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Insulin-dependent glycogen synthesis is delayed in onset in the skeletal muscle of food-deprived aged rats

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Abstract

Insulin resistance with aging may be responsible for impaired glycogen synthesis in the skeletal muscle of aged rats and contribute to the well-known decreased ability to respond to stress with aging. For this reason, to assess the ability of the skeletal muscle to utilize glucose for glycogen synthesis during aging, the time course of glycogen synthesis was continuously monitored by ^{13}C nuclear magnetic resonance for 2 h in isolated [^{13}C] glucose-perfused gastrocnemius–plantaris muscles of 5-day food-deprived adult (6–8 months; $n=10$) or 5-day food-deprived aged (22 months; $n=8$) rats. [^{13}C] glucose (10 mmol/L) perfusion was carried out in the presence or absence of an excess of insulin (1 μmol/L). Food deprivation only decreased glycogen level in adult rats (8.9±2.4 μmol/g in adults vs. 35.6±2.4 μmol/g in aged rats; $P<.05$). In the presence of an excess of insulin, muscle glycogen synthesis was stimulated in both adult and aged muscles, but the onset was delayed with aging (40 min later). In conclusion, this study highlights the important role of glycogen depletion in stimulating glycogen synthesis in muscles. Consequently, the absence of glycogen depletion in response to starvation in aged rats may be the origin of the delay in insulin-stimulated glycogen synthesis in the skeletal muscle. Glycogen synthesis clearly was not impaired with aging.

Keywords: Aging; Starvation; Glucose; Glycogen; ^{13}C NMR; Ex vivo perfusion

1. Introduction

Insulin resistance with aging is well documented in humans [1,2] and rodents [3–6] and impairs whole body glucose tolerance. The skeletal muscle is the major site for the action of insulin on glucose uptake and utilization, and insulin resistance in aging is attributed to an insulin postreceptor defect at this site. Insulin resistance may result from a reduced ability of insulin to promote glucose transport, the rate-limiting step of glucose uptake, or by impaired insulin stimulation of intracellular glucose metabolism [7]. More recently, Basu et al. [8] reported that the severity of the defect in insulin action in healthy elderly individuals may be explained by the degree of fatness rather than age per se.

A principal manifestation of aging is a compromised ability of an organism to adapt to the challenges of its environment. A key component of adaptation is the induction of enzymes responsible for the regulation of the flux of metabolites through major metabolic pathways. Any impairment in the induction of these enzymes could potentially contribute to the well-known decreased ability to respond to stress with aging [9–11]. For example, the induction of phosphoenolpyruvate carboxykinase by fasting does not occur rapidly in old rats, although the magnitude of the induction is similar regardless of the age of rats [12]. We hypothesized that the skeletal muscle in aged rats has an impaired ability to up-regulate glycogen synthesis. In young, growing rats, we reported 5-day (5d) starvation leading to a decrease in muscle glycogen concentration and an increase in muscle ability to up-regulate glycogen synthesis [13,14]. No studies have been made with adult and aged rats. For this reason, we studied the glucose utilization for glycogen synthesis in response to an excess of insulin in isolated and perfused mixed fiber muscle from 5d food-deprived adult and aged rats. ^{13}C nuclear magnetic resonance (NMR) allowed us to continuously and simultaneously compare the ability of the skeletal muscle in adult rats with that of aged rats to use

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glucose for glycogen synthesis. Previous ^{13}C NMR studies in rats [15,16] and humans [17,18] have shown the advantages of directly following glycogen synthesis in intact muscle.

2. Methods and materials

2.1. Materials

[^{13}C] glucose was obtained from Tracer Technologies (Somerville, MA, USA). Porcine insulin was purchased from Novo (Bagsvaerd, Denmark).

2.2. Animal care

All rats and experimental procedures were used in accordance with recommendations from the Institutional Ethics Committee of the University of Clermont-Ferrand (France). Adult (6–8 months old) male Wistar rats were purchased from IFFA-CREDO (L'Arbresle, France). Rats were housed in separate cages in a room with a 12-h light/12-h dark cycle (lights on at 8:00 am) at 22°C. Aged rats were housed in the animal facilities until they reached 22 months. Adults were only fed with A03 pellets ("growth diet"). Aged rats were fed with A03 pellets until they attained 10 months and, then, with AO4 pellets ("maintenance diet"; Usine d'Alimentation Rationnelle, Villemoisson/Orge, France). All rats received water ad libitum. At the beginning of experiments, both adult and aged rats weighed between 500 and 600 g; they were deprived of food for 5 days before NMR experiments were carried out.

2.3. Preliminary experiments

These studies were conducted to ascertain the extent of changes induced by a 5d food deprivation period on body weight, the mass of gastrocnemius–plantaris (GP) group of muscles and the characteristic metabolites of these muscles. Adult and aged rats were used. In order to determine whether possible differences between adult and aged rats were due to aging or starvation or both, we also made measurements in fed rats (see Table 1). Body composition of all the rats was determined by dual-energy X-ray absorptiometry measurements on a Hologic QDR-4500 X-ray densitometer (Hologic France, Massy, France) [19] in the teaching hospital (CHU) of Clermont-Ferrand (France). The rats were then anesthetized with sodium pentobarbital (45 μg/g body wt. ip). The GP group of muscles was dissected in situ, weighed, frozen in liquid nitrogen and then analyzed for muscle glycogen, lactate, total creatine (TCr) and adenosine triphosphate (ATP) as previously reported [14]. At the end of the muscle dissection, blood samples were drawn from the heart for determination of plasma glucose level after 5d food deprivation.

2.4. Perfusion experiment

Adult ($n=10$) or aged ($n=8$) 5d food-deprived rats were anesthetized with sodium pentobarbital (45μg/g body wt. ip). The perfused muscle was prepared according to a modified method of Meyer et al. [20]. The small superfused epitrochlearis, as previously used in aging studies [6], was not practical because of the low sensitivity of NMR. The GP group of muscles and the popliteal artery and vein were exposed. Branches of the artery supplying other structures were doubly ligated. Finally, the artery was cannulated and the veins draining the muscle were cut. Immediately, muscles were perfused with Krebs–Henseleit buffer pH 7.35, isolated and tied by proximal and distal tendons at resting length on a support that was specifically designed for a 20-mm NMR tube. The preparation was then placed in the NMR tube. At time 0 of the experiment, the perfusion medium was replaced by a Krebs–Henseleit buffer containing 3.5% bovine serum albumin, 20% fresh ovine red cells, all amino acids from plasma at physiological levels [21], 0.7 mmol/L papaverine chloride and [^{12}C] glucose (10 mmol/L) for 20 min. The tube was quickly placed in the magnet. [^{12}C] glucose was then changed by [^{13}C] glucose with or without an excess of insulin (1 μmol/L) for 2 h (insulin level was very high in order to obtain maximum response of glycogen synthesis). The

Table 1
Effect of 5d food deprivation on animal characteristics from adult and aged rats[a]

	Fed		Fasted		Significant effects[b]
	Adult	Aged	Adult	Aged	
Whole body					
Body weight on the day of experiment[c] (g)	537±18	603±33	486±15	517±20	F
Fat mass (%)	18.4±9.0	27.6±2.9	19.0±11.0	31.7±4.7	A
Lean body mass (%)	78.8±8.6	68.7±2.3	77.8±11.3	65.7±4.9	A
Muscle					
GP muscle mass[d] (% body weight)	0.56±0.01	0.47±0.02	0.57±0.01	0.50±0.02	A
Glycogen (μmol/g)	29.1±2.4[a]	32.3±2.4[a]	8.9±2.4[b]	35.6±2.4[a]	F, A×F
Plasma					
Glucose (mmol/L)	8.9±0.5	9.1±0.5	5.6±0.5	5.0±0.5	F

Means without a common letter differ ($P<.05$).

[a] Values are means±S.E. ($n=5$ in each group).

[b] A indicates aging; F, food deprivation; A×F, interactions between aging and food deprivation.

[c] The day of experimentation corresponded to the last day of fasting (or d5) for food-deprived rats and the day of sacrifice for fed rats.

[d] Muscle mass was the mass of the GP muscle group at d5.

perfusion medium was warmed at 37°C and recirculated throughout the experiment. The stability of this preparation was previously verified by phosphorus 31 (^{31}P) NMR as described elsewhere [22]. Although there was a significant increase in muscle water (as shown by an increased wet weight of 24±2% for the perfused muscle compared with the control muscle), no changes appeared in muscle pH (about 7) or in ATP or phosphocreatine (PCr) content (taking into account the volume increase) throughout the 2-h experiment (in fed rats, 5.21±0.53 vs. 5.19±0.44 μmol/g and 25.7±2.9 vs. 19.6±1.7 μmol/g for ATP and PCr, respectively, on perfused vs. control muscles). These findings are consistent with those of Meyer et al. [20] in the perfused mixed mammalian muscle. We also verified that glycogen and lactate levels were not altered after surgery [e.g., in fed rats (n=5), 31.9±4.9 and 29.4±0.3 μmol/g for glycogen in adult and aged rats, respectively, and 2.30±0.54 and 4.61±2.7 μmol/g for lactate in adult and aged rats, respectively). Criteria of metabolic viability used to validate the model were in good agreement with those reported by Bonen et al. [23].

2.5. Ex vivo ^{13}C NMR experiment with perfused muscle

The experiments were performed at 100.13 MHz in a Bruker AMX400 spectrometer equipped with a thermostated 20-mm ^{13}C/^{1}H probe. Shimming was performed on the water resonance and a line width of 55 Hz was typically obtained. ^{13}C spectra of muscle ex vivo were acquired with complete proton decoupling mode in 19-min blocks and consisted of 512 scans with an acquisition time of 55 ms, a recycle time of 2 s, pulse angles of 60° and a sweep width of 32 kHz. Spectra were routinely processed with an exponential line broadening of 20 Hz. Basal ^{13}C spectrum was acquired during the perfusion of [^{12}C] glucose. The time course of glycogen synthesis from [1-^{13}C] glucose in muscle ex vivo was measured from the change in the peak height of the [1-^{13}C] glycogen signal (100.6 ppm) over time (about 2 h). Experiments were realized in the presence (n=5 adults; n=4 aged rats) or the absence (n=5 adults; n=4 aged rats) of insulin excess. At the end of the last spectrum of each experiment, muscles of adult and aged rats were quickly freeze clamped and stored in liquid nitrogen pending deproteinization for further analysis.

2.6. Biochemical analysis

Glycogen, lactate, TCr and ATP of muscles were determined enzymatically as previously described [14,22]. Glucose was measured in the blood from fed and food-deprived rats by the glucose oxidase technique using a Beckman Glucose Analyser (Beckman Instruments, Palo Alto, CA, USA).

2.7. Calculations

Free [1-^{13}C] glucose into the muscle and synthesized [1-^{13}C] glycogen were normalized to the size of the total and labeled creatine (Cr) concentration. PCr and Cr, which were present in the muscle at a natural abundance level (1.1%) and remained unchanged throughout the experiment, resonated in the same peak at 55 ppm.

2.8. Statistical analysis

Values are given as means±S.E. (n=4–5 in each group). Two-way ANOVA was performed to discriminate either between the effects of aging (A) and food deprivation treatment (F) and their interactions (A×F) or aging(A) and insulin treatment (I) and their interactions (A×I). Two-way ANOVA with repeated measurements was performed to discriminate between the effects of aging (A), time (T) and their interactions (A×T) on kinetics of glycogen synthesis. Comparisons between two means were made using unpaired Student's t tests. The level of significance used was .05.

3. Results

3.1. Animal characteristics

Body weight was similar in adult and aged rats (Table 1). It was decreased by starvation to a smaller extent in aged rats than in adult rats (11% vs. 15%; P<.05). As expected, aged rats showed an increase in body fat (P<.05) along with a decrease in lean body mass (P<.05) and in the mass of GP muscle (about 15%; P<.05). However, these parameters were not altered by fasting, as previously reported in female food-deprived rats [24]. Muscle lactate (∽2 μmol/g), ATP (∽5 μmol/g) and TCr (∽32 μmol/g) always remained at the same levels in all groups, whereas glycogen showed a drastic decrease (P<.05) in food-deprived adult rats when compared with the other 3 groups (Table 1). Plasma glucose was similar in adult and aged rats and showed a large decrease under starvation regardless of their age.

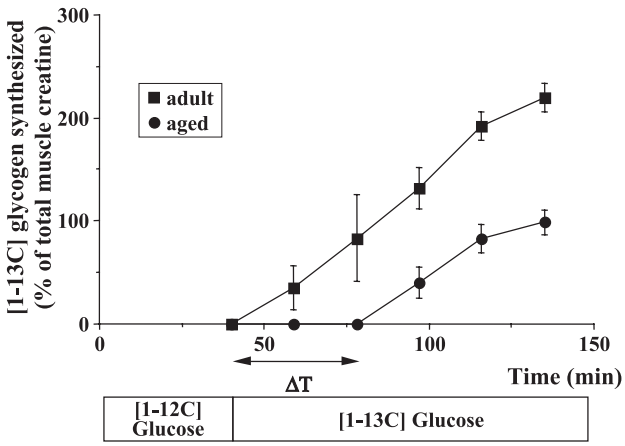

Fig. 1. Mean time course of glycogen synthesis measured by ^{13}C NMR in food-deprived adult and aged muscles perfused with insulin (1μmol/L). Values were expressed in % of muscle TCr, which gave a peak that remained stable during [1-^{13}C] glucose perfusion. Values are means±S.E. (n=4–5 independent experiments in each group). Two-way ANOVA with repeated measurements was performed to discriminate between the effects of aging (A), time (T) and their interactions (A×T) on glycogen synthesis. A significant effect of A×T was observed (P<.0001).

Table 2
Effect of insulin on glycogen level in the glucose-perfused muscle of food-deprived adult and aged rats[a]

Treatment[b]	Adult		Aged	
	Without insulin	With insulin	Without insulin	With insulin
Glycogen (μmol/g)	9.6±1.9	21.8±1.9	6.4±1.9	20.1±1.9
Significant effects[c]	A (NS), I (P<.05), A×I (NS)			

[a] Values are means±S.E. (n=4–5 in each group).
[b] The GP group of muscles was 2-h glucose perfused in the presence (1 μmol/L) or in the absence of insulin.
[c] A indicates aging; I, supply of insulin; A×I, interactions between aging and insulin; NS, not significant (P>.05).

3.2. Time course of glycogen synthesis

Glycogen synthesis from [1-^{13}C] glucose appeared in the presence of insulin whatever the age of the rats (Fig. 1), whereas it remained near zero in the absence of insulin (data not shown). Indeed, the peak of [1-^{13}C] glycogen occurred within 78 min in adults while glycogen synthesis started a further 40 min later in aged rats and was lower than in adult rats (Fig. 1). Both curves were significantly different because a cross-effect of aging and time was determined (P<.0001). However, a plateau of [1-^{13}C] glucose was obtained within 78 min in adult and aged muscles (not shown).

3.3. Effect of insulin excess on total glycogen level

In the presence of an excess of insulin, total glycogen level was significantly repleted in the adult muscle after 135-min perfusion of D[1-^{13}C] glucose but had not reached the value of in situ-fed muscle (see Table 1). In the absence of insulin, the glycogen level in the adult muscle remained low, whereas in the aged muscle it decreased to the same value as in adults (Table 2). After 135-min perfusion of D[1-^{13}C] glucose with insulin, glycogen concentrations were similar in both adult and aged muscles (Table 2).

4. Discussion

In the present study, we used ^{13}C NMR spectroscopic techniques to evaluate continuously nonoxidative disposal of glucose for glycogen synthesis in the presence or absence of an excess of insulin in the rat skeletal muscle during aging. The use of perfused muscle, which is difficult to perform in rats because of the small size of their arteries, allowed us to specifically study the role of insulin in the ability of muscles to use glucose after glucose deprivation induced by 5d starvation without other hormonal interactions. We also observed that, in the presence of an insulin excess, the pathway of muscle glycogen synthesis was not impaired with aging but just delayed in onset. This work provides the first evidence, to our knowledge, of the time-dependent response of glycogen synthesis in the rat skeletal muscle with aging.

Glycogen synthesis in the skeletal muscle of adult and aged rats was dependent on the presence of insulin. This confirms that insulin resistance does occur in adults [3,4,6]. One possible explanation is that impairment occurs early in intracellular glucose metabolism concomitantly with an initial, rapid and disproportionate increase in fat mass compared with lean body mass (rats, >300 g) as reported by Barzilai and Rossetti [25]. In our experiment, rat body weight was greater than 500 g despite a constant fat mass ≥20% in adults. Gupta et al. [26] demonstrated that the greatest decrease in insulin responsiveness in rats occurs when fat mass is >14% of body weight. Unlike caloric restriction, 5d starvation, before muscle perfusion, was not sufficient to reduce the fat mass in either adult or aged Wistar rats. Consequently, the insulin resistance of glycogen synthesis that we observed in perfused fasted muscle may have been due, to a large extent, to the prior excess fat mass in our adult and aged rats.

In summary, this study illustrates the use of ex vivo muscle perfusion as a model to directly investigate the kinetics of glucose utilization in glycogen synthesis in adult and aged starved rats. One particular advantage of this approach is the ability to yield information on the specific contribution of insulin excess to skeletal muscle glycogen synthesis with aging. This study highlights the important role of glucose in stimulating glycogen synthesis in muscles after depletion, a result which seems to be consistent with recent studies on human muscle in vivo [27] and on human muscle cells in culture [28]. Furthermore, the absence of glycogen depletion may be the origin of the delay in insulin-stimulated glycogen synthesis in the muscle of aged fasted rats. A glycogen level similar to that in the fed state may play the role of a limiting factor in glycogen synthesis, as reported by Laurent et al. [27].

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