# Aging Changes Tissue-Specific Glucose Metabolism in Rats

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**This study defines the tissue-specific changes in glucose metabolic flux that occur over time prior to the onset of whole-body insulin resistance in rats. Rats at 6 weeks of age were maintained on a high-carbohydrate diet for either 12 or 26 weeks, at which time euglycemic clamps were performed at basal and midphysiological plasma insulin concentrations. Following death, insulin-sensitive tissues were excised and frozen until assayed for the rate of glucose uptake, glycogenesis, and lipogenesis. Glucose metabolic flux, particularly through glycogenesis, was reduced between 18 and 32 weeks of age in all tissues except the adipose tissues. For example, the rate of glycogenesis in liver at 18 weeks (117 ± 10 nmol glucose incorporated/min/g) was more than double that observed at 32 weeks (54 ± 8 nmol glucose incorporated/min/g, $P < .01$). Despite this, animals in the 32-week group displayed no impairment in whole-body glucose disposal, due to compensatory glucose uptake in white adipose tissue (WAT) and increased glucose flux through lipogenesis in brown adipose tissue (BAT). At 32 weeks, the rate of glucose uptake in WAT (85.0 ± 5.6 nmol 2-deoxy-D-glucose phosphate accumulated/min/g) was approximately double that at 18 weeks (46.6 ± 5.6 nmol 2-deoxy-D-glucose phosphate accumulated/min/g) was approximately double that at 18 weeks (46.6 ± 5.6 nmol 2-deoxy-D-glucose phosphate accumulated/min/g, $P < .01$). These changes in insulin responsiveness in adipose tissue of older animals may underlie the increased adiposity that is currently thought to be the causative factor in the development of age-related insulin resistance.**



IT HAS LONG BEEN established that insulin resistance is a common feature of aging in both humans and rats.[1] The evidence suggests that these defects are associated with changes in body composition, that is, increased adiposity and decreased fat-free mass, rather than with aging per se.[2-5] Indeed, studies that have normalized subjects according to fat-free mass have discovered that there is no difference in insulin sensitivity between young and old subjects.[2,4] Conversely, it has been demonstrated that diets which promote adiposity cause peripheral insulin resistance in rats.[3] Also, age-related insulin resistance in older humans may be ameliorated by exercise training that acts to decrease adiposity.[6]

The present study was performed to examine tissue-specific glucose metabolism in pre–insulin-resistant young and old rats to define any changes that may cause or contribute to the onset of age-related insulin resistance.

## MATERIALS AND METHODS

### *Animals*

All animals used were of the species *Rattus norvigus,* strain Australian Albino Wistar (AAW). Male AAW rats (Combined Universities Laboratory Animal Services, Sydney, Australia) were housed in groups of three at the University of Sydney Department of Biochemistry Animal House. Rats were maintained at 22°C on a 12-hour light/dark cycle.

Animals at 6 weeks of age were fed a high-carbohydrate, low-fat diet[7] for either 12 or 26 weeks. All animals received and completely consumed 315 kJ/d in two equivalent feedings and were weighed at weekly intervals. As a percentage of total energy, the high-carbohydrate diet contained 67% carbohydrate (57% waxy maize starch and 10% sucrose), 22% protein, and 11% fat. Waxy maize starch was donated by Goodman Fielder Ingredients (Gladesville, Australia). Vitamin and mineral mixes were a gift from Millmaster Feeds (Enfield, Australia).

All studies were performed according to the guidelines and with the prior approval of the Sydney University Animal Care and Ethics Committee (protocol no. L02/4-94/3/961).

### *Hyperinsulinemic-Euglycemic Clamp*

At 18 or 32 weeks of age, the animals were cannulated in the right and left jugular veins. Two full days postsurgery, the rats were starved overnight. Euglycemic clamps were performed as described previously,[11] with the blood glucose concentration clamped at 4 to 5 mmol/L. Rats were assessed at either basal (overnight-starved) insulin (25 to 50 μU/mL) or steady-state physiological (150 μU/mL) plasma insulin. Once euglycemia was maintained for approximately 30 minutes, a bolus of [U-$^{14}$C]glucose (50 μCi, 250 mCi/mmol) plus 2-deoxy-D-[2,6-$^{3}$H]glucose (50 μCi, 30 Ci/mmol) was infused and blood samples were withdrawn at regular intervals.[8] Plasma was stored at −20°C prior to analysis for insulin and glucose concentration and [$^{3}$H] and [$^{14}$C] content.

Rats were killed by intravenous infusion of a lethal dose of pentobarbitone sodium (150 mg/kg). Samples of liver, soleus muscle, epididymal white adipose tissue (WAT), and interscapular brown adipose tissue (BAT) were excised within 5 minutes of death and immediately frozen in liquid nitrogen. The tissues were stored at −80°C until processed.

### *Plasma Analyses*

Tracer disappearance curves ($^{3}$H and $^{14}$C specific activity *v* time) were used to calculate the rates of glucose uptake, lipid synthesis, and glycogen synthesis as described previously.[9] Plasma glucose was assayed spectrophotometrically according to the glucose oxidase/peroxidase method developed by Huggett and Nixon,[10] except that 8 U/mL glucose oxidase and 8 U/mL peroxidase were used and 4-amino-antipyrine was used as the dye.

The final plasma sample from each rat was assayed for insulin by a double-antibody radioimmunoassay technique using rat insulin standards (Novo Biolabs, Bagsbaevard, Denmark) and Linco (St Louis, MO) antibodies (guinea pig anti–rat insulin antibody, goat anti–guinea pig IgG serum antibody, and guinea pig carrier IgG) according to the manufacturer's instructions.

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**Table 1. Whole-Body Insulin Sensitivity at 18 and 32 Weeks of Age**

| | $R_d$ (mg · kg$^{-1}$ · min$^{-1}$) | | GIR (mg · kg$^{-1}$ · min$^{-1}$) | |
|---|---|---|---|---|
| Age Group | Basal | Physiological | Basal | Physiological |
| 18 weeks | 7.68 ± 0.76 | 27.2 ± 1.4 | 0 | 24.7 ± 1.8 |
| 32 weeks | 7.30 ± 0.86 | 22.8 ± 3.2 | 0 | 23.5 ± 1.7 |

NOTE. Basal and physiological refer to the insulin concentration.

Abbreviations: $R_d$, rate of tracer disappearance from the blood; GIR, glucose infusion rate.

### *Tissue Processing*

All tissues (100 to 200 mg) were digested in 1 mol/L KOH as described previously.[7] Hydroxide digests were assayed for the rate of glucose uptake, lipogenesis, and glycogenesis. An estimation of the glucose uptake rate was made by separating 2-[$^3$H]deoxy-D-glucose phosphate from 2-[$^3$H]deoxy-D-glucose using ion-exchange chromatography. The rate of glycogenesis was estimated by measuring the amount of $^{14}$C-labeled glycogen that accumulated within each tissue. Glucose uptake and glycogen synthesis were measured as described by Colwell et al.[11]

### *Measurement of Fatty Acid Synthesis*

The rate of lipogenesis within individual tissues was estimated by measuring the amount of $^{14}$C-labeled fatty acids that accumulated within the tissues. Each hydroxide-digest aliquot was saponified by the addition of 1 mL 100% ethanol followed by incubation at 70°C for 20 minutes. Protonation of the fatty acids was effected by the addition of 9 mol/L $H_2SO_4$ (200 μL). Fatty acids were extracted into hexane (two 5-mL washes). Each of the hexane layers was washed with 50 mmol/L NaCl/50 mmol/L $H_2SO_4$ (5 mL) to ensure removal of any hydrophilic $^{14}$C-labeled metabolites. The hexane layers were pooled in a scintillation vial, and the solvent was evaporated. The resultant lipid residue was dissolved in 5 mL toluene/2,5-diphenyloxazole scintillant (6 g/L) and counted for $^{14}$C.

### *Statistical Analysis*

The results are expressed as the mean ± SEM unless otherwise stated. Statistical analysis of the data was performed using one-way ANOVA and the Fisher protected least-significant difference post hoc test (Statview Student; Abacus Concepts, Berkeley, CA).

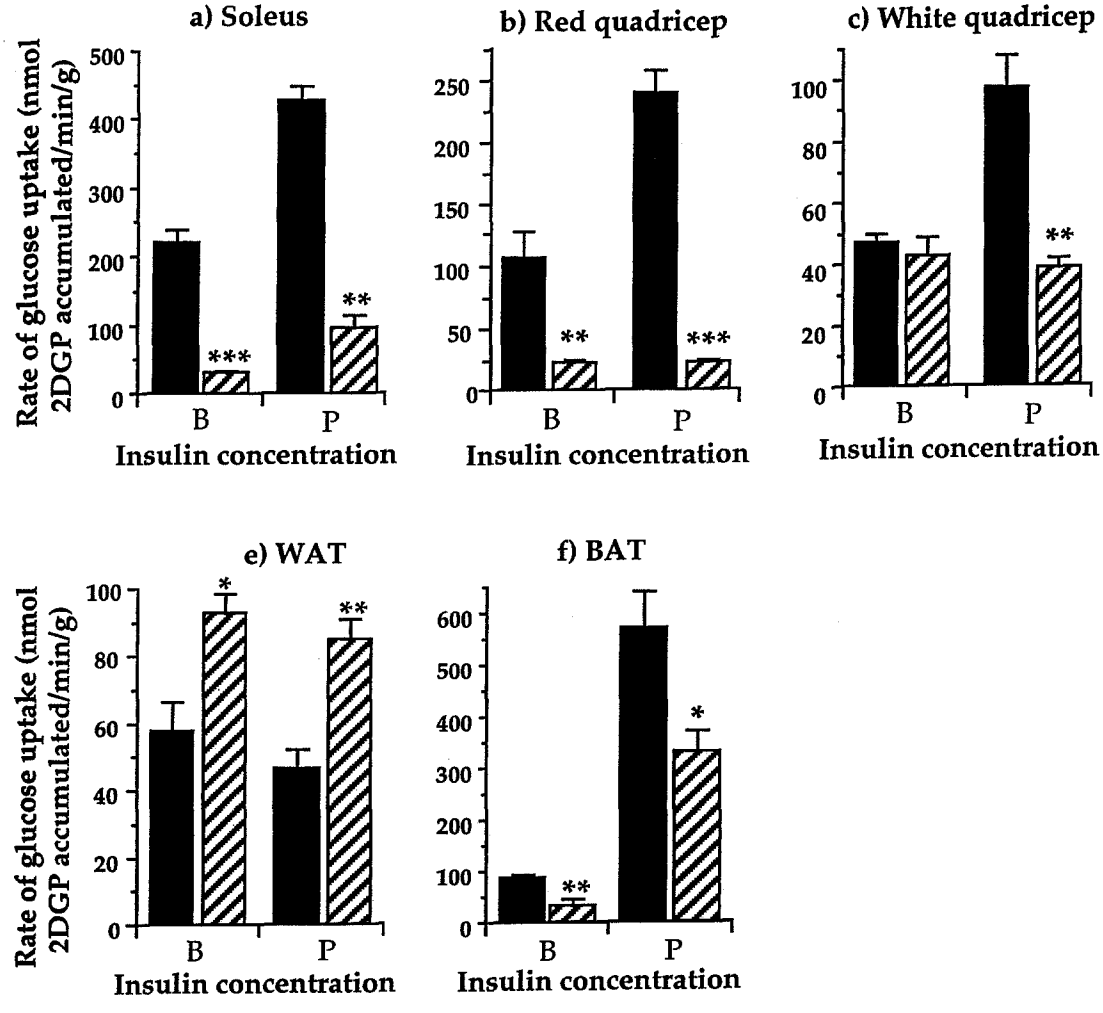


**Fig 1. Rate of glucose uptake at 18 (■) and 32 (▨) weeks of age. Hyperinsulinemic-euglycemic clamps were performed at basal (B) or physiological (P) plasma insulin concentrations. Blood glucose at euglycemia was approximately 4 to 5 mmol/L depending on the fasting blood glucose concentration. Following infusion of a 50-μCi [$^3$H]-2-deoxyglucose bolus, the animals were killed and the (a) soleus, (b) red quadriceps, (c) white quadriceps, (d) WAT, and (e) BAT were excised and subsequently assayed for [$^3$H]-2-deoxyglucose phosphate accumulation. Results are the mean ± SEM. *$P < .03$, **$P < .01$, ***$P < .001$ *v* 18-week group at the same plasma insulin concentration (n = 5 for all but the 18-week physiological group, for which n = 7).**

## RESULTS

### *Whole-Body Insulin Sensitivity*

Age had no effect on the glucose infusion rate required to maintain euglycemia during a hyperinsulinemic-euglycemic clamp. In addition, there was no significant difference in the rate of tracer disappearance between these groups at basal or physiological plasma insulin concentrations (Table 1). These data indicate that whole-body insulin sensitivity was the same at both 18 and 32 weeks of age.

### *Tissue-Specific Insulin Sensitivity*

*Rate of glucose uptake.* Glucose uptake cannot be measured in the liver using 2-deoxyglucose since this tissue has significant glucose-6-phosphatase activity. This enzyme catalyzes the conversion of glucose-6-phosphate to glucose such that 2-deoxyglucose 6-phosphate is not obligatorily accumulated within liver cells. Thus, glucose uptake can only be measured in peripheral tissues using the accumulation of 2-deoxyglucose 6-phosphate as a measure of the rate of glucose uptake.

At basal insulin concentrations, glucose uptake was reduced in the soleus ($P < .001$), red quadriceps ([RQ] $P < .01$), and BAT ($P < .01$) in the 32-week group compared with the 18-week group, but it was increased in WAT ($P < .03$). Insulin administration increased the rate of glucose uptake in all tissues but WAT at 18 weeks of age. In contrast, insulin administration in the 32-week group increased the rate of glucose uptake in soleus and BAT only. At physiological plasma insulin concentrations, the rate of glucose uptake decreased in soleus ($P < .01$), RQ ($P < .001$), white quadriceps ([WQ] $P < .01$), and BAT ($P < .03$) between 18 and 32 weeks of age, but increased in WAT ($P < .01$) (Fig 1).

*Rate of glycogenesis.* At basal insulin concentrations, there was no significant difference in the rate of glycogen synthesis between the 18- and 32-week groups. At 18 weeks of age, insulin administration increased the rate of glycogen synthesis at least threefold in liver, soleus, RQ, WQ, and WAT and more than 10-fold in BAT (Fig 2). However, at 32 weeks of age, an increase in plasma insulin had little or no effect on the rate of glycogen synthesis except in RQ. Hence, the rate of glycogen synthesis was much greater at 18 versus 32 weeks of age at

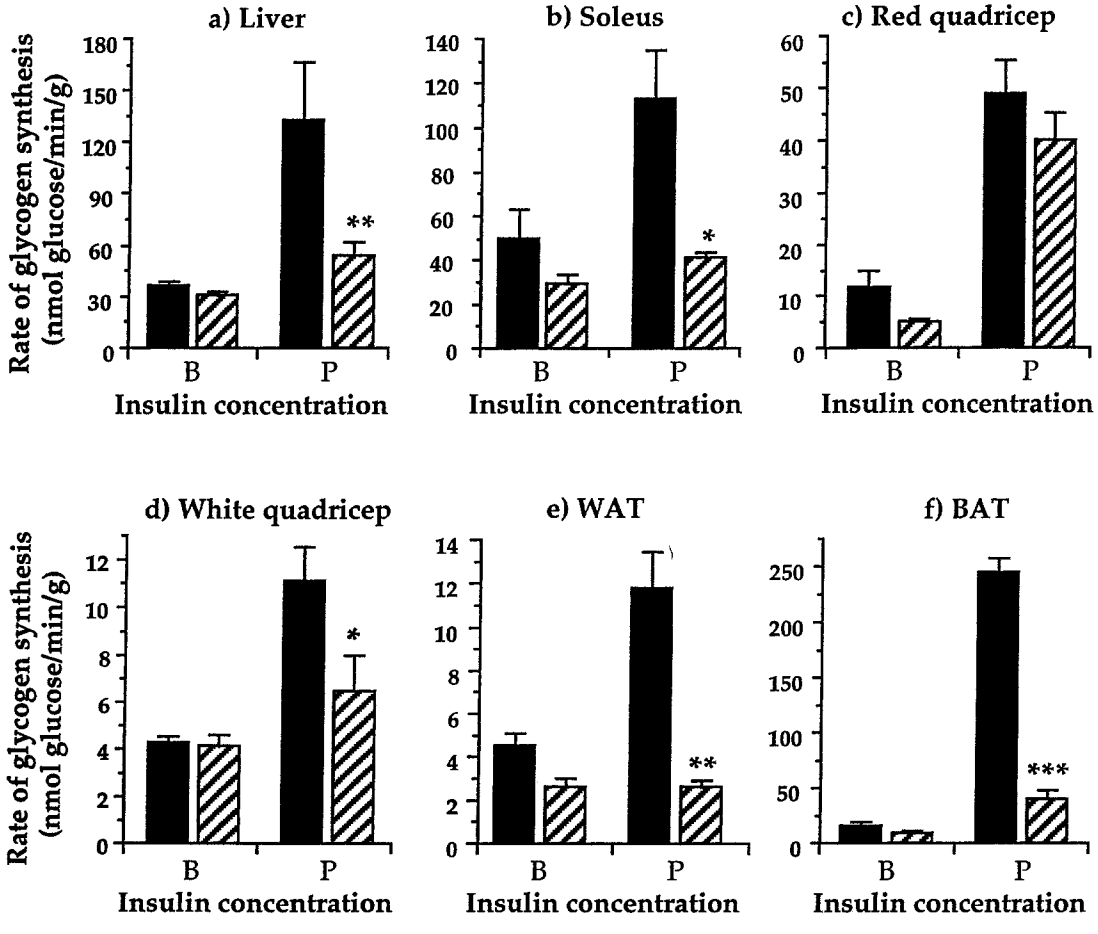


**Fig 2. Rate of glycogen synthesis at 18 (■) and 32 (▨) weeks of age. Hyperinsulinemic-euglycemic clamps were performed at basal (B) or physiological (P) plasma insulin concentrations. Following infusion of a 50-μCi [U-$^{14}$C]-glucose bolus, the animals were killed and the (a) liver, (b) soleus, (c) red quadriceps, (d) white quadriceps, (e) WAT, and (f) BAT were excised and frozen at −180°C until assayed for [$^{14}$C]-glycogen content. Results are the mean ± SEM. *$P < .05$; **$P < .01$, ***$P < .001$ *v* 18-week group at the same plasma insulin concentration (n = 5 for all but the 18-week group at physiological plasma insulin, for which n = 7).**

physiological plasma insulin concentrations in all tissues studied except RQ.

*Rate of lipogenesis.* At basal insulin concentrations, there was no difference in the rate of lipogenesis at 18 or 32 weeks of age. An increase in plasma insulin stimulated the rate of lipid synthesis in both the 18- and 32-week groups in all tissues investigated except RQ (Fig 3). The rate of insulin-stimulated lipogenesis in BAT from 32-week animals was more than double the rate for 18-week animals ($P < .01$). The rate of insulin-stimulated lipogenesis in liver and muscle tended to be lower at 32 weeks versus 18 weeks but did not reach statistical significance.

## DISCUSSION

Although there was no change in whole-body insulin sensitivity between the 18- and 32-week groups, aging caused a dramatic and disparate change in the metabolic profile of the different tissues examined. Glucose uptake and utilization declined in the liver and muscle but increased in the adipose tissues as the animals aged. These changes may cause the increased adiposity that is currently proposed to be responsible for the onset of age-related insulin resistance.[2-5]

At physiological insulin concentrations, a dramatic decrease in the rate of glycogenesis was observed between 18 and 32 weeks of age in all tissues examined, although this did not reach statistical significance in RQ. On a whole-body level, glucose disposal through glycogenesis was reduced more than threefold between 18 and 32 weeks of feeding. These data clearly indicate that glycogen synthesis has lost insulin responsiveness at 32 weeks of age. This is an important discovery, as glycogen synthesis is reportedly more influential than glycolysis in determining the rate of whole-body glucose disposal.[11]

The rate of glucose uptake was severely reduced between 18 and 32 weeks of age in all tissues except WAT (Fig 1). Also, elevated plasma insulin concentrations did not stimulate glucose uptake in WAT. These data may indicate that glucose uptake occurs at a maximal rate even in the fasting state in WAT or, more likely, that a higher insulin concentration is required to stimulate this process in WAT.

Very little of the glucose entering WAT was stored as lipid,

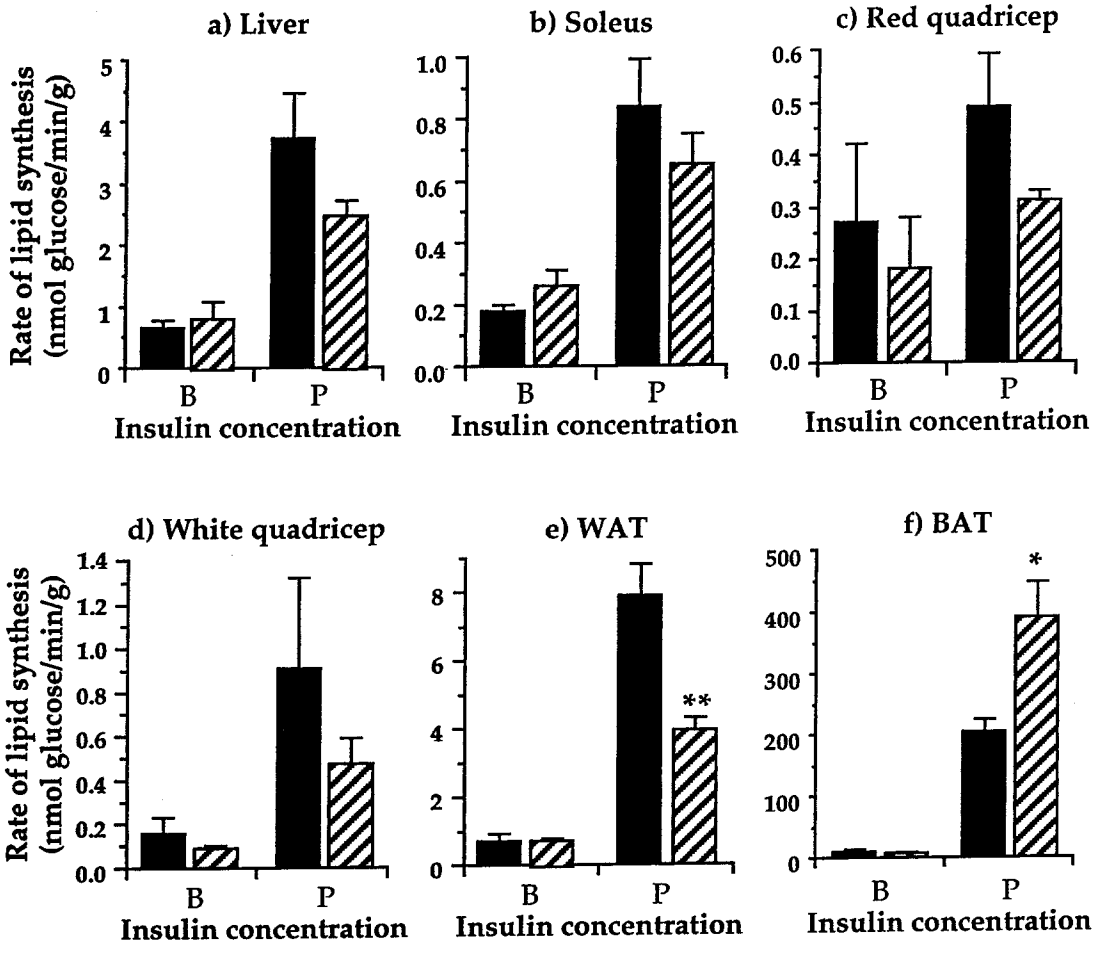


Fig 3. Rate of lipogenesis at 18 (■) and 32 (▨) weeks of age. Hyperinsulinemic-euglycemic clamps were performed at basal (B) or physiological (P) plasma insulin concentrations. Following infusion of a 50-μCi [U-$^{14}$C]-glucose bolus, the animals were killed and the (a) liver, (b) soleus, (c) red quadriceps, (d) white quadriceps, (e) WAT, and (f) BAT were excised, immediately frozen, and subsequently assayed for [$^{14}$C]-lipid content. Results are the mean ± SEM. *$P < .01$, **$P < .001$ *v* 12-week group at the same insulin concentration (n = 5 for all but the 18-week group at physiological plasma insulin, for which n = 7).

particularly at 18 weeks of age. In addition, there was no difference between the rate of glycogenesis and lipogenesis in this tissue at this time. The low rate of glucose storage in WAT relative to glucose uptake may be indicative of an elevated rate of glucose oxidation. It may be that an increased rate of glucose uptake in WAT acts to increase the proportion of glucose undergoing oxidation such that WAT may continue to remove glucose from the bloodstream at a high rate. Perhaps in this way, WAT may contribute to the maintenance of normoglycemia despite decreased insulin responsiveness in other peripheral and/or hepatic tissues.

The operation of a glucose–fatty acid cycle in WAT would dictate that increased glucose utilization in this tissue would prevent lipid oxidation,[12] thereby allowing increased flux through pyruvate dehydrogenase. However, this is unlikely, as the energy demands of WAT are low and there must be a distinct mechanism that increases energy expenditure in this tissue before a higher rate of glucose oxidation can occur. Rather, the expression and/or activity of GLUT4 may have increased in WAT due to age. Determining whether these changes occur in conjunction with an increase in the expression/activity of phosphofructokinase (a rate-limiting step in the glycolytic pathway) requires further investigation at the molecular level. In addition, the rate of lipolysis was not measured in this study but should be investigated in future studies, as it is the balance between lipolysis and lipogenesis that determines fat mass accumulation.

The rate of lipid synthesis in BAT increased twofold between 18 and 32 weeks of age ($P < .001$; Fig 3), although the trend for all other tissues involved a decrease in insulin-stimulated lipogenesis. Thus, it appears that lipogenesis in BAT acquires increased insulin responsiveness between 18 and 32 weeks of age, which is an interesting observation that warrants further investigation, since glucose uptake decreased in this tissue between 18 and 32 weeks.

Changes in tissue-specific insulin responsiveness similar to those observed in this study are associated with the development of insulin resistance in obesity. Early in obesity, some tissues, particularly WAT, exhibit hypersensitivity to insulin, whereas insulin sensitivity in other tissues is reduced.[13,14] Eventually, all tissues become insulin-resistant and insulin resistance can be detected on a whole-body level.[14] In the adult carbohydrate-fed rat used in the present study, there is a deterioration in the insulin responsiveness of all tissues but the adipose tissues between 18 and 32 weeks. However, whole-body glucose homeostasis remains unaffected due to a compensatory increase in glucose uptake in WAT. These animals may be in a "pre–insulin-resistant" state similar to that which occurs during the onset of obesity, as previous studies show that they display whole-body insulin resistance at 52 weeks of age.[7]

Increased rates of glucose uptake and lipogenesis in adipose tissues may play a crucial role in the development of age-related insulin resistance. Current data suggest that a decrease in insulin sensitivity over time is strongly associated with body mass and body composition rather than age per se.[2-5] In light of this evidence, the insulin resistance that has been traditionally associated with aging may be attributed to the decline in physical activity and the increase in body mass and body fat that generally occur with aging. Thus, our discovery that glucose metabolic flux decreases in muscle and liver but increases in the adipose tissues with increasing age provides a mechanism to explain how increased adiposity arises during aging.

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