in body
composition (Table 1) overcomes previous obstacles in
examining this question. First, we have previously
shown in a rodent model with percent FM ranging from
1 to 35%, when FM is over 14% of the body weight, that
insulin responsiveness is fully decreased when ex-
pressed per LBM (1–4). Moreover, several studies in
CR animals have demonstrated that insulin responsive-
ness was restored to levels of younger ages only when
FM was no more than 14%(1, 3). In this study design, a
more stringent CR was implemented to attain lower
levels of percent FM. Second, rodents demonstrated an
increase in body weight and LBM during a substantial
part of their life span such that there can be a twofold
difference between young and old rats (19). LBM was
similar between 8- and 20-mo-old AL rats and between
8- and 20-mo-old CR, although CR was ~30% lower
than that of AL. Therefore, matching LBM in each
intervention throughout aging, LBM is not a major
determinant of insulin action in aging. Interestingly,
although FM was mildly increased in 20- compared
with 8-mo-old AL rats, leptin levels were nearly doubled.
In fact, leptin levels doubled between 8- and 20-mo-old
CR animals that had a similar amount of fat and
increased from 2-mo-old AL to 8-mo-old CR with the
same percent FM (although the difference was not
statistically significant). This contributes to other data
(30) suggesting that aging may be a “leptin-resistant”
state, and its levels do not always simply reflect FM (18).

In this study, insulin action on R_d with aging was
greatly determined by FM. Although increased FM in
20-mo-old AL was not associated with a further de-
crease in insulin action (expressed per LBM), low FM in
the CR animals restored insulin action to levels of
young adulthood (2 mo old). Changes in FM between
the groups were reflected in another important marker of
fat storage, i.e., muscle triglyceride content, previously
suggested to be a direct cause to decrease insulin
action in muscle in obesity (23). Although improvement
in insulin responsiveness by CR was previously demon-
strated (1), this study was performed at physiological
hyperinsulinemia (postmeal levels) and reflects the
ture sensitivity rather than responsiveness (maximally
stimulating insulin levels) to insulin (17). Because it
has been suggested that total LBM may determine
insulin action (10), this study measured insulin action
with aging in young and old animals with the same
LBM. Although it confirms that FM has an effect on
insulin action independent of LBM, it is still possible
that a subsequent decrease in LBM later in life may be
associated with impaired insulin action (11). In such a
case, whether the effect is due to the failing muscle or to
an increase in fat relative to muscle still needs to be
determined. In addition, this study was performed in a
conscious, longer-living aging rat model, whereas many
previous studies were performed in Wistar or Sprague-
Dawley rat models (1).

Several lines of evidence suggest that the improve-
ment in R_d seen in the CR rats is largely due to
enhanced rates of GS. When calculated as the differ-
ce between R_d and glycysis, insulin-mediated GS
rates were significantly improved in the CR compared
with the AL groups and were restored to the rates of
2-mo-old rats (Fig. 2). In addition, the [3H]glucose
incorporation in muscle glycogen was increased in both
CR groups compared with the controls (Table 3). Fi-
ally, the insulin-induced increase in muscle glycogen-
compared with saline-infused controls was also higher
in the CR rats (Table 3). This occurred with enhanced
activation of muscle glycogen synthase (decrease in K_m)
by insulin in both CR groups. This activation was
unopposed because the activity of glycogen phosphor-
lase did not change. Earlier studies in humans have
characterized the insulin resistance of obesity and type
2 diabetes and suggested defective glycogen storage
(23, 27). These studies, utilizing indirect calorimetry or

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<th>Table 3. Muscle glycogen characteristics</th>
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<tr>
<td>ΔGlycogen, µmol/g wet wt</td>
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<td>GSY V_max, µmol·g wet wt</td>
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<td>GP V_max, µmol·g wet wt</td>
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<td>27 ± 2*</td>
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<td>22 ± 8</td>
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<td>30 ± 5</td>
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<td>25 ± 5</td>
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<td>26 ± 6</td>
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<td>Data are presented as means ± SE. Results are expressed per g of muscle wet weight. Increase in muscle glycogen with insulin (ΔGlycogen), glycogen synthase (GSy) maximal velocity (V_max), and glycogen phosphorylase (GP). Muscle was obtained in situ at the end of insulin infusion in all rats. Increase in muscle glycogen with insulin is the increment above basal muscle glycogen obtained from age-matched AL and CR rats that were infused with saline. GSy V_max of muscle GS is in the presence of 7.2 mM Glc-6-P concentration. GP activity is of the AMP-independent form. *P &lt; 0.01 vs. AL.</td>
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estrated to contribute to the insulin resistance of normoglycemic obese rats (1, 4). Increased plasma FFA levels have been shown to mediate a decrease in insulin-induced GS (13), and some of the improvement in insulin action may be due to a decrease in FFA levels during hyperinsulinemia. However, this indirect effect is not involved in insulin-mediated $R_d$ in 8- and 20-mo-old AL rats because their FFA levels, at this degree of hyperinsulinemia, are not suppressed. It is possible, however, that decreased FFA levels may have some effect on increasing $R_d$ in 2-mo-old AL and 8- and 20-mo-old CR rats where the percent suppression of their plasma FFA was similar. Fat-derived peptides such as leptin and tumor necrosis factor-$\alpha$ may be involved in the "cross-talk" between FM and skeletal muscle and have been shown to be involved in insulin action (5, 15). Taken together with this background, our data confirm the fact that an increase in FFA is associated with a decrease in the ability of insulin to store glycogen, and this effect overwhemns other biological effects associated with aging.

While keeping FM low, negating its effects on insulin action, it also provided an opportunity to examine whether the muscle’s capability to store glycogen deteriorates with aging. To delineate this, insulin-mediated GS was determined in young AL and old CR rats with similar FM. Our results clearly demonstrate a similar capacity in all groups, since $[^{14}C]$glucose incorporation in muscle glycogen, the insulin-induced increase in muscle glycogen, and the kinetics of muscle glycogen synthase were similar. Thus we suggest that biological changes described in enzymes with aging in humans (7) and animals, such as in the oxidative (20) and metabolic pathways of glycolysis (29) and GS (8), might not be observed when controlled for the metabolic consequences associated with increased FM.

We demonstrated that chronic CR designed to prevent fat accretion maintained peripheral insulin action on $R_d$ and muscle GS at youthful levels. We conclude that, through a significant part of a life span, aging per se is not associated with a decreased capacity of skeletal muscle to store energy into glycogen.

Perspectives

One of the most robust observations in the biology of aging is that CR extends life in a variety of species. Although CR results in a several-fold decrease in FM, its multisystemic effects (neurological, endocrine, reproductive, immunological, and antigenic) could not historically be linked to fat. However, recently, an explosion of evidence has demonstrated that fat tissue is a very active endocrine gland that secretes a variety of peptides (such as leptin and plasminogen activating inhibitor-1), cytokines (such as tumor necrosis factor), and complement factors (such as D, C3, and B). This is in addition to the presence of substrates, such as glycerol and FFAs, which are stored and released by fat cells and which are known to affect peripheral glucose metabolism. We propose that many of the systemic effects of CR can now be explained by effects related to decreased plasma levels of peptides, cytokines, complement factors, and substrates. We demonstrated specific benefits of CR on the improvement in glucose homeostasis, and we suggest that it may be attributed to a decrease in adipose cells and/or their products. Because FFA did not seem to have significant effects in this study, leptin and tumor necrosis are candidates that have been shown to modulate insulin action.

From a comparative point of view, epidemiological data in human obesity support the role of FM and its distribution as a risk factor for morbidity and mortality in humans due to impaired glucose metabolism (similar to rodents), for cancer (similar to rodents), and for the development of atherosclerotic vascular disease (in humans). Thus we suggest that the role of fat and expression of fat-derived proteins be “front” candidates to explore the benefits of CR.

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REFERENCES


