Age and Gender Effects on Glucose Utilization in Skeletal Muscle and Brown Adipose Tissue of Cold-Exposed Rats (43798)

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Abstract. **We hypothesized that glucose utilization by skeletal muscle is less in cold-exposed older male versus female Fischer 344 (F344) rats and that this reduction may contribute to the poorer cold-exposed thermoregulatory ability of the older males. To test this hypothesis, the rates of *in vivo* glucose utilization of skeletal muscle in 6-, 12-, and 26-month-old male and female F344 rats were estimated during cold exposure by measuring the cellular incorporation of [^{14}C]-2-deoxyglucose ([^{14}C]-2DG) after its conversion to [^{14}C]-2DG-6-phosphate ([^{14}C]-2DGP). Comparable measurements were also made in the interscapular brown fat depot (IBAT). Four-hour fasted rats, that had been fitted with femoral arterial and venous cannulae 24 hr earlier, were exposed to either 26° or 6°C for 2 hr and then received a bolus infusion of [^{14}C]-2DG (125 μCi/kg body wt). Arterial plasma glucose and [^{14}C]-2DG concentrations were measured periodically for an additional 45 min. Rats were sacrificed, and various skeletal muscles and IBAT were removed and immediately frozen in liquid N_2 for subsequent analysis of [^{14}C]-2DGP content. Cold-exposed male rats had significantly lower rectal temperatures than comparably treated females (26-month-old male, 34.8° ± 0.3°; female, 36.1° ± 0.2°C). There was no main effect of age, gender, or cold exposure on skeletal muscle glucose utilization when the data were expressed as nmol/min/g. In contrast, when data were expressed relative to total tissue weight (nmol/min), skeletal muscle glucose utilization was significantly higher in male than in female rats. Although estimated glucose utilization in IBAT (nmol/min/g) isolated from cold-exposed rats was significantly greater than that in brown fat isolated from non-cold-exposed animals, there was no main effect of age or gender. However, glucose utilization per IBAT depot (nmol/min) was significantly less in older male than in female rats, and reflected the fact that IBAT weight of older males was more than 50% lower than that of comparably aged females. Thus, our hypothesis that reduced skeletal muscle glucose utilization contributes to the blunted thermoregulatory ability of older male versus female F344 rats is negated. Rather, our data suggest that while the ability of brown adipocytes to utilize glucose does not appear to decline with age (as indicated by the absence of an age-related decrease in glucose utilization per gram of IBAT), the lower total depot glucose utilization of cold-exposed older males versus females may partially explain the previously reported gender difference in the thermogenic capacity of this tissue.** [P.S.E.B.M. 1994, Vol 207]

Body temperatures of cold-exposed 26-month-old Fischer 344 (F344) rats decrease to a greater degree in males than in age-matched females (1). Associated with this gender effect are differences in the thermogenic capacity of brown adipose tissue, the major site of nonshivering thermogenesis in rats. Specifically, brown fat thermogenic quality is less in older male versus female rats as indicated by significantly lower mitochondria protein and lower cold-induced GDP-binding to BAT mitochondria, one index of thermogenic capacity (1, 2). However, heat production of the cold-exposed rat reflects the additive thermogenic contributions from brown adipose tissue and from skeletal muscle. It is, therefore, possible that the gender differences in the ability to maintain appropri-

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ate cold-exposed body temperature could also be partially explained by a reduction in skeletal muscle thermogenesis. The suggestion of an age-related diminution of cold-induced skeletal muscle thermogenesis is consistent with previous observations describing lower muscle oxidative capacity in older versus younger rats (3–5). However, we did not find attenuated muscle blood flow in cold-exposed 24- versus 12-month-old male F344 rats, suggesting that cold-exposed skeletal muscle oxygen consumption may not be significantly altered with age (6). Clearly, questions remain as to the contribution of skeletal muscle to the cold-induced heat production of the older male rat.

One of the mechanisms whereby skeletal muscle thermogenesis could be compromised with age and/or gender involves alterations in glucose utilization. Supporting such a possibility are the numerous investigations reporting attenuated skeletal muscle glucose uptake/utilization in older rats and humans (7–10). Furthermore, previous investigations have demonstrated a decrease in plasma insulin during cold exposure (11–14). Thus, cold exposure may exacerbate an age and/or gender-related attenuation of skeletal muscle glucose utilization resulting in declining rates of substrate oxidation and, thus, heat production.

The purpose of this investigation was to evaluate the interaction of age, gender, and cold exposure on glucose utilization. We hypothesized that glucose utilization of skeletal muscle is lower in cold-exposed older male vs female rats, and that this difference may contribute to the reduced thermoregulatory ability of the older males. To test this hypothesis, we measured *in vivo* non-cold- and cold-exposed glucose utilization of skeletal muscle in male and female rats, ages 6, 12, and 26 months using the glucose analog 2-deoxyglucose (2DG). We also measured 2DG uptake in the interscapular brown fat depot (IBAT).

Materials and Methods

Animals and Animal Care. Male and female inbred Fischer 344 (F344) rats 6, 12, and 26 months of age were obtained from the National Institute on Aging's animal colony maintained by Harlan Sprague Dawley Laboratory (Indianapolis, IN). Rats were housed individually in wire bottom hanging cages (20 × 25 × 18 cm) and maintained on a 12:12-hr light:dark cycle (lights on at 06:00 hr, off at 18:00 hr) at a room temperature of 25°–26°C. Rats were allowed *ad libitum* access to food (NIH-31 chow, Teklad Research Diets, Indianapolis, IN) and water. Animals were maintained in our colony for approximately 2 weeks prior to glucose utilization measurements.

Rats were examined for signs of external disease; none had overt signs of disease. At the time of death, a systematic, visual necropsy was performed on all rats. Many of the male 26-month-old rats had testicular tumors, and some small tumors were present in other areas of all 26-month-old rats (pathologies were not obtained on any of these tumors). None of these rats were excluded from the study.

Experimental Design. Twenty-four hours prior to glucose utilization measurements, halothane-anesthetized rats were fitted with femoral arterial and venous cannulae and allowed to recover with access to food and water. In a previous investigation, we reported that rats consumed food and water within 18 hr following a surgery (halothane anesthetic) of a similar length (15). Loss in body weight following surgery was less than 1%. At 09:00 hr the following morning, the food was removed from the rats. At 11:00 hr, body weight and colonic temperature were measured, and rats were placed in a room maintained at 26°C (non-cold-exposed) or 6°C (cold-exposed) for 2 hr. At 13:00 hr, blood was collected from the arterial cannula for hematocrit determination, and the rats received a bolus infusion, 0.25–0.70 ml, of [^{14}C]-2DG (125 μCi/kg body wt; New England Nuclear, Boston, MA) through the femoral vein cannula. Approximately 150–200 μl arterial blood was collected at 10, 45, and 90 sec, and 3, 5, 8, 10, 15, 25, 35, and 45 min after infusion of [^{14}C]-2DG and separated by centrifugation into plasma for determination of glucose and [^{14}C]-2DG concentrations. At 13:45 hr, blood was collected for hematocrit determination, and colonic temperature was measured. All hematocrit values were above 37.0% packed cell volume. Rats were killed by lethal injection of sodium pentobarbital through the femoral vein, and IBAT, trapezius, biceps femoris, gastrocnemius, soleus, and pancreas were removed, immediately frozen in liquid N_2, and stored at −70°C until subsequent analysis of [^{14}C]-2DG-phosphate ([^{14}C]-2DGP) content.

Analysis of Tissue Glucose Utilization. Glucose utilization of skeletal muscle and IBAT was estimated by methods described previously (16, 17) using the glucose analog 2DG. This glucose analog is transported into the cell like glucose and is phosphorylated by hexokinase to 2DGP. However, unlike glucose-6-phosphate, 2DGP is not metabolized further. An estimate of glucose utilization which has been previously referred to as the glucose utilization index (GUI; [18]) was calculated as follows:

$$\mathrm{GUI} = \frac{C_p \cdot C_m^*}{\int C_p^*(t)dt} \qquad [1]$$

where C_p is the steady state arterial plasma glucose (mmol/l; see below for definition of steady state); C_m^* is tissue [^{14}C]-2DGP content (dpm/g) at 45 min after bolus injection; and $C_p^*(t)$ is the plasma concentration

of [^{14}C]-2DG at time t. The integral term was evaluated using nonlinear regression (SAS Institute Inc, Cary, NC) to estimate the model parameters. A double exponential equation was found to be the best fit. That is, $C_p^*(t)$ was estimated as follows:

$$C_p^*(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad [2]$$

where A and B are the intercepts and α and β are the slopes. Derivation and assumptions related to the use of Eq. 1 and 2 have been described previously (17). Steady state plasma glucose was defined as values that did not differ by more than 10% from resting values during the 45-min experimental procedure. Two animals (one 12- and one 26-month-old male) were excluded from the study because they failed to meet this criteria.

The GUI may differ from the true glucose utilization rate by a factor that represents the difference between the affinity of hexokinase for glucose compared with that for 2DG (i.e., the lumped constant). We did not include a lumped constant in our calculation of GUI, and therefore the data should be interpreted as an estimate of true tissue glucose utilization. However, we believe that differences between the rate of tissue utilization of glucose and 2DG are functionally insignificant. For example, Kraegen *et al.* (17) observed that the pattern of insulin-stimulated rates of [^{14}C]-glucose incorporation into muscle is similar to that of [^{3}H]-2DG.

Analytical Procedures. Plasma glucose concentration was measured using a glucose colorimetric kit (Sigma, St. Louis, MO) and plasma [^{14}C]-2DG by liquid scintillation counting. Tissue content of [^{14}C]-2DG and [^{14}C]-2DGP were determined from whole frozen tissues that were ground to a fine powder with a liquid nitrogen-cooled mortar and pestle set on dry ice. The tissues were homogenized in 40% ethanol/8% $HClO_4$, using a Branson Cell Disruptor and centrifuged at 22,000g for 30 min at 4°C. The supernatant was removed, neutralized with 3 $M \cdot L^{-1}$ potassium carbonate and 0.5 $M \cdot L^{-1}$ triethanolamine, pH 8.0, and centrifuged at 1500g for 15 min at 4°C. The resulting supernatants were applied to Bio Rad AG × 8 200–400 mesh (acetate form) anion exchange resin columns to separate the [^{14}C]-2DGP from the [^{14}C]-2DG. The columns were washed with H_2O to elute the unbound [^{14}C]-2DG, and then washed with 2 M NaCl and 1 M HCl to collect the [^{14}C]-2DGP. One milliliter samples were taken from each fraction and counted on a scintillation counter.

Statistics. Three- and two-way analysis of variance (ANOVA) were used to evaluate main effects. When a significant main effect was found, Fisher's least significant difference post-hoc test was used to determine differences between groups. Paired t tests were used to determine differences between pre- and postexposure samples for glucose. Differences were considered significant at $P < 0.05$.

Results

Colonic Temperature and Plasma Glucose. There was a significant main effect of gender, but not age, on the colonic temperatures of the rats at the

Table I. Colonic Temperatures (°C) and Plasma Glucose Concentrations (mg/dl) of F344 Rats

	Female			Male		
	Age (months)			Age (months)		
	6	12	26	6	12	26
Temperature						
Non-Cold (26°C)						
Pre	37.8 ± 0.3^{a,b}	38.1 ± 0.2^{a}	37.7 ± 0.2^{a,b}	37.6 ± 0.2^{a,b}	37.5 ± 0.1^{b}	37.3 ± 0.2^{b}
Post	38.8 ± 0.3^{a,*}	38.8 ± 0.2^{a,*}	38.0 ± 0.3^{b}	38.5 ± 0.3^{a,b,*}	38.3 ± 0.2^{a,b,*}	38.0 ± 0.2^{b,*}
Cold (6°C)						
Pre	37.5 ± 0.2	37.8 ± 0.3	37.6 ± 0.1	37.5 ± 0.2	37.6 ± 0.2	37.3 ± 0.2
Post	37.2 ± 0.4^{a}	37.2 ± 0.3^{a}	36.1 ± 0.2^{b,*}	37.1 ± 0.2^{a}	37.4 ± 0.3^{a}	34.8 ± 0.3^{c,*}
Glucose						
Cold (6°C)						
Pre	106.5 ± 1.9^{a}	112.2 ± 2.7^{a}	109.1 ± 4.5^{a}	108.5 ± 3.7^{a}	106.3 ± 3.0^{a}	121.3 ± 5.0^{b}
Post	107.3 ± 4.7^{a}	115.1 ± 2.6^{b,c}	116.6 ± 6.1^{b,c}	114.8 ± 4.0^{b}	107.7 ± 1.7^{a}	128.1 ± 6.1^{b,c}

Note. Values are means ± SE; n = 7–9 rats per group; Pre and Post values represent temperature taken before and after the 2.75 hr experimental treatment period, respectively. Because plasma glucose values did not differ between non-cold- and cold-exposed groups, only cold-exposed data are presented. Within a row, values without or values sharing a common superscript are not significantly different ($P < 0.05$).

* Value is significantly different from age-matched pre value ($P < 0.05$).

beginning of the experiment (Pre values, Table I) or after 2.75 hr (Post values) in the chamber at 26°C. This reflected a small but generally greater colonic temperature in the female versus male rats in both pre- and post measurements of cold- and non-cold-exposed rats. In addition, pre-sampling period colonic temperatures of non-cold-exposed female and male animals were lower than those in the post-sampling period (pre versus post 26-month-old female did not differ significantly). In cold-exposed animals, there were significant main effects of age, gender, and ambient temperature. These effects reflected the significantly lower colonic temperatures of the male and female 26-month-old rats compared with their younger counterparts. However, the degree of hypothermia of the 26-month-old rats was considerably greater in the males compared with the females (Fig. 1).

There were no significant main effects of age, gender, or temperature on plasma glucose (Table I; because plasma glucose values did not differ between non-cold- and cold-exposed groups, only the cold-exposed data are presented). However, one-way ANOVA revealed significantly greater pre-exposure plasma glucose values in 26-month-old male rats compared with younger male, and in all groups of female animals.

IBAT and Muscle Weight. Three-way ANOVA revealed a significant main effect of age and gender, and a significant interaction of age × gender on IBAT weight (Table II). That is, in female rats, IBAT weight increased significantly with age (26 vs 6 month) whereas in males, IBAT tended to be heavier in 12- vs 6-month-old rats, but then declined significantly in the 26-month-old animals. When IBAT weight was expressed relative to body weight, significant effects of age and gender were observed (Fig. 2). This reflected significantly greater values in female versus male rats and the general trend of the IBAT weight/body weight

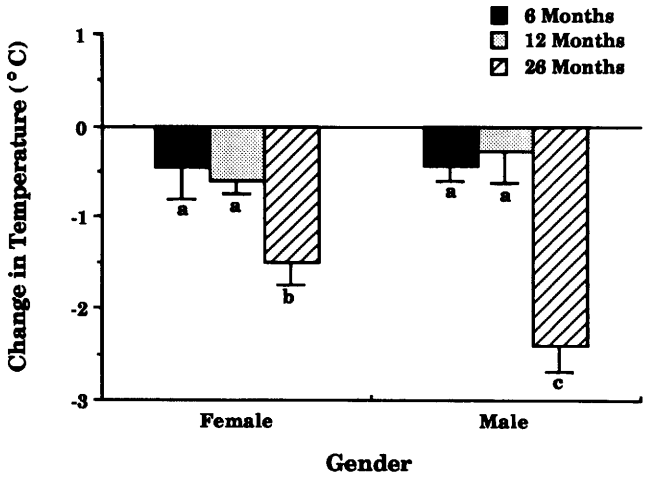

Figure 1. Change in colonic temperature (means ± SE) after 2.75 hr of cold exposure. Values sharing common letters do not differ significantly ($P < 0.05$).

to decrease with age, a trend that became significant in the 26-month-old male rats.

Although mean muscle weights differed among the four various muscle types examined, statistical differences and/or trends were similar. Therefore, only the gastrocnemius weights are presented. At all ages, gastrocnemius weight was greater in male versus female rats (Table II). The interaction effect reflected significantly lower gastrocnemius weights in 26- versus 6-month-old male rats in contrast to stable weights in the females. When gastrocnemius weight was expressed relative to body weight, there were significant main effects of age and gender (Fig. 2). The latter reflected greater gastrocnemius weights (mg/g body weight) of the 6- and 12-month-old female versus male rats. This gender difference was not present in the 26-month-old rats where both males and females had significantly lower relative gastrocnemius weights than did their 6- and 12-month-old counterparts (i.e., there was a main effect of age).

Glucose Utilization in Muscle and Pancreas. Three-way ANOVA of glucose utilization in all muscles and pancreas, expressed as nmol/min/g, indicated no effect of cold-exposure (Table III). Although some effects of age and gender were observed in skeletal muscle GUI (nmol/min/g), no consistent trends were noted. However, when skeletal muscle GUI was expressed as nmol/min, there was a significant effect of gender (Fig. 3; for ease of reading, only the gastrocnemius data are presented). This reflected greater utilization in males versus females, and could be explained by the greater tissue weights of the males. Notably, there was no reduction in muscle GUI in the older versus younger male rats; nor did the 26-month-old males exhibit lower glucose utilization in response to cold.

Glucose Utilization in IBAT. There was a significant main effect of environmental temperature on IBAT glucose utilization expressed as nmol/min/g (Table III). This reflected the significantly greater GUI values of cold-exposed rats compared with those observed in non-cold-exposed animals (only in the 6-month-old male rats did the cold-induced values not differ significantly). A significant main effect of gender reflected the generally greater GUI of females, but was influenced greatly by values for the 12-month-old female rats. The reason(s) for the greater glucose utilization in IBAT from 12-month-old female rats is not clear. However, it appears to be an accurate observation as both the non-cold- and cold-exposed rats displayed the enhanced utilization. Because the error associated with the 12-month-old female rats was proportionately greater than that in the other age and gender groups, ANOVA was performed excluding this age/gender group. Results of this analysis were not, in

Table II. Body, Interscapular Brown Adipose Tissue (IBAT), and Gastrocnemius Weights (g) of Male and Female F344 Rats

	Female			Male		
	Age (months)			Age (months)		
	6	12	26	6	12	26
Body						
Non-Cold	197.7 ± 2.4[a]	230.5 ± 4.9[b]	293.6 ± 7.0[c]	358.9 ± 5.1[d]	418.7 ± 6.7[e]	390.9 ± 11.4[f]
Cold	194.9 ± 4.7[a]	228.3 ± 5.7[b]	279.7 ± 9.6[c]	372.0 ± 6.3[d,f]	413.3 ± 14.8[e]	383.2 ± 11.1[f]
IBAT						
Non-Cold	0.24 ± 0.02[a]	0.25 ± 0.01[a]	0.31 ± 0.02[b]	0.25 ± 0.01[a]	0.27 ± 0.01[a]	0.19 ± 0.01[c]
Cold	0.23 ± 0.01[a]	0.25 ± 0.02[a,b]	0.29 ± 0.01[b]	0.23 ± 0.01[a]	0.28 ± 0.03[a,b]	0.18 ± 0.01[c]
Gastrocnemius						
Non-Cold	1.06 ± 0.03[a]	1.13 ± 0.04[a]	1.04 ± 0.03[a]	1.65 ± 0.05[b]	1.86 ± 0.06[c]	1.38 ± 0.07[d]
Cold	1.03 ± 0.03[a]	1.13 ± 0.03[a]	1.01 ± 0.03[a]	1.72 ± 0.08[b]	1.84 ± 0.08[b]	1.40 ± 0.08[c]

Note. Values are means ± SE; n = 7–9 animals per group; Non-Cold is 26°C; Cold is 6°C. Although the mean muscle weights differed among the various tissues, statistical differences and/or trends were similar. Therefore, for ease of reading only the gastrocnemius weights are presented. Within a row, values sharing a common letter superscript are not significantly different ($P < 0.05$).

general, different from those obtained when the 12-month-old group was included in the analysis.

When the data were expressed as total glucose utilization per IBAT depot (nmol/min), there was a significant main effect of age and gender and an interaction of ambient temperature and gender (Fig. 3). That is, cold-exposed 26-month-old male and female rats had, in general, significantly greater GUI than did their non-cold-exposed counterparts. Moreover, cold-exposed 26-month-old male rats had significantly lower glucose utilization (nmol/min) than did aged-matched females.

Discussion

The factors accounting for the age- and gender-related differences in the ability to maintain homeothermy during cold exposure, observed in this and previous investigations (1), have yet to be elucidated. In this study, we tested the hypothesis that reduced glucose utilization in skeletal muscle (which would be accompanied by attenuated rates of substrate oxidation and heat production) contributes to the 26-month-old male's hypothermia during cold exposure. Our data negate this hypothesis. That is, we observed that the GUI of skeletal muscle, expressed either as nmol/min/g or nmol/min, was not lower in the older versus younger males or in the older males versus females in response to 2.75 hr of exposure to 6°C (Table III and Fig. 3). In fact, total glucose utilization (nmol/min; Fig. 3) of skeletal muscle in cold-exposed 26-month-old males was significantly greater than that of females despite lower body temperatures. These data suggest that, if attenuated skeletal muscle thermogenesis contributes to the age- and gender-related differences in the ability to maintain homeothermy during cold exposure, it is not associated with decreased skeletal muscle glucose utilization.

On the other hand, while the GUI of the IBAT

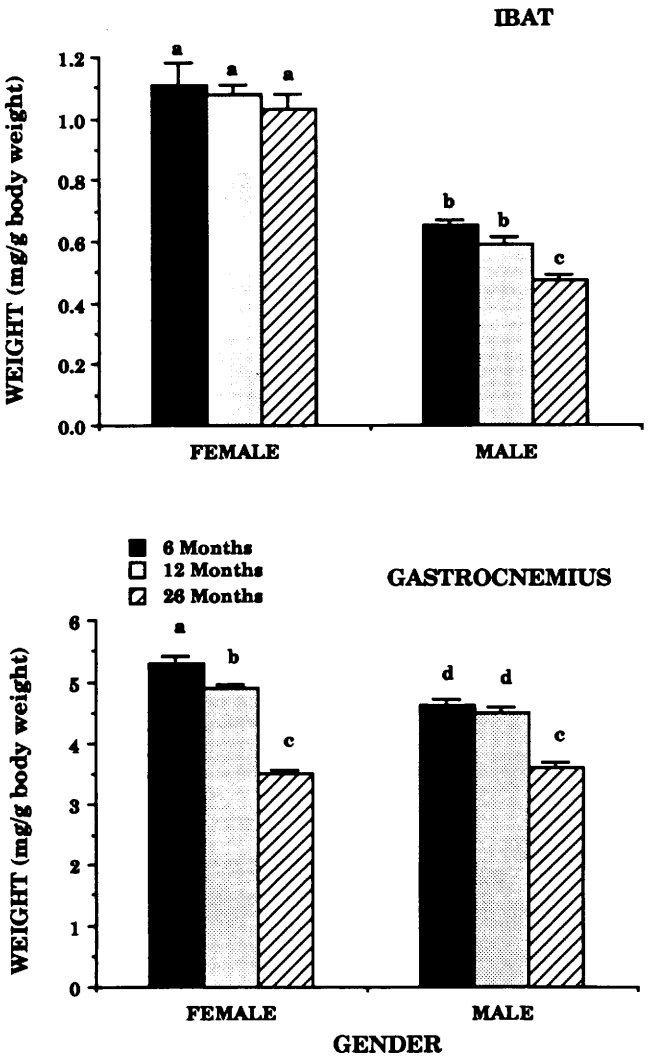

Figure 2. Interscapular brown adipose tissue (IBAT) and gastrocnemius weight expressed as mg/g body weight (means ± SE). Because the weights of IBAT and gastrocnemius did not significantly differ between non-cold- and cold-exposed rats, the values for both groups were combined. Values sharing common letters do not differ significantly ($P < 0.05$).

Table III. Glucose Utilization (GUI) of Interscapular Brown Adipose Tissue (IBAT), Skeletal Muscle, and Pancreas (nmol/min/g) During Non-Cold (26°C) or Cold (6°C) Exposure in F344 Rats

	Female			Male		
	Age (months)			Age (months)		
	6	12	26	6	12	26
IBAT						
Non-Cold	23.9 ± 4.8[a]	68.1 ± 5.7[b]	17.5 ± 6.5[a]	18.8 ± 7.5[a]	13.3 ± 2.9[a]	17.5 ± 4.8[a]
Cold	58.1 ± 9.9[a,*]	133.7 ± 24.2[b,*]	54.2 ± 8.0[a,*]	31.5 ± 6.2[c]	48.4 ± 5.9[a,*]	57.7 ± 9.2[a,*]
Gastrocnemius						
Non-Cold	5.1 ± 1.4[a]	5.6 ± 1.1[a]	12.7 ± 1.4[b]	7.6 ± 0.7[a]	7.2 ± 1.8[a]	8.2 ± 1.5[a]
Cold	4.5 ± 1.4[a]	11.7 ± 2.2[b,*]	8.8 ± 0.9[a,b]	7.6 ± 1.7[a,b]	8.4 ± 1.4[a,b]	10.9 ± 1.9[b]
Biceps femoris						
Warm	5.1 ± 1.4[a]	3.4 ± 0.7[a]	9.6 ± 1.3[b]	4.7 ± 0.6[a]	5.2 ± 1.3[a]	5.1 ± 0.9[a]
Cold	2.9 ± 0.7[a]	4.5 ± 0.5[a,b]	5.5 ± 0.7[b,*]	2.9 ± 0.5[a,b]	4.8 ± 0.7[a,b]	5.0 ± 1.0[a,b]
Soleus						
Non-Cold	11.2 ± 1.1[a]	14.3 ± 2.6[a,b]	15.8 ± 2.4[a,b]	19.3 ± 3.1[a,b]	21.6 ± 4.9[b]	16.3 ± 2.7[a,b]
Cold	9.1 ± 1.4[a]	19.3 ± 2.0[b,c]	10.2 ± 1.5[a,d]	14.5 ± 1.8[a,c]	17.5 ± 2.3[b,c]	13.0 ± 2.5[a,c,d]
Trapezius						
Non-Cold	4.3 ± 1.0	4.2 ± 0.6	3.7 ± 0.4	4.2 ± 0.6	4.8 ± 0.8	5.8 ± 1.0
Cold	2.7 ± 0.7[a]	6.9 ± 0.5[b,c,*]	4.7 ± 0.5[a,c]	3.6 ± 0.5[a]	4.8 ± 0.6[a,c]	6.7 ± 1.5[b,c]
Pancreas						
Non-Cold	4.4 ± 1.2[a,b]	3.1 ± 0.5[a]	4.6 ± 0.6[a,b]	3.2 ± 0.7[a]	4.2 ± 0.9[a,b]	6.0 ± 1.0[b]
Cold	2.9 ± 0.6[a]	3.2 ± 0.3[a,b]	2.4 ± 0.2[a,*]	3.1 ± 0.6[a]	4.1 ± 0.5[a,b]	5.0 ± 0.6[b]

Note. Values are means ± SE; n = 7–9 rats per group. Within a row, values without or values sharing a common letter superscript are not significantly different ($P < 0.05$).
* Value is significantly different from age-matched non-cold-exposed value.

depot (nmol/min) increased significantly in cold-exposed 26-month-old male and female rats, the magnitude of this increase was significantly less in the males. This difference could be explained by the smaller amount of IBAT in the older males versus females (Table III and Fig. 2). Thus, although tissue specific GUI (nmol/min/g) of IBAT did not decline with age, a reduction in IBAT weight of the older male rat resulted in attenuated glucose utilization. This, in turn, may contribute to lower brown fat thermogenesis in the older males versus females and help to explain the greater thermoregulatory "deficit" in the males.

Although past (1, 2) and present data strongly imply that the deficient thermoregulatory response of cold-exposed male rats involves altered BAT thermogenesis, we cannot completely eliminate a possible contribution of skeletal muscle. However, if there is attenuated skeletal muscle thermogenesis in the older cold-exposed male rat, it does not reflect defects in insulin-mediated metabolic pathways. That is, although we (19) as well as others (11–14) have observed lower plasma insulin levels during cold exposure, skeletal muscle GUI reported here was either unchanged or slightly increased. Furthermore, recent investigations have observed no significant differences in glucose tolerance (20, 21) or in skeletal muscle insulin resistance (21, 22) of senescent rats. Thus, if a reduction in cold-exposed skeletal muscle thermogenesis does occur in the aging male rat as a result of lower carbohydrate oxidation, it more likely reflects changes in rate or amount of glycogenolysis. This possibility is consistent with previous investigations describing enhanced use of skeletal muscle glycogen in humans during cold exposure (23). We have also observed increased glycogen utilization during cold-exposure in young and old male rats, although initial muscle glycogen concentration is reduced in the aged rats (15, 24).

The significant increase in brown fat GUI of cold-exposed male and female rats observed in the present investigation strongly implies that, unlike skeletal muscle, plasma glucose is utilized for cold-induced BAT thermogenesis. However, the fact that plasma insulin decreases during cold exposure indicates that the enhanced BAT glucose uptake is not dependent on insulin. A likely stimulant is norepinephrine as indicated by several lines of evidence. Among these are the observations of increased plasma levels of norepinephrine, but not epinephrine, in older (as well as younger) rats that have been cold-exposed for 4 hr at 6°C (19); cold-induced elevated IBAT norepinephrine turnover and frequency of nerve impulses to IBAT (25–27); norepinephrine-enhanced glucose uptake and thermogenesis (as measured by oxygen consumption) in isolated brown adipocytes in the absence of insulin (12, 28); and beta adrenergic agonist-induced increases in the total number of GLUT4 glucose transporters and induced translocation of glucose transporter to the plasma membrane (11, 29). Further research is warranted to define more clearly the role of norepinephrine in glucose transport to brown adipocytes of older animals.

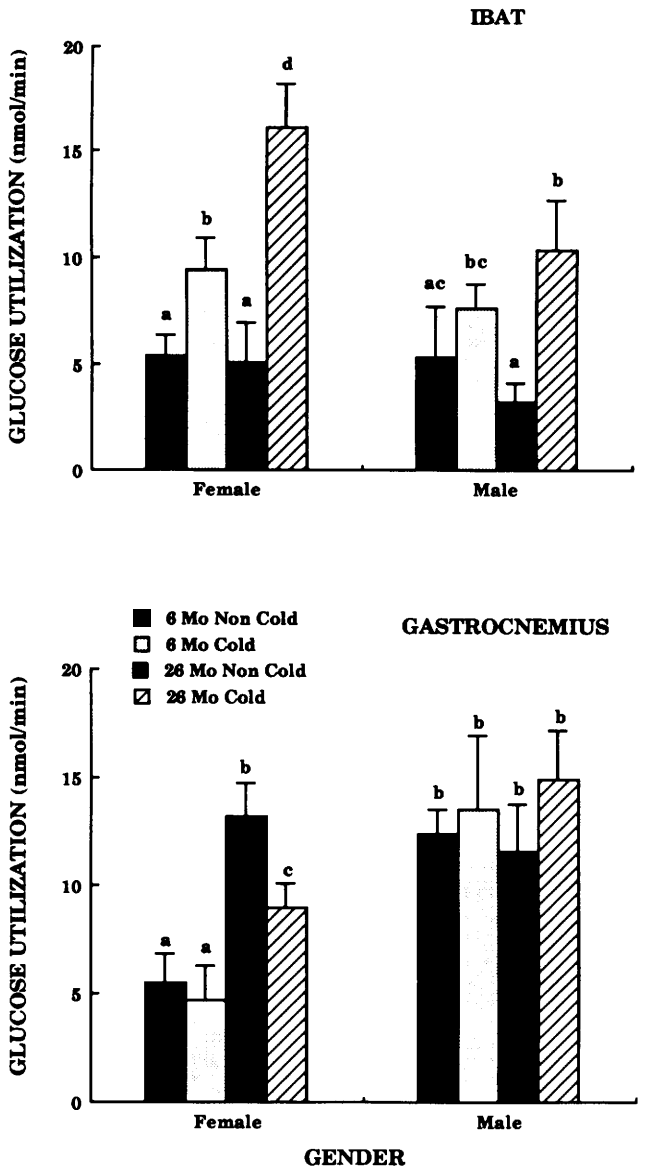

Figure 3. Total glucose utilization index (GUI) of interscapular brown adipose tissue (IBAT) and gastrocnemius in 6- and 26-month-old rats. Values sharing common letters do not differ significantly ($P < 0.05$).

Several investigations have suggested that biological aging is characterized by declining glucose homeostasis. This hypothesis has been supported by observations of age-related increases in peripheral insulin resistance (30, 31), decreased glucose tolerance (7, 32), and insulin secretion (33, 34). However, the data reported here as well as that of some recent investigations have raised questions concerning the validity of this hypothesis (1, 20, 21, 35–38). For example, we found no significant differences in the IBAT and skeletal muscle glucose utilization in old versus young male and female rats (cold-exposed or non-cold-exposed). Eiffert *et al.* (39) did not observe significant differences in skeletal muscle insulin receptor number, binding constant, or tyrosine kinase activity in aging Sprague-Dawley rats; and Ivy *et al.* (21) and Goodman *et al.* (40) found no significant alteration in glucose uptake of aging rats. Although these data do not invalidate the declining glucose homeostasis hypothesis, we suggest that factors such as obesity, inactivity, and diet (37) have more impact on glucose metabolism than aging *per se*.

In summary, the estimated glucose utilization of IBAT, but not skeletal muscle, increased during cold exposure in young and old, female and male rats. Moreover, the values of GUI were substantially higher in skeletal muscle in the cold-exposed older males versus females. This gender difference was reversed for GUI in IBAT. Thus, our hypothesis that a reduction in skeletal muscle glucose utilization contributes to the poorer thermoregulatory ability of the older male versus female rats is negated. Rather, our data suggest that while the ability of brown adipocytes to utilize glucose does not appear to decline with age (as indicated by the absence of an age-related decrease in glucose utilization per gram of IBAT), the lower total depot glucose utilization of cold-exposed older males versus females may partially explain the previously reported gender difference in the thermogenic capacity of this tissue.

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