Chronic Intake of Sucrose Accelerates Sarcopenia in Older Male Rats through Alterations in Insulin Sensitivity and Muscle Protein Synthesis

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Abstract

Background: Today, high chronic intake of added sugars is frequent, which leads to inflammation, oxidative stress, and insulin resistance. These 3 factors could reduce meal-induced stimulation of muscle protein synthesis and thus aggravate the age-related loss of muscle mass (sarcopenia).

Objectives: Our aims were to determine if added sugars could accelerate sarcopenia and to assess the capacity of antioxidants and anti-inflammatory agents to prevent this.

Methods: For 5 mo, 16-mo-old male rats were starch fed (13% sucrose and 49% wheat starch diet) or sucrose fed (62% sucrose and 0% wheat starch diet) with or without rutin (5 g/kg diet), vitamin E (4 times), vitamin A (2 times), vitamin D (5 times), selenium (10 times), and zinc (+44%) (R) supplementation. We measured the evolution of body composition and inflammation, plasma insulin-like growth factor 1 (IGF-I) concentration and total antioxidant status, insulin sensitivity (oral-glucose-tolerance test), muscle weight, superoxide dismutase activity, glutathione concentration, and in vivo protein synthesis rates.

Results: Sucrose-fed rats lost significantly more lean body mass (−8.1% vs. −5.4%, respectively) and retained more fat mass (+0.2% vs. +33%, respectively) than starch-fed rats. Final muscle mass was 11% higher in starch-fed rats than in sucrose-fed rats. Sucrose had little effect on inflammation, oxidative stress, and plasma IGF-I concentration but reduced the insulin sensitivity index (divided by 2). Meal-induced stimulation of muscle protein synthesis was significantly lower in sucrose-fed rats (+7.3%) than in starch-fed rats (+22%). R supplementation slightly but significantly reduced oxidative stress and increased muscle protein concentration (+4%) but did not restore postprandial stimulation of muscle protein synthesis.

Conclusions: High chronic sucrose intake accelerates sarcopenia in older male rats through an alteration of postprandial stimulation of muscle protein synthesis. This effect could be explained by a decrease in insulin sensitivity rather than by changes in plasma IGF-I, inflammation, and/or oxidative stress.

Keywords: sarcopenia, protein synthesis, fructose, antioxidant, insulin sensitivity, rats

Introduction

Aging is associated with a progressive loss of skeletal muscle mass and function (sarcopenia), which leads to a decrease in mobility and independence and an increase in morbidity (1, 2). It can result from decreased physical activity, endocrine changes, neural factors, inadequate nutrition, and inflammation but also occurs in well-nourished active healthy elderly subjects. Consistent findings show that this could be partly explained by a decreased ability of aged muscle to respond appropriately to food intake; in adults, the meal stimulates muscle protein synthesis and inhibits muscle proteolysis, which results in a net gain of muscle protein mass called anabolism. Daily, this anabolism compensates fasting net protein loss. During aging, a defect in muscle protein synthesis response to the anabolic effect of the meal develops (3–5), leading to an imbalance between protein anabolism and catabolism during the day and a slow erosion of muscle protein mass.

Meal-induced stimulation of muscle protein synthesis is mainly caused by combined action of insulin and absorbed amino acids.
Amino acids, in particular, Leu (a substrate for protein synthesis and a signaling molecule), stimulate protein synthesis; insulin has a permissive effect on this stimulation and facilitates muscle nutrient delivery through an increase in blood flow (6, 7). During aging, there is a decrease in the sensitivity of muscle protein synthesis to amino acids/Leu (4, 5, 8) and an impaired endothelial responsiveness, explaining an altered insulin action on muscle blood flow (9).

In addition to "normal" aging, factors that increase insulin resistance, inflammation, and oxidative stress (these factors being related) aggravate muscle anabolism defect. Low-grade inflammation accelerates sarcopenia (10) and triggers, in old rats, the defect in muscle protein synthesis response to the meal (11). Its prevention restores meal response and improves muscle mass (8, 12). Antioxidant supplementation normalizes the sensitivity of muscle protein synthesis to stimulation by Leu in old rats (13). Inflammation and oxidative stress reduce endothelial responsiveness to insulin (9). Consistently, insulin resistance is associated with lean mass loss in older men (14), and in older diabetic patients, this lean mass loss can be attenuated by insulin sensitizers (15).

Other factors are now in motion in our societies that could add to normal aging and accelerate age-related deterioration of meal-induced muscle anabolism. Indeed, several authors (16–18) consider that "added sugars," i.e., glucose, fructose, sucrose, etc., which are added in food products, are responsible for the development of dyslipidemia, hypertension, insulin resistance, oxidative stress, and inflammation. Mechanisms involved are complex, but fructose is supposed to play a central role (18, 19). Since 1949, US sugar deliveries for human consumption have increased by ~75% (20). In 1970, the situation worsened with the introduction of high-fructose corn syrups, which increased fructose proportion in sugar intake. At the world level, added sugar intake is still increasing (18). Many studies evaluated the effect of added sugar intake on metabolic syndrome in humans or animal models. However, very few focused on aging, and, to our knowledge, no studies analyzed added sugar effects on sarcopenia, which was the aim of this study. Our hypothesis was that added sugars can induce insulin resistance, inflammation, and oxidative stress, this could, in turn, aggravate the age-related defect in meal-induced muscle anabolism and accelerate sarcopenia.

In addition, added sugar-induced metabolic disturbances are reduced by polyphenols/antioxidants such as resveratrol (21), allium sativum (16), quercetin (22), cranberry pomace (23), or grape seed extract (24). Similarly, we showed that rutin; vitamins E, A, and D; selenium; and zinc (R) supplementation restored muscle protein synthesis sensitivity to Leu in old rats (13). We proposed that R supplementation could also be efficient to counteract our hypothetical accelerating effect of added sugars on sarcopenia. Thus, to test the effect of added sugars on sarcopenia, and the interaction with R supplementation, we fed 16-mo-old Wistar rats for 5 mo with a control diet, with or without R supplementation, or with a diet in which starch was replaced by sucrose, with or without R supplementation. For 5 mo, we followed the evolution of body weight, food intake, lean body mass, fat mass, and plasma markers of inflammation, and after 5 mo, we compared muscle mass, insulin sensitivity, plasma insulin-like growth factor 1 (IGF-I) concentration, oxidative stress, and the meal response of in vivo muscle protein synthesis.

Methods

Animals and diet. This experiment was conducted in accordance with institutional guidelines on animal experimentation in France and was approved by the Ethics Committee in Animal Experiments of Avignon (registration number CE 56-12). Male 16-mo-old Wistar rats (n = 120; Janvier) were housed under controlled environmental conditions (21°C, 55% hygrometry, 12-h dark period starting at 0700) and were allowed free access to water and food (standard pellets, UAR 04). After 1 mo of acclimation, rats were randomly divided into 4 groups (n = 30/group) and were fed 4 different diets as dry powders: starch-fed [St (13% sucrose and 49% wheat starch diet)] rats were fed a control diet [AIN-93 (23)]; St + R supplementation rats were fed the control diet supplemented with rutin (5 g/kg of dry matter in substitution with cellulose), vitamin E (all-rac-a-tocopherol acetate, 300 IU/g, 60 g/kg vitamin mixture instead of 14.2), vitamin A (all-trans-retinyl palmitate, 300,000 IU/g, 1.6 g/kg vitamin mixture instead of 0.8), vitamin D (cholecalciferol-D3, 500,000 IU/kg vitamin mixture instead of 100,000), selenium (sodium selenite anhydrous, 0.14 g/kg mineral mixture instead of 0.01025), and zinc (zinc carbonate, 2.95 g/kg mineral mixture instead of 1.65); sucrose-fed [Su (62% sucrose and 0% wheat starch diet)] rats were fed the control diet in which wheat starch was replaced by sucrose; Su + R supplementation rats were fed the sucrose diet with R supplementation. Food was freely available between 0830 and 1600 every day. During the first 2 mo, rats consumed food ad libitum, and then, because of small differences in food intake, the amount of food offered was adjusted to ~22 g/d, which was totally consumed in all groups. Food intake was monitored every 2–3 d during the first 2 mo, and then every day. Body weight was measured once a week. Body composition was recorded before and at the end of the experimental period using MRI (Echo MRI International). At 0, 3, and 5 mo, a blood sample was drawn from a lateral tail vein to measure plasma acute-phase proteins (α-2-macroglobulin and fibrinogen). The penultimate week, oral-glucose-tolerance tests (OGTTs) were performed. Rats were fed glucose by gavage (2 g of glucose/kg body weight using a 50% glucose solution in tap water). Blood samples were collected in tubes containing EDTA from the tail vein at −2, 15, 30, 60, and 120 min after glucose administration. Finally, in the fifth month of the experiment, in vivo muscle protein synthesis rates were measured either in the postabsorptive state (after overnight food deprivation) for one-half of the rats or in the postprandial state (2 h after food distribution) for the other half of the rats. Thus, rats studied during the postprandial state had continuous access to food during 2 h. They consumed ~16 of the 22 g of food offered.

The final number of rats per group ranged from 8 to 10.

Measurements of in vivo protein synthesis and tissue sampling. Protein synthesis rates were measured using the flooding dose method. Twenty minutes before death, each rat was injected intravenously with i-Val (150 μmol/100 g body weight) containing 80% of L-[1-13C] Val (Eurisko-Top) to flood the precursor pool. Rats were then killed after anesthesia with pentobarbital sodium (6 mg/100 g body weight) by exsanguination through the abdominal aorta. Posterior leg skeletal muscles (gastrocnemius, tibialis anterior, extensor digitorum longus, and soleus) were quickly excised, weighted, and frozen in liquid nitrogen until further analysis. Protein synthesis rates were measured in the tibialis anterior as described previously (26) by measuring muscle-free amino acid incorporation (μmol/100 g tissue/60 min) in rats after bilateral hindlimb perfusion. We measured muscle-free amino acid incorporation (μmol/100 g tissue/60 min) in rats after bilateral hindlimb perfusion. This was done to ensure an accurate measurement of muscle protein synthesis rates.

Calculations. In vivo muscle fractional synthesis rates (FSRs; percent per day) were calculated as described previously (3); FSR = 100 ×
(EP - EN)/(EA × t), where t is incorporation time, expressed in days; EP and EA (atom %) are 13C enrichments of protein-bound Val and muscle-free Val, respectively; and EN (atom %) is an estimation of the natural 13C enrichment of protein-bound Val. It was determined in rats that were not injected with the flooding dose (2–3/group). Absolute synthesis rates (ASRs) were calculated from the product of FSRs with protein content and expressed in milligrams per day. Ribosomal capacity was calculated as the ratio of total RNA (most of the RNA in tissues is ribosomal) to protein (milligrams of RNA per gram of protein). Ribosomal efficiency was calculated as the amount of protein synthesized (milligrams) per day per milligram of RNA.

**Plasma measurements.** Plasma fibrinogen was measured by turbidimetry on an automated chemistry analyzer (ABX Pentra 400; Horiba). Plasma concentration of α-2-macroglobulin was measured by ELISA (ELISA kit from Immunology Consultants Laboratory, Inc.). For OGTTS, insulin concentration was determined with a commercial ELISA kit (10-1250-01; Mercodia AB) and glucose concentration was determined using the ABX Pentra 400 analyzer and glucose PAP kit (Horiba). Plasma total antioxidant capacity was measured using the TAS kit (Randox Laboratories) and ABX Pentra 400. Plasma IGF-I concentration was also measured at the end of the experiment by ELISA (R&D Systems).

**Muscle oxidative status.** Total glutathione content (reduced + oxidized) was determined in the tibialis anterior as previously described by Malmzæt (27) on the ABX Pentra 400. In the same muscle, superoxide dismutase activity was measured using the RANSOD kit (Randox Laboratories) after homogenization of an aliquot of frozen muscle powder in the SD124 buffer of the kit (phosphate buffer) and centrifugation.

**Statistical analysis.** Data were analyzed 1) by 2-factor ANOVA, carbohydrate (starch or sucrose) × R supplementation (with or without R supplementation); 2) by 3-factor ANOVA, carbohydrate × R supplementation × nutritional state (postabsorptive or postprandial) for muscle protein metabolism; 3) by repeated-measures 2-factor ANOVA, carbohydrate × R supplementation × time, when measurements were repeated over time (body weight, food intake, and inflammation); and 4) by 3-factor ANCOVA, carbohydrate × R supplementation × nutritional state × total muscle protein content for muscle FSRs. Fisher’s test was used for post hoc comparisons of means. The t test was used to assess if differences in body composition before and after the experiment were different from 0. Survival was compared between groups using log-rank test. We used SAS software. Data are expressed as means ± SEs. The level of significance was set at P < 0.05.

**Results**

**Food intake, body weight, and survival.** During the ad libitum intake periods, sucrose feeding induced a 12% decrease in food intake (P < 0.01; Supplemental Figure 1). Although these differences were small, we adjusted food intake of all groups to the intake of Su + R supplementation rats (~22 g/d, which is sufficient). Thus, during this pair-feeding period (Supplemental Figure 1), there was no longer any significant difference in food intake between groups.

Weight was similar in all groups at the beginning of the experiment (St: 607 ± 13 g; St + R supplementation: 611 ± 14 g; Su: 605 ± 13 g; Su + R supplementation: 608 ± 14 g). It was stable in all groups during the first 2 mo, and then, in all groups, it decreased significantly (P < 0.01) during months 3, 4, and 5 (from month 1 to 5, respectively: St, 609 ± 15 g, 615 ± 15 g, 583 ± 14 g, 564 ± 12 g, and 537 ± 11 g; Su + R supplementation, 618 ± 14 g, 626 ± 14 g, 600 ± 14 g, 586 ± 11 g, and 558 ± 11 g; Su, 606 ± 11 g, 606 ± 11 g, 585 ± 9 g, 571 ± 11 g, and 568 ± 10 g; Su + R supplementation, 605 ± 15 g, 610 ± 15 g, 589 ± 13 g, 578 ± 13 g, and 574 ± 14 g). This time-related decrease in weight was significantly different in sucrose- and starch-fed rats (P < 0.01): the rate of decrease was lower in sucrose-fed rats (~2%/mo) than in starch-fed rats (~4%/mo), especially between months 4 and 5 (~5% in starch-fed rats, ~1% in sucrose-fed rats). Consequently, month 5 was the only month where a significant difference was observed between groups: weight was significantly higher in sucrose-fed rats than in starch-fed rats (P = 0.04). There was never any significant effect of R supplementation.

The total number of spontaneous deaths over 5 mo was 9 in St, 10 in St + R supplementation, 11 in Su, and 11 in Su + R supplementation groups. There was no significant difference in survival between groups. This mortality rate (34% at 22 mo) was close to what is usually observed, 50% mortality at 23–24 mo (28).

**FIGURE 1** Evolution of body composition in older male rats fed diets containing 13% or 62% sucrose with or without R supplementation for 5 mo. We calculated for each rat the percent change = 100 × (final mass - initial mass)/initial mass. (A) Lean body mass. (B) Fat mass. Two-factor ANOVA, C × R supplementation, were conducted, and significant effects are given. Values are means ± SEs (n = 18–20). *Significantly different from 0. Labeled means without a common letter differ, P < 0.05. C, carbohydrate; R, rutin, vitamin E, vitamin A, vitamin D, selenium, and zinc.
Body composition and tissue weight. Lean body mass decreased significantly (P < 0.01) in all groups during the experimental period (Figure 1A). This loss was significantly higher in sucrose-fed than in starch-fed rats (P = 0.03). There was no effect of R supplementation.

Fat mass decreased significantly during the experimental period only in St rats (Figure 1B). A significant effect of carbohydrate feeding (P < 0.01) was detected: sucrose prevented the age-related loss of fat mass. There was also a significant interaction between the effects of carbohydrate and R supplementation: the effect of R supplementation was significantly different (P = 0.03) in starch-fed rats (slowing the age-related loss of fat mass) and in sucrose-fed rats (preventing sucrose-induced increase in fat mass).

When expressed in proportion to body weight, in all muscles, muscle weight was significantly lower in sucrose-fed than in starch-fed rats (Table 1). This was also the case for absolute muscle weight in tibialis anterior and soleus muscles. R supplementation had no effect on muscle weight in any of the muscles. However, in the extensor digitorum longus muscle, for values expressed in proportion to body weight, there was a significant interaction in carbohydrate × R supplementation: the effect of R supplementation was significantly different (P = 0.03) in starch-fed rats (decrease) and in sucrose-fed rats (increase). In any case, it seems clear that sucrose feeding accelerated the age-related loss of muscle mass.

Muscle oxidative stress markers. Sucrose feeding had no effect on tibialis anterior muscle total glutathione content and concentration (Table 2). However, there was a significant interaction between the effects of carbohydrate and R supplementation: R supplementation significantly increased glutathione (micromoles and micromoles per gram) in starch-fed rats, whereas it had no effect in sucrose-fed rats. Regarding muscle superoxide dismutase activity, the only significant effect detected was a decrease induced by R supplementation (Table 2).

Systemic antioxidant and inflammation markers. Plasma antioxidant activity was significantly increased by R supplementation in starch-fed rats (St = 0.76 ± 0.03 mmol/L vs. St + R supplementation = 0.85 ± 0.03 mmol/L) as well as in sucrose-fed rats (Su = 0.79 ± 0.03 mmol/L vs. Su + R supplementation = 0.91 ± 0.03 mmol/L).

Repeated-time ANOVA showed that plasma α2-macroglobulin values tended to be higher (P = 0.06) in sucrose-fed than in starch-fed rats (Figure 2A). Values in all groups decreased during the first 3 mo and then increased during the last 2 mo (P < 0.01). This increase in the last 2 mo was significantly higher in sucrose-fed than in starch-fed rats (P-interaction = 0.02). Over the 5 mo of the experiment, plasma α2-macroglobulin increased by 0.05% and 5.8% in starch-fed rats (St and St + R supplementation, respectively, significant) and by 28% and 35% in sucrose-fed rats (Su and Su + R supplementation, respectively, significant with P = 0.01 only in the Su + R supplementation group). However, final α2-macroglobulin concentrations were not significantly different between groups.

Neither carbohydrate nor R supplementation had an effect on plasma fibrinogen concentrations (Figure 2B), even though there was an effect of time (P < 0.01).

OGTT. Plasma glucose concentrations in feed-deprived rats did not differ among the groups (overall mean ± SE: 5.97 ± 0.26 mmol/L). The plasma glucose concentration reached its peak 15 min after glucose gavage in all groups without significant differences between groups. Plasma glucose AUCs also did not differ between groups.

<table>
<thead>
<tr>
<th>Muscle absolute weight</th>
<th>St</th>
<th>St + R supplementation</th>
<th>Su</th>
<th>Su + R supplementation</th>
<th>ANOVA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius, g</td>
<td>2.19 ± 0.06</td>
<td>2.13 ± 0.09</td>
<td>1.97 ± 0.10</td>
<td>2.15 ± 0.09</td>
<td>ns</td>
</tr>
<tr>
<td>Tibialis anterior, g</td>
<td>0.64 ± 0.02</td>
<td>0.65 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>0.61 ± 0.03</td>
<td>C, P = 0.02</td>
</tr>
<tr>
<td>EDL, mg</td>
<td>215 ± 4</td>
<td>214 ± 6</td>
<td>197 ± 8</td>
<td>214 ± 6</td>
<td>ns</td>
</tr>
<tr>
<td>Soleus, mg</td>
<td>228 ± 10a</td>
<td>228 ± 10a</td>
<td>198 ± 10b</td>
<td>216 ± 11b</td>
<td>C, P = 0.04</td>
</tr>
</tbody>
</table>

**TABLE 1** Muscle weights of older male rats fed diets containing 13% or 62% sucrose with or without R supplementation for 5 mo1

<table>
<thead>
<tr>
<th>Muscle relative weight</th>
<th>St</th>
<th>St + R supplementation</th>
<th>Su</th>
<th>Su + R supplementation</th>
<th>ANOVA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius, %BW</td>
<td>0.41 ± 0.01</td>
<td>0.39 ± 0.02</td>
<td>0.35 ± 0.01</td>
<td>0.37 ± 0.01</td>
<td>C, P &lt; 0.01</td>
</tr>
<tr>
<td>Tibialis anterior, %BW</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>C, P &lt; 0.001</td>
</tr>
<tr>
<td>EDL, % BW</td>
<td>0.41 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.35 ± 0.01</td>
<td>0.37 ± 0.01</td>
<td>C, P &lt; 0.001</td>
</tr>
<tr>
<td>Soleus, % BW</td>
<td>0.43 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.35 ± 0.01</td>
<td>0.37 ± 0.02</td>
<td>C, P &lt; 0.001</td>
</tr>
</tbody>
</table>

**TABLE 2** Tibialis anterior muscle glutathione concentration and SOD activity of older male rats fed diets containing 13% or 62% sucrose with or without R supplementation for 5 mo1

<table>
<thead>
<tr>
<th>Glutathione concentration, μmol/g</th>
<th>St</th>
<th>St + R supplementation</th>
<th>Su</th>
<th>Su + R supplementation</th>
<th>ANOVA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71 ± 0.03b</td>
<td>0.80 ± 0.04b</td>
<td>0.82 ± 0.04b</td>
<td>0.74 ± 0.04b</td>
<td>C × R supplementation, P = 0.03</td>
<td></td>
</tr>
<tr>
<td>Total glutathione, μmol</td>
<td>0.46 ± 0.02c</td>
<td>0.53 ± 0.03b</td>
<td>0.48 ± 0.03c</td>
<td>0.43 ± 0.04b</td>
<td>C × R supplementation, P = 0.05</td>
</tr>
<tr>
<td>SOD activity, U/mg protein</td>
<td>5.39 ± 0.17a</td>
<td>5.09 ± 0.17b</td>
<td>5.72 ± 0.17a</td>
<td>5.23 ± 0.17b</td>
<td>R supplementation, P = 0.01</td>
</tr>
</tbody>
</table>

1 Values are means ± SE (n = 17–19). Labeled means in a row with a common letter differ, P < 0.05. C, carbohydrate; R, rutin, vitamin E, vitamin A, vitamin D, selenium, and zinc; SOD, superoxide dismutase; St, starch fed (13% sucrose and 49% wheat starch diet); Su, sucrose fed (62% sucrose and 0% wheat starch diet).

2 ANOVA significant effects.
Fasting plasma insulin concentrations were higher in sucrose-fed than in starch-fed rats (P = 0.03). Group means ± SEs were as follows: St, 1.01 ± 0.30 μg/L; St + R supplementation, 0.97 ± 0.31 μg/L; Su, 1.38 ± 0.19 μg/L; and Su + R supplementation, 1.87 ± 0.28 μg/L. The mean value in the Su + R supplementation group was significantly different from values in the St and St + R supplementation groups. After glucose gavage, significant differences appeared at 30 and 60 min (higher levels in sucrose-fed than in starch-fed rats) and the AUC was also significantly higher. As a result, insulin sensitivity, calculated according to Matsuda and DeFronzo (29), was lower in sucrose-fed than in starch-fed rats (P < 0.01; Figure 3). R supplementation had no significant effects on insulin sensitivity, glycemia, or insulin concentrations before or during OGTT.

IGF-I. Plasma IGF-I concentrations did not differ among the groups (overall mean ± SE: 899 ± 22 μmol/L).

**Muscle protein metabolism.** Sucrose induced a significant loss of protein mass (P = 0.05) without affecting muscle protein concentration (Table 3). R supplementation slightly but significantly increased muscle protein concentration (P < 0.01) but had no effect on protein mass (Table 3). Ribosomal capacity (RNA content per gram of protein) was increased by sucrose feeding (P = 0.03); in response to R supplementation, it remained stable in starch-fed rats, whereas it decreased in sucrose-fed rats; thus, there was a significant interaction of carbohydrate × R supplementation (P = 0.05). FSRs (percent protein synthesized per day − FSR), ASRs (amount of protein synthesized per day − ASR), and ribosomal efficiency (RE; amount of protein synthesized per day and per gram of RNA − RE), which are the only markers sensitive to the nutritional state, were, as expected, stimulated by feeding (Table 3). It was the only significant effect for ASR and RE. For FSR, we showed previously that this marker is highly correlated to the age-related loss of muscle mass, which introduces “noise” when studying the effect of feeding (30). To take this factor into account, a good solution is to use a variance-covariance analysis using total protein as a covariate (measured independently of FSR and highly significant, P < 0.001). This analysis detected a significant interaction between carbohydrate and nutritional state (P = 0.04), showing that muscle protein synthesis was less stimulated by the meal in sucrose-fed than in starch-fed rats (Figure 4). ASR, FSR, and RE mean values seemed specifically low in Su + R supplementation postprandial rats, suggesting an effect of R supplementation on the postprandial response of muscle protein synthesis in sucrose-fed rats. However, this would require a significant carbohydrate × R supplementation × nutritional state interaction and it was not the case (P > 0.3). In addition, such an effect is not consistent with other results (evolution of lean body mass and lack of effect of R supplementation). Sucrose was responsible for the decreased postprandial response of muscle protein synthesis (Figure 4).

**Discussion**

The aim of this study was to determine if added sugars can modulate the age-related loss of muscle mass (sarcopenia). Additionally, we tested the capacity of R supplementation to prevent hypothetical deleterious effects of added sugars. We showed that feeding old rats over 5 mo with a diet in which all starch was replaced by sucrose induced an accelerated age-related loss of lean body mass and hind limb muscle mass and that R supplementation had little influence on this phenomenon.

The aging rat is a convenient model to study sarcopenia because sarcopenia is rapid and well characterized in this animal (3). Because fructose is considered central in the deleterious effects of added sugars on metabolic syndrome, authors often used “rodent added sugar model” diets containing only fructose and no starch or glucose (18). However, it seemed more relevant to the human situation to use a sucrose model; we estimate that our rats consumed ~4 times the average intake of added sugars observed in humans, with a proportion of fructose close to what is observed in humans (50% in our rats, ~47% in humans).
Although many studies analyzed the effects of added sugar intake on liver, adipose tissue, brain, or systemic homeostasis (31), few studies focused on muscle, especially during aging. To our knowledge, no other studies analyzed the effect of added sugars on sarcopenia. We showed that sucrose accelerated the age-related loss of lean body mass and hind limb muscle mass. In accordance with our results, in growing rats, high sucrose intake during 48 d also reduced gastrocnemius muscle mass (22). Thus, it seems clear that added sugars can accelerate sarcopenia.

Because the evolution of muscle mass is dependent on the change in muscle protein synthesis, we analyzed the effects of sucrose on muscle FSRs, ASRs, and RE. Although there were tendencies, it was only for FSRs that a significant interaction was detected between sucrose and nutritional state, showing that sucrose reduced postprandial stimulation of FSRs. This lower meal-induced stimulation of FSRs could explain the observed sucrose-induced accelerated loss of muscle proteins. A similar phenomenon has been observed during normal aging in rats (3) and in humans (32). It is now recognized that a decrease in the sensitivity of muscle protein synthesis to meal stimulation develops during aging (8). Our results suggest that added sugars potentiate this phenomenon.

This could be caused by insulin sensitivity modifications. Indeed, we showed, using OGTT, that sucrose-fed rats had a lower sensitivity to insulin. After glucose load, glycemia was not different in starch- and sucrose-fed rats, but more insulin was necessary in sucrose-fed rats to achieve this. No similar OGTT study was performed in sucrose- or fructose-fed aging rats. Fasting glycemia was measured after 9, 18, or 26 mo of high sucrose feeding in rats (33); fasting glycemia was increased in sucrose-fed rats compared with starch-fed rats only after 26 mo. The effect of added sugars on insulin resistance was more extensively studied in growing rats. Clamp studies performed after 1, 2, 5, and 8 wk of sucrose feeding showed that hepatic insulin resistance appears after 1 wk, and muscle insulin resistance appears progressively, beginning at week 2 (34). Other studies confirmed that evolution of glycemia after OGTT was close to normal in sucrose- or fructose-fed growing rats (23, 35). Interestingly, the use of an intraperitoneal glucose tolerance test (16) led to more marked differences in glycemia between growing fructose-fed rats and control rats. This
could be because of the effects of glucagon-like peptide 1 and gastric inhibitory polypeptide that are bypassed when using an intraperitoneal injection. Thus, added sugars clearly induce alterations in insulin sensitivity, but glycemia is often still maintained.

However, to our knowledge, the effect of added sugars on insulin action on protein metabolism was never studied. Insulin has a permissive effect on the stimulation of muscle protein synthesis by amino acids (6). It also increases muscle blood flow (7). Muscle mass is decreased in many insulin-resistant disease states (diabetes, cancer, and renal failure). In a model of muscle insulin receptor knockout mice, it was shown that muscle mass (and function) was also reduced and that it was not due to oxidative stress or increased proteolysis; it seemed only caused by the lack of insulin signaling toward protein synthesis (36). Thus, in our study, it is possible that sucrose altered insulin-mediated protein synthesis activation and that resulted in sarcopenia acceleration. This could start a vicious cycle because reduced muscle mass (a major site for substrate oxidation) promotes insulin resistance.

We wondered whether alterations of growth hormone/IGF-I axis could be induced by sucrose feeding and be partly responsible for acceleration of lean body mass loss in sucrose-fed rats. It seems that it was not the case because plasma IGF-I concentrations were not different in sucrose- and starch-fed rats, which is consistent with results obtained in young rats fed a 60% fructose diet for 12 wk (37). Thus, modification in insulin sensitivity remains our main hypothesis to explain the observed effect of fructose on sarcopenia acceleration.

Another way for added sugars to affect muscle protein synthesis would be inflammation. Indeed, we showed previously that it was not the case because plasma IGF-I concentrations were 78% of previously observed values and α-2-macroglobulin concentration only 3% of previously observed values. Similarly, sucrose had also a marked effect on body fat mass; it increased in Su rats whereas it decreased in St rats. Such an increase in fat mass after high-sucrose (38) or -fructose (39, 40) feeding was already described in growing rats. It seems recognized that a high-sucrose diet is able to upregulate the lipogenesis pathway (38). The decrease observed in St rats is consistent with results observed in aging rats (41). It must be noted that in our study, R supplementation reduced the age-related decrease in fat mass and prevented the fructose-induced increase in fat mass. The effects were not marked, but it is remarkable to see that they were opposite in starch- and sucrose-fed rats, although it always tended to normalize fat mass. This brings to mind the ponderostat hypothesis (42). Indeed, the ponderostat hypothesis suggests that a ponderostat signal (which could be estrogen, an estrogen synthesized in white adipose tissue and stored as oestyl-estrone) could be released in the bloodstream in proportion to adipose tissue mass, and the brain could then trigger the release of diverse hormones and signals to bring back adipose tissue mass to a "normal" value. Glucocorticoids could be specifically responsible for alterations in this ponderostat setting (42). A possible interaction between antioxidants and estrogen is unknown. However, polyphenols were already shown to have an influence on body fat mass regulation (43–45) and to modulate glucocorticoid action (46).

Apart from this effect on fat mass, R supplementation had the expected effects on plasma antioxidant value and small effects on muscle oxidative stress. It had no effect on inflammation but inflammation was low. In addition, it had no effects on sucrose-induced alterations in insulin sensitivity. However, for similar doses, rutin was shown to reduce glycemia in streptozotocin-induced diabetes (47) and to improve OGTT in insulin-resistant mice (48). Other polyphenols (green tea polyphenols and grape seed extracts) improved insulin signaling or insulin sensitivity during high fructose intake (24, 49). It is thus surprising to have no effect of rutin in our experiment. It could be related to the fact that the rats were older than in other published experiments.

In addition, R supplementation had no effects on lean body mass, muscle mass, or muscle protein synthesis rates. However, there was a tendency for a positive effect on muscle total protein content (P = 0.08) and a significant positive effect on muscle protein concentration. It has been previously shown that a similar supplementation was able to restore a normal stimulation of muscle protein synthesis by Leu (13, 50). Thus, it is possible that a small positive effect of such supplementation on muscle protein mass exists, maybe through stimulation of muscle protein synthesis or through inhibition of muscle proteolysis.

In conclusion, sucrose feeding accelerated the age-related loss of lean body mass and muscle mass. The mechanism involved could be alterations in insulin sensitivity leading to a weaker stimulation of muscle protein synthesis by the meal. R supplementation could not prevent this phenomenon, although it had a small effect on muscle protein mass in starch- and sucrose-fed rats. The main effect of R supplementation was a ponderostat-like effect on body fat mass. Thus, long-term consumption of added sugars can accelerate sarcopenia and should be avoided.

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