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Increased Insulin Mediated Glucose Metabolism in Fat Cells from I versus C57BL Mice (42494)

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Abstract. The purpose of this study was to determine whether adipocytes from I strain mice, which are characterized by a greater *in vivo* glucose tolerance than most other strains, had a higher capacity to utilize glucose in response to physiological concentrations of insulin. Using C57BL mice as a control strain, we examined the effect of insulin on glucose metabolism in epididymal and inguinal adipocytes from 2-month-old male mice. Body weight was only slightly less (7%) for the I mice than for the C57BL mice, but fat pad sizes were 60 and 20% less for epididymal and inguinal depots, respectively, in the I mice. Fat cell size was also smaller in epididymal adipocytes from the I mice than from the C57BL mice. Fat cell size of inguinal adipocytes was similar in the two strains. Without insulin the rates of [U-^{14}C]glucose incorporation into CO_2 or lipids were twofold higher in cells from the I mice than in those from the C57BL mice. Maximal insulin concentration (2.5 n*M*) increased glucose metabolism by 140 and 500% in epididymal and inguinal adipose cells, respectively, in the I mice versus 30 and 50% in the C57BL mice. The maximal effect of insulin was reached at a much higher insulin concentration in the I mice than in the C57BL mice. The activity of fatty acid synthetase was four- to sixfold higher in fat cells from I than in those from C57BL mice. These results demonstrate an increased insulin responsiveness of glucose metabolism in fat cells from the I mice related to an increased lipogenic capacity. Furthermore, they show that adipose tissue in mice exhibits significant regional differences in terms of insulin responsiveness of glucose metabolism.

I strain mice have a lower body fat content (1, 2) compared with C57BL, C3H, or A mice (nonobese strains). I strain mice, unlike these nonobese mouse strains, are resistant to fat accumulation with age (3) and high fat diets (4). Food efficiency is less and oxygen consumption is greater in I than in C57BL and C3H mice (2, 5). In addition, *in vivo* glucose tolerance is greater in I mice than in the other nonobese mouse strains (6, 7), such that I strain mice have low serum glucose concentrations accompanied by low serum insulin concentrations after an oral or intravenous dose of glucose (unpublished results). The tissue site of the increased glucose uptake and utilization in I mice has not been determined. Previous studies (7, 8) indicated glucose metabolism in response to insulin of isolated diaphragm from I and C57BL mice is similar. A preliminary study indicated glucose metabolism of epididymal tissue incubated *in vitro* is greater for I strain mice than for C57BL mice, suggesting adipose tissue could be the site of enhanced glucose metabolism in the I mice.

Therefore, the objective of this study was to determine whether adipocytes from I strain mice had a higher capacity to utilize glucose in presence of physiological concentrations of insulin. Since glucose metabolism in fat cells from different sites varies in both rats (9, 10) and humans (11), we chose to characterize the insulin response of adipocytes from two adipose depots, epididymal and the subcutaneous inguinal fat pads, from the I and C57BL mice.

Materials and Methods. *Animals.* Male mice, I/Crgl and C57BL/6 Crgl strains, were obtained from the Cancer Research Laboratory (CRL), University of California (Berkeley, CA) or from first generation litters of parents from the CRL mated at San Diego State University (SDSU). The mice were maintained at SDSU in a room at 23–26°C with a 12-hr alternate light and dark cycle and had access to water and pelleted Wayne Lab-Blox F6[1] (Continental Grain Company, Chicago, IL) *ad*

[1] Guaranteed analysis: crude protein, 24%; crude fat, 6%; crude fiber, 4.5%. Average vitamin and mineral content meets or exceeds NRC recommendations for mice.

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libitum. Age-matched groups of mice were transported (air cargo) to Paris, France (Institut Biomédical des Cordeliers) where environmental conditions for the mice were maintained as closely as possible to those at SDSU and the mice were fed the same diet. The experiments were conducted in Paris after at least a 2-week adaptation period when the mice were 8 weeks of age. A 2-week adaptation period has been shown to be sufficient for rodents to resume normal food and water intake, locomotor activity, and metabolic patterns when the light cycle is reversed (12).

Preparation of isolated adipocytes. Mice were killed between 9 and 10 AM by cervical dislocation. For each experiment the total epididymal and inguinal adipose tissues from four to seven mice of each strain were removed and weighed. The tissues from each site were then pooled and cut into small pieces. The fat cells were isolated by digestion for 1 hr at 37°C in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 3% serum albumin (Fraction V, Sigma Chemical Co., St. Louis, MO), 5 m*M* glucose, half the recommended Ca^{2+} concentration (1.25 m*M*), and 1 mg/ml of collagenase (Boehringer Mannheim France SA, Meylan) by a modification of the method of Rodbell (13). The fat cells were then filtered through a 190-μm nylon screen, washed twice with collagenase-free buffer by allowing the cells to float and aspirating away the infranatant buffer, and suspended in fresh buffer.

Determination of fat cell size and number. Fat cell size and number were determined by using a photomicrographic method previously described (14).

Glucose metabolism studies. Two major pathways of glucose metabolism in adipocytes (incorporation into CO_2 and total lipids) were studied as previously described (15). Duplicate aliquots (0.5 ml containing between 0.4 and 0.7×10^6 cells) of adipocytes were incubated without or with insulin (porcine, Sigma Chemical Co.) at concentrations in the range of 0.06 to 2.5 n*M*. After a 2-hr incubation, CO_2 was collected in hyamine, the total lipid contents of the incubation flasks were extracted (16), and radioactivity was determined. Blanks without cells were run in parallel with each experiment to correct for nonmetabolic ^{14}C labeling.

Determination of fatty acid synthetase activity. Fat cells suspended in ice-cold 0.25 *M* sucrose containing 3 m*M* dithiothreitol and 1 m*M* EDTA at pH 6.8 were sonicated 15 sec and then centrifuged at 100,000*g* at 0°C for 60 min. Fatty acid synthetase activity was measured at 37°C in duplicate, spectrophotometrically (Perkin–Elmer model 555, Uberlingen, Germany), in the supernatants by following the oxidation of NADPH in the presence of acetyl-coenzyme A (CoA) and malonyl-CoA according to the method of Martin *et al.* (17). Conditions of the assay were selected to ensure linear rates with respect to time (8–10 min) and sample concentration.

Statistical analysis. Results are expressed as means ± SEM. All *P* values were obtained by using nonpaired or paired (as indicated) *t* tests (18). Values of $P \leq 0.05$ were considered significant.

Results. *Experimental animals.* Body weight (Table I) is lower (7%) for I mice than for C57BL mice. Marked differences between the two strains are observed in the size of the fat pads. In I mice the size of the fat pads are substantially less (epididymal, 60%; inguinal, 20%) than the respective tissues from C57BL mice. Epididymal fat cells (Table II) from I

TABLE I. BODY WEIGHT AND TISSUE WEIGHTS FROM MALE I AND C57BL MICE AT 8 WEEKS OF AGE[a]

	I	C57BL	P^b
Body weight (g)	21.20 ± 0.30 (40)	22.80 ± 0.30 (31)	<0.001
Epididymal (mg)	120.60 ± 7.20 (30)	300.50 ± 11.50 (25)	<0.001
(% Body weight)	0.57 ± 0.03 (30)	1.31 ± 0.04 (25)	<0.001
Inguinal (mg)	160.90 ± 6.30 (34)	203.30 ± 6.40 (31)	<0.001
(% Body weight)	0.75 ± 0.03 (34)	0.88 ± 0.15 (31)	<0.005

[a] Values are the means ± SEM; number in parentheses is the number of mice.
[b] Means between mouse strains compared by *t* test.

TABLE II. CELL SIZE OF ISOLATED ADIPOCYTES FROM EPIDIDYMAL AND INGUINAL DEPOTS FROM I AND C57BL MICE[a]

	μg lipid/cell		
	I	C57BL	P^b
Epididymal	0.054 ± 0.010 (4)	0.089 ± 0.010 (4)	<0.050
Inguinal	0.033 ± 0.003 (5)	0.037 ± 0.003 (5)	>0.050

[a] Values are the means ± SEM; number in parentheses is the number of experiments. In each experiment isolated fat cells were prepared from the pooled tissue of four to seven mice. Cell size values were obtained from the same experiments used for the [U-^{14}C]glucose metabolism studies.

[b] Means between mouse strains compared by *t* test.

mice are smaller than those from C57BL mice, but the size of the inguinal fat cells is similar for both strains.

In addition, site-to-site variations in fat pad weights and fat cell sizes differ between the two strains. In mice from the control strain, epididymal fat pads ($P < 0.001$) and epididymal adipocytes ($P < 0.005$) are greater than inguinal adipose tissue and cells. In contrast, in the I mice, epididymal fat pads are smaller ($P < 0.001$) than inguinal fat pads, but fat cell size is similar ($P > 0.05$) in the two sites.

Glucose metabolism studies. The basal (no insulin) and maximally stimulated (2.5 n*M* insulin) [U-^{14}C]glucose incorporation into CO_2 and total lipids in epididymal and inguinal adipose cells from the two strains of mice are illustrated in Fig. 1, as well as the absolute effect of insulin (insulin-stimulated minus basal). The basal and insulin-stimulated rates of glucose conversion to CO_2 and lipids are 2- to 9-fold higher in adipocytes from the I mice than in those from the C57BL mice. Furthermore, the absolute effect of the hor-

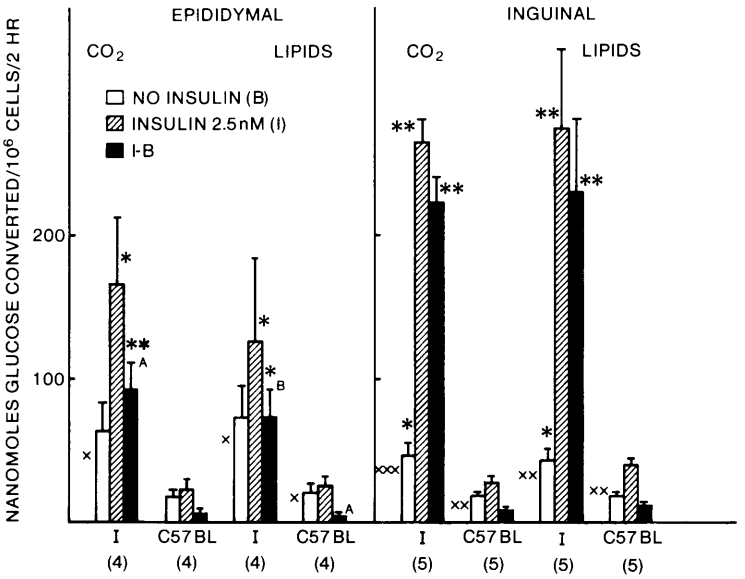

FIG. 1. [U-^{14}C]Glucose metabolism in epididymal and inguinal adipocytes from 8-week-old I and C57BL mice incubated in the absence (□) or presence (▨) of insulin (2.5 n*M*). The absolute effect (increment over basal) of insulin is also shown (■). Adipocytes were incubated for 2 hr at 37°C in Krebs–Ringer bicarbonate buffer containing 3% albumin and 5 m*M* glucose. Bars represent the mean ± SEM of four (epididymal) or five (inguinal) experiments with adipocytes from four to seven mice of each strain. Statistical differences between mouse strains is indicated by (*) $P < 0.025$ or (**) $P < 0.005$, differences between inguinal and epididymal by (A) $P < 0.05$ or by (B) $P < 0.025$, and differences between basal and insulin-stimulated values (X) $P < 0.03$, (XX) $P < 0.01$, or (XXX) $P < 0.001$.

mone is more than 10-fold higher in the fat cells from the I mice than from C57BL mice. In epididymal adipocytes insulin increases glucose incorporation into CO_2 by 70 nmole (per 2 hr per 10^6 cells) in the I mice and only by 6 nmole in the control strain. The difference is even more marked for inguinal fat cells, where insulin increases glucose incorporation into CO_2 by 220 nmole in the I mice versus 9 nmole for the C57BL mice. Similar differences are observed for glucose incorporation into total lipids which parallels glucose incorporation into CO_2.

These results further show the presence of regional differences in the insulin stimulatory effect on glucose metabolism in the two strains of mice, where insulin response is higher in adipocytes from the inguinal fat depot than in those from the epididymal adipose tissue.

Since basal values differed between the two strains, we elected to express the insulin dose–response curves of glucose metabolism (CO_2 and lipid) as a percentage increase over basal (insulin-stimulated minus basal × 100/basal) as shown in Fig. 2. These data provide evidence that the effectiveness of insulin (-fold increase) in stimulating the total glucose metabolism (CO_2 + lipid) was markedly increased in I strain mice as compared to C57BL mice across a range of hormone concentrations, encompassing the physiological value of insulin concentrations. These curves also show that the maximal insulin effect was clearly achieved at 0.36 n*M* insulin in fat cells from the C57BL mice in both inguinal and epididymal sites. In contrast, the shapes of the dose–response curve in I strain mice suggest that maximal stimulation may not be reached yet at 2.5 n*M* insulin, the highest concentration tested here, especially in inguinal adipocytes, and, there-

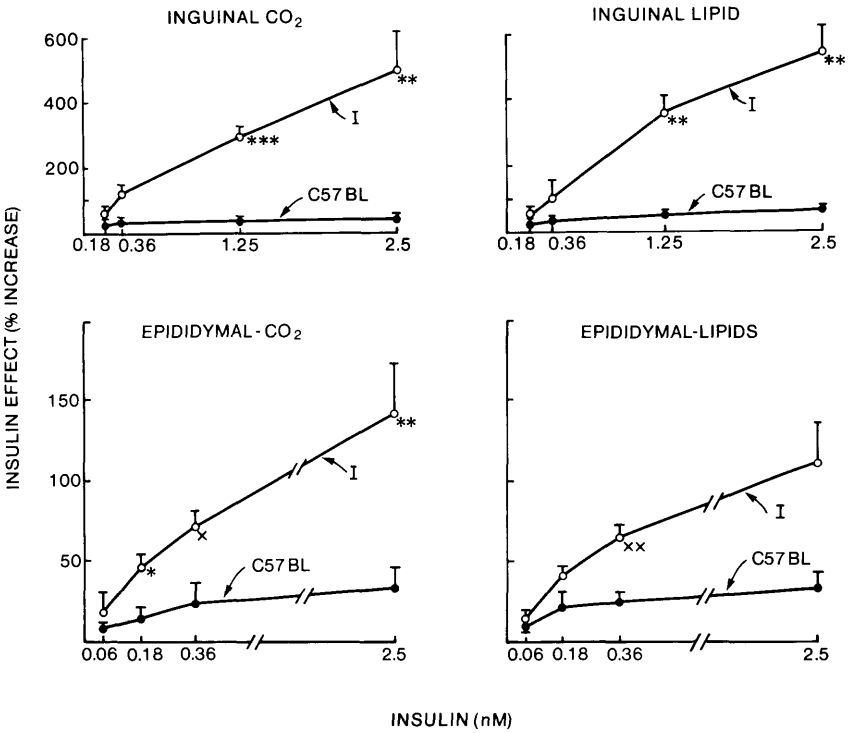

FIG. 2. Insulin dose–response curves for [U-^{14}C]glucose incorporation into CO_2 and lipids in epididymal and inguinal adipocytes from I (○) and C57BL (●) mice. The same fat cell suspensions as in Fig. 1 were used. Adipocytes were incubated as indicated in the legend of Fig. 1. Data are expressed as insulin-stimulated minus basal × 100/basal. Each point represents the mean ± SEM of four (epididymal) or five (inguinal) experiments with adipocytes pooled from four to seven mice of each strain. Statistical differences between mouse strains is indicated by (X) $P < 0.05$, (*) $P < 0.025$, (XX) $P < 0.01$, (**) $P < 0.005$, or (***) $P < 0.001$.

fore, an accurate estimation of ED_{50} (insulin concentration eliciting half-maximal stimulation) was not possible. However, this shift to the right of the dose–response curves is a clear indication that the sensitivity to insulin of glucose metabolism was lower in the two types of adipose cells from the I mice than from the C57BL mice and that inguinal adipocytes were less sensitive than epididymal in the I mice.

Fatty acid synthetase activity. The increased insulin responsiveness of adipocytes from I mice as compared to adipocytes from C57BL suggested the presence of an enhanced intracellular capacity to utilize glucose. We therefore measured the activity of one of the rate-limiting lipogenic enzymes, fatty acid synthetase (FAS). FAS activity (Table III) is dramatically higher in both epididymal (six-fold) and inguinal (fourfold) fat cells from I than in those from C57BL mice.

Discussion. Relative to the number of studies with rat adipocytes, only a few have been conducted utilizing isolated mouse cells. The present study shows that the insulin effect on glucose metabolism in control mice, C57BL, is low (only 2-fold the basal rates) as compared with the insulin effect usually reported (9, 19) in adipocytes from rats (2- to 10-fold the basal rates). Although considerable variation in insulin response of adipocytes occurs between laboratories, the values found in this study are comparable to other studies. Using epididymal adipocytes from C57BL mice, Batchelor *et al.* (20) found less than a 2-fold increase in labeled glucose conversion to CO_2 and lipids with maximum insulin stimulation (3.4 n*M*), and Carnie *et al.* (21) found a 2-fold increase in CO_2 labeling with 7 n*M* insulin. The results of the present study indicate that not only is glucose metabolism response to insulin low in epididymal fat cells from C57BL mice as reported previously (20, 21), but also the response is low in inguinal fat cells.

TABLE III. FATTY ACID SYNTHETASE ACTIVITY IN ISOLATED FAT CELLS OF EPIDIDYMAL AND INGUINAL TISSUE FROM I AND C57BL MICE[a]

	U/10^6 cells		
	I	C57BL	P^b
Epididymal	41.6 ± 13.9 (4)	6.6 ± 0.4 (4)	<0.050
Inguinal	36.4 ± 9.2 (5)	8.3 ± 2.3 (5)	<0.025

[a] Values are the means ± SEM; number in parentheses is the number of experiments. These values were obtained from the same experiments used for the [U-^{14}C]glucose metabolism studies. One unit (U) is defined as the amount of enzyme needed to catalyze the oxidation of 1 nmole of NADPH/min.

[b] Means between mouse strain compared by *t* test.

In contrast to the weak effect of insulin on glucose metabolism in epididymal adipocytes from the C57BL mice, a much higher insulin effect is observed in epididymal adipocytes from the I mice and an even greater one in inguinal fat cells. In good agreement with the large increase in the rates of glucose incorporation into lipids, fatty acid synthetase is drastically enhanced in adipocytes from the I mice.

Differences in insulin sensitivity of glucose metabolism, as evaluated by the shift in insulin dose–response curves, are also evident between the two strains. Although more responsive to insulin, adipocytes from the I mice are much less sensitive than those from the C57BL mice. Nevertheless, even the lowest insulin concentrations tested produce a higher increment of glucose metabolism in fat cells from I mice than from C57BL mice. This indicates that each dose of insulin is much more efficient in the I mice. *In vivo* plasma insulin concentration ranges from 0.07 to 0.25 n*M* for the I strain mice and from 0.13 to 0.42 n*M* for the C57BL mice. Thus, a decrease in insulin sensitivity would be of no physiological consequence in view of the magnitude of the effect of the increased insulin responsiveness of glucose metabolism in the I mice.

The strain-associated differences in the insulin effect observed here cannot be explained by differences in fat cell size, a factor known to influence the magnitude of the insulin response in adipocytes (18), since cell size is similar (inguinal) or only slightly different (epididymal) in the two strains of mice.

In addition, the results clearly show differences in the effect of insulin on glucose metabolism in adipocytes according to the site of adipose tissue. Interestingly, in contrast to that observed in mice, subcutaneous rat adipocytes are reported to be much less responsive to insulin than adipocytes from other intercavity fat depots (9, 22).

Although lipogenesis is greater in response to insulin in I mice, body fat content of I mice is approximately two times less than for

C57BL mice (2). This paradox could be explained by higher rates of lipolysis in I mice. Nearly a threefold increase in basal glycerol release has been observed for epididymal adipose tissue in I mice compared with C57BL mice (8). In addition, in I mice *in vivo* oxygen consumption is increased (2), and GDP binding to brown adipose tissue mitochondria is higher than in C57BL mice (23), suggesting thermogenesis is greater in I mice, and, consequently, overall energy expenditure as well.

The 5- and 10-fold greater glucose utilization in response to insulin in the epididymal and inguinal tissues, respectively, from I mice could be quantitatively important in accounting for their increased glucose tolerance, particularly if other adipose tissue sites exhibit the same increase in glucose utilization in I mice compared to those of C57BL mice. Lipogenic enzyme activity (fatty acid synthetase and citrate cleavage enzyme) were recently determined (unpublished) in total epididymal and inguinal adipose tissues and in liver. While enzyme activities in the epididymal and inguinal tissues were three- to sixfold higher in I mice than in C57BL mice, no difference between the two strains was found in enzyme activities in the liver, suggesting adipose tissue may be a crucial site of altered glucose metabolism in the I mice.

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