Neutral glyceride synthesis from glucose in human adipose tissue: comparison between growing and mature subjects

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Abstract Basal and insulin-stimulated neutral glyceride syntheses from glucose were studied in fat cells of different size (fat cell volume, 0.07–0.20, 0.20–0.60, 0.60–1.00, 1.00–1.50 $\mu m^3 \times 10^6$) obtained from subcutaneous adipose tissues in 20 subjects aged 3 months to 67 years. In 0.07–0.20 or 0.20–0.60 $\mu m^3 \times 10^6$ fat cells, the basal rate of glucose conversion to neutral glyceride was significantly lower in mature (36 to 67 years old) than in growing (0 to 12 years old) subjects. In 0.60–1.00 or 1.00–1.50 $\mu m^3 \times 10^6$ fat cells, however, basal rate was not significantly different between the two groups. The stimulating effect of insulin on conversion of glucose to neutral glyceride was not significantly different from the basal rate in fat cells of each size taken from the mature subjects, whereas in fat cells from growing subjects, it was significantly different from the basal rate in each fat cell size category. • These results indicate that when fat cell size is taken into account, not only is the rate of basal glucose conversion to neutral glyceride higher in growing subjects but also its responsiveness to exogenous insulin, and that insulin insensitivity of large fat cells, reported previously, may be influenced by age. — **Taniguchi, A., T. Kono, H. Okuda, F. Oseko, I. Nagata, K. Kataoka, and H. Imura.** Neutral glyceride synthesis from glucose in human adipose tissue: comparison between growing and mature subjects. *J. Lipid Res.* 1986. **27:** 925–929.

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Several investigators have shown that human adipose tissue is insensitive to exogenous insulin in vitro (1–5). On the other hand, Gries and Steinke (6) reported that there was an age-dependent change in basal and insulin-stimulated lipid synthesis in adipose tissue in subjects aged 5 to 80 yr. In their study, however, fat cell size was not taken into account. It is well known that fat cell size increases with advancing age in humans (7, 8). Recent studies have shown that larger fat cells have a greater rate of basal lipid synthesis than smaller fat cells of the same specimen (5, 9–13), indicating that fat cell size influences the rate of lipid synthesis. It is thus important to analyze the effect of age on the rate of lipid synthesis, taking the fat cell size into account.

In this experiment, we studied basal and insulin-stimulated neutral glyceride synthesis using fat cells of different size in eleven subjects aged 3 months to 12 yr and nine subjects aged 36 to 67 yr. In adipose tissues obtained from nine subjects aged 3 months to 12 yr, the relationship between fat cell size and insulin-stimulating effect on neutral glyceride synthesis was also investigated.

MATERIALS AND METHODS

Specimens of subcutaneous adipose tissue from the periumbilical area were obtained from 20 patients aged 3 months to 67 yr undergoing abdominal surgery. They were divided into two groups by age. One group consisted of 8 males and 3 females aged 3 months to 12 yr. The other comprised 3 males and 6 females aged 36 to 67 yr. They had no history or signs of diabetes mellitus, obesity, and hypertension. Prior to the study, all patients fasted and did not receive any fluid supplementation overnight before surgery. General anesthesia was performed with halothane and nitrous oxide. Subcutaneous adipose tissues were removed between 30 and 90 min after the induction of anesthesia. On the basis of previous reports (14, 15), it was assumed that variations in duration and depth of anesthesia did not significantly affect metabolism of fat cells in vitro.

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Adipose tissue samples weighing about 100 mg were preincubated for 30 min at 37°C in 1.4 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 2.5% bovine serum albumin and 0.9 mM glucose. Porcine insulin (20 mIU/ml) was added to half of the incubation test tubes, and 0.5 μCi of [U-^{14}C]glucose was then added. After incubation for 60 min, 7 ml of 2% osmium tetroxide in 50 mM collidine-HCl buffer, pH 7.4, was added to each test tube, and each tube was incubated for at least 96 hr at 37°C. Fixed fat cells were obtained by filtering osmium-treated tissue slices between 250-μm and 25-μm stainless mesh screens and were separated into several fractions according to diameter by successive filtration through stainless mesh screens (10, 12, 13, 16, 17). A portion of each fraction was used for measuring fat cell diameter with a texture analyzer system (Leitz, West Germany) (12, 18). The average fat cell volume was calculated by the formula of Goldrick (19). The remaining portion of each fraction was washed with water, lyophilized, and weighed, and the number of fat cells in each fraction was calculated from both the dried weight of each fraction and the average volume of fat cells in each fraction, assuming the density of osmium-fixed fat cells to be 1.1 (17). Osmium-treated fat cells in each fraction were decolorized with hydrogen peroxide (30%, 60°C) (20) and dried with air. One ml of water was added to each test tube, and the neutral glycerides were extracted by the method of Dole (21). Then, radioactivity in the glyceride fraction was determined in a liquid scintillation counter. The rate of uptake of labeled glucose by fat cells was calculated from fat cell number and the radioactivity in the fat cells.

Statistical analyses

All data are shown as mean ± SEM. The significance of differences was analyzed by Student's t test, taking a value of $P < 0.05$ as significant. In addition, the relationship between fat cell volume and the rate of neutral glyceride synthesis from glucose was analyzed by the coefficient of correlation, taking a value of $P < 0.05$ as significant.

RESULTS

In adipose tissue of growing subjects (0-12 yr old), insulin stimulated glucose conversion to neutral glyceride in a dose-dependent manner, reaching the plateau at concentrations of 5 to 20 mIU/ml (data not shown). Hence, we used 20 mIU/ml of insulin in the following studies.

There was a positive correlation between fat cell volume and basal or insulin-stimulated neutral glyceride synthesis in all subcutaneous adipose tissues examined, when the correlation was calculated with cells isolated from an adipose tissue (representative data are shown in **Fig. 1**). This indicates that fat cell volume influences the rate of neutral

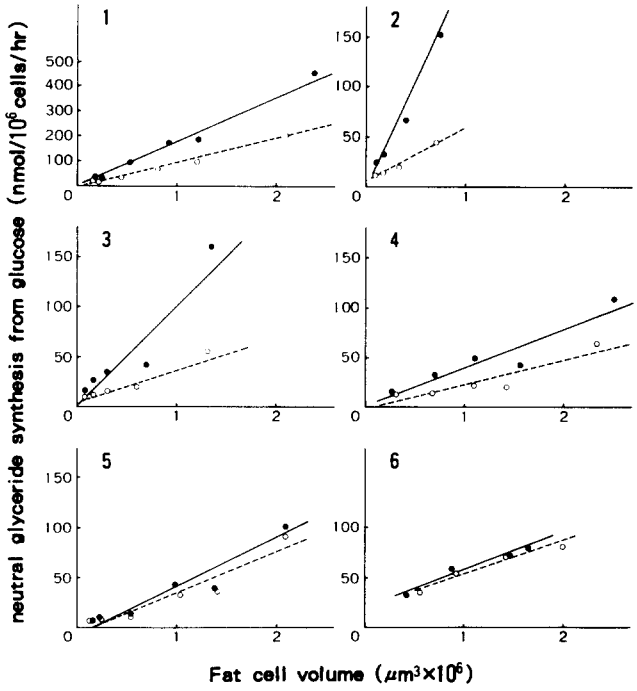

Fig. 1. Relationship between fat cell volume and neutral glyceride synthesis from glucose in human adipose tissue from subjects aged 6 months to 64 years. Closed circles and solid lines: addition of insulin (20 mIU/ml) to the incubation medium; open circles and broken lines: no addition of insulin.

No.	Age	Sex	Insulin	Regression Equation	r	P
1	0.5	F	(−)	y = 93.8x − 4.2	0.991	<0.01
			(+)	y = 194x − 14.1	0.993	<0.01
2	3	F	(−)	y = 54.7x + 5.8	0.994	<0.01
			(+)	y = 224x − 4.0	0.993	<0.01
3	6	M	(−)	y = 37.1x + 4.9	0.994	<0.01
			(+)	y = 108x + 1.2	0.972	<0.01
4	12	F	(−)	y = 24.8x − 1.7	0.911	<0.01
			(+)	y = 38.6x + 3.1	0.947	<0.01
5	59	F	(−)	y = 42.2x − 7.9	0.945	<0.01
			(+)	y = 48.4x − 6.9	0.978	<0.01
6	64	F	(−)	y = 31.0x + 21.9	0.976	<0.01
			(+)	y = 36.1x + 21.2	0.976	<0.01

glyceride formation from glucose. To exclude the effect of fat cell volume, we measured the rate of glyceride formation in fat cells of similar size (0.07-0.20, 0.20-0.60, 0.60-1.00, 1.00-1.50 $\mu m^3 \times 10^6$) after selective screening of osmium-fixed fat cells. This procedure enabled us to compare the rate of glyceride synthesis in fat cells of the same size from growing (0 to 12 yr old) and mature (36 to 67 yr old) subjects (**Table 1**). The basal rate tended to decrease in mature subjects compared with that in growing subjects. The decrease was statistically significant in fat cells with sizes of 0.07-0.20 and 0.20-0.60 $\mu m^3 \times 10^6$, but not statistically significant in fat cells with sizes of 0.60-1.00 and 1.00-1.50 $\mu m^3 \times 10^6$ (Table 1). The stimulating effect of insulin on the rate of conversion of glucose to neutral glyceride was clearly seen in growing subjects, but it was less or not significant in mature subjects. In

TABLE 1. Basal and insulin-stimulated neutral glyceride synthesis from glucose in fat cells of similar sizes from growing (0-12 yr) and mature (36-67 yr) subjects

	Range of Fat Cell Volume ($\mu m^3 \times 10^6$) (Fat Cell Diameter, μm)			
	0.07-0.20 (50-70)	0.20-0.60 (70-105)	0.60-1.00 (105-125)	1.00-1.50 (125-145)
Basal lipid synthesis (nmol per 10^6 cells per hr)				
Growing subjects (n = 11, 4 ± 1 yr)	16.3 ± 1.2[a]	24.3 ± 2.1[b]	36.0 ± 6.1	57.0 ± 12.9
Fat cell volume ($\mu m^3 \times 10^6$)	0.13 ± 0.01	0.36 ± 0.03	0.70 ± 0.03	1.25 ± 0.05
Mature subjects (n = 9, 53 ± 4 yr)	6.8 ± 0.5	16.0 ± 3.3	33.1 ± 9.3	45.0 ± 5.6
Fat cell volume	0.13 ± 0.02	0.38 ± 0.05	0.77 ± 0.06	1.26 ± 0.06
Insulin-stimulated lipid synthesis				
Growing subjects	25.5 ± 1.4[a,c]	38.1 ± 5.2[b,d]	78.1 ± 16.8[b,d]	116.0 ± 26.8[a,d]
Fat cell volume	0.14 ± 0.01	0.33 ± 0.02	0.72 ± 0.03	1.21 ± 0.04
Mature subjects	7.7 ± 1.6	21.3 ± 6.3	31.0 ± 12.2	46.9 ± 9.6
Fat cell volume	0.11 ± 0.02	0.37 ± 0.05	0.71 ± 0.07	1.23 ± 0.08

P values between growing and mature subjects in basal or insulin-stimulated lipid synthesis: [a], $P < 0.01$, [b], $P < 0.05$; *P* values between basal and insulin-stimulated lipid synthesis in growing or mature subjects: [c], $P < 0.01$, [d], $P < 0.05$.

addition, in growing subjects, the insulin effect was demonstrated in fat cells of all sizes, and the insulin effect was more remarkable in large than in small fat cells (Fig. 1, Table 1); in the presence of insulin, the average increases in the rates of conversion of glucose to neutral glyceride in 0.07-0.20, 0.20-0.60, 0.60-1.00, 1.00-1.50 $\mu m^3 \times 10^6$ fat cells were 56%, 57%, 117%, and 104%, respectively (Table 1). On the other hand, the rate of glucose conversion to fatty acids was negligible in all adipose tissues examined, being less than 2% in our studies, suggesting that most of the conversion of glucose to neutral glyceride was via conversion to glycerol.

DISCUSSION

It is well known that lipid synthesis in adipose tissue is greater in large than in small fat cells, both in humans (5, 9) and animals (10-13), and our present study has confirmed these observations. It has been reported that, in animal adipose tissue, there is an age-dependent change in lipid synthesis among fat cells of similar size; with the advance of age, both the rate of lipid synthesis (10, 11) and insulin responsiveness (11) are considerably decreased. In human adipose tissue, Gries and Steinke (6) reported an age-dependent decrease in lipid synthesis. In their study, however, fat cell size was not taken into account. Since fat cell size increases with age (7, 8), the rate of conversion of glucose to neutral glyceride was measured in the present study by collecting fat cells of similar size after fixation with osmium tetroxide (10, 12, 13). The present study demonstrates that the basal rate of conversion of glucose to neutral glyceride and the response to insulin are much greater in fat cells from growing subjects than in mature subjects even when compared in fat cells of the same size. One might argue that results from our present study were influenced by anesthesia or preoperative care; however, this was not probable, since anesthesia and preoperative care were similar in growing and in mature human subjects. Moreover, the difference in the basal rate was significantly larger in small fat cells, and the insulin responsiveness was more pronounced in large fat cells. These results indicate that not only basal but also the insulin-stimulated rate of conversion of glucose to neutral glyceride is influenced by fat cell size.

The reason for the diminished neutral glyceride synthesis in fat cells from mature subjects is unclear. It is known that lipid synthesis is influenced by fat cell size (5, 9, 10-13), duration of fast (22), and glucose or fat cell concentration in the incubation medium (23). In this experiment, the rate of neutral glyceride synthesis from glucose was compared by eliminating these factors, in that fat cells of the same size were studied under the same conditions, although the effect of variable fat cell concentrations on the rate of conversion of glucose to neutral glyceride cannot be ruled out completely. This possibility seems unlikely, however, because in rat adipose tissue, the rate of conversion of glucose to glyceride-glycerol and the insulin effect were reported to change toward the reverse direction (23) and because this was not the case in our study. It is still unknown which step of neutral glyceride synthesis from glucose is impaired in adipose tissue of mature subjects. Recently, Kashiwagi et al. (24) have shown that lactate accounts for 50-70% of glucose carbons taken up by human fat cells. It is possible, therefore, that there is a shift in the metabolic fate of glucose from neutral glyceride to some other metabolites, such as lactate, in adipose tissue of mature subjects, although we did not measure lactate. Moreover, Novak, Melichar, and Hahn (25) have demonstrated that the rate of lipolysis is enhanced in newborn infants and then rapidly declines. This suggests that diminished neutral glyceride synthesis in mature subjects might result from a decreased lipolytic rate, i.e., a decreased lipolytic rate reduces intracellular free fatty acids,

thus reducing the rate of conversion of glucose to glyceride-glycerol (24).

Insulin responsiveness of fat cells of the same size in terms of the neutral glyceride synthesis declined in mature subjects. This suggests that insulin resistance at the cellular level is seen in fat cells of mature subjects. Insulin resistance is caused by alterations of insulin binding, glucose transport, or any metabolic steps distal to glucose transport. Insulin binding is reported to be influenced by fat cell size (26), but this factor was ruled out in our experiments by collecting fat cells of the same size. With regard to the change of glucose transport, Andrews et al. (22) demonstrated that glucose transport was stimulated by insulin, but that glucose incorporation into triglyceride was not enhanced in adipose tissues in subjects of ages 18 to 29 yr. The discrepancy between glucose transport and conversion of glucose to triglyceride may suggest an impairment at some steps distal to glucose transport in adipose tissue of mature subjects.

Finally, the present study also showed that the insulin effect was greater in large than in small fat cells. This is in contrast to previous studies showing insulin insensitivity of large fat cells (27, 28). The reason for such discrepancy is not known, but one possible explanation is the difference in the methods that were used. Most previous studies compared large fat cells from old, obese animals with small fat cells from young, lean animals, and factors of obesity, fat cell size, and age were not eliminated in these experiments. In our study, these factors could be separately taken into account, indicating that insulin insensitivity of large fat cells reported previously (27, 28) may be influenced by age rather than by the enlargement of fat cell size.

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