Communication

ATP Citrate Lyase in Human Adipose Tissue¹

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The question of whether the *de novo* synthesis of fatty acids in human adipose tissue is physiologically important has been under discussion for a long time.

Shrago *et al.* (1) showed that the incorporation of citrate-1, 5-¹⁴C into fatty acids in human adipose tissue, was only one-sixteenth of its rate in rats and that there was no significant amount of ATP citrate lyase [EC 4.1.3.8] in a homogenate of human adipose tissue. Thus, he concluded that adipose tissue is not a physiologically important site of fatty acid synthesis in humans. Moreover, there are many reports that after incubation of glucose-1-¹⁴C with human adipose tissue *in vitro*, the radioactivity is mainly recovered in glyceride-glycerol and ¹⁴CO₂, with only 7-17% of the total in fatty acids, in basal conditions (2, 3).

Conversely, adipose tissue is thought to be an important site of fatty acid synthesis, judging from the occurrence of *de novo* fatty acid synthesis in human adipose tissue during prolonged incubation *in vitro* (4), the presence of a fatty acid synthesis pathway similar to that found in liver and yeast (5), and variation in the conversion of glucose-1-¹⁴C or acetate-U-¹⁴C to ¹⁴C-fatty acids resulting from incubation with insulin or preceding glucose infusion into patients (2, 3).

Recently, we determined ATP citrate lyase activity in human abdominal subcutaneous adipose tissue obtained at surgery and found that the activity varied greatly among patients. In particular, adipose tissues from patients who had been supplied with nutrients only by intravenous injection (IVI group) had a much higher activity than tissues from patients who had taken a meal orally (Oral-feeding group).

The adipose tissues were homogenized in an equal volume (per g wet weight) of cold 0.25 M sucrose. The homogenate was centrifuged at 12,000 × *g* and 4°C for 10 min and the resulting supernatant was again centrifuged at 100,000 × *g* and 4°C for 60 min. The final supernatant was used as the enzyme extract.

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ATP citrate lyase activity was measured by a modification of the photometric method of Srere (6). The reaction mixture contained the following components in a total volume of 1.0 ml : 20 mM Tris-HCl buffer, pH 7.3, 10 mM MgCl₂, 20 mM potassium citrate, 190 μ M NADH, 390 μ M CoA, 5 mM ATP, 20 units of malate dehydrogenase and enzyme extract. The complete assay mixture except CoA was used as a blank in our experiment, because oxidation of NADH by a pathway other than the citrate cleavage pathway actually occurred in the assay mixture. The mixture was incubated at 25°C and the enzyme activity was calculated taking the molar absorption coefficient as 6.22×10^3 . Enzyme activity was expressed as μ mol of product formed per hr on a wet weight basis, because determination of protein in the crude enzyme solution was disturbed by the presence of blood attached to the adipose tissue.

Table 1. ATP citrate lyase activity in human adipose tissue.

Sample No.	Sex age	Disease	Activity (μ mol/hr/g tissue)
1 ^a	49	Gastric cancer	0.075
2 ^a	20	Gastric cancer	0.041
3 ^a	42	Cholecystitis	0.035
4 ^a	39	Uterine cancer	0.092
5 ^a	65	Gastric cancer	0.097
6 ^b	64	Cancer of esophagus	0.586 [21 days] ^c
7 ^b	76	Gastric cancer	1.254 [7 days] ^c
8 ^b	62	Gastric cancer	0.228 [4 days] ^c

^a Oral-feeding 2,000–2,100 kcal/day, ^b IVI 1,800–2,500 kcal/day, ^c Period of IVI treatment.

The enzyme activity in human adipose tissue was found to be less than 0.1 (μ mol/hr/g tissue) in the Oral-feeding group, who had orally taken a meal contained the following nutrients in a total calorie count of 2,000–2,100 kcal/day: 90–100 g of protein, 40–45 g of lipid, 330 g of carbohydrate and 10–15 g of NaCl. On the other hand, the enzyme activity was found to be 0.2 to 1.3 (μ mol/hr/g tissue) in patients in the IVI group, who had been supplied with glucose, essential amino acids (1 g/kg body weight), 500 kcal of fat (only on patient No. 6) and vitamins by intravenous injection in a total calorie count of 1,800–2,500 kcal/day for 4 to 21 days (Table 1). Now, it should be noted that patients in the Oral-feeding group were fasted overnight prior to surgery and those in the IVI group were supplied with nutrients till just before surgery. Thus, there was difference of nutritional status between the Oral-feeding, and the IVI group at surgery. It remains to be solved in future whether the difference between the two groups in enzyme activity was caused by the methods of nutrition and/or starvation before surgery in the Oral-feeding group. For comparison, we also determined ATP citrate lyase activity in rat epididymal adipose

tissue and obtained the value of 0.212 ± 0.042 $\mu\text{mol/hr/g}$ tissue ($n=4$), which was about 3-fold higher than those of human in the Oral-feeding group in Table 1.

It is well known that ATP citrate lyase activity in rat adipose tissue greatly fluctuates after a period of starvation and refeeding (7). On the other hand, Shrago *et al.* (8) reported that the ATP citrate lyase was virtually absent in human adipose tissue and there was no change in enzyme activity under conditions of starvation and refeeding. Conversely, our results suggest that ATP citrate lyase activity in human adipose tissue may be influenced by the nutritional condition. Bray reported detection of lipogenesis from glucose or pyruvate in adipose tissues from patients in positive caloric balance, but not in those during caloric restriction (9). He also found that stimulation of lipogenesis by insulin was augmented by force-feeding and abolished by food deprivation (9). So, he advocated that it is difficult to draw conclusions on lipogenesis from studies on human adipose tissue obtained without control of caloric intake (10).

The present investigation demonstrated that the ATP citrate lyase actually exists in human adipose tissue and that its activity may be influenced by nutritional conditions: namely, the enzyme activity in the adipose tissue was considerably higher in patients who had been supplied with nutrients by intravenous injection than in patients who had taken a meal orally and then fasted overnight prior to surgery. Our results support Bray's opinion described above: namely, it may be difficult to draw conclusions on lipogenesis from studies on adipose tissue which has been obtained without control of caloric intake, since the synthesis of fatty acids in human and rat is influenced by prior nutritional status (10).

SUMMARY

ATP citrate lyase [EC. 4.1.3.8] activity in human adipose tissue was assayed. The activity was considerably higher in tissue from patients who had been supplied with nutrients only by intravenous injection than that from patients who had orally taken a meal and then fasted overnight. These results suggest that ATP citrate lyase activity in human adipose tissue is greatly influenced by the nutritional status.

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