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Participation of β-adrenergic activity in modulation of GLUT4 expression during fasting and refeeding in rats

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Abstract

Through in vitro studies, several factors have been reported as modulators of GLUT4 gene expression. However, the role(s) of each potential GLUT4 modulator is not completely understood in the in vivo setting. The present study has investigated the hypothesis that β-adrenergic stimulation participates in modulation of GLUT4 expression during fasting and refeeding. As such, GLUT4 messenger RNA (mRNA) and protein were investigated in insulin-sensitive tissues during a 48-hour fast. In addition, the effects of 8-hour refeeding on GLUT4 mRNA in the gastrocnemius muscle and interscapular brown adipose tissue (BAT) were investigated. Whether β-adrenoceptor blockade by propranolol (20 mg/kg) treatment influenced the responsiveness to fasting/refeeding was also investigated. The results show that fasting repressed GLUT4 gene and protein expression in BAT, white adipose tissue, and soleus muscle, but had no effect on the gastrocnemius muscle. Refeeding induced a rapid overexpression of GLUT4 mRNA in both gastrocnemius (~25%, $P < .05$) and BAT (~200%, $P < .001$). Propranolol treatment induced an increase (~60%, $P < .05$) in GLUT4 mRNA at the end of the fasting period. In contrast, propranolol treatment attenuated GLUT4 mRNA induction after refeeding; the latter may be due to attenuation of postprandial insulin levels. These results suggest that sympathetic activity is important for the repression of GLUT4 gene expression during fasting. In contrast, sympathetic control of the GLUT4 gene seems to be overbalanced by metabolic/hormonal modulators during refeeding stage. Taken together, the results suggest that feeding behavior influences GLUT4 gene expression pattern through changes in sympathetic activity, especially during long-term starvation periods.

1. Introduction

The "thrifty" gene hypothesis was proposed 40 years ago [1] in an attempt to explain the increasing incidence of obesity and diabetes. This theory proposes that genes allowing conservation of glucose and efficient storage of energy during periods of food abundance aid survival during subsequent food scarcity. This genetic profile was considered to have been beneficial to the survival of the Paleolithic hunter-gatherer and fundamental to human evolution. However, these previously beneficial genes became deleterious as food availability increased in modern life.

The GLUT4 gene is a thrifty gene that codifies a glucose transporter expressed in tissues with high glucose uptake capacity when the substrate is abundantly disposable and insulin levels are increased, as occurs in the postprandial period [2]. On the other hand, whole-body insulin resistance occurs during fasting [3], conserving glucose that may be consumed by other tissues. In white adipose tissue (WAT), GLUT4 gene expression decreases during fasting and, during refeeding, rapidly increases to levels above those observed in the fed state [4,5], a response that typifies thrifty genes. In skeletal muscle, the primary tissue involved in whole-body GLUT4-mediated glucose uptake [6], reduced glucose utilization is observed in the fasting state [3], although the manner in which the GLUT4 gene is regulated remains controversial [7,8].

Recently, Stannard and Johnson [9] highlighted that physical activity is required before dietary nutrients could be utilized or stored in humans, suggesting that thrifty genes might have a secondary role in survival when compared with genes that preserved physical capacity during food deprivation. Physical activity of a strenuous nature would be

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required for hunting, gathering, and avoidance of predation [9]. Moreover, physiologic cold defenses involving high-energy consumption may also have been fundamental to human evolution [10]. As such, the regulation of the GLUT4 gene in skeletal muscle and brown adipose tissue (BAT) is undoubtedly complex because preservation of glucose uptake by these tissues during fasting or cold exposure is of paramount importance.

High sympathetic outflow is fundamental to heat production by BAT during cold exposure [11,12]. In addition, if a stimulus such as strenuous physical activity is imposed during fasting, a high sympathetic activity response occurs. These observations suggest that sympathetic activity may be a modulator of GLUT4 gene expression, thereby playing an important role in glucose homeostasis during fasting, physical activity, and cold exposure. Consistent with such a hypothesis, in vitro studies have clearly shown that cyclic adenosine monophosphate (cAMP) represses GLUT4 gene expression in both cultured adipocytes [13-15] and myotubes [16]. However, the true role of sympathetic activity in vivo as a modulator of GLUT4 gene during fasting and refeeding had never been investigated.

The goal of the present study was to investigate (1) whether 48-hour fasting modulated GLUT4 messenger RNA (mRNA) and protein abundance in insulin-sensitive tissues, (2) whether 8-hour refeeding modulated GLUT4 mRNA expression in the gastrocnemius skeletal muscle and interscapular BAT, and (3) whether β-adrenergic blockade by propranolol treatment attenuates the effects of fasting and/or refeeding on GLUT4 expression.

2. Materials and methods

2.1. Animals

Male Wistar rats were purchased from the Animal Center of the Institute of Biomedical Sciences, University of São Paulo (São Paulo, Brazil), and were studied at 2 months of age. The animals were maintained at 23° ± 2°C on a 12:12-hour light-dark cycle (lights on from 6 AM to 6 PM) and allowed free access to water and standard rodent chow (Nuvilab CR-1, Nuvital, Curitiba, Brazil) until the day of the experiments. One week before the experiments, animals were transferred to individual cages. All experiments were carried out at 8 AM unless stated otherwise. Blood samples were either collected from conscious animals (blood drops from tail) or from anesthetized rats (sodium pentobarbital, 50 mg/kg body weight, intraperitoneally) at the time of tissue collection. The experimental protocol (163/02) was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of São Paulo.

2.2. Effects of fasting

Fasted rats submitted to 48 hours of food deprivation (food withdrawal at 8 AM), were compared with ad libitum fed rats, both studied at 8 AM. Blood was collected, and plasma was stored for later analysis of insulin, glucose, and nonesterified fatty acid (NEFA) levels. Samples of epididymal WAT, interscapular BAT, and gastrocnemius and soleus skeletal muscle tissue were collected and immediately processed for total RNA or protein extraction. Additional experiments were performed in rats submitted to 60 hours of food deprivation (food withdrawal at 8 PM and rats killed at 8 AM) or in rats submitted to 48 hours of food deprivation and killed at 12 AM.

2.3. Effects of refeeding

A group of 48-hour–fasted rats were refed ad libitum (food offered at 8 AM). After refeeding (0, 2, 4, or 8 hours), blood, gastrocnemius skeletal muscle, and interscapular BAT samples were collected as described above. Additional groups of conscious rats were used solely for time course blood glucose evaluation on samples obtained from the tail vein by using a glucometer (Precision QID, Medisense, São Paulo, SP, Brazil) at 0, 30, 60, and 90 minutes and 2, 3, 4, 6, and 8 hours of refeeding (refed rats). Rats for which the fast was prolonged served as controls.

2.4. Effect of propranolol on fasting and refeeding

Fasted rats received a single intraperitoneal injection of the β-adrenoceptor antagonist propranolol (20 mg/kg body weight) [17] or the same volume of saline 2 hours before the end of the fast (ie, at 46 hours of food deprivation). These animals were studied at 0 (end of 48-hour fast), 2, 4, and 8 hours of refeeding, and individual food intake was measured at each time point. Samples of blood, gastrocnemius skeletal muscle, and interscapular BAT were collected as described above, and additional groups of conscious rats previously given injections of propranolol or saline were used for blood glucose analysis (glucometer) as described above.

2.5. Northern blotting for GLUT4 mRNA

GLUT4 mRNA was analyzed as previously described [18]. Total RNA was extracted from 100 mg of snap-frozen

Table 1
General characteristics of ad libitum fed and 48-hour–fasted rats

	Fed	Fasted
Body weight (g)	213 ± 4.3 (18)	183 ± 2.9 (25)***
Soleus muscle weight (mg)	92.1 ± 6.8 (6)	80.2 ± 6.1 (6)
Gastrocnemius muscle weight (g)	1.21 ± 0.03 (12)	1.03 ± 0.04 (15)**
BAT weight (mg)	230 ± 9.1 (5)	198 ± 9.0 (5)*
WAT weight (g)	1.35 ± 0.09 (9)	1.09 ± 0.07 (7)*
Plasma glucose (mmol/L)	6.4 ± 0.2 (18)	4.0 ± 0.1 (25)***
Plasma insulin (μU/mL)	6.1 ± 0.9 (11)	3.9 ± 0.6 (14)*
Plasma free fatty acids (mmol/L)	0.171 ± 0.04 (7)	0.424 ± 0.06 (8)**

Data were obtained at 8 AM. Initial body weight (48 hours earlier) was 201.8 ± 1.8 and 204.8 ± 2.0 g, respectively, for ad libitum fed and 48-hour–fasted rats. Data are mean ± SEM, and the number of animals is indicated between parentheses.

* $P < .05$ vs fed rats, Student t test.

** $P < .01$ vs fed rats, Student t test.

*** $P < .0001$ vs fed rats, Student t test.

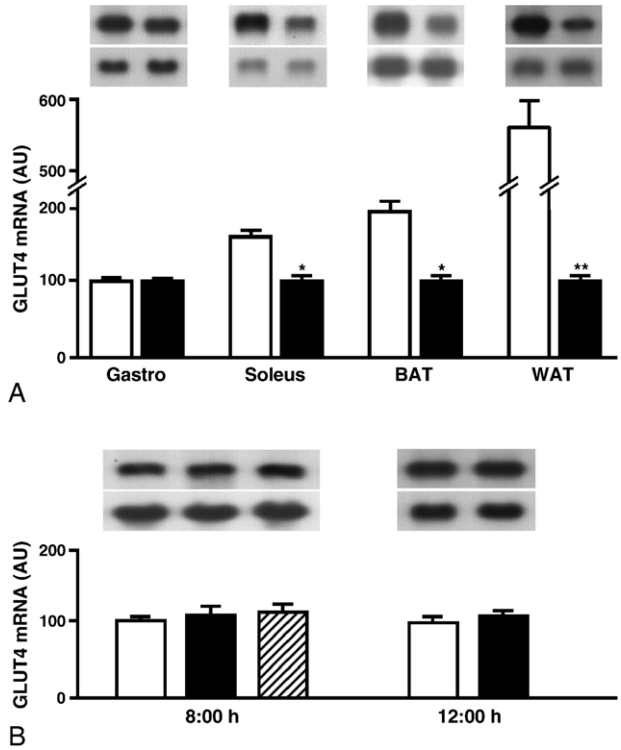

Fig. 1. GLUT4 mRNA in the gastrocnemius muscle (Gastro), soleus muscle (Soleus), interscapular BAT, and epididymal WAT in ad libitum fed and fasted rats. A, GLUT4 mRNA was analyzed in ad libitum fed (open bars) and 48-hour–fasted (closed bars) rats in tissues harvested at 8 AM: top, typical images of the GLUT4 and respective β-actin mRNAs; bottom, the relative content of GLUT4 mRNA was normalized by the β-actin value. Data are mean ± SEM of 5 to 15 animals evaluated in at least 4 different experiments. B, GLUT4 mRNA was analyzed in the gastrocnemius muscle from ad libitum fed (open bars), 48-hour–fasted (closed bars), and 60-hour–fasted (shaded bars) rats in tissues harvested at 8 AM, or in ad libitum fed (open bars) and 48-hour–fasted (closed bars) rats in tissues harvested at 12 AM. The relative content of GLUT4 mRNA was normalized by the β-actin value. Data are mean ± SEM of 5 animals, evaluated in at least 4 different experiments. *$P < .05$ and **$P < .01$ vs fed rats (Student t test).

tissues (except for WAT, 500 mg) by using TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Twenty micrograms of total RNA was fractionated by 1% agarose-formaldehyde gel electrophoresis, transferred to nylon membranes, and prehybridized in 0.5 mol/L Na_2HPO_4 (pH 6.8), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, 1 mmol/L EDTA, and 100 μg/mL salmon sperm DNA solution for 3 hours at 65°C. Subsequently, the membranes were probed with an [α-^{32}P]2′-deoxycytidine 5′-triphosphate (dCTP)-labeled rat GLUT4 complementary DNA by random priming (Random Primers DNA Labeling System, Invitrogen Life Technologies), 2×10^6 cpm/mL overnight at 65°C. The membranes were washed under high-stringency conditions and exposed to Hyperfilm (Amersham Pharmacia Biotech, Amersham, UK) at −70°C for 5 days. After that, the membranes were stripped and rehybridized with β-actin probe. The blots were analyzed by scanner densitometry (Image Master 1D, Pharmacia Biotech, Uppsala, Sweden), and the results were expressed as arbitrary units (AU) after normalization by the respective β-actin value, considering the mean of fasted rats 100%.

2.6. Western blotting for GLUT4 protein

Immunoblotting was carried out as previously described [18]. Equal amounts of total membrane protein (30 μg) were solubilized in sample buffer (0.5 mol/L TRIS-HCl [pH 7.5], 9% SDS, 15% glycerol, 0.05% bromphenol blue, 6% 2-mercaptoethanol), heated in a boiling water bath, and resolved by SDS-polyacrylamide gel (10%) electrophoresis. Proteins were electrotransferred to nitrocellulose membrane, and nonspecific protein binding to the nitrocellulose was reduced by preincubating the membrane in blocking buffer containing 3% nonfat milk. The nitrocellulose membrane was then incubated with anti–GLUT4 antiserum (Polyclonal Rabbit Raised Anti-GLUT4, AB1346, Chemicon International, Temecula, CA) and submitted to enhanced chemiluminescent detection (ECL Western Blotting Detection, RPN2106, Amersham Pharmacia Biotech). The intensity of the blots was quantified by densitometry (Image Master 1D, Pharmacia Biotech), and the protein expression in control rat tissues was normalized as 100%.

2.7. Analytical procedures

Plasma glucose obtained from the animals at sacrifice was determined with a kit from CELM (Barueri, SP, Brazil),

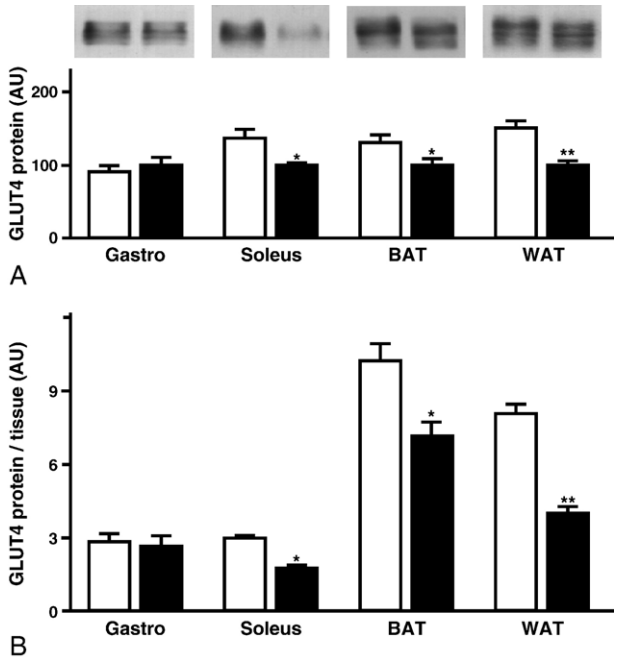

Fig. 2. GLUT4 protein in total membrane fraction from gastrocnemius muscle (Gastro), soleus muscle (Soleus), interscapular BAT, and epididymal WAT of ad libitum fed (open bars) and 48-hour–fasted (closed bars) rats. A, Typical images of the GLUT4 protein (top) and relative content of GLUT4 protein (bottom) in 30 μg of protein subjected to electrophoresis. B, Total tissue GLUT4 protein content, based on the total tissue protein recovery. Data are mean ± SEM of 4 to 8 animals evaluated in at least 4 different experiments. *$P < .05$ and **$P < .01$ vs fed rats (Student t test).

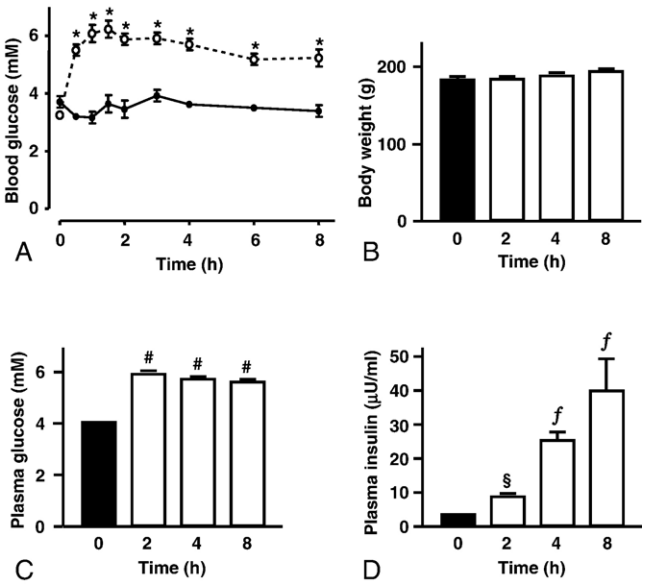

Fig. 3. Effects of refeeding on blood glucose, body weight, and plasma insulin concentration. Rats were fasted for 48 hours (time 0) and then refed for 2, 4, or 8 hours. A, Blood glucose was analyzed in the same conscious rats during refeeding after 48 hours of fasting (open circles) or in those that remained in fasting state (closed circles). Data are mean ± SEM of 6 (fasted) and 10 (refed) animals. Time, $P < .0001$; treatment, $P < .0001$; and interaction, $P < .0001$ (2-way ANOVA); $^{*}P < .0001$ vs time 0 (Student t test). Body weight (B), plasma glucose (C), and plasma insulin (D) in 48-hour–fasted rats (closed bars, time 0) or 48-hour–fasted rats refed for 2, 4, or 8 hours (open bars). Data are mean ± SEM of 14 fasted and 5 to 10 refed animals. $^{\#}P < .001$ vs 0, $^{\S}P < .05$ vs 4 and 8 hours, $^{f}P < .05$ vs all groups (1-way ANOVA, SNK post hoc test).

plasma insulin by radioimmunoassay with a kit from DPC (Los Angeles, CA), and plasma NEFAs were measured based on the method of Chromy et al [19].

2.8. Data analysis

All values were reported as mean ± SEM. At least 4 different experiments of Western and Northern analysis were performed, evaluating at least 5 different animals in each group. Detailed information regarding sample size is included in the legends of the figures and tables. Two means were compared by unpaired 2-tailed Student t test, and 3 or more means were analyzed by 1-way analysis of variance (ANOVA), with Student-Newman-Keuls (SNK) post hoc test. Time course studies involving 2 experimental groups were analyzed by 2-way ANOVA (no repeated measures), and unpaired 2-tailed Student t test for comparison in each time point. Statistical analyses were performed by using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA).

3. Results

3.1. Effects of fasting

Fasting (48 hours) caused a 15% body weight reduction, which was accompanied by a similar reduction in the gastrocnemius muscle and BAT and WAT weights (Table 1). Fasting also decreased plasma glucose and insulin levels by ~37%, whereas it increased plasma NEFA levels by 150% (Table 1).

The GLUT4 mRNA content (Fig. 1A) was significantly reduced in soleus muscle and BAT and WAT of fasted rats.

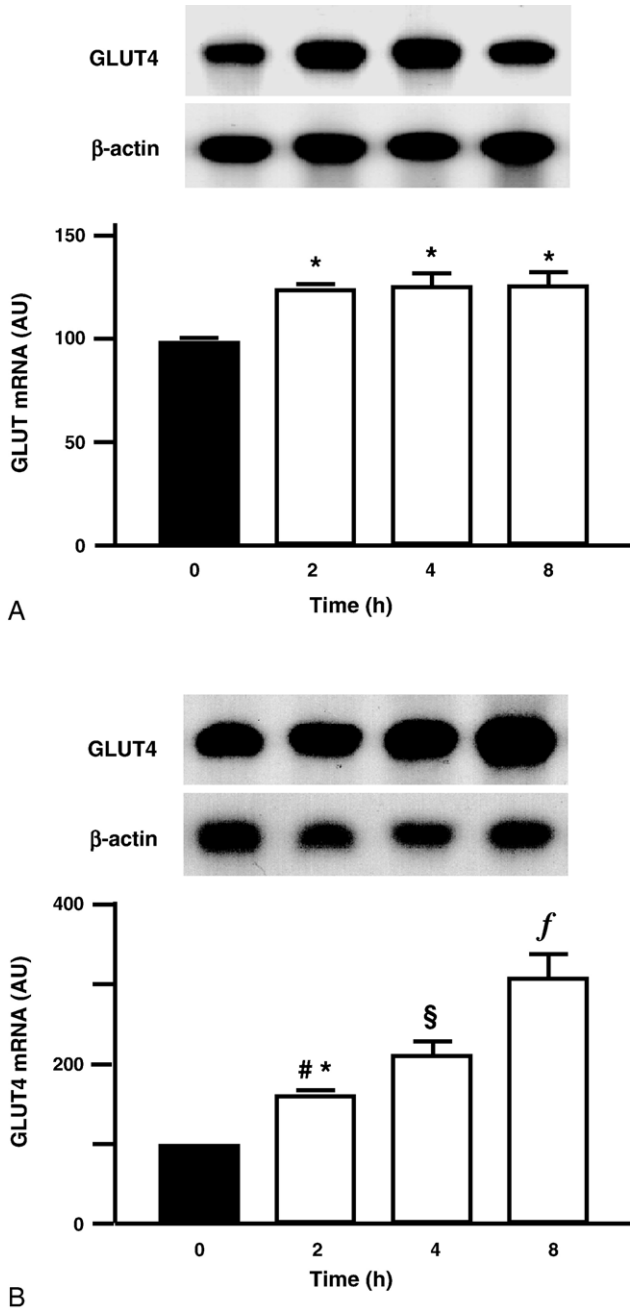

Fig. 4. GLUT4 mRNA in gastrocnemius muscle (A) and interscapular BAT (B) of 48-hour–fasted rats (closed bars, time 0) or 48-hour–fasted rats refed for 2, 4, or 8 hours (open bars). Top, typical images of the GLUT4 and respective β-actin mRNAs; bottom, the relative intensity of GLUT4 mRNA expression was normalized by the β-actin value and expressed as percentage of the fasting value. Data are mean ± SEM of 5 to 15 animals evaluated in at least 4 different experiments. $^{*}P < .05$ vs 0, $^{\#}P < .001$ vs 8 hours, $^{\S}P < .01$ vs 0 and 8 hours, and $^{f}P < .001$ vs 0, 2, and 4 hours (1-way ANOVA, SNK post hoc test).

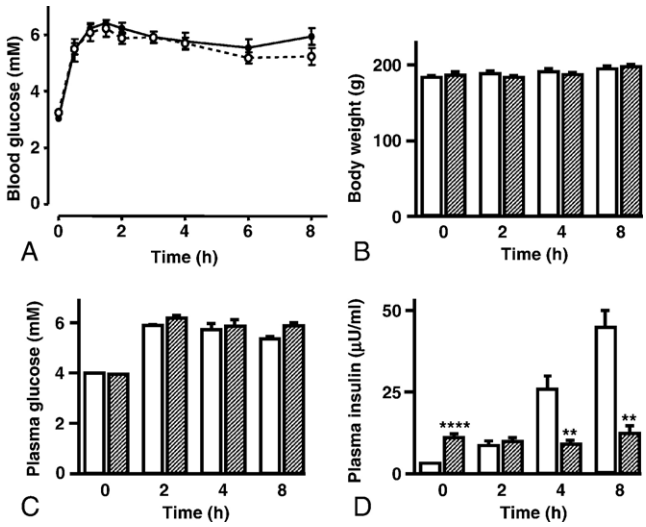

Fig. 5. Effects of refeeding on blood glucose, body weight, and plasma insulin concentration. Rats fasted for 48 hours (time 0) were injected with saline or propranolol 2 hours before the end of fasting and then refed for 2, 4, or 8 hours. A, Blood glucose in conscious rats injected with saline (closed circles) or propranolol (open circles) during 8 hours of refeeding. Data are mean ± SEM of 10 animals. Body weight (B), plasma glucose (C), and plasma insulin (D) in rats given injections of saline (open bars) or propranolol (shaded bars) at the end of fasting (time 0) and after 2, 4, or 8 hours of refeeding. Data are mean ± SEM of 5 to 10 animals. In plasma insulin analysis: time, $P < .0001$; treatment, $P < .001$; and interaction, $P < .0001$ (2-way ANOVA). $^{**}P < .01$ and $^{****}P < .0001$ vs saline-injected rats at the same time (Student t test).

The greatest effect was observed in WAT. However, no significant difference was observed for GLUT4 mRNA of the gastrocnemius muscle between control and 48-hour–fasted rats (Fig. 1A). Similarly, prolongation of the fast to 60 hours or killing of rats at different times of the day (12 AM vs 8 AM) had no significant effects on gastrocnemius GLUT4 mRNA (Fig. 1B).

Consistent with the data presented in Fig. 1, GLUT4 protein measured in a total membrane fraction was reduced in the soleus muscle and BAT and WAT, but not in the gastrocnemius muscle, of 48-hour–fasted rats (Fig. 2A). The total tissue GLUT4 was analyzed by using the total protein recovery from the tissues; again GLUT4 protein was significantly decreased in the soleus muscle and BAT and WAT, but not in the gastrocnemius muscle (Fig. 2B).

Analyzing the different tissues on the same Western experiment (ie, the same membrane) and expressing the data as per gram of tissue, we found that the level of GLUT4 protein expression was soleus > gastrocnemius > BAT > WAT (data not shown).

3.2. Effects of refeeding

The effect of refeeding on glycemic excursions was first analyzed in groups of conscious animals, which were ad libitum refed or maintained under fasting state. The same animals were used for analysis throughout the 8-hour period (Fig. 3A). Refeeding induced a rapid increase in glycemia (to 5.5 ± 0.1 mmol/L at 30 min), remaining stable for the subsequent 8 hours (between 6.2 ± 0.3 and 5.2 ± 0.2 mmol/L at 1.5 and 6 hours, respectively). On the other hand, animals that extended their fasting state maintained glycemia between 3.2 ± 0.2 and 3.9 ± 0.2 mmol/L, values achieved at 1 and 3 hours after the previous 48 hours of food deprivation, respectively.

Eight hours of refeeding was not enough to recover the body weight loss induced by fasting (Fig. 3B). The change in body weight gain at 2, 4, and 8 hours of refeeding was 5.4 ± 0.2, 6.8 ± 0.3, and 11.7 ± 0.9 g, respectively. These values of change in body weight are the same as for food intake measured in each period (5.3 ± 0.9, 6.7 ± 0.9, and 11.6 ± 1.2 g, respectively, for 2-, 4-, and 8-hour refed rats).

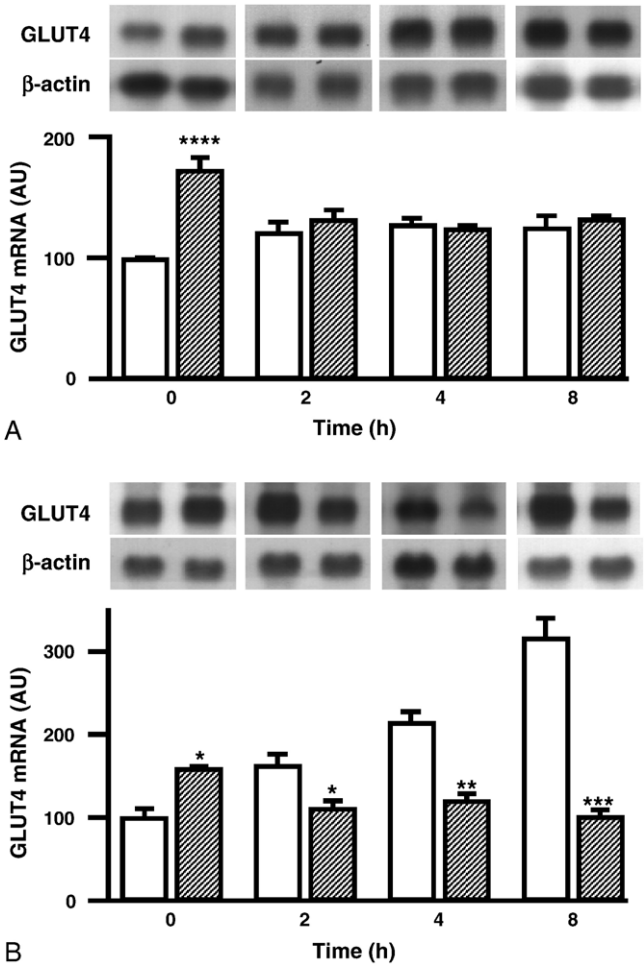

Fig. 6. GLUT4 mRNA in gastrocnemius muscle (A) and interscapular BAT (B). Rats fasted for 48 hours (time 0) were given injections of saline (open bars) or propranolol (shaded bars) 2 hours before the end of fasting and then refed for 2, 4, or 8 hours. Top, typical images of the GLUT4 and respective β-actin mRNAs; bottom, the relative intensity of GLUT4 mRNA expression was normalized by the β-actin value and expressed as percentage of the saline-injected fasted rats. Data are mean ± SEM of 4 to 10 animals, evaluated in at least 4 different experiments. In gastrocnemius analysis: time, $P = .57$; treatment, $P < .001$; and interaction, $P < .0001$; in BAT analysis: time, $P < .001$; treatment, $P < .0001$; and interaction, $P < .0001$ (2-way ANOVA). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, and $^{****}P < .0001$ vs saline-injected rats at the same time (Student t test).

There were no differences between tissue weights within 8 hours of refeeding (data not shown).

Plasma glucose, measured in blood samples collected at the time of sacrifice, was similar to the blood glucose profile observed in conscious rats (Fig. 3C). Plasma insulin increased progressively within 8 hours ($P < .01$) of refeeding (Fig. 3D).

GLUT4 mRNA content in the gastrocnemius was similarly increased (to ~125%) at all times of refeeding (Fig. 4A). On the other hand, in BAT, the GLUT4 mRNA showed a progressive increase to 162% at 2 hours and to 313% at 8 hours of refeeding (Fig. 4B). In BAT, the GLUT4 mRNA in 8-hour refed rats was higher than that of fed rats (313 ± 27 vs 196 ± 15 AU, $n = 5$, $P < .01$). In this regard, both tissues showed refeeding-induced overexpression of the GLUT4 mRNA.

3.3. Effects of propranolol on fasting and refeeding

The effect of propranolol on glycemic homeostasis was analyzed in groups of conscious animals that received a single injection of propranolol or saline 2 hours before starting the 8-hour refeeding. The propranolol-induced β-adrenoceptor blockade did not induce any significant change in glycemic profile (Fig. 5A) nor in body weight evolution (Fig. 5B) during refeeding. In blood samples collected from animals that were killed, plasma glucose was similar between propranolol- and saline-injected rats at each time point (Fig. 5C), and plasma NEFA levels measured at the end of fasting was also unchanged (0.43 ± 0.07 and 0.52 ± 0.05 mmol/L in saline- and propranolol-injected rats, respectively). However, plasma insulin concentration (Fig. 5D) was strongly affected by propranolol. Compared with saline-injected rats, in propranolol-injected rats, plasma insulin at the end of fasting (time 0) was 2.7-fold higher, but it remained suppressed, being 2.7- and 3.1-fold lower at 4 and 8 hours of refeeding, respectively.

Propranolol treatment modulated GLUT4 mRNA expression (Fig. 6) in both gastrocnemius muscle and BAT. At time 0, the propranolol injection 2 hours earlier was able to induce a significant increase in GLUT4 mRNA of 69% (Fig. 6A) in the gastrocnemius and 62% (Fig. 6B) in BAT. During the refeeding progression, the GLUT4 mRNA content in propranolol-injected rats was suppressed, remaining similar to the values of saline-injected rats in the gastrocnemius (Fig. 6A) and becoming significantly lower in BAT (Fig. 6B).

4. Discussion

Prolonged periods of starvation followed by abundant refeeding have been proposed as recurrent feeding behavior in the early times of human evolution. We therefore hypothesized that regulation of GLUT4 gene expression would allow efficient storage of energy during the food abundance period, as well as preserve energy during subsequent periods of food scarcity. In fact, starvation- and refeeding-induced suppression and overexpression, respectively, of the GLUT4 gene have been reported previously in WAT. The latter changes were closely correlated with circulating insulin levels [4,5]. However, subsequent studies have shown that chronic hyperinsulinemia, such as in insulin-resistant states, is associated with a reduction of GLUT4 expression [20,21], suggesting that insulin is not the sole regulator of this gene. Several modulators of the GLUT4 gene expression have been characterized in isolated myocytes and adipocytes, including substrates, hormones, nuclear receptors, as well as oxidative stress and muscle contraction. However, the true role of these potential modulators in the in vivo setting has been difficult to determine because of the redundant control mechanisms that operate to maintain glucose homeostasis.

During fasting, GLUT4 expression has been shown to be modulated in a tissue-specific manner. Fasting causes reduced GLUT4 expression in both white [4,5,8] and brown [8] adipose tissues, but appears to have varying effects in skeletal muscle (likely related to the fiber type) [7]. In the present study, we observed that fasting caused repression of GLUT4 gene in WAT and BAT, as well as in soleus red muscle. On the other hand, GLUT4 expression was unaltered in the gastrocnemius muscle after fasting for varying durations. These observations suggest a greater effect on GLUT4 expression in oxidative vs nonoxidative fiber types after prolonged starvation. Furthermore, these observations are consistent with the hypothesis that glycolytic fibers would be recruited during sudden bursts of physical activity (ie, during hunting), such that maintenance of GLUT4 levels will allow rapid glucose utilization when required.

We next investigated GLUT4 gene expression during refeeding after a period of fasting. Here, only mRNA was analyzed because protein changes were not expected in a short time. Besides, we did not investigate the refeeding effects on WAT because it was previously reported [4,5], and we chose the gastrocnemius muscle because its fiber composition represents the total body skeletal muscle composition [22].

Refeeding induced a rapid increase in GLUT4 mRNA in the gastrocnemius muscle and BAT, highlighting GLUT4 as a thrifty gene because it is rapidly induced during periods of increased food availability.

Modulation of GLUT4 gene expression during both fasting and refeeding may be the consequence of a cluster of metabolic, hormonal, and neural regulators. One purpose of the present study was to investigate the potential role of β-adrenergic sympathetic activity in the fasting- and refeeding-induced modulations of GLUT4 gene expression. As Stannard and Johnson [9] highlighted for the hunter-gatherer, before the food energy could be utilized or stored physical activity of a strenuous nature would be required for hunting, gathering, and resisting predation, conditions in which stress-induced activation of sympathetic activity is expected to be triggered.

Although acute starvation had been considered as a state of stress [23], it is important to point out that increased sympathetic activity has not been observed in several tissues when evaluated by methods such as catecholamine turnover rate analysis, microdialysis, or microneurography. In contrast, fasting-induced decrease in the sympathetic outflow has been reported in BAT [24,25], heart [24,26], pancreas and liver [27], kidney [28], and skeletal muscle [29]. In addition, it has been shown that fasting induces a reduction of central sympathetic nervous outflow [30], reduced sympathoadrenal activity [31,32], plasma epinephrine [32], and urinary excretion of cathecolamines [27]. Fasting has been shown to increase the sympathetic outflow only in WAT [25,33]. Conversely, refeeding markedly increases central sympathetic nervous outflow [30] and BAT and skeletal muscle sympathetic activity [11,12,34].

Treatment of fasted rats with the β-adrenergic blocker propranolol had no effect on fasting glycemia, postfasting food intake, or postfasting glycemic profiles (during the 8 hours of refeeding). However, propranolol did significantly influence plasma insulin and GLUT4 gene expression in both fasting and refeeding states. These results suggest that β-adrenergic activity, even in the fasting state, is participating in the glucose homeostasis control.

At the end of the fasting period, propranolol-injected rats exhibited increased plasma insulin levels. β-Adrenergic activity has previously been shown to amplify glucose-induced insulin secretion from pancreatic β cells. Furthermore, parasympathetic activity, a stimulator of insulin secretion independently of glucose stimulus, may be involved in the results observed because sympathovagal balance has shown to be reduced in fasting [35] and parasympathetic activity was proposed to be increased in norepinephrine- and epinephrine-deficient mice [36]. In addition, other factors including neuropeptides released by autonomic fibers, incretins released from the gut, and somastotatin paracrine action may be involved in the β-adrenoceptor blockade effect on plasma insulin in fasting.

The β-adrenoceptor blockade at the end of the fasting induced a significant increase in GLUT4 mRNA in both skeletal muscle and BAT, indicating that in spite of a low sympathetic flow, remaining β-adrenergic activity was still maintaining a suppressive effect on the GLUT4 gene expression. In addition, the β-adrenoceptor blockade might increase α-adrenergic activity, which by reducing the generation of intracellular cAMP could stimulate GLUT4 gene expression, considering that the repressive effect of cAMP on GLUT4 expression, clearly reported in vitro [13-16], seems to be involved in the results observed. Besides, propranolol-induced increase in muscle glucose uptake [37] and cAMP inhibition of GLUT4 translocation and glucose uptake in L6 myoblasts [38] were already reported, reinforcing the participation of the sympathetic activity.

Whether strenuous physical activity is required and stress-induced activation of sympathetic activity is triggered in the fasting state, increased glucose disposal for muscle would be guaranteed because muscle contraction per se acutely increases GLUT4 protein translocation and glucose uptake [2]. In BAT, thermogenic activity and the correlated glucose utilization [12] are decreased in fasting, also preserving energy substrates; however, if stress-induced increases in body temperature occur, such as for fighting, thermogenesis can rapidly increase [11], in that case induced by sympathetic stimulation [39].

During refeeding, the food intake induced a progressive increase in the insulin levels of saline-injected rats, whereas the propranolol-injected rats showed unchanged insulin levels. Considering that during refeeding the central sympathetic nervous outflow is enhanced [30] and the parasympathetic activity is decreased [35], the result observed is probably a consequence of the dominant α-adrenergic suppressive effect on insulin secretion.

When β-adrenoceptor blockade was imposed, the GLUT4 mRNA expression during refeeding showed a decrease compared with the high levels observed at the end of fasting, achieving similar levels in skeletal muscle and significantly lower levels in BAT than those in saline-injected rats. Considering that the expected direct effect of β-adrenoceptor blockade is an increase of the GLUT4 expression, these results showed that during refeeding, metabolic and hormonal regulations of GLUT4 gene override the sympathetic effect. Regarding this, one exciting hypothesis may involve the thyroid hormone T3, a stimulator of GLUT4 transcription [40], which is reduced by fasting and rapidly increases during refeeding. It was reported that a single injection of propranolol, in the same dose used in the present study, prevents the refeeding-induced plasma T3 increase [41]. Besides, we cannot exclude the participation of insulin as a modulator of the observed GLUT4 regulation. Considering both saline- and propranolol-injected rats, the insulin levels correlated with BAT GLUT4 mRNA levels (Pearson $r = 0.9363$, $P = .0006$; data not shown); however, they did not correlate with gastrocnemius GLUT4 mRNA levels, highlighting the tissue-specific control of GLUT4 gene expression.

In summary, the present study reports that fasting modulates GLUT4 expression in a tissue-specific manner with a general repression of the gene. Refeeding rapidly increases GLUT4 mRNA in the gastrocnemius muscle and BAT to a level greater than that observed before fasting, highlighting GLUT4 as a typical thrifty gene. In addition, we demonstrate sympathetic activity participation in GLUT4 gene expression control in vivo. During fasting, sympathetic activity seems to be important for continued repression of the GLUT4 gene. However, during refeeding, the sympathetic control of GLUT4 gene expression seems to be overbalanced by metabolic/hormonal modulators. All together, the results suggest that during evolution, food behavior may have imprinted a characteristic GLUT4 gene expression pattern, which is influenced by autonomic sympathetic activity, especially during a long-term starvation period.

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