

## Glucose Metabolism in Isolated Adipocytes from *ad Libitum*- and Restricted-Fed Lean and Obese Zucker Rats at Two Different Ages<sup>1</sup> (42680)

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**Abstract.** Chronic food restriction in Sprague-Dawley rats has been shown to alter adipose glucose metabolism. In the present study, lean and obese male Zucker rats were food restricted from 5 weeks until either 10 or 26 weeks of age and adipocyte glucose metabolism was measured. Adipocytes from restricted-fed lean and obese Zucker rats converted more glucose to CO<sub>2</sub> and fatty acids than those from their *ad libitum*-fed counterparts in both the absence and the presence of increasing doses of insulin at 10 weeks of age. At the highest insulin dose, adipocytes from restricted-fed obese rats converted significantly more glucose to CO<sub>2</sub> and fatty acids than did those from restricted-fed lean rats. Basal glyceride-glycerol values were similar in all groups at this age. At the 0.4 and 2.0 ng/ml insulin levels, glyceride-glycerol production was highest in restricted-fed lean rats; restricted-fed obese and *ad libitum*-fed lean rats had similar values; and *ad libitum*-fed obese rats had the lowest. At the 20 ng/ml dose, glyceride-glycerol values of restricted-fed rats were higher than those of *ad libitum*-fed rats. Basal and insulin-stimulated values were compared within each group. Most basal versus insulin-stimulated values were significantly different for the two lean groups. For *ad libitum*-fed obese rats, only 0 versus 20 ng/ml insulin values were significant. Restricted-fed obese rats had significant increases in 0 versus both 2 and 20 ng/ml insulin values. Restricted-fed obese rats had significantly lower serum insulin levels relative to *ad libitum*-fed obese rats at 10 weeks of age. Adipocytes from all rats at 26 weeks of age had similar basal rates of conversion of glucose metabolism to all three metabolites. In the presence of insulin, adipocytes from restricted-fed lean rats metabolized significantly more glucose to CO<sub>2</sub> and glyceride-glycerol than adipocytes prepared from the three other groups. Fatty acid production was similar in all groups at each insulin level. Only restricted-fed lean rats showed consistent significant responses to insulin stimulation for the three metabolites. Whether these results are due to age, length of food restriction, or serum insulin levels remains to be determined. © 1988 Society for Experimental Biology and Medicine.

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The genetically obese Zucker rat has provided an opportunity to study the morphology and physiology of early-onset hyperplastic-hypertrophic obesity in an animal model. There are numerous similarities in metabolic disturbances found in the obese Zucker rat and the juvenile-onset form of human obesity (1). These include hypertriglyceridemia (2), elevated plasma insulin levels (3, 4), and insulin resistance in peripheral tissues (5-7). These disturbances in insulin metabolism have led to the suggestion that the Zucker rat would also be a useful animal model for insulin-independent diabetes (8).

Serum insulin is elevated from weaning in

obese rats (4, 9) and may also be higher during suckling (10). Serum glucose levels are usually similar to those of lean rats. The presence of hyperinsulinemia in these rats has led to their being classified as insulin resistant. Despite the early appearance of high serum insulin levels and enlarged fat cell size, isolated adipocytes from 6-week-old obese Zucker rats have been found to bind more insulin than adipocytes from lean rats of the same age (11) and to utilize more glucose in both the basal and the insulin-stimulated states (11, 12). Adipocytes from 10-week-old obese rats have also been reported to metabolize more glucose than adipocytes from lean rats (13). However, fat cells from older obese rats no longer show these characteristics in comparison to cells from lean rats (11, 14).

In Sprague-Dawley rats, severe, long-term food restriction has been shown to prevent the development of the age-related adipose

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tissue insulin resistance (15). York and Bray (16) examined the effects of lowered food intake in the obese Zucker rat on adipose tissue glucose metabolism and concluded that food restriction did not correct adipocyte insulin resistance in obese rats 15 to 18 weeks of age following 8 to 10 weeks of food restriction.

The purpose of the present study was to determine the effects of both short- and long-term food restriction on glucose metabolism in isolated adipocytes from obese and lean Zucker rats. Food restriction was initiated at 5 weeks of age when obese and lean rats could be clearly identified and at a time when food restriction should not interfere with organ growth of the rats. In addition, it would allow for integration of results with previous studies (17, 18) as well as other ongoing studies in this laboratory. Tissue was removed at 10 weeks of age when adipocytes from *ad libitum*-fed obese rats were reported to be quite metabolically active (13) and at 26 weeks of age when they have been reported to be severely insulin resistant (7).

**Materials and Methods.** *Animals.* Rats were purchased from the Biology Department, Vassar College, Poughkeepsie, New York. Male Zucker rats at 35 days of age were divided into four groups: (i) *ad libitum*-fed lean, (ii) restricted-fed lean, (iii) *ad libitum*-fed obese, and (iv) restricted-fed obese. The rats were individually housed with a 12-hr light/12-hr dark cycle at a temperature of 72–74°F. A purified diet similar in nutrient composition and caloric value to chow was provided to all rats. The restricted-fed rats received approximately 30% less food per day than their respective *ad libitum*-fed counterparts. The contents of this diet have been described in detail previously (19, Diet A).

Rats were fed in the late afternoon preceding the initiation of the dark cycle so that restricted-fed rats would eat according to their normal nocturnal pattern. Food intakes of the restricted groups were readjusted when changes in food intakes occurred in the *ad libitum*-fed groups. Food intakes were determined daily and body weights weekly. Rats were allowed *ad libitum* access to water. Groups of rats were killed after either 5 or 21 weeks of food restriction at 9–10 and 26 weeks of age, respectively. Numbers of rats

used at the two time points are given in the tables.

*Preparation of isolated adipocytes.* After a 12-hr fast, the rats were weighed and killed by decapitation. Blood was collected and centrifuged, and the serum was frozen. Right and left epididymal and retroperitoneal fat pads from each rat were quickly removed, placed in warm saline, and weighed. Isolated adipocytes were prepared by the method of Rodbell (20), as previously described (18). After digestion, the cells were strained through a 250- $\mu$ m mesh filter and washed three times by flotation in a collagenase-free buffer. The adipocytes were resuspended in this buffer at a cell concentration of 1–5  $\times 10^5$  cells/ml. Separate preparations were prepared from the two fat depots. However, since similar responses were found for epididymal and retroperitoneal depots, only data from epididymal fat cells are presented.

*Glucose metabolism studies.* A  $\frac{1}{2}$ -ml aliquot of the cell suspension was added to  $\frac{1}{2}$ -ml KRB with glucose (final concentration of 1 mM glucose, 0.250  $\mu$ Ci D-[U- $^{14}$ C]glucose). Insulin was added to cell samples from each rat at a concentration of 0.0, 0.4, 2, or 20 ng/ml. All determinations were made in triplicate. Following 1-hr incubation at 37°C, the reaction was terminated and incorporation of glucose into CO<sub>2</sub>, glyceride-glycerol, and fatty acids determined as previously described (18).

*Serum determinations.* Insulin concentration was determined by radioimmunoassay using a rat insulin standard (21). Serum glucose was determined in a Beckman glucose analyzer by an oxygen electrode technique (22).

*Determination of fat cell size.* A  $\frac{1}{2}$ -ml aliquot of the original fat cell suspension was fixed in OsO<sub>4</sub> by the method of Cushman and Salans (23). The cells were counted using a Coulter Counter, Model ZB. Total lipid in the sample was determined (24), and this value was divided by the cell number to give the cell size.

*Statistical analysis.* Data are presented as means  $\pm$  SE. Data at each age were analyzed for statistical significance by two-way analysis of variance (ANOVA) followed by the *F* test to determine the significance between specific groups (25). At both ages, within

each experimental group, data for each metabolite were analyzed by one-way ANOVA. If a significant *F* value was obtained, further comparisons were made using the "Least Significant Difference" as described by Snedecor and Cochran (26) to determine differences between pairs of means. This was done primarily to determine significance between basal (0 insulin) and the three different insulin levels. A *P* value of less than 0.05 was considered significantly different. *P* values of less than 0.1 have been noted.

**Results.** *Ten weeks of age.* After 5 weeks of food restriction, as expected, restricted-fed rats had significantly lower food intake and body weight than their respective *ad libitum*-fed group. *Ad libitum*-fed lean rats weighed an amount similar to that of restricted-fed obese rats. Food restricted-lean rats had greater serum glucose and insulin levels than *ad libitum*-fed lean rats (Table I). The precise reason for this is not known, but we have noted similar findings in earlier experiments with food-restricted rats (19, 27). The two obese groups and the restricted-fed lean rats had similar glucose values that were also significantly greater than those of *ad libitum*-fed lean rats. Serum insulin levels of restricted-fed obese rats were lowered by 50% compared to *ad libitum*-fed obese rats. Serum insulin levels for both obese groups were significantly higher than those for the two lean groups.

There was a trend toward lowered fat pad weights and fat cell sizes in restricted-fed lean rats compared to *ad libitum*-fed lean rats, but these values did not reach statistical significance when analyzed by ANOVA. Restricted-fed obese rats had significantly lower fat pad weights than *ad libitum*-fed obese rats, but there were no differences in fat cell sizes. Fat pad weights and cell sizes were greater in obese than in lean rats.

In the absence of insulin and at the three levels of insulin used, fat cells from restricted-fed rats metabolized significantly more glucose to CO<sub>2</sub> than did fat cells from *ad libitum*-fed rats (Fig. 1A). At the 20 ng/ml insulin level, CO<sub>2</sub> production in adipocytes of restricted-fed obese rats was significantly higher than that of restricted-fed lean rats. Basal incorporation of glucose into fatty acids was higher in restricted-fed compared

TABLE I. BODY WEIGHT, CUMULATIVE FOOD INTAKE, SERUM GLUCOSE AND INSULIN VALUES, AND EPIDIDYMAL AND RETROPERITONEAL FAT PAD WEIGHT AND CELL SIZE IN *AD LIBITUM*-FED AND RESTRICTED-FED LEAN AND OBESE MALE ZUCKER RATS AT 10 WEEKS OF AGE

	Body weight (g)	Food intake (g)	Glucose (mg/dl)	Insulin (ng/dl)	Epididymal		Retroperitoneal	
					Pad weight (g)	Cell size (μg lipid/cell)	Pad weight (g)	Cell size (μg lipid/cell)
<i>Ad libitum</i> -fed (n = 13)	231.2 ± 5.8 <sup>a</sup>	588.1 ± 27.5 <sup>a</sup>	90.0 ± 3.0 <sup>a</sup>	1.01 ± 0.13 <sup>a</sup>	1.95 ± 0.15 <sup>a</sup>	0.40 ± 0.03 <sup>a</sup>	1.33 ± 0.12 <sup>a</sup>	0.54 ± 0.07*
Restricted-fed (n = 13)	186.2 ± 0.33 <sup>b</sup>	411.4 ± 14.1 <sup>b</sup>	117.8 ± 3.6 <sup>b</sup>	1.38 ± 0.09 <sup>a</sup>	1.54 ± 0.08 <sup>a</sup>	0.31 ± 0.01 <sup>a</sup>	0.94 ± 0.10 <sup>a</sup>	0.42 ± 0.08*
<i>Ad libitum</i> -fed (n = 6)	285.0 ± 18.5 <sup>c</sup>	727.8 ± 48.4 <sup>c</sup>	114.8 ± 3.6 <sup>b</sup>	4.57 ± 0.61 <sup>b</sup>	6.62 ± 0.61 <sup>b</sup>	0.84 ± 0.08 <sup>b</sup>	4.55 ± 0.50 <sup>b</sup>	0.77 ± 0.12
Restricted-fed (n = 6)	215.6 ± 4.8 <sup>a</sup>	518.6 ± 17.2 <sup>d</sup>	114.5 ± 4.6 <sup>b</sup>	2.64 ± 0.27 <sup>c</sup>	4.99 ± 0.19 <sup>c</sup>	0.97 ± 0.17 <sup>b</sup>	3.14 ± 0.25 <sup>c</sup>	0.76 ± 0.03

Note. Values are means ± SE. Within a column, values with different superscripts are significantly different at *P* < 0.05.

\* Lean values significantly different from obese by ANOVA at *P* < 0.05.

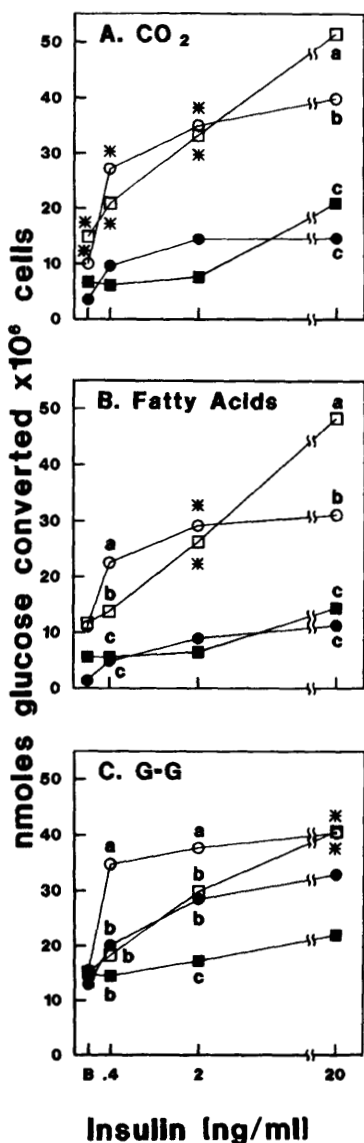


FIG. 1. CO<sub>2</sub> (A), fatty acid (B), and glyceride-glycerol (C) production from [U-<sup>14</sup>C]glucose (1 mM) in the absence (B) or presence of insulin (X axis) over 60 min at 37°C in isolated epididymal adipose cells from 10-week-old male Zucker rats. *Ad libitum*-fed lean rats (●—●); restricted-fed lean rats (○—○); *ad libitum*-fed obese rats (■—■); and restricted-fed obese rats (□—□). Results are presented as means. Asterisks indicate restricted-fed rats significantly different from *ad libitum*-fed rats at  $P < 0.05$  by  $2 \times 2$  ANOVA at this insulin level. Data points with different letters are significantly different from each other at  $P < 0.05$  by the  $F$  test. See Table II for comparison within each group for each metabolite.

to *ad libitum*-fed rats (Fig. 1B). In the presence of 0.4 ng/ml insulin, fatty acid synthesis from glucose was highest in the restricted-fed lean rats followed by restricted-fed obese rats. Fatty acid synthesis at 0.4 ng/ml insulin was similar for the two *ad libitum*-fed groups. At the 2 ng/ml insulin level, restricted-fed rats had significantly higher values than did *ad libitum*-fed rats. At the highest dose used, 20 ng/ml insulin, restricted-fed obese rats had the highest fatty acid synthesis from glucose followed by the restricted-fed lean rats and then the two *ad libitum*-fed groups. Basal glyceride-glycerol values were similar in all four groups (Fig. 1C). In the presence of the two intermediate levels of insulin, restricted-fed lean rats had values for glyceride-glycerol production significantly greater than the three other groups. At the 20 ng/ml insulin levels, restricted-fed rats had higher values than *ad libitum*-fed rats.

As described under Materials and Methods, the relationships among basal and the three insulin-stimulated values for each metabolite of glucose within each group were analyzed by one-way ANOVA. All ANOVA results were significant within each group for each metabolite (Table II). The "Least Significant Difference" determinations comparing results between specific insulin levels were then done (Table II). For both lean groups, all insulin-stimulated values were significantly greater than basal values except for the 0 versus 0.4 ng/ml insulin dose for fatty acid and glyceride-glycerol synthesis from glucose for adipocytes from *ad libitum*-fed lean rats. For *ad libitum*-fed obese rats, only the 0 versus 20 ng/ml insulin level comparisons for the three metabolites were significantly different. For restricted-fed obese rats, all comparisons were significantly different except for 0 versus 0.4 ng/ml insulin. Comparisons among the other insulin levels within each group for each metabolite are also shown in Table II.

*Twenty-six weeks of age.* At 26 weeks of age, body weights of restricted-fed lean rats were significantly lower than body weights of the other three groups of rats (Table III). Body weights of the *ad libitum*-fed obese rats were the highest and were significantly

TABLE II. SUMMARY OF COMPARISONS FOR INSULIN RESPONSE<sup>a</sup> WITHIN EACH GROUP FOR EACH METABOLITE AT 10 WEEKS OF AGE

		ANOVA	Insulin levels (ng/ml)					
			0 vs 0.4	0 vs 2	0 vs 20	0.4 vs 2	0.4 vs 20	2 vs 20
<i>Ad libitum</i> -fed lean	CO <sub>2</sub>	S <sup>b</sup>	S	S	S	S	S	NS <sup>c</sup>
	FA	S	NS	S	S	S	S	NS
	GG	S	NS	S	S	<0.1	S	NS
Restricted-fed lean	CO <sub>2</sub>	S	S	S	S	NS	S	NS
	FA	S	S	S	S	<0.1	S	NS
	GG	S	S	S	S	NS	NS	NS
<i>Ad libitum</i> -fed obese	CO <sub>2</sub>	S	NS	NS	S	NS	S	S
	FA	<0.1	NS	NS	S	NS	NS	S
	GG	S	NS	NS	S	NS	S	NS
Restricted-fed obese	CO <sub>2</sub>	S	NS	S	S	S	S	S
	FA	S	NS	S	S	S	S	S
	GG	S	NS	S	S	S	S	S

<sup>a</sup> Data are presented in Figs. 1A, 1B, and 1C for CO<sub>2</sub>, FA (fatty acids), and GG (glyceride-glycerol), respectively. Data obtained at 0, 0.4, 2, and 20 ng/ml insulin were analyzed by one-way ANOVA. If the *F* value for ANOVA was *P* < 0.05 (unless otherwise noted) then it was followed by test for "Least Significant Difference" (26) to test for differences between specific pairs of means.

<sup>b</sup> S = *P* < 0.05.

<sup>c</sup> NS = *P* > 0.1.

greater than those of *ad libitum*-fed lean and restricted-fed obese rats. These two groups had similar body weights. Serum glucose was highest in *ad libitum*-fed lean rats followed by restricted-fed obese rats and then by restricted-fed lean and *ad libitum*-fed obese rats. Insulin levels were significantly higher in obese than in lean rats (Table III). Epididymal fat pad weight and fat cell size were greater in obese than in lean rats (Table III). Both groups of obese rats had lowered retroperitoneal pad weight when compared to their respective control group (Table III), but obese values were greater than lean values.

Basal values for glucose conversion to CO<sub>2</sub> were not significantly different among the four groups of rats (Fig. 2A). However, insulin-stimulated CO<sub>2</sub> release was threefold higher in restricted-fed lean rats than in the other three groups of rats at each insulin level (Fig. 2A). Incorporation of glucose into fatty acids was similar in all groups in the absence of insulin as well as at each insulin level used (Fig. 2B). Basal glyceride-glycerol synthesis was also not significantly different among any of the groups (Fig. 2C). Insulin-stimulated glyceride-glycerol production was significantly greater in restricted-fed lean rats

than in all other rats at this age for all three insulin levels.

As with data from 10-week-old rats, comparisons were made within each group for each metabolite. For *ad libitum*-fed lean rats, only the CO<sub>2</sub> results were significantly different (*P* < 0.1) (Table IV). For restricted-fed lean rats at 26 weeks of age, both CO<sub>2</sub> and glyceride-glycerol production were significantly different. For obese rats only, glyceride-glycerol in *ad libitum*-fed obese rats was significantly different (*P* < 0.1). Specific differences by the "Least Significant Difference" method are shown in Table IV.

**Discussion.** It has previously been reported by DiGirolamo and Rudman (15) that Sprague-Dawley rats fed one-third the calories of *ad libitum*-fed rats from weaning until over 1 year of age maintained insulin sensitivity in adipose tissue pieces tested *in vitro*. Our results for lean Zucker rats indicate that this effect of chronic food restriction on glucose metabolism can also be found using isolated adipocytes and, in addition, show that a more moderate degree of restriction can also improve this tissue's response to insulin when compared to tissue from *ad libitum*-fed lean rats. We have

TABLE III. BODY WEIGHT, CUMULATIVE FOOD INTAKE, SERUM GLUCOSE AND INSULIN VALUES, AND EPIDIDYMAL AND RETROPERITONEAL FAT PAD WEIGHT AND CELL SIZE IN *AD LIBITUM*-FED AND RESTRICTED-FED LEAN AND OBESE MALE ZUCKER RATS AT 26 WEEKS OF AGE

	Body weight (g)	Food intake (g)	Glucose (mg/dl)	Insulin (ng/dl)	Epididymal		Retroperitoneal	
					Pad weight (g)	Cell size ( $\mu\text{g}$ lipid/cell)	Pad weight (g)	Cell size ( $\mu\text{g}$ lipid/cell)
<i>Ad libitum</i> -fed (n = 5)	430.4 $\pm$ 18.1 <sup>a</sup>	2677.2 $\pm$ 18.1 <sup>a</sup>	135.3 $\pm$ 5.9 <sup>a</sup>	2.58 $\pm$ 1.01*	5.84 $\pm$ 0.66*	0.69 $\pm$ 0.15*	6.36 $\pm$ 1.00 <sup>a</sup>	0.79 $\pm$ 1.24
Restricted-fed (n = 5)	331.8 $\pm$ 6.8 <sup>b</sup>	1943.6 $\pm$ 8.9 <sup>b</sup>	88.8 $\pm$ 7.0 <sup>b</sup>	2.40 $\pm$ 0.80*	4.34 $\pm$ 0.90*	0.85 $\pm$ 0.09*	3.28 $\pm$ 0.55 <sup>a</sup>	0.83 $\pm$ 0.12
<i>Ad libitum</i> -fed (n = 3)	539.0 $\pm$ 21.7 <sup>c</sup>	3303.6 $\pm$ 108.7 <sup>c</sup>	96.0 $\pm$ 0.0 <sup>b</sup>	5.30 $\pm$ 0.10	9.23 $\pm$ 1.21	1.00 $\pm$ 0.10	20.63 $\pm$ 3.72 <sup>c</sup>	0.84 $\pm$ 0.17
Restricted-fed (n = 3)	432.0 $\pm$ 8.1 <sup>a</sup>	2246.0 $\pm$ 44.5 <sup>d</sup>	118.5 $\pm$ 2.5 <sup>c</sup>	5.60 $\pm$ 0.00	10.63 $\pm$ 1.68	0.97 $\pm$ 0.12	14.57 $\pm$ 2.69 <sup>d</sup>	0.79 $\pm$ 0.11

Note. Values are means  $\pm$  SE. Within a column, values with different superscripts are significantly different at  $P < 0.05$ .  
\* Lean values significantly different from obese by ANOVA at  $P < 0.05$ .

shown similar findings for adipocytes from lean Zucker rats food restricted until 1 year of age (17). However, for obese rats, this response to food restriction on adipocyte glucose metabolism was only apparent at 10 weeks of age following 5 weeks of food restriction. Results presented here for 26-week-old and previously for 1-year-old rats (17) showed no differences between food-restricted and *ad libitum*-fed obese rats. This finding does not appear to be attributable to length of food restriction, as another study showed that 3 weeks of food restriction from 20 to 23 weeks of age also had no effect on similar measurements in obese rats (unpublished observation).

In the present study, serum insulin levels were lowered in obese rats at 10 but not at 26 weeks of age. Possibly the age-related differences in adipocyte glucose metabolism may be explained by this lack of lowering of serum insulin in the older obese rats. However, a number of factors would suggest that this is not the case. Restricted-fed lean rats had no changes in serum insulin levels relative to *ad libitum*-fed rats, yet the restricted-fed lean rats had high rates of adipocyte glucose conversion at both ages. At 10 weeks of age, glucose metabolism values for restricted-fed obese rats were similar to or higher than those of restricted-fed lean rats, yet their serum insulin values were significantly greater than those of the lean rats. Furthermore, when serum insulin levels have been lowered by fasting, food restriction, or dehydroepiandrosterone treatment in obese rats of similar age, i.e., 26 weeks, no changes in adipocyte glucose metabolism relative to *ad libitum*-fed obese rats were observed (18, 28). York and Bray also did not report any differences in adipose tissue metabolism from 15- to 18-week-old obese rats food restricted from 8 weeks of age when results were compared to those of *ad libitum*-fed obese rats (16). This was found despite markedly lower serum insulin levels in the food-restricted group and use of more severe food restriction than that used in the present experiment. This suggests that another serum factor(s) is involved, or that there is some characteristic associated with the fat cells themselves responsible for the eventual development of insulin resistance. We realize

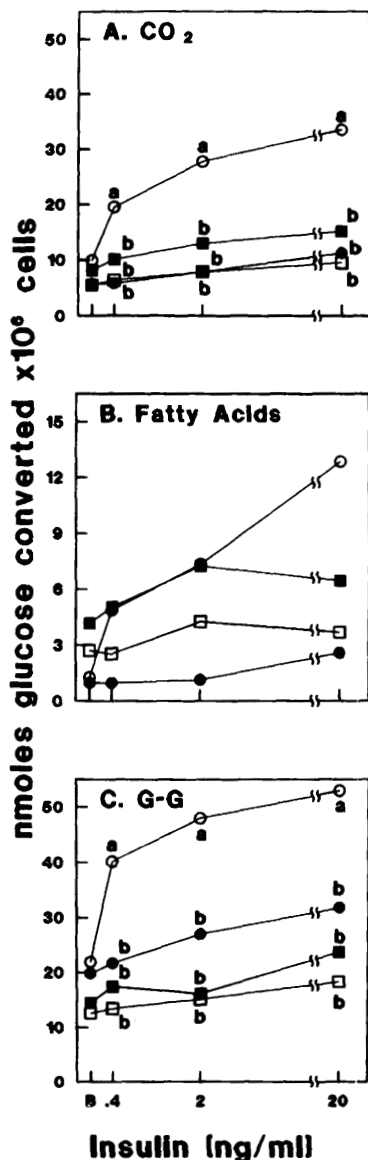


FIG. 2. CO<sub>2</sub> (A), fatty acid (B), and glyceride-glycerol (C) production from [U-<sup>14</sup>C]glucose (1 mM) in the absence (B) or presence of insulin (X axis) over 60 min at 37°C in isolated epididymal adipose cells from 26-week-old male Zucker rats. *Ad libitum*-fed lean rats (●—●); restricted-fed lean rats (○—○); *ad libitum*-fed obese rats (■—■); and restricted-fed obese rats (□—□). Results are presented as means. Asterisks indicate restricted-fed rats significantly different from *ad libitum*-fed rats at  $P < 0.05$  by  $2 \times 2$  ANOVA at this insulin level. Data points with different letters are significantly different from each other at  $P < 0.05$  by the  $F$  test. See Table IV for comparison within each group for each metabolite.

that these conclusions are based on data from a relatively small group of rats, but we have found similar results in a study continued to 1 year of age (17). Furthermore, for these types of studies, small sample sizes are not unusual (11, 13) nor are results even statistically analyzed in most studies presenting these types of data (11, 13, 16).

Whether or not this metabolic change found in adipocytes in relation to food restriction is due to a meal-feeding effect is difficult to ascertain. Although the intent in the present experiment was not to limit access to food over any 24-hr period, restricted-fed rats tended to eat most of their food overnight and always had empty feed dishes at feeding time late the following afternoon. Leveille (29) reported that meal-fed rats incorporated more [U-<sup>14</sup>C]glucose into CO<sub>2</sub>, fatty acids, and glyceride-glycerol in adipose tissue pieces than did the tissue from nibblers. However, these data were expressed per 100 mg tissue. Since meal-fed rats ate less and also weighed less than nibblers, the rats were clearly also food-restricted and presumably had smaller fat cells. Data expressed on a per weight basis would thus reflect different fat cell numbers for the two groups. In addition, meal-fed rats were killed immediately after consuming their 2-hr meal of a diet that was high in glucose (50% or more). Nibblers were killed several hours into the light period and, thus, in a very different postabsorptive state. In the present study, rats were in a similar postprandial state and results were expressed on a per cell basis. It would, therefore, appear that regardless of whether the effects found are due to food restriction per se or to the manner of food restriction, i.e., meal feeding, either age or duration of the intervention plays some role in determining how the fat cells of obese rats metabolize glucose.

In a number of previous studies, Zucker obese rats have been shown to respond differently to food restriction than do lean rats. For example, chronically food-restricted rats preserve body fat at the expense of lean body mass (17, 30-32). It is possible that the changes in adipose tissue glucose metabolism noted here for 10-week-old obese rats play a role in this maintenance of body fat. However, due to the similar response found in

TABLE IV. SUMMARY OF COMPARISONS FOR INSULIN RESPONSE<sup>a</sup> WITHIN EACH GROUP FOR EACH METABOLITE AT 26 WEEKS OF AGE

		ANOVA	Insulin levels (ng/ml)					
			0 vs 0.4	0 vs 2	0 vs 20	0.4 vs 2	0.4 vs 20	2 vs 20
<i>Ad libitum</i> -fed lean	CO <sub>2</sub>	<0.1	NS <sup>b</sup>	NS	S <sup>c</sup>	NS	S	NS
	FA	NS						
	GG	NS						
Restricted-fed lean	CO <sub>2</sub>	S	NS	S	S	S	S	NS
	FA	NS						
	GG	S	<0.1	S	S	NS	NS	NS
<i>Ad libitum</i> -fed obese	CO <sub>2</sub>	NS						
	FA	NS						
	GG	<0.1	NS	NS	S	NS	S	S
Restricted-fed obese	CO <sub>2</sub>	NS						
	FA	NS						
	GG	NS						

<sup>a</sup> Data are presented in Figs. 2A, 2B, and 2C for CO<sub>2</sub>, FA (fatty acids), and GG (glyceride-glycerol), respectively. Data obtained at 0, 0.4, 2, and 20 ng/ml insulin were analyzed by one-way ANOVA. If the *F* value was *P* < 0.05 (unless otherwise noted) "Least Significant Difference" (26) determination was done between specific pairs of means.

<sup>b</sup> NS = *P* > 0.1.

<sup>c</sup> S = *P* < 0.05.

adipocytes of the restricted-fed lean rats this appears to be an unlikely explanation. What happens to insulin response and glucose metabolism in other tissues as a consequence of chronic food restriction may be of more significance. We have recently examined glucose metabolism in muscle of similarly treated lean and obese rats at 15 weeks of age (33). CO<sub>2</sub> release was similar in muscle of all groups except for basal CO<sub>2</sub> release in *ad libitum*-fed obese rats which was significantly lower than that of all other groups. Muscle glycogen synthesis was highest with or without insulin in restricted-fed lean rats followed by *ad libitum*-fed lean rats and then the two obese groups that had similar values. Adipocyte response for the four groups was similar to that reported here for 26-week-old rats. Thus, it would appear necessary to study obese rats at a younger age to obtain a more comprehensive picture of what occurs in other tissue in response to food restriction.

The present results from 9- to 10-week-old lean and obese *ad libitum*-fed rats do not agree with the findings of Czech *et al.* (13). They reported that adipocytes from 10-week-old obese Zucker rats metabolized more glucose than those from lean rats in the basal state and following insulin stimulation. However, it is unclear what the prandial state

of rats in that study was. In addition, rats in the two studies were fed different diets and both lean and obese rats in the Czech *et al.* study weighed considerably less than *ad libitum*-fed lean and obese rats in the present study. Therefore, it is difficult to make direct comparisons between the two studies.

Our results presented in Fig. 1, in general, show similar results for adipocytes from *ad libitum*-fed lean and obese rats at 10 weeks of age. If one examines these dose-response curves, there is some suggestion of both a rightward shift and a lowering of maximal response for glyceride-glycerol production and possibly also for CO<sub>2</sub> release for the *ad libitum*-fed obese versus lean rats, although by two-way ANOVA these differences were not significant. This type of curve is suggestive of both receptor and postreceptor defects (34). Examination of Table II shows that *ad libitum*-fed obese rats did not have statistically greater insulin-stimulated versus basal values until the highest insulin dose, while for *ad libitum*-fed lean rats most basal versus insulin-stimulated comparisons were significant. This would further suggest receptor defects in the obese rats. The glyceride-glycerol results for the restricted-fed obese rats would also be suggestive of a receptor defect at this age. We did not recalculate data on a per-

centage of maximal response basis as is frequently done due to the differences in basal and maximum values measured in the different groups (34). Results for the older lean and obese *ad libitum*-fed groups are basically in agreement with previously published studies (11, 16–18).

A number of studies have shown that glucose transporters in association with serum insulin levels are involved in the regulation of adipocyte cellular glucose uptake (35–39). It has also been shown that insulin resistance is associated with a relative depletion of glucose transporters in the intracellular pool in fat cells of both diabetic and old rats. Recently, young (30-day-old) obese Zucker rats have been found to have a marked increase in adipocyte glucose transporters relative to age-matched lean rats (40). Glucose transporter cellular location has been shown to be altered by changes in dietary constituents (38). Perhaps determination of the cellular location of adipose glucose transporters in restricted-fed lean and obese rats and in older obese rats will provide an explanation for the glucose metabolism and insulin response findings presented in this manuscript for adipose tissue.

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