Effect of insulin upon the cellular character of rat adipose tissue

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Abstract The effect of insulin upon the lipid content, and the number and size of fat cells in the epididymal, retroperitoneal, and subcutaneous adipose tissue of a large number of rats were examined. Insulin administration began either in early life (birth, 1, or 3 wk of age) or during adulthood (age 10 wk). At different times during growth, groups of treated and control animals were killed and the size and number of fat cells in each of the three adipose depots were determined. Insulin-treated animals gained weight at an increased rate and had fatter epididymal, retroperitoneal, and subcutaneous adipose depots than untreated controls. In each site the expanded adipose tissue was accompanied by an increase in the lipid content per cell (cell size), but in no case was there an increase in the number of adipose cells. This was the case regardless of whether insulin treatment was initiated before weaning (birth, 1 wk of age), at weaning (3 wk), or post weaning (10 wk) and irrespective of the duration of the insulin treatment.

Supplementary key words adipose depots · cell number · cell size · early life · adipocyte

The growth of adipose tissue is accomplished by an increase in the size and number of its constituent fat cells. Early in life new cell formation predominates, but in adult life the adipose depot appears to grow almost exclusively by deposition of more fat in existing cells, i.e., by cellular enlargement (1–5). In obesity, the expanded adipose tissue mass may be accompanied either by a marked increase in the number of adipose cells (1, 4, 5) or by cellular enlargement without an increase in cell number (3, 6). The factors which control adipose cellular proliferation and enlargement and which, therefore, may be responsible for the alterations in cell number and size in obesity are unknown. However, it is important to elucidate these factors, since they could provide insight into normal growth and development of adipose tissue as well as abnormal growth in the obese state.

Since insulin enhances DNA (7, 8), RNA (9), protein (10, 11), and lipid synthesis (12) in a variety of cells, this hormone may be one factor influencing adipose tissue growth by stimulating either new cell formation (increasing cell number) or deposition of lipid in preexisting cells (increasing cell size). Moreover, hyperinsulinemia often accompanies excessive adiposity (13, 14), an observation which has led to speculation that insulin may play a role in the excessive growth of the adipose depot in obesity. Several investigators have demonstrated that insulin administration to rats results in weight gain and increased adiposity (15–17). Vost and Hollenberg (18), in their studies on the effect of insulin on DNA synthesis in rat fat cells, reported that when insulin is administered to adult animals there is stimulation of the growth of the epididymal and lumbar fat depots by an increase in fat cell size (lipid content per cell) rather than by new cell formation (18). However, several recent studies in man (1, 3, 6) and in experimental animals (4, 5, 19–21) indicate that cell number may not be susceptible to change in adult life; adipose tissue grows by a process that is regulated very early in life by factors which influence both cellular mitosis and enlargement, but which in later life influence cell size only. Therefore, it remains possible that insulin may influence adipose tissue growth through an early effect on cellular mitosis and thus lead to adipose hypercellularity.

In the present study the influence of insulin upon the postnatal growth of rat adipose tissue has been examined. Insulin was administered at various times in the early and adult life of normal rats, and its effect upon fat cell size and number in three different adipose depots was determined.

METHODS

Animals

Charles River (CD strain) rats were used in all experiments. A large number of females were mated with one

of nine proven male breeders. At birth, female offspring were removed from each litter; the males were then identified, weighed, and redistributed so that each mother suckled six newborn male rats. Animals were handled gently with sterile gloves by the same individual throughout the experiment. At weaning, rats were caged individually and allowed free access to food (standard Purina Rat Chow) and to water containing 5% glucose. All animals were weighed at weekly intervals beginning at birth.

Insulin administration

Rats treated with insulin received twice daily subcutaneous injections of ultralente insulin, 0.025 U/g body wt (U-40, Eli Lilly & Co., Indianapolis, Ind.). The insulin was diluted in 5% glucose in saline so that the desired amount could be administered in a volume of 0.2 ml. Injections were made under the dorsal skin of the neck.

Experimental design

In the first experiment insulin was administered to a large number of rats at various stages of their early and adult life, and its effect on the growth and cellular character of the epididymal adipose depot was determined. Animals were separated into four groups according to when insulin treatment was begun: group I at birth, group II at age 1 wk, group III at weaning (3 wk), and group IV at 10 wk of age. Each group contained 72 rats, 12 litters of 6 animals. In each litter, two animals received insulin, two an equivalent volume of 5% glucose in saline, and two no injection. Once insulin or glucose–saline injections were begun, they were continued until the animals were killed. In groups I, II, and III, insulin-treated rats and an equal number of untreated and glucose–saline-treated control animals were killed at 3, 5, 8, and 15 wk of age; their epididymal fat pads were removed, and the lipid content and fat cell number and size in this tissue were determined. Group IV rats were killed at 3, 5, and 10 wk of age (before treatment) and at age 15 wk (after treatment), and the lipid content and cellular character of their epididymal adipose depots were determined.

In the second experiment, insulin administration was begun early in life and its effect on the *adult* cellular character of three different adipose depots was examined. Eight litters of six animals each were studied. In each litter, three animals received insulin and the remaining three served as untreated controls. In four litters, insulin administration was begun at birth (group V) and in the remaining four at weaning (age 3 wk, group VI). Once begun, insulin was administered until the animals were killed. At 10 wk of age, one-half of the insulin-treated and control rats in each group were killed. Three separate fat depots were examined; the epididymal, retroperitoneal, and dorsal interscapular subcutaneous adipose tissues were removed and their lipid content and fat cell size and number were determined. The remaining rats in each treatment and control group were killed at 20 wk of age and identical studies were undertaken.

Isolation of tissues and determination of fat cell size and number

Animals were killed by decapitation after receiving a stunning blow to the head. Epididymal and/or retroperitoneal and dorsal interscapular subcutaneous fat pads were removed as described by Johnson et al. (4) and placed in bicarbonate buffered Krebs-Ringer medium at 37°C. For every animal the entire fat pad from each site was weighed, and a representative portion of tissue from each site was taken for lipid extraction (22), determination of lipid content (23), and measurement of fat cell size and number (24). Fat cell size, expressed as the average lipid content per cell, was calculated as follows:

$$\mu\text{g lipid/cell} = \frac{\text{wet weight of osmium-fixed tissue } (\mu\text{g})}{\text{total number of cells in osmium-fixed tissue}} \times \frac{\text{lipid weight of unfixed tissue } (\mu\text{g})}{\text{wet weight of unfixed tissue } (\mu\text{g})}$$

The total number of fat cells in each individual adipose depot was calculated by multiplying the number of cells in a known amount of lipid by the total amount of lipid in the depot.

RESULTS

Cell size and number are expressed as the mean for each group ± the standard error of the mean. Significance testing was performed by the *t* test and significant values are reported at $P < 0.05$.

Effect of insulin administration on the growth of the epididymal fat pad

Since no difference in body weight, epididymal fat pad lipid content, or fat cell size and number was observed between untreated and glucose–saline-treated rats, these two non-insulin-treated subgroups were combined to constitute the control group.

Figs. 1*a*–4*a* indicate the growth curves for the four groups of insulin-treated and control animals. In each experimental group, insulin treatment produced a significant increase in body weight over that of controls. This effect of insulin was not observed until the 5th wk of life irrespective of whether treatment was begun at birth (Fig. 1*a*), 1 wk (Fig. 2*a*), or 3 wk of age (Fig. 3*a*). Thereafter, differences in body weight became more marked as the animals continued to receive insulin for the remainder of the experimental period. No differences

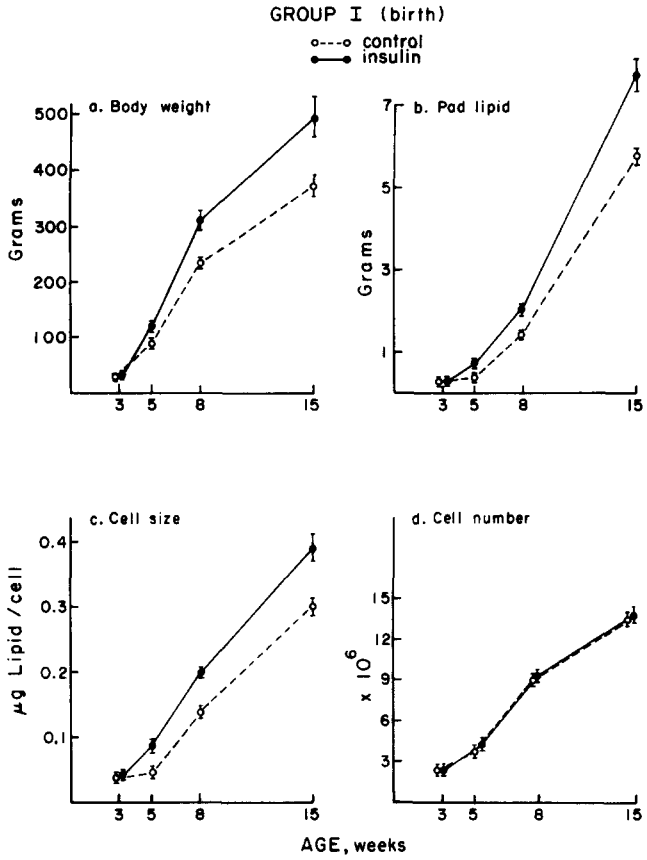

FIG. 1. Insulin administration began at birth; rats were killed at ages 3, 5, 8, and 15 wk and body weight (*a*), lipid content (*b*), fat cell size (*c*), and total fat cell number (*d*) in the epididymal fat pad were determined. Each point represents the mean ± SEM of 6–12 animals.

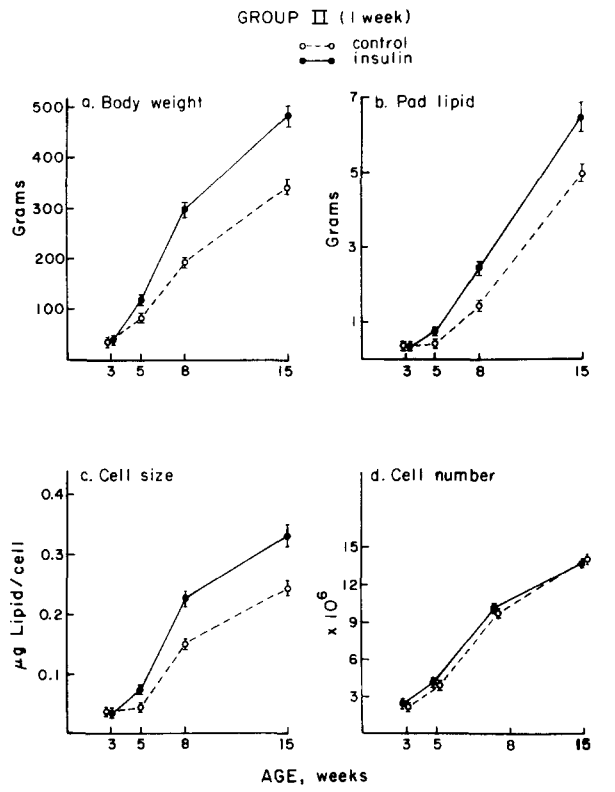

FIG. 2. Insulin administration began at 1 wk of age; rats were killed at ages 3, 5, 8, and 15 wk and body weight (*a*), lipid content (*b*), fat cell size (*c*), and total fat cell number (*d*) in the epididymal fat pad were determined. Each point represents the mean ± SEM of 6–12 animals.

were observed in the growth curves of animals in group IV prior to the initiation of insulin administration at 10 wk (Fig. 4*a*). However, within 2 wk a significant increase in the body weight of insulin-treated animals was observed and this difference became progressively more marked until age 15 wk.

The increased body weight of insulin-treated rats over that of controls was, at each age, paralleled by an increase in the weight of their epididymal fat pads. Most (88–92%) of the difference in the weight of this tissue between treated and control rats was due to an increased accumulation of lipid in the epididymal pads of animals receiving insulin. The increasing adiposity of the epididymal fat pad with age closely paralleled the growth curve in each experimental group (Figs. 1*b*–4*b*). The lipid content of the epididymal fat pads of the treated and control animals of group I was, as a group, similar at weaning even though insulin had been administered for 3 wk (Fig. 1*b*). However, at 5 wk of age the epididymal pads of the insulin-treated animals in this group, as well as of those in groups II and III (Fig. 3*b*), were fatter than controls of the same age, a difference which became more pronounced in each group as the animals continued to grow and to be exposed to insulin. Administration of insulin to rats of group IV, beginning at age 10 wk, resulted in the development of fatter epididymal pads than those of the controls at 15 wk (Fig. 4*b*).

The differences in the adiposity of the epididymal fat pads between insulin-treated and control animals could be attributed solely to differences in adipose cell size (Figs. 1*c*–4*c*). At weaning, the size of the epididymal fat cells of treated rats was, as a group, similar to that of the controls even in those animals receiving insulin from birth (Fig. 1*c*) or from the 1st wk of life (Fig. 2*c*). From the 3rd to the 5th wk of life, the epididymal fat cell size of control animals increased only slightly or not at all. However, cellular enlargement did occur during this period in the epididymal adipose cells of animals receiving insulin from birth, age 1 wk, or 3 wk of life. Thus, at 5 wk, the age at which group differences in the adiposity of this depot were first noted, there was a significant increase in fat cell size in the epididymal fat pads of insulin-treated animals of groups I (Fig. 1*c*), II (Fig. 2*c*), and III (Fig. 3*c*) over that in the controls. Enlarged adipose cells were observed in the epididymal fat pads of the insulin-treated rats of group IV at 15 wk even though insulin

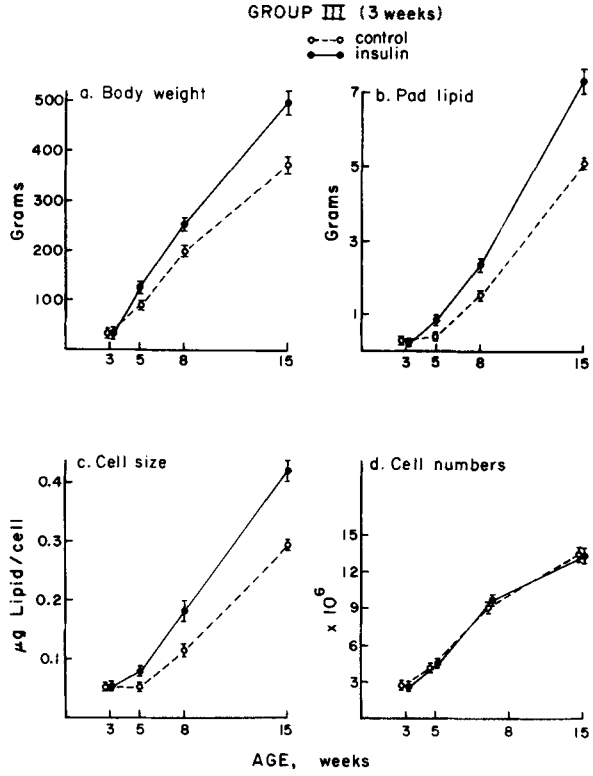

FIG. 3. Insulin administration began at 3 wk of age; rats were killed at ages 3, 5, 8, and 15 wk and body weight (*a*), lipid content (*b*), fat cell size (*c*), and total fat cell number (*d*) in the epididymal fat pad were determined. Each point represents the mean ± SEM of 6–12 animals.

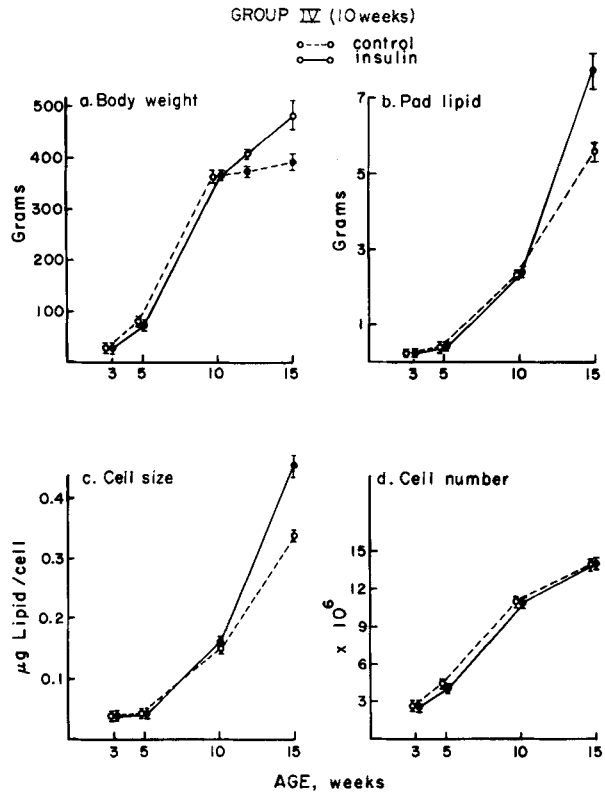

FIG. 4. Insulin administration began at 10 wk of age; rats were killed at ages 3, 5, 10, and 15 wk and body weight (*a*), lipid content (*b*), fat cell size (*c*), and total fat cell number (*d*) in the epididymal fat pad were determined. Each point represents the mean ± SEM of 6–12 animals.

administration was not begun until 10 wk of age (Fig. 4*c*).

Figs. 1*d*–4*d* indicate that total cell number in the fat pads of the insulin-treated animals of each group was similar to that of controls at every age examined. At weaning, cell number was not increased in the epididymal fat pads of group I (Fig. 1*d*) and II (Fig. 2*d*) insulin-treated animals relative to the controls even though they received treatment from birth or age 1 wk. Total fat cell number in the epididymal pads of treated and control animals in these two groups and in group III (Fig. 3*d*) was similar after 5, 8, and even 15 wk of insulin administration. In animals receiving insulin beginning at age 10 wk, epididymal fat cell number was similar to that of controls even though the tissue contained more lipid (Fig. 4*d*).

At weaning, two of the insulin-treated rats in group II (insulin treatment beginning at 1 wk) and three of the insulin-treated animals in group I (insulin treatment beginning from birth) did weigh more than any of the other treated or control animals in those groups (Fig. 5). The differences persisted until age 5 wk, when these five animals were killed. These particular insulin-treated rats had significantly larger epididymal fat pads (0.703 ±

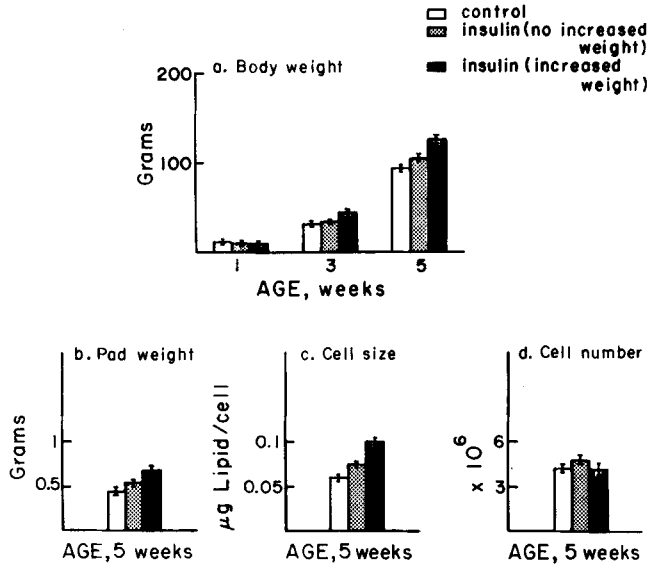

FIG. 5. Body weight at ages 1, 3, and 5 wk (*a*), and lipid content (*b*), fat cell size (*c*), and total fat cell number (*d*) in the epididymal adipose tissue of 5-wk-old animals with and without increased body weight at weaning. Values are means ± SEM of the control rats (open bars), the five insulin-treated animals of groups I and II with the greatest weight at weaning (solid bars), and the remaining insulin-treated rats (stippled bars).

0.05 g) than did either the controls (0.419 ± 0.021 g) or the other insulin-treated rats (0.530 ± 0.04 g) in these groups. This increase in epididymal adiposity was accompanied by significantly larger fat cells (0.100 ± 0.01 vs. 0.060 ± 0.01 vs. 0.830 ± 0.01 μg of lipid/cell). There were, however, no differences in cell number.

Effect of insulin treatment begun early in life on adult cell size and number in three fat depots

Fig. 6*a* indicates that rats receiving insulin from birth (group V) weighed significantly more than their matched controls, beginning at age 5 wk. This difference became progressively more marked at 10 and 20 wk of age. The increase in body weight of the insulin-treated rats over that of the controls is reflected in the ncreased adiposity of each of the three fat depots examined (Table 1). At 10 wk of age, the epididymal, retroperitoneal, and subcutaneous fat pads of insulin-treated group V animals were significantly fatter than those of the controls. After 20 wk of insulin treatment, these differences between insulin-treated and control animals were even greater. The increased adiposity of each depot was accompanied by an increase in fat cell size; no significant increase in total cell number was noted even though insulin administration was begun at birth. The fat cells of insulin-treated rats in group V were larger in the epididymal, retroperitoneal, and subcutaneous depots than in these sites in control animals. At each age tested, the cell size of insulin-treated rats compared with controls was increased to a similar degree in retroperitoneal (70–90%) and epididymal fat (80–88%). However, in the subcutaneous depot, insulin treatment increased cell size above control to a greater degree (100–120%) than in either of the other two depots.

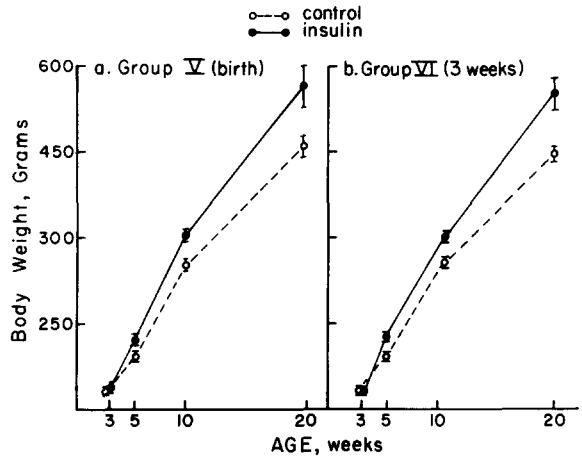

FIG. 6. Body weight of insulin-treated and control rats at 3, 5, 10, and 20 wk of age. (*a*) group V, insulin treatment began at birth; (*b*) group VI, insulin treatment began at age 3 wk. Points represent means ± SEM.

Fig. 6*b* illustrates the growth curves of the animals in group VI that received insulin beginning at age 3 wk. Again, a significant increase in the body weight of the insulin-treated rats over that of the controls was first noted at age 5 wk, a difference which became more marked with continued exposure to insulin. The lipid content of each of the three fat depots was greater in the

TABLE 1. Effect of insulin administration on body weight and lipid content in three adipose depots of adult rats

Experimental Group	Age	Body Weight	Pad Lipid: Epididymal	Pad Lipid: Retroperitoneal	Pad Lipid: Subcutaneous	Cell Size: Epididymal	Cell Size: Retroperitoneal	Cell Size: Subcutaneous	Cell Number: Epididymal	Cell Number: Retroperitoneal	Cell Number: Subcutaneous
	wk	*g*		*g*			*μg lipid/cell*			× 10^6	
Group V											
Insulin	10	294	2.42	2.57	0.55	0.1886	0.1976	0.1104	11.0	9.1	3.1
		±2[a]	±0.09[a]	±0.24[b]	±0.05[b]	±0.0104[a]	±0.0015[a]	±0.0010[a]	±0.36	±0.76	±0.06
Control		268	1.59	1.04	0.20	0.1097	0.1120	0.0570	12.0	10.5	3.0
		±2	±0.07	±0.19	±0.02	±0.0087	±0.0094	±0.0037	±1.48	±0.75	±0.13
Insulin	20	560	9.80	11.21	2.34	0.5702	0.6322	0.3016	12.6	11.2	7.0
		±31[b]	±1.00[b]	±1.82[b]	±0.37[b]	±0.0312[b]	±0.0290[b]	±0.0313[b]	±1.16	±0.44	±0.80
Control		460	3.79	3.92	0.56	0.3015	0.3543	0.1363	13.9	10.6	6.6
		±13	±0.37	±0.58	±0.11	±0.0332	±0.0822	±0.0138	±0.84	±1.22	±0.33
Group VI											
Insulin	10	299	2.24	1.89	0.38	0.1478	0.1535	0.0907	12.3	8.1	3.8
		±10[a]	±0.10[a]	±0.08[a]	±0.06[b]	±0.0046[a]	±0.0104	±0.0032[b]	±0.50	±0.37	±0.17
Control		260	1.34	0.91	0.16	0.0999	0.1062	0.0436	11.2	7.2	3.4
		±9	±0.21	±0.02	±0.03	±0.0024	±0.0022	±0.0026	±1.05	±0.55	±0.19
Insulin	20	547	6.46	8.72	2.14	0.4324	0.5998	0.2706	13.7	10.5	6.9
		±33[b]	±0.60[b]	±1.93[b]	±0.53[b]	±0.0337[b]	±0.0495[b]	±0.0080[b]	±1.61	±1.70	±1.29
Control		433	3.89	4.17	0.05	0.2629	0.3335	0.0943	13.7	11.4	6.5
		±8	±0.09	±0.48	±0.09	±0.0223	±0.0290	±0.0176	±0.94	±1.40	±0.43

Values are means ± SEM of six animals. Insulin administration began either at birth (group V) or at 3 wk of age (group VI) and continued until animals were killed at 10 and 20 wk of age.

[a] Significantly different from control, $P < 0.05$.

[b] Significantly different from control, $P < 0.02$.

insulin-treated animals than in the controls at both 10 and 20 wk of age (Table 1). As in animals treated with insulin from birth, the increased adiposity in each fat depot of the insulin-treated rats of group VI was accompanied by an increase in fat cell size but no detectable change in cell number. Once again, insulin's effect of increasing fat cell size over that of the control was most marked in the subcutaneous depot (100–190%).

DISCUSSION

The present investigation demonstrates that insulin increases the adipose tissue mass of postnatal rats and that this increased adiposity is the result of enlargement, and not proliferation, of fat cells. This effect of insulin was observed in three different fat depots and was irrespective of whether the hormone was administered in the early or the adult life of the animal. Vost and Hollenberg (18) reported a similar effect of insulin on adipose tissue, but since the hormone was administered in that study only to *adult* animals, the absence of an effect on cell number might have been expected. The current study demonstrates that even if insulin is administered to rats early in life, at a time when a stimulation of adipose cellular mitosis might be anticipated, it does not increase the cellularity of the adipose tissue. Under these circumstances, the excessive growth of the epididymal fat pad at each stage of its development and the enlargement of each of three different fat depots in the adult animal are clearly results of adipose cellular enlargement only. Even when insulin produces increased body weight prior to weaning, the expanded epididymal fat depot contains a normal number of enlarged fat cells. This failure of insulin to stimulate new cell formation does not appear to be a function of the duration of its administration, for hypercellularity was not observed even after 20 wk of daily exposure to the hormone. Johnson et al. (4) have observed that the subcutaneous depot of lean Zucker rats continues to add new cells through the 26th wk of life. If a similar phenomenon occurs in the subcutaneous fat of the Charles River (CD strain) rat, insulin does not appear to enhance the process, at least during the first 20 wk.

The hazards of characterizing adipose tissue cellularity on the basis of data derived from only one fat depot have been emphasized previously (4–6). The observations that insulin increases the lipid content of three different adipose depots and that this is accomplished by stimulating the accumulation of fat per cell (increasing cell size) suggest that this pattern may be representative of the action of this hormone on adipose tissue as a whole. Since the three sites examined in this study constitute the major fat depots of the rat (4), it is likely that the increased adiposity of insulin-treated animals can be attributed to the action of this hormone to produce adipose cellular enlargement without a change in fat cell number.

The detailed examination of the growth of the epididymal fat pad at each stage of its development, both pre- and postweaning, indicates that the first observable effect of insulin to increase the adiposity of this depot occurs between the 3rd and 5th wk of life. During this period of life there is a tendency for the epididymal fat cells of control animals to increase in size only slightly or not at all. In contrast, the epididymal adipose cells of insulin-treated animals show considerable enlargement during this period. This observation may be analogous to that of Johnson et al. (4), who reported that adipose cell size in the lean Zucker rat showed a transient decrease at weaning, a decrease which did not occur in the genetically obese animal. The difference in epididymal adiposity and cell size between insulin-treated and control animals became progressively more marked as the rats grew and continued to receive insulin. A detailed examination of the effect of insulin on the growth of the subcutaneous and retroperitoneal adipose tissue was not undertaken. However, the observations that in adult life these depots contained enlarged fat cells in normal numbers when insulin was administered from birth and that the adult cellular character of all three fat depots was similar suggest that the influence of this hormone on each of these three sites is qualitatively similar at each stage of their development.

It remains possible that insulin did stimulate the formation of new adipocytes but that these new cells were so small that they could not be detected by the methods used in this study. This is particularly possible in studies done on epididymal fat at 3 wk of age and on subcutaneous sites where cells are small. However, the failure to detect changes in cell number after 15–20 wk of insulin, when many of these small cells should have enlarged to a measurable size, suggests that this is not the case.

Insulin produced cellular enlargement in all three fat depots, but it appears to have increased cell size in the subcutaneous depot to a greater degree than in the other two. Whether this is indicative of site-to-site differential sensitivity of adipocytes or their precursors to insulin is not known. Although insulin markedly increased the adiposity of each depot, lipid did not account completely for the total increase in the wet weight of each pad. Thus, from 8 to 15% of the insulin-produced increase in wet weight of each depot was due to unidentified nonlipid material. This is not surprising, since insulin is known to stimulate the synthesis of protein and other nonlipid substances in adipocytes as well as the proliferation of connective tissue cells (18).

The current study does not establish whether the adipose cellular enlargement produced by insulin is due

to a primary action of the hormone upon cellular lipid metabolism or is secondary to hypoglycemia-induced hyperphagia and consequent increased delivery of substrate to the cell. Food intake was not monitored, but in other studies in rats, insulin in similar doses has been demonstrated to enhance eating (17, 25). Moreover, the failure of insulin to increase adipose cell number in rats treated from birth could reflect the limited ability of suckling animals to increase food intake sufficiently to influence cellular multiplication (19). When food intake is no longer restricted at weaning, the only remaining mechanism for adipose depot expansion is cellular enlargement. Additional studies in which food intake and the nutritional status of suckling and weanling rats are monitored and controlled are required to resolve this question.

In addition, before firm conclusions can be drawn regarding the effect of insulin on adipose cellularity, it is necessary to examine the influence of hyperinsulinemia in utero upon the growth and development of adipose tissue. The possibility that hyperinsulinemia in utero may produce changes in adipose cell number is suggested by the observation of excessive growth and adiposity in the fetus and newborn of diabetic mothers (26). Whether these fat infants have increased numbers of cells in their expanded adipose depots and, if so, whether this is a permanent alteration remain to be determined.

Nevertheless, these studies provide strong evidence that insulin enhances the postnatal growth of epididymal adipose tissue by increasing the amount of lipid stored in preexisting cells rather than by stimulating cellular proliferation. These data support the concept of Cheek and Hill (27) that the predominant action of insulin at the cellular level is to stimulate cytoplasmic growth. The recent observations by Therriault and Melin (21) of increased cell numbers in cold-acclimated rats and by Knittle et al. (28) of decreased fat cell number in children with growth hormone deficiency indicate that other factors may be more important determinants for nuclear increase and hence for regulating cell number than insulin.

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