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A tissue-specific increase in lipogenesis in rat brown adipose tissue in hypothyroidism

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1. Rats were made hypothyroid by feeding them with propylthiouracil together with a low-iodine diet for 4 weeks. 2. [U-^{14}C]Glucose conversion into fatty acids was substantially enhanced in brown adipocytes isolated from hypothyroid rats. Incorporation of ^3H_2O into fatty acids in vivo was enhanced in hypothyroidism in interscapular brown fat, but not in epididymal white fat or in liver. Hypothyroidism increased the activities of fatty acid synthase and ATP citrate lyase in brown, but not in white, adipocytes. 3. Glycolytic flux in brown adipocytes, quantified by [3-^{3}H]glucose detritiation, was increased by hypothyroidism. This change was accompanied by increased maximum activity of phosphofructokinase. In white adipocytes a large increase in phosphofructokinase maximum activity was observed in hypothyroidism, but this change was accompanied by only small increases in the rate of glucose detritiation by incubated cells. It is suggested that in the brown adipocyte the overall conversion of glucose into fatty acids is enhanced in thyroid deficiency, but that this change is muted in the white adipocyte, possibly because of limitation of glucose transport. 4. Fatty acid synthesis in brown adipocytes from hypothyroid animals was considerably less sensitive to inhibition by exogenous fatty acids than is the process in cells from euthyroid animals. Consequently, the effect of hypothyroidism to enhance lipogenesis is amplified in the presence of physiological concentrations of fatty acid.

INTRODUCTION

Hypothyroidism causes profound changes in the handling of long-chain fatty acids by white and brown adipose tissues. In white fat lipolysis is suppressed [see the Introduction to Saggerson (1986) and Milligan et al. (1987) for references] and triacylglycerol synthesis is enhanced (Baht & Saggerson, 1988). Qualitatively the same changes are also seen in brown fat (Woodward & Saggerson, 1986; Baht & Saggerson, 1988), together with decreased noradrenaline-stimulated respiration (Woodward & Saggerson, 1986), a process which is mainly dependent on β-oxidation (Ma & Foster, 1986; Isler et al., 1987; Saggerson et al., 1988). In the course of a previous study under a particular set of experimental conditions, it was noted somewhat surprisingly, that hypothyroidism increased fatty acid synthesis in rat brown adipocytes by 11-fold (see Table 3 of Baht & Saggerson, 1988). There appear to have been few studies of the effect of hypothyroidism on fatty acid synthesis in lipogenic tissues; rather, studies have concentrated on the effects of hyperthyroidism induced by thyroid-hormone administration. In rat liver, thyroid-hormone administration increased lipogenesis and the activities of key lipogenic enzymes such as acetyl-CoA carboxylase and fatty acid synthase (Diamant et al. 1972; Roncari & Murthy, 1975; Volpe & Marasa, 1975; Gnoni et al., 1980; Sugden et al., 1981, 1983), whereas thyroidectomy or propylthiouracil treatment decreases fatty acid synthase activity (Baquer et al., 1976; Gnoni et al., 1980). By contrast, although it is generally found that hyperthyroidism and hypothyroidism also increase and decrease respectively these parameters in white adipose tissue (Diamant et al., 1972; Volpe & Marasa, 1975; Baquer et al., 1976; Gnoni et al., 1980), the amplitude of these changes is smaller than in liver, and Roncari & Murthy (1975) have even reported a decrease in acetyl-

CoA carboxylase, fatty acid synthase and lipogenic rate in vivo in white fat on thyroid-hormone administration. Brown adipose tissue has received less attention in this regard, although Sugden et al. (1983) reported that tri-iodothyronine slightly decreased fatty acid synthesis in vivo in interscapular brown adipose tissue. The implication therefore is that lipogenesis in these three tissues may show tissue-specific differences in its response to changes in thyroid hormone status.

In this study we have made parallel measurements of metabolic fluxes and enzyme activities in brown and white adipose tissues from euthyroid and hypothyroid rats. We show that hypothyroidism results in appreciable increases in glucose utilization in brown adipocytes that are not seen in white adipocytes and that, in particular, thyroid-hormone insufficiency results in a substantial increase in lipogenesis in brown fat that is not seen in white fat or in liver. Furthermore we have shown that physiological concentrations of non-esterified fatty acids appear to amplify the increase in brown-fat lipogenesis that is observed in hypothyroidism.

MATERIALS AND METHODS

Chemicals

These were obtained and treated as described by Saggerson (1972) and Woodward & Saggerson (1986).

Animals

Both hypothyroid rats and their euthyroid controls were 9–10 weeks old. These were male animals of the Sprague–Dawley strain bred at University College London and kept at approx. 21 °C on a 13 h-light/11 h-dark cycle, with light from 06:00 to 19:00 h. Control animals were maintained on Rat and Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex,

U.K.) supplied *ad libitum*. Hypothyroidism was induced over 4 weeks by feeding rats on a low-iodine version of the same diet, with 6-n-propyl-2-thiouracil (0.01 %, w/v) added to the drinking water (Chohan *et al.*, 1984; Woodward & Saggerson, 1986).

Isolation of adipocytes

White and brown cells were invariably obtained from the same animals. White adipocytes were isolated from the epididymal depot by disaggregation in collagenase (1.25 mg/ml) essentially as described by Rodbell (1964). Brown adipocytes were isolated from the interscapular depot by the procedure originally described by Fain *et al.* (1967) and elaborated by Nedergaard & Lindberg (1982).

Measurements of flux rates in incubated adipocytes

Freshly isolated cells were dispensed (approx. 2×10^6 cells/ml) into 25 ml silicone-treated Erlenmeyer flasks containing 4 ml of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) and fatty acid-poor albumin (40 mg/ml). Other additions are indicated in individual Table or Figure legends. The flasks were incubated at 37 °C for 1 h with shaking under O_2/CO_2 (19:1). For measurement of lipogenesis the flasks contained 5 mM-[U-^{14}C]glucose (0.1 μCi/μmol). Incorporation of ^{14}C into acylglycerol fatty acids and glycerol was measured as described by Saggerson & Greenbaum (1970a) and Saggerson (1972). For measurement of phosphofructo-kinase flux as glucose detritiation, the flasks contained 10 mM-[3-^{3}H]glucose (0.1 μCi/μmol). ^{3}H$_2$O formed during the incubation was separated chromatographically from [^{3}H]glucose and measured as described by Bontemps *et al.* (1978) and Rider & Hue (1985).

Measurement of lipogenesis *in vivo*

The procedure was similar to that described by Hems *et al.* (1975), Stansbie *et al.* (1976) and McCormack & Denton (1977). Conscious rats were injected intraperitoneally with 0.3–0.5 ml of 0.15 M-NaCl containing ^{3}H$_2$O (35 mCi/kg body wt.) and glucose (800 mg/kg body wt.). After 60 min the rats were decapitated and blood samples taken to determine the specific radioactivity of plasma water. Liver, epididymal adipose tissue and interscapular adipose tissue were rapidly removed, weighed and extracted in 10 ml of propan-2-ol/hexane/1 M-H$_2$SO$_4$ (40:10:1, by vol.) (Dole, 1961), with an Ultra-Turrax tissue disintegrator. After addition of 6 ml of hexane and 7 ml of water and thorough mixing, the resulting hexane layer, containing tissue lipids, was washed twice with 10 ml of 1 mM-H$_2$SO$_4$. A 4 ml portion of the washed hexane extract was evaporated to dryness, saponified and treated to measure ^{3}H in acylglycerol fatty acids as described by Saggerson & Greenbaum (1970a).

Measurements of enzyme activities in adipocyte extracts

Adipocytes were isolated as described above, washed in albumin-free Krebs–Ringer bicarbonate and transferred to ice-cold sucrose medium [0.25 M-sucrose/10 mM-Tris/HCl buffer (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol] and homogenized in an ice-cooled Potter–Elvehjem homogenizer with ten strokes of a rotating Teflon pestle (450 rev./min) with a radial clearance of 0.2 mm. The homogenates were centrifuged briefly in a Sorvall RC5B centrifuge (integrated field-

Table 1. Effects of hypothyroidism on enzyme activities and metabolic fluxes in brown and white adipocytes

Cells were incubated as described in the Materials and methods section for measurement of metabolic fluxes. Insulin (4 nM) was present throughout for measurement of lipogenesis and as indicated for measurement of glucose detritiation. The values are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses; [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ respectively for effects of hypothyroidism.

Adipocyte type	Thyroid status	[U-^{14}C]Glucose conversion to fatty acids (μg-atoms/h per 100 μg of DNA)	Enzyme activities (nmol/min per 100 μg of DNA)				[3-^{3}H]Glucose detritiation (μg-atoms of H/h per 100 μg of DNA)	
			Fatty acid synthase	ATP citrate lyase	Phosphofructo-kinase		Without insulin	With insulin (4 nM)
Brown	Euthyroid	2.6±0.4 (4)	22.1±4.1 (3)	52.5±10.0 (3)	221±35 (3)		0.50±0.02 (3)	2.48±0.25 (4)
	Hypothyroid	21.7±2.5[c] (4)	68.4±12.1[a] (3)	396±35[c] (3)	789±23[c] (3)		1.82±0.12[c] (3)	6.10±0.66[b] (3)
White	Euthyroid	—	46.0±4.9 (3)	122±14 (3)	48.1±2.3 (3)		0.56±0.05 (3)	0.74±0.11 (4)
	Hypothyroid	—	43.1±2.5 (3)	149±13 (3)	429±25[c] (3)		1.09±0.08[b] (3)	1.06±0.24 (3)

time = 30000 g-min) to remove floating fat and some particulate material. The resulting supernatants were then centrifuged at 4 °C for 1 h at 100000 $g_{av.}$ to yield cytosolic fractions, which were stored frozen at -20 °C until assayed for enzyme activities. ATP citrate lyase (EC 4.1.3.8) was assayed at 25 °C as decribed by Martin & Denton (1970), and fatty acid synthase (EC 2.3.1.85) as described by Saggerson & Greenbaum (1970b). 6-Phosphofructo-1-kinase (EC 2.7.1.11) was assayed at 25 °C under conditions of maximum activity at pH 7.8 (i.e. in the presence of 5 mM-potassium phosphate/5 mM-fructose 6-phosphate/1.5 mM-ATP/1.25 mM-AMP) as described by Gualberto et al. (1987).

Measurement of DNA

The DNA content of adipocytes or tissue pieces was measured by the method of Switzer & Summer (1971), with calf thymus DNA as standard.

RESULTS AND DISCUSSION

Baht & Saggerson (1988) showed that hypothyroidism substantially increased fatty acid synthesis in brown adipocytes, while having only a minimal effect in white adipocytes. Tables 1 and 3 confirm this effect of hypothyroidism seen when brown cells are incubated with glucose and insulin with or without palmitate. Table 1 also shows that hypothyroidism caused significant increases in the activities of the synthesis-pathway enzymes fatty acid synthase and ATP citrate lyase. By contrast, in white adipocytes these two activities were unaffected by hypothyroidism. This tissue-selectivity of the effect of hypothyroidism was also apparent when fatty acid synthesis was measured in vivo (Table 2). The animals were injected with a glucose load to elevate insulin concentrations and hence to achieve as far as possible maximal rates of lipogenesis; 3–4-fold increases in fatty acid synthesis were observed in brown fat from the hypothyroid rats, but hepatic synthesis rates were unchanged and only small, non-significant, increases were observed in white fat.

Baht & Saggerson (1988) observed that brown adipocytes from hypothyroid rats have increased activities of all enzymes in the glycerolipid synthesis pathway and exhibit increased rates of conversion of [^{14}C]glucose into acylglycerol glycerol when the cells are incubated with insulin and palmitate. It might be expected that this increased flux in hypothyroidism should be accompanied by increased glucose uptake and increased triose phosphate formation. Table 1 suggests that this is so, since brown adipocytes from hypothyroid rats showed approx. 3-fold higher rates of [3-^{3}H]glucose detritiation (a measure of flux through phosphofructokinase) when incubated with or without insulin. In accord with this, 100000 g supernatants from these cells contained approx. 3-fold increased activities of phosphofructokinase assayed under conditions that gave values approximate to $V_{max.}$. In white adipocytes, however, hypothyroidism again had a far more modest effect, in that [3-^{3}H]glucose detritiation was only slightly increased and, furthermore, insulin had little effect on this process. It is therefore possible that in white adipocytes fatty acid synthesis (and perhaps the induction of more fatty acid synthase and ATP citrate lyase activities) cannot be appreciably increased in hypothyroidism, because there is no concomitant expansion of glycolytic flux. Table 1 shows that, somewhat anomalously, phosphofructokinase activity in white-adipocyte 100000 g supernatants was appreciably increased in hypothyroidism. It therefore seems likely that the constraint in the glucose metabolism in these cells in hypothyroidism is before this enzyme, presumably at the stage of glucose transport into the cell. It is also noteworthy that the large increases in phosphofructokinase activity observed here in both adipocyte types in hypothyroidism differ substantially from the situation in rat heart, where this activity is decreased in the hypothyroid state (Gualberto et al., 1987), or in liver, where only a small increase is seen on thyroidectomy (Baquer et al., 1976).

Saggerson et al. (1988) showed that, in the presence of insulin, fatty acid synthesis from [^{14}C]glucose by brown adipocytes was extremely sensitive to inhibition by

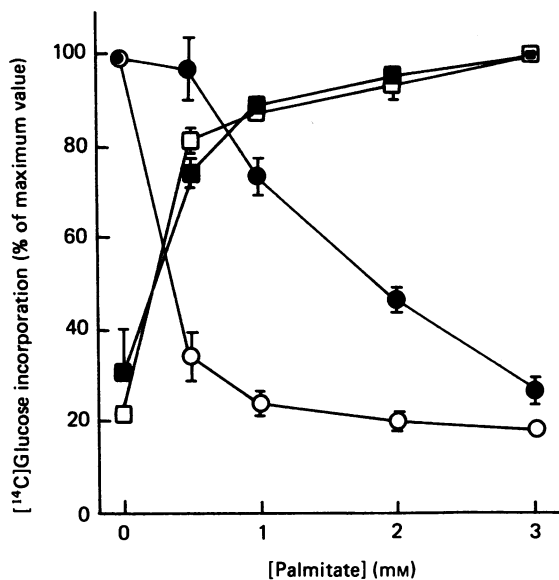**Table 2. Effects of hypothyroidism on fatty acid synthesis in vivo**

Rats were injected with ^{3}H$_2$O plus a glucose load and ^{3}H incorporation into tissue fatty acids was determined as described in the Materials and methods section. The values are means $\pm$ S.E.M. for the numbers of experiments shown in parentheses; [a]$P < 0.05$ for effects of hypothyroidism.

Tissue	Thyroid status	^{3}H$_2$O incorporation into fatty acids	
		μg-atoms of H/h per g wet wt.	μg-atoms of H/h per 100 μg of DNA
Brown adipose tissue	Euthyroid	18.9 $\pm$ 3.5 (6)	1.74 $\pm$ 0.43 (6)
	Hypothyroid	71.3 $\pm$ 21.7[a] (6)	6.04 $\pm$ 1.52[a] (6)
White adipose tissue	Euthyroid	15.0 $\pm$ 3.9 (6)	5.78 $\pm$ 0.86 (6)
	Hypothyroid	22.9 $\pm$ 2.8 (6)	10.77 $\pm$ 2.29 (6)
Liver	Euthyroid	22.6 $\pm$ 3.7 (3)	–
	Hypothyroid	25.0 $\pm$ 7.0 (3)	–

Table 3. Comparison of insulin-responsiveness of glycerolipid synthesis in brown adipocytes from euthyroid and hypothyroid rats

Cells were incubated for 1 h with 5 mM-[U-^{14}C]glucose and the indicated additions. The values are means±s.e.m. for the numbers of experiments shown in brackets. The numbers in parentheses show the mean stimulation (fold) by insulin. Significance of effects of hypothyroidism is indicated by [a]$P < 0.05$, [b]$P < 0.02$, [c]$P < 0.01$ respectively.

Thyroid status	Insulin (4 nM)	Palmitate (1 mM)	[^{14}C]Glucose incorporation (μg-atoms/h per 100 μg of DNA)	
			Into fatty acid	Into acylglycerol glycerol
Euthyroid	−	−	0.09 ± 0.03 [4]	0.17 ± 0.02 [4]
	+	−	1.95 ± 0.56 [5]	0.86 ± 0.19 [5]
			(17.2 ± 2.5)	(4.6 ± 0.8)
	−	+	0.07 ± 0.02 [5]	0.66 ± 0.06 [5]
	+	+	0.45 ± 0.13 [5]	3.00 ± 0.46 [5]
			(6.6 ± 0.8)	(4.4 ± 0.4)
Hypothyroid	−	−	1.66 ± 0.20[c] [3]	0.38 ± 0.02[c] [3]
	+	−	19.6 ± 5.8[a] [3]	2.39 ± 0.39[b] [3]
			(11.9 ± 3.4)	(6.3 ± 1.2)
	−	+	1.02 ± 0.30[b] [4]	1.58 ± 0.09[c] [4]
	+	+	17.9 ± 1.6[c] [4]	11.5 ± 0.7[c] [4]
			(22.4 ± 6.3)[a]	(7.3 ± 0.3)[c]

Fig. 1. Effect of palmitate on [^{14}C]glucose incorporation into glycerolipids by brown adipocytes from euthyroid and hypothyroid rats

Cells were incubated for 1 h with 5 mM-[U-^{14}C]glucose and 4 nM-insulin. The values are means±s.e.m. for four experiments and are normalized relative to the highest measured rates, which were set at 100 in each case. At zero [palmitate], rates of incorporation into fatty acids were 2.6±0.4 and 21.7±2.5 μg-atoms/h per 100 μg of DNA for the euthyroid and hypothyroid states respectively. At 3 mM-palmitate, rates of incorporation into acylglycerol glycerol were 4.8±0.4 and 12.3±1.4 μg-atoms/h per 100 μg of DNA for the euthyroid and hypothyroid states respectively. ○, □, Euthyroid rats; ●, ■, hypothyroid rats. ○, ●, Fatty acid formation; □, ■, acylglycerol glycerol formation.

exogenous fatty acids, a noteworthy difference from what is observed with white cells (Saggerson, 1972; Saggerson et al., 1988). Table 3 and Fig. 1 show that this effect of exogenous fatty acids on brown adipocytes is

interactive with the effect of insulin and is significantly lessened in hypothyroidism. Fig. 1 shows that 0.3–0.4 mM exogenous palmitate caused 50% inhibition of fatty acid synthesis in brown cells from euthyroid rats. By contrast, the far higher fatty acid synthesis in cells from hypothyroid rats was half inhibited by approx. 1.8 mM-palmitate (4% albumin was present). Hence the effect of hypothyroidism on fatty acid synthesis absolute rate is amplified in the presence of physiological concentrations of exogenous fatty acid, rising from an 8-fold increase at zero [palmitate] to a 27-fold increase at 1 mM-palmitate. Fig. 1 also shows that, although glycerolipid synthesis is expanded in hypothyroidism (also see Baht & Saggerson, 1988), the shape of the dependence of this process on [palmitate] is not altered. Table 3 shows the interaction of this fatty acid effect with that of insulin. In cells from euthyroid animals, exogenous palmitate had little effect in the absence of insulin, but substantially decreased synthesis in the presence of the hormone. As a consequence, the 17-fold effect of insulin on fatty acid synthesis seen in euthyroid cells in the absence of palmitate was significantly decreased ($P < 0.01$) to 6.6-fold in the presence of fatty acid. Since effects of palmitate are less in hypothyroidism (Fig. 1), insulin effects on fatty acid synthesis were retained in the presence of fatty acid (Table 3). Table 3 also shows that palmitate had no effect on the degree of stimulation of acylglycerol glycerol synthesis by insulin, and that insulin effects on this process were slightly increased in hypothyroidism.

At present it is not known why, in the euthyroid state, fatty acid synthesis is very sensitive to exogenous fatty acid concentration in brown, but not in white, adipocytes. Nor is it understood why hypothyroidism should lessen the effect in brown adipocytes. Fatty acid handling by brown adipocytes is appreciably altered in hypothyroidism, in that maximum rates of oxidation are decreased (Woodward & Saggerson, 1986), whereas fatty acid esterification is increased (Baht & Saggerson, 1988; and see Fig. 1 and Table 3). It remains to be investigated whether these changes might form the basis of the effect reported here.

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