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Evidence that Fatty Acid Synthesis in the Interscapular Brown Adipose Tissue of Cold-Adapted Rats is Increased *in vivo* by Insulin by Mechanisms Involving Parallel Activation of Pyruvate Dehydrogenase and Acetyl-Coenzyme A Carboxylase

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Plasma insulin concentrations in cold-adapted rats were altered acutely by administration of glucose or anti-insulin serum. Rates of fatty acid synthesis in interscapular brown adipose tissue were determined from the incorporation of ^3H from ^3H_2O into tissue lipid. Rates of synthesis were greatly elevated after glucose administration and markedly decreased after injection with anti-insulin serum. Parallel changes in the initial activities of both acetyl-CoA carboxylase and pyruvate dehydrogenase were observed under these conditions, but no changes in total activities were evident. The results suggest that this tissue is an important site of fatty acid synthesis in the cold-adapted rat and that this feature of the tissue is sensitive to changes in plasma insulin concentrations.

In a previous study (Stansbie *et al.*, 1976*b*) changes in rates of fatty acid synthesis in liver and epididymal adipose tissue of fed rats were induced *in vivo* by acutely altering plasma insulin concentrations by treatment with glucose or anti-insulin serum. It was found in the white adipose tissue that there were parallel changes in the rate of fatty acid synthesis and the initial activities of both pyruvate dehydrogenase (EC 1.2.4.1) and acetyl-CoA carboxylase (EC 6.4.1.2). These observations thus supported earlier work which indicated that the marked stimulation of fatty acid synthesis observed in samples of rat epididymal adipose tissue incubated *in vitro* in the presence of insulin involved not only enhancement of glucose transport (Crofford & Renold, 1965; Vinten *et al.*, 1976) but also parallel activation of pyruvate dehydrogenase and acetyl-CoA carboxylase [for brief reviews see Denton, (1975) and Denton *et al.* (1977)].

Pyruvate dehydrogenase from mammalian tissues is inactivated by phosphorylation brought about by a tightly bound MgATP-linked kinase, and re-activation is achieved through the action of a specific phosphatase [for review see Denton *et al.* (1975)]. Acetyl-CoA carboxylase from mammalian sources can exist in interconvertible inactive protomeric and active polymeric forms [for review see Volpe & Vagelos (1976)]. Exposure of epididymal adipose tissue to insulin results within minutes in 2–3-fold increases in the proportion of both enzymes in their respective active forms. However, in liver, although some alteration in the rate of fatty acid synthesis *in vivo* can be induced by treatment with glucose or anti-insulin serum, the activity of pyruvate dehydrogenase does not appear to be altered and only small changes in acetyl-CoA carboxylase activity are observed (Stansbie *et al.*, 1976*b*).

There have been a number of reports that insulin *in vitro* stimulates fatty acid synthesis from glucose in a number of brown-adipose-tissue preparations, including interscapular tissue from rats (Joel, 1965; Himms-Hagen, 1965; Fain *et al.*, 1967; Fain & Loken, 1969; Knight & Myant, 1970). In preliminary experiments with small pieces (5–10mg) of interscapular tissue from cold-adapted rats we have been able to confirm the effect of insulin on fatty acid synthesis from glucose *in vitro*. However, this preparation is highly unsatisfactory, as the ATP content falls within 10min of incubation to less than 20% of the content *in vivo* (J. G. McCormack & R. M. Denton, unpublished work). In the present study we have therefore confined our attention to investigating the effects of insulin on fatty acid synthesis in the tissue *in vivo*. As in the earlier study on liver and white adipose tissue of normal animals (Stansbie *et al.*, 1976*b*), plasma insulin concentrations have been raised and lowered acutely by administration of glucose and anti-insulin serum respectively. We have found that the rate of fatty acid synthesis and the activities of both pyruvate dehydrogenase and acetyl-CoA carboxyl-

ase change in parallel with alterations in plasma insulin concentrations. The results also indicate that in cold-adapted rats brown adipose tissue may be an important site of conversion of carbohydrate into fat.

Methods

Female albino Wistar rats were transferred to a room at 5–8°C about 1 week after weaning and cold-adapted for a period of 3–4 weeks. Rats were allowed free access to a stock laboratory diet (modified 41B; Oxoid Ltd., Basingstoke, Hants, U.K.) and weighed between 180 and 220g at use. After the first week, weight gain in the cold was at least as great as that of matched animals kept in the animal house. The rats were always removed from the cold and brought into the laboratory (at approx. 20°C) 1h before each experiment. The interscapular brown fat-pads were removed from the rats as quickly, and with as little contamination from surrounding tissues, as possible. The excision of some of the surrounding white adipose and other tissues (about 10–20% of the total by visual inspection) was unavoidable to allow rapid freezing of the tissue. However, these tissues were shown to make a negligible contribution to the parameters under study.

Sources of materials including anti-insulin serum (which bound about 5μg of ^{125}I-labelled bovine insulin/ml at 4°C) and methods for the measurement of rates of fatty acid synthesis and the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase were as described by Stansbie *et al.* (1976*b*). One unit of enzyme is defined as the amount that will catalyse the formation of 1μmol of product/min at 30°C.

Results and Discussion

As explained in Stansbie *et al.* (1976*b*), intraperitoneal injection of glucose can be used to ensure high plasma insulin concentrations. With this technique the concentrations of insulin attained are in the physiological range, but are not accompanied by the hypoglycaemia that, for example, injection of insulin would cause. In the present study, we have taken the extra precaution of administering anti-insulin serum to glucose-treated animals in order to check that any changes seen after glucose injection are, in fact, the result of increased plasma insulin concentrations and not some other effect of increased glucose availability. Administration of anti-insulin serum and glucose to normal male rats results in activities of pyruvate dehydrogenase in epididymal adipose tissue close to those in tissue of rats treated with anti-insulin serum alone (G. L. Evans & R. M. Denton, unpublished work).

Table 1. *Effects of anti-insulin serum and glucose administration on rates of fatty acid synthesis in interscapular brown adipose tissue of cold-adapted rats*

Rats were lightly anaesthetized with diethyl ether, and, where indicated, anti-insulin serum (0.3ml) was administered by intravenous injection. All animals were then injected intraperitoneally with water (0.5ml) containing 5mCi of ^3H_2O with or without glucose (200mg) and allowed to recover from the anaesthesia. After 1h at room temperature (about 20°C), the animals were again anaesthetized with ether for 3min, and the interscapular brown adipose tissue was removed and quickly frozen with liquid N_2. Subsequently, incorporation of ^3H into tissue fatty acids was determined as described by Stansbie *et al.* (1976*b*). Rates of fatty acid synthesis were calculated as μg-atoms of 'H' incorporated/h per g wet wt. from the radioactivity in the fatty acid fraction, and the specific radioactivity of plasma water was measured in blood samples taken at the same time as the tissue samples (no correction being made for isotope effects of ^3H versus 'H'). Results are shown as means ± S.E.M. for seven observations. $*P<0.05$ and $**P<0.01$ versus control values; $\dagger P<0.05$ and $\dagger\dagger P<0.01$ versus values observed in tissue of rats injected with glucose and ^3H_2O.

Injections	Rate of fatty acid synthesis (μg-atoms of 'H' incorporated/h per g wet wt.)
^3H_2O (control)	63.3±8.3
^3H_2O+glucose	133.4±25.1*
^3H_2O+anti-insulin serum	25.5±2.3**††
^3H_2O+glucose+anti-insulin serum	51.3±13.5†

For the measurement of rates of fatty acid synthesis in brown adipose tissue (Table 1), the cold-adapted rats were subjected to light ether anaesthesia, since this is necessary for the intravenous injection of anti-insulin serum. Such a brief period of anaesthesia has little or no effect on subsequent rates of fatty acid synthesis in liver and adipose tissue of normal male animals (Stansbie *et al.*, 1976*b*).

The rate of fatty acid synthesis in interscapular brown adipose tissue of cold-adapted rats was greatly decreased after administration of anti-insulin serum and elevated after treatment with glucose. There was more than a 5-fold difference between these two conditions of low and high plasma insulin concentrations. Administration of anti-insulin serum to glucose-treated rats led to a rate of fatty acid synthesis that was less than 40% of the value found in the glucose-treated rats; thus the increase with glucose would seem to be largely the result of the high plasma insulin concentration. Some measurements were also made of rates of fatty acid synthesis in samples of mesenteric white adipose tissue of the cold-adapted rats. Injection

Table 2. *Effects of anti-insulin serum and glucose administration on initial and total activities of pyruvate dehydrogenase and acetyl-CoA carboxylase in interscapular brown adipose tissue of cold-adapted rats*

Rats were under Nembutal (60mg/kg) anaesthesia throughout. Injections of glucose and anti-insulin serum were as indicated in the legend of Table 1. Tissues were removed and immediately frozen 15min after the injections. Enzyme activities were assayed as described by Stansbie *et al.* (1976*b*). Results are given as means ± S.E.M. for the numbers of observations given in parentheses. *$P<0.05$ and **$P<0.01$ versus control values; †$P<0.05$ and ††$P<0.01$ versus values observed in tissue of rats injected with glucose.

	Pyruvate dehydrogenase activity		Acetyl-CoA carboxylase activity	
Injections	Initial activity (% of total)	Total activity (units/g wet wt.)	Initial activity (% of total)	Total activity (unit/g wet wt.)
None (control)	19.2 ± 1.7 (8)	4.7 ± 0.59 (8)	21.2 ± 1.5 (8)	0.87 ± 0.09 (8)
Glucose	31.5 ± 4.6 (10)*	4.0 ± 0.22 (10)	50.9 ± 4.2 (5)**	0.83 ± 0.06 (5)
Anti-insulin serum	13.2 ± 1.7 (11)*††	4.0 ± 0.31 (11)	17.6 ± 1.6 (7)††	0.97 ± 0.07 (7)
Anti-insulin serum + glucose	16.7 ± 1.0 (3)†	4.8 ± 0.22 (3)	15.1 ± 2.3 (3)††	0.92 ± 0.05 (3)

of anti-insulin serum decreased the rate from 8.68±2.17 (7) in control rats to 4.40±0.73 (7), whereas glucose treatment of a separate batch of rats increased the rate from 5.24±0.35 (3) in control animals to 11.10±2.21 (3). (The results are given as μg-atoms of 'H' incorporated/h per g wet wt. and are means ± S.E.M. for the numbers of observations in parentheses.) These rates are 30–50% of the corresponding rates found in white adipose tissue of normal rats (Stansbie *et al.*, 1976*b*), but the changes with glucose and anti-insulin serum are similar.

In Table 1, rates of fatty acid synthesis are given without correction for isotope effects of ^3H versus ^1H. In both liver and white adipose tissue, it has been estimated that between 13 and 14 g-atoms of 'H' are incorporated/mol of fatty acid synthesized (Windmueller & Spaeth, 1966, 1967; Jungas, 1968). Assuming this holds true for brown adipose tissue, then the rates observed in tissue of glucose-treated rats are about 10 μmol of fatty acid synthesized/h per g wet wt. This is a very high rate. It is about four times the rates observed in white adipose tissue or liver of normal male rats after glucose injection (Stansbie *et al.*, 1976*b*) and six to ten times the corresponding values observed in mesenteric white adipose tissue and liver of the cold-adapted female rats (see above; also J. G. McCormack & R. M. Denton, unpublished work). The rate is also much higher than that observed in small pieces of brown adipose tissue from cold-adapted female rats incubated *in vitro* with glucose and insulin. However, these low rates *in vitro* are probably the result of the very low concentrations of ATP found in this preparation (see the introduction).

The activities of pyruvate dehydrogenase and acetyl-CoA carboxylase measured in brown adipose tissue after treatment with glucose and anti-insulin serum are shown in Table 2. Initial activities are those observed in extracts immediately after extraction under conditions that restrict the interconversion of the active and inactive forms of the two enzymes. Total activities are those observed after treatment of extracts such that inactive forms of the enzymes are converted into the active forms. For pyruvate dehydrogenase, this was achieved by incubation of extracts with pig heart pyruvate dehydrogenase phosphate phosphatase in the presence of Mg^{2+} and Ca^{2+} (Stansbie *et al.*, 1976*a*), and for acetyl-CoA carboxylase by incubation of extracts with citrate and albumin (Halestrap & Denton, 1974). It is evident that the proportions of both enzymes in their respective active forms change in parallel with changes in rates of fatty acid synthesis. The 5-fold difference in fatty acid synthesis observed between rats treated with anti-insulin serum and those treated with glucose is associated with nearly 3-fold changes in the initial activities of both enzymes. Treatment with both anti-insulin serum and glucose gave values very close to those obtained with anti-insulin serum alone. No appreciable changes in total activities of either enzyme were evident. The activity of glutamate dehydrogenase was also assayed in all extracts as a convenient index of recovery of mitochondrial enzymes (Severson *et al.*, 1976). Expression of initial activity of pyruvate dehydrogenase in terms of glutamate dehydrogenase activity resulted in the same pattern of changes as that given in Table 2. The overall mean tissue activity of glutamate dehydrogenase was 5.6±0.40 units/g of wet tissue (mean ± S.E.M. for 32 observations). Our results conflict with some findings of Bailey *et al.* (1976), who concluded from studies *in vitro* on brown adipose tissue from foetal rats that 30min exposure to insulin increased the total activity of pyruvate dehydrogenase, but did not alter the proportion of the enzyme in its active form. We are not aware of any other measurements of acetyl-CoA carboxylase activity in this tissue. In some cold-adapted

animals measurements were also made of activities of pyruvate dehydrogenase in samples of mesenteric white adipose tissue, but in others this was difficult because of the small amounts of tissue. It was found that total activity was unaltered by the treatments (overall mean 0.32 ± 0.04 unit/g of tissue: mean $\pm$ S.E.M. for 11 observations); initial activities were 13 ± 2.3 (4), 22 ± 5.8 (3) and 41 ± 3.3 (4)% of total activity (means $\pm$ S.E.M. for the numbers of observations in parentheses) in mesenteric adipose tissue of anti-insulin-serum-treated, control and glucose-treated rats respectively. These values are similar to those obtained in epididymal adipose tissue of normal male rats (Stansbie *et al.*, 1976*b*).

Overall, the present study indicates that insulin is important in the regulation of rates of fatty acid synthesis in brown adipose tissue of cold-adapted rats. Moreover, it lends further support to the conclusion previously made from studies *in vivo* and *in vitro* on white adipose tissue that parallel changes in the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase are important in the regulation of rates of fatty acid synthesis by changes in the concentration of insulin in the physiological range (Denton, 1974, 1975).

The high rates of fatty acid synthesis and the high total activities of pyruvate dehydrogenase and acetyl-CoA carboxylase found in brown adipose tissue in the present study are worthy of further comment. The total activity of pyruvate dehydrogenase is as high as that found in any other mammalian tissue that has been examined, including heart muscle (Hennig *et al.*, 1975; Kerbey *et al.*, 1976), and is some five to ten times that found in white adipose tissue or liver of normal male rats (Stansbie *et al.*, 1976*b*) or of the cold-adapted female rats used in this study. The total activity of acetyl-CoA carboxylase was three to five times the corresponding activities in liver and white adipose tissue. It is widely held that brown adipose tissue is not an important site of fatty acid synthesis and that the tissue relies heavily on fatty acids derived from other sources (Joel, 1965; Himms-Hagen, 1965; Knight & Myant, 1970). The results of the present study suggest that this is not the case for brown adipose tissue of the cold-adapted rat, and that, indeed, in this animal it is an important site of fatty acid synthesis. It would seem reasonable to expect this to hold true in other cold-adapted non-hibernating animals fed *ad libitum* on high-carbohydrate diets.

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