Hepatic carbon flux after re-feeding

Hyperthyroidism blocks glycogen synthesis and the suppression of glucose output observed in response to carbohydrate re-feeding

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1. The work investigated hepatic glycogen synthesis and glucose output after the intragastric administration of glucose or glycerol or the provision of chow *ad libitum* to 48 h-starved euthyroid or hyperthyroid rats. 2. After glucose administration, glycogen synthesis via the indirect pathway [Newgard, Hirsch, Foster & McGarry (1983) *J. Biol. Chem.* **258**, 8046–8052] occurred concomitantly with reversal of glucose flux across the liver and re-activation of pyruvate kinase in the euthyroid controls. Glycogen synthesis was decreased and net glucose output continued in the hyperthyroid rats, but normal re-activation of pyruvate kinase was observed. 3. Use of 3-mercaptopicolinate indicated that the glucose released from liver of hyperthyroid rats was synthesized from substrates metabolized via the gluconeogenic pathway. 4. Hepatic glycogen synthesis was also impaired in hyperthyroid rats after administration of glycerol or chow. Measurements of portal-minus-hepatovenous concentration differences and arterial glucose concentrations after the administration of glycerol in combination with 3-mercaptopicolinate indicated that flux from triose phosphate to glucose 6-phosphate was not decreased. 5. Inhibited glycogen synthesis after chow re-feeding was associated with accelerated re-activation of hepatic pyruvate dehydrogenase complex in the hyperthyroid rats. 6. The results indicate three distinct and independent actions of hyperthyroidism after re-feeding: (i) it inhibits the reversal of glucose flux across the liver normally observed in response to carbohydrate; (ii) it affects glycogen deposition at a site distal to glucose 6-phosphate; (iii) it allows more rapid re-activation of liver pyruvate dehydrogenase complex in response to a mixed diet.

INTRODUCTION

The effects of hyperthyroidism on carbohydrate metabolism in the starved state include accelerated glucose turnover [1,2], together with increased activities of a number of gluconeogenic enzymes, including pyruvate carboxylase [3,4], PEPCK [3,5] and G6Pase [6–8]. These changes in hepatic gluconeogenic capacity have been correlated with increased gluconeogenic flux from pyruvate (see, e.g., [9]; for reviews see [10,11]). In addition, gluconeogenesis from glycerol is increased [11]. This arises because the normal feedback inhibition of glycerol kinase by glycerol 3-phosphate is not observed [11].

Increased hepatic gluconeogenesis is normally associated with decreased glycolysis and lipogenesis, but in the hyperthyroid state both glycolytic and lipogenic capacities are increased (for reviews, see [10,11]). As a result, substrate cycling occurs at 6-phosphofructokinase-1 and fructose 1,6-bisphosphatase, and at glucokinase and G6Pase [1,2]. In view of these metabolic changes, it might be expected that hyperthyroidism would influence the response to the provision of nutrients after starvation, and indeed, this may be the case. Glucose administration to starved hyperthyroid rats stimulates hepatic lipogenesis, whereas no stimulation is observed in euthyroid rats [12,13]. Conversely, glycogen synthesis is observed in the euthyroid, but not the hyperthyroid, state [1,12]. The objective of the present work was to investigate the

factors responsible for this impaired hepatic glycogen synthesis. A preliminary account of some aspects of this work has been given elsewhere [14].

MATERIALS AND METHODS

Materials

Sources of materials were as in [15]. Standard laboratory chow was purchased from E. Dixon and Sons (Ware) Ltd., Ware, Herts., U.K., and contained 52% (w/w) digestible carbohydrate.

Treatment of rats

Female albino Wistar rats (180–220 g) were starved for 48 h (in grid-bottomed cages). Rats were made hyperthyroid by the subcutaneous injection of tri-iodothyronine (T_3) dissolved in 10 mM-NaOH/0.03% bovine serum albumin (100 μ g/100 g body wt. per day for 2 consecutive days during the period of starvation, as in [13]). Experiments were starved between 08:30 and 09:30 h, when glucose (2 mmol/100 g body wt.), glycerol (4 mmol/100 g body wt.) or water (1 ml/100 g body wt.) was administered intragastrically under light diethyl ether anaesthesia as described previously [12,13], or standard laboratory chow was provided *ad libitum*. In some experiments an inhibitor of PEPCK, 3-mercaptopicolinate (MPA; 20 mg/100 g body wt., in 0.15 M-NaCl, pH 7.4) or 0.15 M-NaCl (see [16]) was injected

Abbreviations used: PK, L-pyruvate kinase; PEPCK, phosphoenolpyruvate carboxykinase; G6P, glucose 6-phosphate; G6Pase, glucose-6-phosphatase; MPA, 3-mercaptopicolinate; PDH, pyruvate dehydrogenase complex; PEP, phosphoenolpyruvate; FBP, fructose 1,6-bisphosphate; DCA, dichloroacetate; T_3 , tri-iodothyronine.

Table 1. Chow intakes and liver weights after the provision of chow *ad libitum* to control or T₃-treated 48 h-starved rats

For experimental details see the text. Results are means $\pm$ S.E.M. for six to ten rats. Statistically significant effects of T₃ treatment are indicated by $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$, and of chow re-feeding by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Time after chow administration (h)	Chow intake (g)		Liver weights			
	Control	T ₃ -treated	(g)		(g/100 g body wt.)	
			Control	T ₃ -treated	Control	T ₃ -treated
0	—	—	5.37 $\pm$ 0.23	5.31 $\pm$ 0.22	2.98 $\pm$ 0.12	3.24 $\pm$ 0.18
2	3.45 $\pm$ 0.30	3.48 $\pm$ 0.26	5.69 $\pm$ 0.20	6.49 $\pm$ 0.17 ^{***†}	3.30 $\pm$ 0.08	3.90 $\pm$ 0.10 ^{***††}
4	4.44 $\pm$ 0.38	4.53 $\pm$ 0.75	6.41 $\pm$ 0.34*	7.26 $\pm$ 0.07 ^{***†}	3.75 $\pm$ 0.16 ^{**}	4.17 $\pm$ 0.11 ^{**}
6	6.60 $\pm$ 0.11	4.61 $\pm$ 0.58 ^{††}	7.41 $\pm$ 0.31 ^{***}	7.12 $\pm$ 0.24 ^{***}	4.03 $\pm$ 0.14 ^{***}	4.27 $\pm$ 0.13 ^{***}
8	7.05 $\pm$ 0.33	6.59 $\pm$ 0.17	7.00 $\pm$ 0.32 ^{**}	7.64 $\pm$ 0.25 ^{***}	4.12 $\pm$ 0.22 ^{***}	4.52 $\pm$ 0.15 ^{***}
24	14.73 $\pm$ 0.89	13.50 $\pm$ 0.88	8.73 $\pm$ 0.52 ^{***}	8.03 $\pm$ 0.29 ^{***}	5.19 $\pm$ 0.28 ^{***}	4.77 $\pm$ 0.14 ^{***}

intraperitoneally at the time of intragastric intubation. Rats were sampled at intervals for up to 2.0 h after the intragastric load, or for up to 24 h after the provision of chow, after being anaesthetized for 5 min with sodium pentobarbital (6 mg/100 g body wt.). Chow intake and liver weights after the provision of chow are shown in Table 1.

Enzyme assays

PDH complex (active form, PDH_a) and citrate synthase activities were measured in freeze-clamped liver extracts as described in [17] and [18]. PDH_a activities are expressed relative to citrate synthase to correct for possible differences in mitochondrial content/extraction (see, e.g., [17,19,20]). Total PDH activity is unaffected by hyperthyroidism [4,20]. A unit of enzyme activity is that which converts 1 μ mol of substrate into product/min at 30 °C.

Fig. 1. Hepatic glycogen concentrations after the intragastric administration of glucose to 48 h-starved euthyroid (▲) or hyperthyroid (△) rats

Statistically significant effects of hyperthyroidism are indicated by $\dagger P < 0.05$, and of glucose administration by $**P < 0.01$, $***P < 0.001$.

L-Pyruvate kinase (PK) was partially purified and assayed at sub-maximal and maximal (5 mM) concentrations of PEP exactly as described in [21,22]. The activity ratio (v/V) refers to the ratio of the activity measured at 0.15 mM-PEP in the absence of FBP (an allosteric activator) to that measured at 5.0 mM-PEP in the presence of 5 μ M-FBP (see [22]). The activity ratio was also measured at 0.15 mM-PEP in the presence of FBP.

Metabolite assays

Glucose and lactate concentrations were routinely determined in KOH-neutralized HClO₄ extracts of arterial blood: in some cases blood was sampled from the hepatic portal and hepatic vein before the arterial sample was taken. Samples of liver were freeze-clamped, and frozen tissue, powdered under liquid N₂, was extracted with HClO₄ for the determination of lactate, pyruvate and glycogen concentrations (see [23] for details of methods).

Statistics

The results are presented as means $\pm$ S.E.M. for the numbers of rats (n) specified. Statistical differences were assessed by Student's unpaired t test.

Table 2. Hepatic PDH activities at 2 h after the administration of glucose or chow to 48 h-starved euthyroid or hyperthyroid rats

For experimental details see the text. Results are means $\pm$ S.E.M. for six to ten rats. Statistically significant effects of T₃ treatment are indicated by $\dagger\dagger P < 0.01$, and of glucose by $**P < 0.01$.

Nutritional status	Treatment	Hepatic PDH activity (munits/unit of citrate synthase)	
		Control	T ₃ -treated
Fed 48 h-starved	Water	82.1 $\pm$ 8.2	83.2 $\pm$ 9.3
	Water	10.1 $\pm$ 1.1	11.7 $\pm$ 0.6
	Glucose	10.1 $\pm$ 0.5	10.5 $\pm$ 0.6
	Chow	11.1 $\pm$ 2.9	36.3 $\pm$ 6.3 ^{***††}

RESULTS

Glycogen synthesis and liver PDH activities after glucose re-feeding

The time course for hepatic glycogen synthesis after an intragastric glucose load is shown in Fig. 1. In euthyroid rats, glycogen concentrations showed a linear increase from 0.5 h to 2 h. As observed previously [19], hepatic PDH remained inactive during this period (Table 2). Glycogen concentrations at 2 h after the administration of glucose were significantly lower in liver of hyperthyroid rats (see also [1,12]). The time course was, however, complex, in that significant inhibition in response to hyperthyroidism was observed only at later time points. Impaired glycogen synthesis was not linked to increased activity of liver PDH, which, as in the euthyroid state, remained inactive (Table 2; see [20]). The redirection of pyruvate from oxaloacetate to acetyl-CoA formation is not therefore a factor causing decreased synthesis of hepatic glycogen in the hyperthyroid state.

Liver glycogen synthesis and PDH activities after chow re-feeding

As it was considered possible that the decreased hepatic glycogen deposition in hyperthyroid rats might result from altered handling of a limited supply of carbohydrate, concentrations were measured in rats re-fed with chow *ad libitum*.

Concentrations at 2 h after the administration of chow to euthyroid rats were almost double those observed at 2 h after glucose administration (2.50 ± 0.32 and $1.14 \pm 0.15\%$ of wet wt. of liver respectively; $P < 0.01$). Doubling the amount of glucose administered (to 4 mmol/100 g body wt.) did not elicit proportional increases in glycogen deposition, even when it was given by intraperitoneal injection. Here blood glucose concentrations exceeded 30 mM, but the hepatic glycogen concentration was only $1.66 \pm 0.23\%$ of wet wt. of liver. Factors other than an increased carbohydrate intake must therefore account for the higher glycogen concentrations observed in chow-re-fed rats.

There were progressive increases in hepatic glycogen from 2 h to 8 h after the provision of chow, with relatively small increases during the following 16 h (Fig. 2a). Total hepatic glycogen content showed a steady increase as liver weights increased during the latter period (Table 1, Fig. 2b). Re-activation of hepatic PDH did not occur within the first 2 h after chow administration (Table 2, Fig. 2c), but re-activation to approx. 50% of the fed value was observed after 4 h (Fig. 2c). The fed value was achieved by 24 h (Fig. 2c).

The possibility that delayed re-activation of hepatic PDH after the administration of glucose is instrumental in directing pyruvate towards glyconeogenesis has been raised previously on the basis of experiments using dichloroacetate (DCA) [19]. Activation of hepatic PDH was associated with decreased glycogen concentrations. In the present experiments, glycogen deposition occurred concurrently with increased activity of liver PDH at 2–8 h after chow administration. Thus the maintenance of a low activity of the enzyme complex is not obligatory for glycogen deposition. Significant glycogen deposition was observed in DCA-treated rats provided with chow (values of $2.50 \pm 0.32\%$ and $1.17 \pm 0.27\%$ of wet wt. at 2 h in control or DCA-treated chow-re-fed rats respectively; $P < 0.01$). It seems likely that the inhibitory

Fig. 2. Hepatic glycogen concentrations (a), contents (b) and PDH activities (c) after the administration of chow *ad libitum* to 48 h-starved euthyroid (▲) or hyperthyroid (△) rats

Statistically significant effects of hyperthyroidism are indicated by †† $P < 0.01$, ††† $P < 0.001$, and of chow re-feeding by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Table 3. Effects of T₃ treatment on hepatic glycogen, and blood and hepatic lactate plus pyruvate concentrations, in glucose-re-fed, MPA-treated, 48 h-starved rats

For experimental details see the text. Results are means $\pm$ S.E.M. for five to ten rats. Statistically significant effects of T₃ treatment are indicated by $\dagger P < 0.05$, $\ddagger P < 0.01$, $\text{***} P < 0.001$, $\text{+++} P < 0.001$, of MPA by $\dagger P < 0.05$, $\ddagger P < 0.01$, $\text{***} P < 0.001$, and of glucose by $\dagger P < 0.05$, $\ddagger P < 0.01$, $\text{***} P < 0.001$.

Treatment	Time after re-feeding (h)	Lactate plus pyruvate				Blood (mm)		Hepatic ($\mu\text{mol/g wet wt.}$)	
		Glycogen (% liver wet wt.)		T ₃ -treated		Control	T ₃ -treated	Control	T ₃ -treated
		Control	T ₃ -treated	Control	T ₃ -treated				
Water	1.0	0.12 $\pm$ 0.01	0.14 $\pm$ 0.04	1.13 $\pm$ 0.17	1.72 $\pm$ 0.14 $\dagger$	0.97 $\pm$ 0.09	1.22 $\pm$ 0.20		
	2.0	0.14 $\pm$ 0.03	0.16 $\pm$ 0.01	1.19 $\pm$ 0.18	1.67 $\pm$ 0.26	0.93 $\pm$ 0.14	1.38 $\pm$ 0.27		
Water + MPA	1.0	0.11 $\pm$ 0.05	0.24 $\pm$ 0.06	1.88 $\pm$ 0.25 $\dagger$	2.55 $\pm$ 0.19 $\ddagger$	1.34 $\pm$ 0.03 $\ddagger$	1.75 $\pm$ 0.15 $\dagger$		
	2.0	0.15 $\pm$ 0.04	0.13 $\pm$ 0.03	2.85 $\pm$ 0.19 $\ddagger$	4.71 $\pm$ 0.30 +++	1.84 $\pm$ 0.21 $\ddagger$	2.89 $\pm$ 0.24 +++		
Glucose	1.0	0.68 $\pm$ 0.05 ***	0.40 $\pm$ 0.06 ***	2.47 $\pm$ 0.39 ***	3.87 $\pm$ 0.37 ***	1.51 $\pm$ 0.20 $\dagger$	3.28 $\pm$ 0.27 ***		
	2.0	1.25 $\pm$ 0.15 ***	0.76 $\pm$ 0.11 ***	2.28 $\pm$ 0.15 ***	3.89 $\pm$ 0.38 ***	1.20 $\pm$ 0.30	2.39 $\pm$ 0.18 ***		
Glucose + MPA	1.0	0.17 $\pm$ 0.04 +++	0.23 $\pm$ 0.05	2.53 $\pm$ 0.21	3.50 $\pm$ 0.17 ***	1.64 $\pm$ 0.17	2.87 $\pm$ 0.23 ***		
	2.0	0.11 $\pm$ 0.07 +++	0.17 $\pm$ 0.05 $\ddagger$	2.48 $\pm$ 0.14	3.81 $\pm$ 0.10 +++	1.64 $\pm$ 0.25	2.92 $\pm$ 0.19 +++		

Table 4. Effect of T₃ treatment on hepatic L-type pyruvate kinase in glucose- or glycerol-re-fed 48 h-starved rats

For experimental details see the text. Results are means $\pm$ S.E.M. for five to 13 rats. Neither glucose nor glycerol re-feeding had any effect on the V_{max} of PK in either control or T₃-treated rats (water-re-fed, control, 48.3 ± 1.9 units/units/g wet wt., T₃-treated, 49.0 ± 1.8 ; glucose-re-fed, 47.3 ± 2.4 , 44.3 ± 3.4 ; glycerol-re-fed, 46.3 ± 2.1 , 49.6 ± 3.7). Statistically significant effects of T₃ treatment are indicated by $\dagger P < 0.05$, $\ddagger P < 0.01$, $\text{***} P < 0.001$, $\text{***} P < 0.001$.

Treatment	Activity ratio of PK							
	0.5 h		1.0 h		1.5 h		2.0 h	
	-FBP	+FBP	-FBP	+FBP	-FBP	+FBP	-FBP	+FBP
Water	12.2 $\pm$ 0.8	65.0 $\pm$ 1.7	11.2 $\pm$ 0.5	66.0 $\pm$ 1.4	12.7 $\pm$ 0.7	68.6 $\pm$ 1.8	10.5 $\pm$ 0.6	65.4 $\pm$ 2.9
Water + T ₃	11.8 $\pm$ 1.2	67.7 $\pm$ 1.5	10.7 $\pm$ 0.6	64.7 $\pm$ 1.8	10.3 $\pm$ 0.7	66.3 $\pm$ 2.1	12.3 $\pm$ 0.5	60.5 $\pm$ 5.3
Glucose	15.8 $\pm$ 1.3 $\dagger$	68.4 $\pm$ 3.2	16.6 $\pm$ 1.6 **	64.5 $\pm$ 1.1	19.6 $\pm$ 1.9 **	65.7 $\pm$ 2.4	20.7 $\pm$ 1.2 ***	65.8 $\pm$ 2.1
Glucose + T ₃	20.2 $\pm$ 0.9 ***	66.1 $\pm$ 2.1	18.2 $\pm$ 0.7 ***	67.7 $\pm$ 1.4	17.3 $\pm$ 1.4 ***	64.1 $\pm$ 3.2	19.5 $\pm$ 0.9 ***	65.7 $\pm$ 1.7
Glycerol	-	-	18.6 $\pm$ 2.6 $\dagger$	70.1 $\pm$ 1.9	-	-	21.0 $\pm$ 2.3 ***	69.0 $\pm$ 1.9
Glycerol + T ₃	-	-	18.3 $\pm$ 1.3 ***	69.0 $\pm$ 2.3	-	-	21.8 $\pm$ 2.7 **	66.9 $\pm$ 1.0

Table 5. Effects of T_3 treatment on blood glucose and lactate plus pyruvate concentrations and hepatic glycogen concentrations in glycerol-re-fed, MPA-treated, 48 h-starved rats

For experimental details see the text. Results are means $\pm$ S.E.M. for six to 11 rats. Statistically significant effects of T_3 treatment are indicated by $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$, effects of MPA-treatment by $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$, and effects of glycerol by $^{**}P < 0.01$, $^{***}P < 0.001$.

Treatment	Blood metabolite concn. (mM)				Glycogen concn. (% liver wet wt.)	
	Glucose		Lactate + pyruvate		Control	T_3 -treated
	Control	T_3 -treated	Control	T_3 -treated		
Control	4.88 $\pm$ 0.29	5.39 $\pm$ 0.29	1.24 $\pm$ 0.11	2.00 $\pm$ 0.30 †	0.15 $\pm$ 0.02	0.16 $\pm$ 0.04
Glycerol	7.18 $\pm$ 0.20	9.32 $\pm$ 0.41 ***†††	1.95 $\pm$ 0.11 ***	2.37 $\pm$ 0.11 *	1.22 $\pm$ 0.15 ***	0.52 $\pm$ 0.06 ***††
MPA	2.41 $\pm$ 0.33 ††	0.93 $\pm$ 0.15 †††	2.66 $\pm$ 0.17 ††	4.71 $\pm$ 0.30 ††††	0.11 $\pm$ 0.02	0.12 $\pm$ 0.03
Glycerol + MPA	6.64 $\pm$ 0.38 ***	4.02 $\pm$ 0.06 ***††††	2.83 $\pm$ 0.16 †††	2.73 $\pm$ 0.36 **	0.53 $\pm$ 0.10 ***†	0.11 $\pm$ 0.03 ††††

effect of DCA on hepatic glycogen synthesis after re-feeding is exerted predominantly via a decreased supply of lactate and pyruvate (see [19]), rather than a direct consequence of activation of hepatic PDH.

Hyperthyroid rats exhibited significant hepatic glycogen deposition in response to chow, but concentrations were less than those in the euthyroid controls throughout the period of study (Fig. 2a). This could not be attributed to decreased chow intake (Table 1). When results were expressed as total liver glycogen content, differences between control and T_3 -treated rats were still statistically significant (Fig. 2b). At 24 h after chow re-feeding, glycogen concentrations were only 58 % of those of the controls. Hepatic PDH activity was increased to 50 % of the fed value within 2 h of chow administration. This contrasts with the delayed re-activation observed in euthyroid controls (Table 2, Fig. 2c) and the lack of response to glucose at 2 h (Table 2). The factors determining the differential response of PDH to glucose and chow in hyperthyroid rats were not further investigated in this study. Previous work has indicated that hyperthyroidism increases PDH phosphatase activity in liver after re-feeding [20], and the present work indicates that, under physiological conditions, liver PDH may be more readily re-activatable in the hyperthyroid state.

Precursors used for glycogen synthesis after glucose administration

In starved (euthyroid) rats, hepatic gluconeogenesis after glucose administration utilizes C_3 derivatives of glucose via the gluconeogenic pathway (for review see [24]). In confirmation of previous results [16], inhibition of gluconeogenesis from oxaloacetate at the level of PEPCK by MPA [25] was associated with impaired glycogen deposition after glucose re-feeding. The limited hepatic glycogen deposition seen after glucose administration to hyperthyroid rats was also inhibited by MPA (Table 3). Thus, despite increased glucokinase activity, C_3 precursors metabolized to oxaloacetate are used in preference to glucose for glycogen synthesis in liver of hyperthyroid rats. Other work has demonstrated an increased hepatic gluconeogenic capacity in hyperthyroidism, and, in view of high blood and hepatic lactate and pyruvate concentrations (Table 3), it is unlikely that a decreased precursor supply accounts for the diminished glycogen deposition. The impairment in glycogen synthesis thus resides in altered handling of the intermediates in the gluconeogenic pathway distal to PEPCK.

Hepatic response to glucose

In starvation, inhibition of hepatic PDH occurs concomitantly with glycogenolysis, decreased glycolysis and increased gluconeogenesis. These changes can be correlated with an increased glucagon/insulin concentration ratio, and covalent modification of glycogen phosphorylase and PK. The phosphorylation state of PK can be assessed from the ratio of activities measured at sub-maximal and maximal PEP concentrations [22]. Claus *et al.* [26] have demonstrated dephosphorylation and re-activation of PK (an increased activity ratio) after glucose administration to starved euthyroid rats. Re-activation of PK was observed in the present study with both euthyroid and hyperthyroid rats (Table 4). The increase in activity ratio was significant throughout the period of study, whereas transient activation was

Table 6. Effects of T_3 treatment on hepatic portal and hepatic venous blood glucose concentrations and on portal minus hepatovenous glucose concentration differences in glucose- or glycerol-re-fed 48 h-starved rats

For experimental details see the text. Results are means $\pm$ S.E.M. for five to ten rats. Statistically significant effects of T_3 treatment are indicated by $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$, and of glucose or glycerol by $**P < 0.01$, $***P < 0.001$.

Treatment		Time after re-feeding			
		1.0 h		2.0 h	
		Control	T_3 -treated	Control	T_3 -treated
Portal glucose concn. (mM)	Water	4.89 ± 0.19	4.90 ± 0.29	4.26 ± 0.19	$5.10 \pm 0.28\dagger$
	Glucose	$8.41 \pm 0.34***$	$11.30 \pm 0.49***\dagger\dagger\dagger$	$8.04 \pm 0.27***$	$8.88 \pm 0.42***$
	Glycerol	$7.49 \pm 0.20***$	$8.80 \pm 0.19***\dagger\dagger\dagger$	$6.81 \pm 0.11***$	$8.57 \pm 0.37***\dagger\dagger\dagger$
Hepatovenous glucose concn. (mM)	Water	5.36 ± 0.22	5.63 ± 0.31	4.74 ± 0.19	$5.98 \pm 0.31\dagger\dagger$
	Glucose	$7.78 \pm 0.40***$	$11.82 \pm 0.49***\dagger\dagger\dagger$	$8.02 \pm 0.31***$	$9.56 \pm 0.54***\dagger$
	Glycerol	$8.89 \pm 0.15***$	$9.90 \pm 0.16***\dagger\dagger\dagger$	$7.59 \pm 0.06***$	$9.45 \pm 0.35***\dagger\dagger\dagger$
Portal minus hepatovenous glucose concn. difference (mM)	Water	-0.47 ± 0.09	-0.73 ± 0.12	-0.48 ± 0.11	-0.88 ± 0.16
	Glucose	$+0.63 \pm 0.24**$	$-0.56 \pm 0.26\dagger$	$+0.02 \pm 0.28$	-0.65 ± 0.34
	Glycerol	$-1.40 \pm 0.21**$	-1.10 ± 0.15	-0.79 ± 0.13	-0.88 ± 0.28

observed by Claus *et al.* [26] when less glucose was provided. As expected, effects of glucose administration were not apparent when the activity ratio was increased by FBP. Glucose re-feeding did not affect the activity measured at 5 mM-PEP. There was no marked difference between the responses of hyperthyroid or euthyroid rats, except that, consistent with accelerated glucose absorption [27], the activity ratio was greater in the hyperthyroid rats at an earlier time point. The values for apparent $K_{0.5}$ for PEP after the administration of water or glucose respectively were 1.40 mM and 0.69 mM in hyperthyroid rats, and 1.4 mM and 0.78 mM in euthyroid rats. There was thus no evidence for insensitivity of the hyperthyroid liver to glucose re-feeding.

Although increased pyruvate carboxylation and PEPCK activities have been reported in hyperthyroidism (see the Introduction), raising the possibility of increased substrate cycling through PK, pyruvate carboxylase and PEPCK, there was no evidence that this could be a major factor inhibiting glycogen synthesis at the later time points after re-feeding.

Flux from triose phosphate to G6P

In liver, glycerol acts both as a gluconeogenic and as a glyconeogenic substrate, entering at the C_3 level. Starved rats were therefore administered glycerol in order to determine whether the effect of thyroid hormone on glycogen synthesis was exerted at a locus distal to triose phosphate.

Significant increases in glycaemia were observed in rats given glycerol or glycerol plus MPA (Table 5). As MPA blocks gluconeogenesis from lactate and pyruvate, increases in glycaemia in the latter group can be specifically attributed to conversion of glycerol into glucose via G6P. Glucose concentrations in hyperthyroid rats treated with MPA were less than those of the corresponding controls, but the increases in glucose concentrations after glycerol administration were similar in the two groups. Conversion of triose phosphate into G6P is therefore not decreased by hyperthyroidism. Nonetheless, hepatic glycogen concentrations after gly-

cerol re-feeding were significantly lower in the hyperthyroid rats (Table 5).

It has been previously reported [28,29] that MPA-treated rats are able to synthesize hepatic glycogen after glycerol re-feeding (Table 5). In our experiments, glycogen deposition was observed, but was significantly decreased by MPA, and at the same time, lactate plus pyruvate accumulated (Table 5). It appears that, as well as being converted into G6P, a proportion of the triose phosphate derived from glycerol enters glycolysis and is converted into pyruvate, which may then be used via PEPCK for glucose or glycogen synthesis. This conclusion is supported by the finding of an increased activity ratio of PK after glycerol administration (Table 4).

As in both glucose- and glycerol-re-fed rats glycogen synthesis occurs concomitantly with activation of PK, it is tempting to suggest independent control mechanisms for the spans of glycolysis distal and proximal to triose phosphate. Such uncoupling may be permitted by the maintenance of low fructose 2,6-bisphosphate concentrations ($< 30\%$ of the fed value [26,30]), at which flux through 6-phosphofructokinase-1 might be expected to be relatively low [31], and it may be relevant that the K_m of the cyclic AMP-dependent protein kinase for 6-phosphofructokinase-2/fructose-2,6-bisphosphatase is lower than that for PK [32].

G6P disposal after glucose re-feeding

Glucose output [a negative portal (P) minus hepatovenous (V) concentration difference] was evident in water-fed rats (both control and hyperthyroid) and in glucose-re-fed hyperthyroid rats (Table 6). However, a large positive value of (P - V) indicated a reversal of carbon flux with net hepatic glucose uptake at 1 h after glucose administration to euthyroid rats. By 2 h after re-feeding this value had decreased (Table 6). Consequently there was no consistent relationship between net glucose uptake and the portal glucose concentration over the 2 h period (see Table 6).

Glucose output was suppressed in water-fed rats by

Fig. 3. Relationship between portal glucose concentrations and portal-minus-hepatovenous concentration differences in glucose-re-fed, MPA-treated, euthyroid rats

Values were obtained at 1 h and 2 h after an intragastric glucose load. MPA was administered at the time of intubation.

inhibition of gluconeogenesis with MPA [a value for (P-V) of 0.219 ± 0.078]. In glucose-fed rats treated with MPA, (P-V) was positive and proportional to (P) at concentrations > 6 mm (Fig. 3). At 1 h after glucose re-feeding, there was a good correlation between the measured value of (P-V) (Table 6) and the value of (P-V) that would be predicted from the portal glucose concentration, given the relationship between these parameters shown in Fig. 3 (values of $+0.63$ and $+0.65$ respectively). This indicates complete diversion of gluconeogenic product from glucose output. At 2 h, the value of (P-V) that would be predicted from the portal glucose concentration if glucose output were inhibited was $+0.57$, whereas the measured value of (P-V) was $+0.02$ (see Table 6 and Fig. 3). The change in (P-V) occurring over the 2 h re-feeding period was not a consequence of variation in the rate of G6P utilization for glycogen synthesis, as rates of glycogen synthesis (estimated by using $^3\text{H}_2\text{O}$ [16]) between 0 and 1 h and between 1 h and 2 h after glucose administration were 161 ± 16 and 164 ± 44 μg -atoms of ^3H /h per g of liver respectively. The difference between these values of (P-V) must therefore be a consequence of glucose output.

It has been proposed that both activation of glycogen synthase and inhibition of G6Pase are necessary for glycogen repletion after re-feeding [33]. Although the present results cannot exclude the possibility of changes in hepatic glucose uptake, they strongly suggest that suppression of glucose output by inhibition of G6Pase may not be obligatory. This suggestion is supported by measurements of (P-V) in glycerol re-fed rats. Consistent with the increased blood glucose concentrations observed after glycerol administration, measurement of concentration differences confirmed that hepatic glucose output was not inhibited (Table 6), even though glycogen deposition was observed.

Recent work has indicated that one of the major factors influencing whether hepatic glycogen is synthesized via gluconeogenesis or directly from glucose is the plasma glucose concentration [24,33,34], the percentage contribution of the direct pathway being progressively increased at higher glucose concentrations. However,

decreased glycogen deposition in the hyperthyroid rats could not be related to relative hypoglycaemia. Portal glucose concentrations either exceeded (1 h) or were similar to (2 h) those found in the euthyroid controls (results not shown).

DISCUSSION

Although conversion of triose phosphates into G6P is not decreased by hyperthyroidism, the present results demonstrate at least two actions which may impede glycogen deposition after re-feeding, both of which are related to the disposal of G6P. Firstly, hyperthyroidism over-rides the redirection of hepatic glucose flux normally observed in response to carbohydrate. As at 1 h after glucose administration there is significant glycogen deposition despite continued net glucose output, it is suggested that this effect does not limit hepatic glycogen deposition in the hyperthyroid state. The inhibition of glycogen synthesis after glycerol re-feeding suggests an additional, critical, site of action of thyroid hormone distal to G6Pase. Investigations of direct effects of hyperthyroidism on the enzymes of glycogen metabolism have yielded conflicting results [35-40]. In one study, under conditions where glycogen was depleted, basal phosphorylase *a* activity was decreased [35], whereas synthase *a* was increased [36]. In other studies phosphorylase *a* was increased [37], while synthase activity was unaffected [38-40]. Clearly, further experiments are required to elucidate the possible mechanisms by which the hyperthyroid state may modify the activities of these enzymes after re-feeding.

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