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Acute Effects *in vivo* of Anti-Insulin Serum on Rates of Fatty Acid Synthesis and Activities of Acetyl-Coenzyme A Carboxylase and Pyruvate Dehydrogenase in Liver and Epididymal Adipose Tissue of Fed Rats

By DAVID STANSBIE, ROGER W. BROWNSEY, MARCO CRETTAZ* and RICHARD M. DENTON
Department of Biochemistry, University of Bristol Medical School, University Walk, Bristol BS8 1TD, U.K.

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Plasma insulin concentrations in fed rats were altered acutely by administration of glucose or anti-insulin serum. Rates of fatty acid synthesis in adipose tissue and liver were estimated from the incorporation of ^3H from ^3H_2O. In adipose tissue, parallel changes in the rate of fatty acid synthesis and initial activities of pyruvate dehydrogenase and acetyl-CoA carboxylase were evident. In liver, although changes in rates of fatty acid synthesis were found, the initial activity of pyruvate dehydrogenase did not alter, but small parallel changes in acetyl-CoA carboxylase activity were observed.

Studies indicate that the marked stimulation of fatty acid synthesis in rat epididymal adipose tissue incubated *in vitro* in the presence of insulin involves not only enhancement of glucose transport (Crofford & Renold, 1965; Vinten *et al.*, 1976) but also the parallel activation of pyruvate dehydrogenase and acetyl-CoA carboxylase (see Denton, 1975). Pyruvate dehydrogenase complexes from mammalian tissues including adipose tissue and liver are inactivated by phosphorylation brought about by a tightly bound MgATP-linked kinase; re-activation is achieved by the action of a specific phosphatase (Linn *et al.*, 1969*a*,*b*; for a review see Denton *et al.*, 1975). Acetyl-CoA carboxylase from mammalian sources can exist in interconvertible inactive protomeric and active polymeric forms (for a review see Volpe & Vagelos, 1973). Brief exposure of rat epididymal adipose tissue to insulin leads to increases in the proportion of both pyruvate dehydrogenase (Jungas, 1971; Coore *et al.*, 1971; Weiss *et al.*, 1971; Taylor *et al.*, 1973; Stansbie *et al.*, 1976) and acetyl-CoA carboxylase (Halestrap & Denton, 1973, 1974) in their respective active forms.

In marked contrast with adipose tissue, the acute effects of insulin on the rate of fatty acid synthesis and the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase in liver are not well established. Insulin has been reported to increase fatty acid synthesis in perfused liver preparations, but these increases have been small and usually only evident in livers from starved animals or animals treated with anti-insulin serum (Haft, 1968; Assimacopoulos-Jeannet & Jeanrenaud, 1975). Insulin appears not to alter the activity of pyruvate dehydrogenase in perfused livers (Patzelt *et al.*, 1973), but Wieland *et al.* (1972) have observed some activation of liver pyruvate dehydrogenase *in vivo* after intraperitoneal injection of high doses of insulin (0.5mg/rat). No effects of the hormone on liver acetyl-CoA carboxylase activity have been reported.

The object of the present studies was to compare the effects of insulin on rat liver and adipose tissue under as near identical conditions as possible. Plasma insulin concentrations in fed rats were altered acutely, on the one hand by intraperitoneal injection of glucose (to ensure high plasma concentrations of insulin without hypoglycaemia), and on the other by intravenous administration of insulin antiserum. Rates of fatty acid synthesis have been estimated by monitoring the incorporation of ^3H_2O into the fatty acids of liver and adipose tissue of the same animals (Windmueller & Spaeth, 1966, 1967; Jungas, 1968; Hems *et al.*, 1975). Measurements have also been made of changes in the proportions of acetyl-CoA carboxylase and pyruvate dehydrogenase in their respective active forms in the two tissues. In adipose tissue, convincing parallel changes in the rate of fatty acid synthesis and the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase were evident. However, in liver, although parallel changes in the rate of fatty acid synthesis and the initial activity of acetyl-CoA carboxylase were found, the changes were smaller than those in adipose tissue and were not associated with any appreciable alterations in the initial activity of pyruvate dehydrogenase.

Methods

Male albino Wistar rats (180–220g) allowed free access to a stock laboratory diet (modified 41B;

* Present address: Laboratoire de Recherches Médicales, Université de Genève, Avenue de la Roseraie 64, 1211 Genève 4, Switzerland.

Oxoid, London S.E.1, U.K.) were used throughout. Sources of chemicals, biochemicals and radiochemicals were as given in Stansbie *et al.* (1976), with the addition that anti-insulin serum, prepared by the method of Robinson & Wright (1961), was a kind gift from Dr. A. Cole of this department. The sera bound approx. 5 μg of ^{125}I-labelled bovine insulin/ml at 4°C.

Rates of fatty acid synthesis were assayed after intraperitoneal injection of ^3H_2O (see the legend of Table 1 for details). Pairs of epididymal fat-pads or samples of liver (about 1 g) were saponified by heating in 3ml of KOH (30%, w/v) at 70°C for 10–15min, followed by the addition of 3ml of ethanol (95%, v/v) and continued heating at 70°C for a further 2h. After cooling and acidification with 3ml of H_2SO_4 (9M), lipids were extracted by shaking with 3×10ml of light petroleum (b.p. 40–60°C). The petroleum fractions were combined, washed with 3× 10ml of water and then evaporated to dryness at room temperature with an overdraught of air. The lipid residues (containing principally fatty acids and cholesterol) were dissolved in 15ml of scintillator [4g of 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole/litre of toluene) and radioactivity was assayed in a Nuclear–Chicago Isocap 300 liquid-scintillation counter. The extensive washing of petroleum fractions with water, and subsequent evaporation to dryness, decreased contamination of the lipid residue by ^3H in water and other non-lipid substances to negligible values. Incorporation into cholesterol has been neglected; this was found to be less than 5% of that into fatty acids, in agreement with earlier studies in the perfused liver (Windmueller & Spaeth, 1966, 1967; Lowenstein, 1971). The rate of fatty acid synthesis was thus calculated as μg-atoms of 'H' incorporated/g wet wt. of tissue from the total radioactivity in the lipid residue and the specific radioactivity of plasma water measured in blood samples taken at the same time as tissue samples (thus no correction was made for the isotope effect of ^3H versus ^1H). In separate experiments, it was found that, after intraperitoneal administration of ^3H_2O, the specific radioactivity of plasma water rapidly increased to reach a peak by 6min; thereafter the specific radioactivity declined by about 20% in the next 10min and subsequently remained constant.

Enzyme activities were measured in tissue samples that had been frozen to the temperature of liquid N_2 (with freeze clamps for the liver samples) immediately after removal from animals under Nembutal anaesthesia. The frozen tissue was extracted at 0°C with 100mM-potassium phosphate buffer, pH7.0, containing 2mM-EDTA and 5mM-2-mercaptoethanol by using a Polytron PT20 tissue homogenizer (position 4) for 30s. Extracts were centrifuged for 15s in an Eppendorf 3200 Microfuge, and samples of infranatant immediately assayed for initial activities of acetyl-CoA carboxylase (Halestrap & Denton, 1974) and pyruvate dehyrogenase (Stansbie *et al.*, 1976). Total activity of acetyl-CoA carboxylase was taken to be the activity present in extracts after incubation with 20mM-potassium citrate and 20mg of defatted bovine serum albumin/ml for 30min at 30°C (Halestrap & Denton, 1974). Total activity of pyruvate dehydrogenase was taken to be the activity present in extracts after incubation with pig heart pyruvate dehydrogenase phosphate phosphatase in the presence of 25mM-$MgCl_2$ and 1mM-$CaCl_2$ (Stansbie *et al.*, 1976). Most results are given as initial activity as a percentage of total activity; a unit of enzyme activity catalyses utilization of substrate at the rate of 1 μmol/min at 30°C.

Results and Discussion

In preliminary experiments, considerable variation in rates of fatty acid synthesis was evident in both liver and adipose tissue of different groups of rats. Presumably this reflected small variations in nutritional status, although care was taken to match animals for size and age and to commence experiments at the same time of day (about 10:00h). Hems *et al.* (1975) have shown very considerable variations in fatty acid synthesis in liver and adipose tissue of mice during the 24h cycle. Treatment with insulin alone proved to be rather unsatisfactory, mainly because the animals tended to tolerate anaesthesia very poorly with the onset of marked hypoglycaemia. It was therefore decided to ensure high plasma insulin concentrations (but still in the physiological range and without hypoglycaemia) by intraperitoneal administration of glucose. This proved to be a satisfactory procedure which usually increased rates of fatty acid synthesis in fed animals and eliminated much of the variation in the rates in adipose tissue and liver of different groups of animals.

In the experiments reported in Table 1, all rats were subjected to light ether anaesthesia at the beginning of the procedure, since this was necessary for the intravenous injection of anti-insulin serum. Control experiments showed that this brief period of anaesthesia had little or no effect on the subsequent rate of fatty acid synthesis in fed rats treated with glucose. Rates of fatty acid synthesis expressed on a wet-weight basis were very similar in both adipose tissue and liver of the fed rats treated with glucose. Windmueller & Spaeth (1966, 1967) and Jungas (1968) have estimated that 13–14g-atoms of 'H' are incorporated per mol of fatty acid in both liver and adipose tissue; thus the rates after glucose treatment correspond to about 2.5 μmol of fatty acid/h per g wet wt. of tissue for both tissues, and this approaches closely the maximum rates observed with preparations *in vitro*. After the administration of anti-insulin serum, the rate of fatty acid synthesis was diminished very considerably in adipose tissue to a value which

was less than one-quarter of the rate seen in glucose-treated animals. In the same animals, the rate of fatty acid synthesis in the liver was diminished only to about one-half.

Activities of pyruvate dehydrogenase and acetyl-CoA carboxylase were measured in adipose tissue and liver of rats treated with glucose or anti-insulin serum as used for the measurement of fatty acid synthesis (Table 2). However, in these experiments, Nembutal anaesthesia was used throughout. Some measurements of the effects of glucose and anti-insulin serum treatment on the rate of fatty acid synthesis were carried out on animals under Nembutal anaesthesia; results very similar to those given in Table 1 were obtained, but it was found very difficult to keep the animals alive for the 1 h needed for the measurement of fatty acid synthesis. Measurements of enzyme activities were made after 10 and 30 min; very similar results were obtained at both times. In adipose tissue, anti-insulin serum treatment resulted in obvious decreases in the initial activities of both pyruvate dehydrogenase and acetyl-CoA carboxylase without any appreciable alteration in the total activities of the enzymes. In the liver, no significant changes in the initial or total activities of pyruvate dehydrogenase were apparent at either 10 or 30 min after glucose or anti-insulin serum treatment. There was a small general increase in the activity of liver pyruvate dehydrogenase in all animals after 30 min. The initial activity of acetyl-CoA carboxylase in liver 30 min after anti-insulin serum was decreased by about 25%.

In adipose tissue, overall, very convincing parallel changes in rates of fatty acid synthesis and initial activities of pyruvate dehydrogenase and acetyl-CoA carboxylase were observed. This lends further support for the conclusion, previously drawn from studies *in vitro*, that changes in the initial activities of pyruvate dehydrogenase and acetyl-CoA carboxylase are important in the acceleration of fatty acid synthesis in adipose tissue by insulin. By contrast, it appears that the regulation of fatty acid synthesis in liver may be rather different from that in adipose tissue in a number of respects. In particular, regulation through alterations in plasma insulin concentrations may be

Table 1. *Effects of anti-insulin serum on the rate of fatty acid synthesis in rat epididymal adipose tissue and liver*

Rats were lightly anaesthetized with diethyl ether, and where indicated anti-insulin serum (0.25 ml) was administered by intravenous injection. All animals were injected intraperitoneally with ^3H_2O (5 mCi in 1 ml), containing (for controls) 200 mg of glucose, and allowed to recover from the anaesthesia. After 1 h, liver pieces and epididymal fat-pads were removed after a fresh exposure of the rats to diethyl ether for 3 min, and incorporation of ^3H into tissue fatty acids was determined as described in the Methods section. Results are given as μg-atoms of 'H' incorporated/h per g wet wt. (no correction being made for isotope effects of ^3H versus ^1H) and are shown as means±S.E.M. for the numbers of observations given in parentheses. $*P<0.05$; $**P<0.001$ versus values for glucose-treated animals (Student's *t* test).

	Fatty acid synthesis (as μg-atoms of 'H' incorporated/h per g wet wt.)	
Injection	Adipose tissue	Liver
Glucose plus ^3H_2O	31.7±3.97	35.7±3.54 (7)
^3H_2O	14.0±2.58*	22.7±3.50* (4)
^3H_2O; anti-insulin serum	7.29±1.79**	17.5±2.19** (9)

Table 2. *Effects of anti-insulin serum on initial activities of pyruvate dehydrogenase and acetyl-CoA carboxylase in rat epididymal adipose tissue and liver*

Rats were under Nembutal (60 mg/kg) anaesthesia throughout. Injections of glucose, 0.9% NaCl and anti-insulin serum were as indicated in the legend of Table 1. Tissues were removed after 10 or 30 min and enzyme activities assayed as given in the Methods section. Results are given as means±S.E.M. for the numbers of observations given in parentheses. No significant changes in total enzyme activities were observed; the mean total activities (as munits/g fresh wt. of tissue) were as follows: pyruvate dehydrogenase, liver 887±51 (29), adipose tissue 290±24 (29); acetyl-CoA carboxylase, liver 223±10 (42), adipose tissue 200±16 (26). $*P<0.05$; $**P<0.01$ versus values for glucose-treated animals.

		Pyruvate dehydrogenase initial activity (% of total)		Acetyl-CoA carboxylase initial activity (% of total)	
Treatment	Time (min)	Adipose tissue	Liver	Adipose tissue	Liver
Glucose	10	35.0±2.7 (4)	5.9±0.9 (4)	19.3±2.7 (4)	46.0±3.3 (4)
0.9% NaCl	10	19.5±4.4 (4)	7.4±2.2 (4)	—	—
Anti-insulin serum	10	9.5±2.6** (4)	5.9±1.3 (4)	7.6±1.4** (6)	43.0±2.5 (6)
Glucose	30	40.0±3.0 (5)	11.4±1.2 (5)	32.0±2.5 (5)	53.7±3.5 (13)
0.9% NaCl	30	21.0±5.1 (6)	15.7±2.6 (6)	12.6±1.5 (6)	57.6±3.9 (6)
Anti-insulin serum	30	17.0±3.6** (6)	12.1±1.0 (6)	18.4±4.0* (6)	41.9±3.1* (13)

less important, for, although alterations in plasma insulin concentrations with glucose and anti-insulin serum were associated with changes in fatty acid synthesis in the liver, the changes were smaller than those in adipose tissue. Moreover, no changes in liver pyruvate dehydrogenase activity were apparent, although in the same animals very marked changes in adipose-tissue pyruvate dehydrogenase were observed. Presumably this difference reflects, in part, the different role of pyruvate dehydrogenase in the two tissues.

There was a modest decrease in the initial activity of acetyl-CoA carboxylase in livers of rats treated with anti-insulin serum. It appears likely that this decrease brings about, at least in part, the diminution in the rate of fatty acid synthesis observed under these conditions.

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