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Mechanisms regulating cardiac fuel selection in hyperthyroidism

Mary C. SUGDEN,*§ Mark J. HOLNESS,* Yong-Ling LIU,* David M. SMITH,† Lee G. FRYER‡ and Yolanta T. KRUSZYNSKA‡

* Department of Biochemistry, Faculty of Basic Medical Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K., † Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, U.K., and ‡ University Department of Medicine, Royal Free Hospital, Pond Street, London NW3 2QG, U.K.

Starvation (48 h) decreases fructose 2,6-bisphosphate (Fru-2,6-P_2) concentrations and the ratio of free to acylated carnitine in hearts of euthyroid rats. These decreases, which are indicative of increased lipid fuel oxidation, are accompanied by decreased rates of glucose uptake and phosphorylation, assessed by using radioactive 2-deoxyglucose. Cardiac concentrations of acylated carnitines were increased at the expense of free carnitine even in the fed state in response to experimental hyperthyroidism, but neither Fru-2,6-P_2 concentrations nor rates of glucose utilization were suppressed. Starvation (48 h) did not further increase the proportion of acylated carnitine in the heart in hyperthyroidism, and suppression of Fru-2,6-P_2 concentrations and glucose utilization rates by starvation was attenuated. Although glucose utilization rates were decreased, starvation did not decrease immunoreactive GLUT 4 protein concentrations. Furthermore, although hyperthyroidism was associated with a statistically significant (30–40 %) increase in relative abundance of GLUT 4 mRNA, the amount of GLUT 4 protein was not increased by hyperthyroidism in either the fed or the starved state. The results demonstrate a significant effect of hyperthyroidism to enhance cardiac glucose utilization in starvation by a mechanism which does not involve changes in GLUT 4 expression but may be secondary to changes in glucose–lipid interactions at the tissue level.

INTRODUCTION

Experimental hyperthyroidism promotes the uptake and phosphorylation of glucose by a range of skeletal muscles *in vivo* (Sugden *et al.* 1990*a*). This action underlies the increases in whole-body glucose utilization observed in hyperthyroidism (Okajima & Ui, 1979*a*,*b*; Huang & Lardy, 1981). We have speculated (Sugden *et al.*, 1990*a*) that at least two post-transport mechanisms underly the effects of hyperthyroidism to increase glucose utilization by skeletal muscle: both involve attenuation of the suppression of glucose utilization normally invoked by competing substrates, i.e. lipid metabolites (which inhibit glucose degradation) and glycogen (which inhibits use of glucose 6-phosphate for glycogen synthesis). The former effect becomes increasingly important during food restriction and is specific to skeletal muscles containing mainly oxidative fibres (Holness & Sugden, 1990). Recent reports have suggested that, in addition to these effects, which are exerted distal to glucose transport, hyperthyroidism may stimulate skeletal-muscle glucose utilization directly by increasing the expression of the insulin-sensitive glucose transporter isoform, GLUT 4 (Casla *et al.*, 1990; Weinstein *et al.*, 1991). This direct effect of hyperthyroidism, which involves increases in relative abundance of both GLUT 4 protein and GLUT 4 mRNA, was suggested to be due either to an increased rate of transcription of the GLUT 4 gene or to stabilization of GLUT 4 mRNA (Weinstein *et al.*, 1991).

The heart is a major target organ for the thyroid hormones. Effects of hyperthyroidism include increased contractile force and heart rate and altered cardiac gene expression (e.g. Garber *et al.*, 1983; Gustafson *et al.*, 1986). As is the case in skeletal muscle, glucose uptake and phosphorylation by the heart *in vivo* in the euthyroid state is suppressed by increased lipid oxidation (Holness & Sugden, 1990). There are, however, major differences between cardiac skeletal muscle in terms of the changes in glucose utilization evoked in response to a variation in the circulating insulin concentration. First, the major fate of the additional glucose which is taken up in response to insulin in skeletal muscle is glycogen (James *et al.*, 1985*a*; see also Holness & Sugden, 1991): this is not the case in heart. Secondly, in contrast with findings in skeletal muscle, exercise-training fails to increase insulin-dependent glucose uptake in hearts of rats fed on a high-fat diet, i.e. exercise can oppose the effects of fat-feeding to decrease insulin-dependent glucose utilization in skeletal muscle, but not in heart (Wake *et al.*, 1991). Furthermore, there are quite marked differences in the dose–response curves for insulin-mediated glucose uptake and phosphorylation *in vivo* in cardiac versus skeletal muscle (James *et al.*, 1985*b*). In the present study, we have examined the effects of experimental hyperthyroidism on cardiac fuel selection and, in particular, glucose uptake and phosphorylation in the heart *in vivo* in relation to changes in cardiac GLUT 4 mRNA expression and the supply and utilization of lipid fuels.

MATERIALS AND METHODS

Materials

Sources of materials for measurements of glucose utilization indices (GUIs) and metabolite concentrations are given in Schofield *et al.* (1985), Holness *et al.* (1988) and Holness & Sugden (1990). Hybond N nylon membranes, [α-^{32}P]dATP and ^{125}I-F(ab′)$_2$ donkey anti-rabbit IgG were obtained from Amersham International (U.K.). Terminal deoxynucleotidyltransferase was obtained from Boehringer Mannheim (U.K.). Oligonucleotides were custom-synthesized by Oswel DNA Services, Edinburgh, U.K. Chemicals used for Northern blotting were of molecular-biology grade.

Experimental procedures

Rats. Female albino Wistar rats (200–250 g) on a 12 h-light/12 h-dark cycle were either fed *ad libitum* on standard rodent chow (supplied by Special Diets Services, Witham, Essex,

Abbreviations used: Fru-2,6-P_2, fructose 2,6-bisphosphate; GLUT 4, the insulin-regulatable glucose transporter isoform; GUI, glucose utilization index; NEFA, non-esterified fatty acid; T_3, tri-iodothyronine.

§ To whom correspondence and reprint requests should be addressed.

U.K.; 52% digestible carbohydrate, 15% protein, 3% lipid and 30% non-digestible residue, all by weight) or starved for 48 h (food removal at the end of the dark phase of the light/dark cycle). Experiments with rats with continuous access to food *ad libitum* (termed 'fed *ad libitum*') were completed within 3 h of the end of the dark (feeding/activity) period. Experimental hyperthyroidism was induced by the subcutaneous injection of triiodothyronine (T_3; 100 μg/100 g body wt. per day) for 3 consecutive days, sampling on the morning of day 3 (in the fed state or after starvation for 48 h; see Holness *et al.*, 1985, for details). Heart weights (g/100 g body wt.) in the control and T_3-treated animals were: fed euthyroid, 0.38 ± 0.03; fed hyperthyroid, 0.47 ± 0.02; 48 h-starved euthyroid, 0.31 ± 0.14; 48 h-starved hyperthyroid, 0.46 ± 0.02 ($P < 0.001$ for the effects of hyperthyroidism in both groups).

Metabolite measurements. Hearts were excised and freeze-clamped while rats were under sodium pentobarbital anaesthesia (6 mg/100 g body wt.; intraperitoneally, 5 min). Free and acylated carnitine (see Schofield *et al.* 1985), glycogen (Keppler & Decker, 1974) and fructose 2,6-bisphosphate (Fru-2,6-P_2) (Richards & Uyeda, 1980) concentrations were measured in heart extracts. Non-esterified fatty acid (NEFA) concentrations were measured in plasma with a C-test kit. The principle of the NEFA assay relies on enzymic coupling of the formation of acyl-CoA to a colour reaction.

Measurement of GUI. Rats used for measurement of GUI were each fitted with an indwelling cannula (Ferré *et al.*, 1985; Issad *et al.*, 1987) at 5–7 days before the start of the experiments. The experimental procedures used for the measurements of GUI values and blood glucose concentrations, given in detail in Sugden *et al.* (1990*b*), involved injection of 2-deoxy[^{3}H]glucose through the indwelling cannula, sampling blood at intervals for up to 60 min and tissues at 60 min. GUI values were calculated as described by Issad *et al.* (1987) by dividing the radioactivity (d.p.m.) of tissue 2-deoxy[^{3}H]glucose 6-phosphate by the calculated integral of blood 2-deoxy[^{3}H]glucose/[glucose]. As in Issad *et al.* (1987), values were not corrected for the discrimination factor (lumped constant) for deoxyglucose in glucose metabolic pathways (Ferré *et al.*, 1985, 1986). Previous studies (Ferré *et al.*, 1985, 1986; Pénicaud *et al.*, 1987) have failed to detect any significant change in the lumped constant with changes in nutritional, developmental or hormonal status.

Northern-blot analysis. Tissues were freeze-clamped in liquid N_2 and stored at -70 °C for subsequent RNA extraction. Total cellular RNA was extracted from approx. 1 g of pooled tissue by the acid guanidinium thiocyanate/phenol/chloroform method (Chomcyznski & Sacchi, 1987). RNA concentration was determined from its A_{260}. RNA (40 μg) was denatured and size-separated by electrophoresis on a Mops/formaldehyde/1% agarose denaturing gel (Maniatis *et al.*, 1982). RNA was transferred to a nylon membrane and fixed by baking for 2 h at 80 °C. The oligonucleotide probe for GLUT 4 [a 43-base oligonucleotide corresponding to bases 1564–1606 in the sequence given by Birnbaum (1989) and containing about 50% G+C bases] was labelled with [α-^{32}P]dATP with terminal deoxynucleotidyltransferase (Roychoudhury & Wu, 1980). Hybridization was carried out for 16 h at 55 °C in a solution of 5 × SSC (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0), 10 × Denhardt's solution [1 × Denhardt's solution = 0.01% (w/v) Ficoll/0.01% (w/v) BSA/0.01% (w/v) polyvinylpyrrolidone], 100 μg of denatured sonicated herring sperm DNA, 20 mM-sodium phosphate, pH 6.8, 10% (w/v) dextran sulphate, 2 ng of labelled oligonucleotide/ml and 7% (w/v) SDS. Membranes were washed at 55 °C for 3 × 20 min in 3 × SSC/0.01 M-sodium phosphate (pH 6.8)/10 × Denhardt's solution/5% SDS, and then twice at 22 °C for 3 min with 1 × SSC/1% SDS. Finally, two stringent washes each of 30 min at 60 °C with 1 × SSC/1% SDS were always used. Blots were normalized for RNA loading by re-probing with oligo(dT).

Signal detection and quantification. Washed membranes were exposed to Kodax XAR-5 film for 24 h at -70 °C and autoradiographs developed. Hybridized bands were sized by comparison with the positions of 18 S and 28 S rRNA. Signal intensities were determined with a direct capture two-dimensional surface counter image system (Autograph; Oxford Positron Systems, Oxford, U.K.). This necessitated the conversion of counts for individual blots into percentages to allow the data from replicate studies to be used in analysis. We determined empirically that the counter was linear between 0 and 22000 c.p.m., which was within the range of radioactivity hybridizing to the filters. The autograph analysis system was used only to provide data on the relative intensities of the signal in individual blots due to differences between blots resulting from probe-labelling, efficiency of hybridization and washing etc.

Western-blot analysis. Crude membranes from rat heart were prepared by a modification of the methods of Klip *et al.* (1987) and Pederson *et al.* (1990). This involved pulverization of hearts under liquid N_2, homogenization (1:10, w/v) in 10 mM-$NaHCO_3$/0.25 M-sucrose/5 mM-NaN_3, pH 7.4 at 4 °C, by using a Polytron tissue homogenizer and a series of centrifugation steps, further details of which are given in Klip *et al.* (1987) and Pederson *et al.* (1990). Protein was determined on the final crude membrane preparation (Bradford, 1976), which was solubilized in 50 mM-Tris/HCl (pH 6.8) / 1 mM-EDTA / 10 mM-dithiothreitol/10% (v/v) glycerol/2% (w/v) SDS/12 mg of pyronin Y/ml before electrophoresis.

Samples (100 μg) were subjected to SDS (0.1%)/PAGE [10% (w/v) polyacrylamide] on 15 cm × 10 cm × 0.15 cm slab gels by the method of Laemmli (1970). Protein was electrophoretically transferred from the gels to nitrocellulose membranes (Sartorius) in 39 mM-glycine/48 mM-Tris/0.0375% SDS/20% (v/v) methanol by using a semi-dry blotting apparatus (Pharmacia–LKB Multiphor 11 system) at a constant current of 1.6 mA/cm^2 for 1 h. Blots were blocked by incubation for 1 h in 20 mM-Tris/HCl (pH 7.5)/500 mM-NaCl/0.2% (v/v) Tween 20 (TTBS) containing 5% (w/v) low-fat milk powder (Marvel) and then incubated overnight with affinity-purified antibodies against residues 494–509 of GLUT 4. Antibodies were dissolved at a concentration of 2 μg/ml in TTBS containing 1% milk powder. Blots were washed extensively with TTBS containing a second antibody (see below) applied at a dilution of 1:400 in TTBS containing 1% milk powder. The second antibody used for these studies was ^{125}I-F(ab')$_2$ donkey anti-rabbit IgG (2.5 μCi in 10 ml). After extensive washing with TTBS, the blots were dried and autoradiographed at -70 °C. Variation between blots was assessed with standards from rat muscle, run and blotted with each gel. Autoradiographs were scanned with a linear densitometer to quantify the bands, each lane being scanned at least three times to assess variation across each band.

Statistics

Statistical significance of differences in GUI was assessed by Student's unpaired *t* test. GUI values are given as means ± S.E.M. for five to eight rats. Each Northern blot was repeated three times in duplicate to ensure that the patterns were reproducible. Western analysis was repeated on five separate samples in each group.

RESULTS AND DISCUSSION

Cardiac indices of fuel utilization

Concentrations of Fru-2,6-P_2, a potent activator of 6-

Table 1. Effects of thyroid status on cardiac carnitine concentrations and plasma NEFA concentrations in fed and 48 h-starved rats

Full details of the treatments and procedures used are given in the Materials and methods section. Statistically significant effects of T_3 treatment are indicated by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Effects of starvation were statistically significant (at least $P < 0.05$) in all cases in the control group. Effects of starvation were statistically significant (at least $P < 0.05$) in all cases except S, F/(S+L) and S/F (which were not statistically significant) in the T_3-treated group.

Nutritional status...	Fed		48 h-starved	
	Control	T_3-treated	Control	T_3-treated
Carnitine concn. (nmol/g)				
Free (F)	704.7±23.2	546.2±19.5***	295.5±30.8	408.2±41.3*
Short-chain acylcarnitine (S)	270.2±27.7	359.4±26.1*	439.7±28.0	310.9±36.7*
Long-chain acylcarnitine (L)	36.5±4.1	79.0±6.9***	51.0±4.6	38.4±5.4
Total carnitine (F+S+L)	1011.4±22.0	984.6±19.7	786.2±46.8	757.5±48.3
Total cardiac carnitine (nmol/100 g body wt.)	386±23	460±19*	248±18	349±24**
Ratio F/(S+L)	2.30±0.34	1.25±0.14*	0.60±0.14	1.17±0.12**
Ratio S/F	0.38±0.04	0.67±0.09*	1.49±0.14	0.76±0.11**
Carnitine acetyltransferase (units/g)	185.7±12.9	219.1±16.6	157.9±3.4	168.7±6.5
Plasma NEFA concn. (mM)	0.18±0.01	0.63±0.04***	0.44±0.03	0.48±0.03

phosphofructo-1-kinase, bear a close correlation with glycolytic flux rates in the perfused heart (Lawson & Uyeda, 1987). *In vitro*, Fru-2,6-P_2 concentrations are elevated by increased heart work (Lawson & Uyeda, 1987), but are decreased by the provision of lipid fuels (Hue *et al.*, 1988), which substitute for glucose as the primary energy substrate when their availability is increased (Randle *et al.*, 1964). Increased oxidation of NEFA by the heart *in vivo* is indicated by an increased proportion of acylated carnitine (Pearson & Tubbs, 1967), an increase in the ratio of the concentration of short-chain acylcarnitine to that of free carnitine (Pearson & Tubbs, 1967) and decreased Fru-2,6-P_2 concentrations (French *et al.*, 1988*b*). The changes in the concentrations of these signal metabolites and rates of glucose uptake and phosphorylation (measured directly by using radiolabelled 2-deoxyglucose and expressed as GUI values) evoked by experimental hyperthyroidism in the fed and starved states are shown in Tables 1 and 2.

Cardiac fuel selection in hyperthyroidism in the fed state

Cardiac concentrations of acid-soluble (short-chain) acylcarnitine and acid-insoluble (long-chain) acylcarnitines were significantly increased by hyperthyroidism in the fed state (by 33% and 116% respectively; Table 1). The concentration of free carnitine was decreased correspondingly (by 23%, Table 1). As a consequence, the ratio of free to acylated carnitine [F/(S+L)] dramatically declined, whereas that of short-chain acylcarnitine to free carnitine (S/F) increased (Table 1). In the heart, short-chain acylcarnitine is predominantly acetylcarnitine (Pearson & Tubbs, 1967). Since the activity of carnitine acetyltransferase is high and unaffected by hyperthyroidism (Table 1), it may be inferred that the acetyl-CoA/CoA concentration ratio is increased by hyperthyroidism in the fed state [see Pearson & Tubbs (1967) for discussion]. The observed pattern of changes in the concentrations of free and acylated carnitines and the implied change in the acetyl-CoA/CoA concentration ratio indicates an increased rate of cardiac fatty acid oxidation in the fed state in hyperthyroidism: this is associated with an increased NEFA supply in the fed state (Table 1; see also Sugden *et al.*, 1990*a*).

We have demonstrated a modest (34%) decline in cardiac Fru-2,6-P_2 concentrations in fed euthyroid rats after acute (2 h) exposure to an elevated NEFA supply *in vivo* (French *et al.*, 1988*b*). Acute (3 h) exposure to an elevated fatty acid supply also suppresses cardiac GUI in fed euthyroid rats (Holness & Sugden, 1990). In marked contrast, despite exposure to high circulating concentrations of NEFA and changes in cardiac concentration ratios of free and acylated carnitine consistent with increased fatty acid oxidation, cardiac GUI values and Fru-2,6-P_2 concentrations were unaffected by hyperthyroidism in the fed state (Table 2). Since cardiac glycogen concentrations are not increased by hyperthyroidism (Table 2), the predominant fate of glucose 6-phosphate derived from circulating glucose is degradation. The failure to suppress either the Fru-2,6-P_2 concentration or the value of GUI in the fed state strongly suggests that rates of cardiac glycolysis remain at least as high in the hyperthyroid as in the euthyroid state. Concomitant degradation of both glucose and fatty acids at high rates is consistent with the known effects of thyroid hormone to increase energy demand as a consequence of increased heart work (inferred from significant cardiac hypertrophy; see the Materials and methods section).

Cardiac fuel selection in the starved state

The proportion of acylated carnitine and the short-chain acylcarnitine/free carnitine concentration ratio increased with 48 h starvation in hearts of euthyroid rats (see also French *et al.*, 1988*a*), but not of hyperthyroid rats (Table 1). However, Fru-2,6-P_2 concentrations and GUI values had declined after 48 h starvation in both groups (Table 2). It has been proposed that the decreases in cardiac GUI values (Issad *et al.*, 1987; French *et al.*, 1988*a*) and Fru-2,6-P_2 concentrations (French *et al.*, 1988*a*) observed after prolonged starvation in the euthyroid state are due to increased rates of utilization of fatty acids and ketone bodies. From the degrees of cardiac hypertrophy induced by hyperthyroidism in fed and starved rats (see the Materials and methods section), it may be inferred that the increases in heart work associated with hyperthyroidism are not less, and may actually be even greater, in the starved than in the fed state. The decline in glucose utilization observed in hyperthyroidism in response to 48 h starvation must therefore be compensated for by the increased use of alternative fuels to glucose, most probably the ketone bodies. There are greatly increased circulating concentrations of the ketone bodies after prolonged starvation, and the rate of ketone-body utilization, which, in general, reflects ketonaemia, is unaffected by hyperthyroidism (Holness *et al.*, 1987).

Compared with the 60% decline in Fru-2,6-P_2 concentrations evoked by 48 h starvation in euthyroid rats, Fru-2,6-P_2 concentrations in hyperthyroid rats were decreased by only 25% (Table 2): corresponding decreases in GUI values were 87% and 47% respectively (Table 2). As a consequence of the approx. 50% attenuation of these responses to prolonged starvation,

Table 2. Effects of thyroid status on cardiac GUI values, Fru-2,6-P_2 concentrations and glycogen concentrations in fed and 48 h-starved rats

Full details of the treatments and procedures used are given in the Materials and methods section. Statistically significant effects of T_3 treatment are indicated by: $*P < 0.05$; $**P < 0.01$. Effects of starvation were statistically significant ($P < 0.001$) in all cases in the control group. Effects of starvation were statistically significant ($P < 0.01$) for GUI values and glycogen concentrations in the T_3-treated group.

Nutritional status...	Fed		48 h-starved	
	Control	T_3-treated	Control	T_3-treated
GUI (ng/min per mg)	71.2±8.9	95.9±10.1	9.2±6.2	51.1±7.5**
Fru-2,6-P_2 (nmol/g)	1.93±0.11	1.82±0.10	0.76±0.06	1.37±0.24*
Glycogen (% wet wt.)	0.33±0.04	0.22±0.04	0.65±0.04	0.51±0.07

Table 3. Northern- and Western-blot analyses of GLUT 4 expression in fed or 48 h-starved control and hyperthyroid rats

Full details of the procedures used are given in the Materials and methods section. Autoradiograms were quantified by scanning densitometry. Results are means±S.E.M. Statistical significance of T_3 treatment is indicated by $*P < 0.05$, and that of starvation by $\dagger P < 0.05$.

	GLUT 4 mRNA abundance (% of fed control)		GLUT 4 protein abundance (% of fed control)	
Nutritional status	Control	T_3-treated	Control	T_3-treated
Fed	100.0±10.7	134.1±6.4*	100.0±6.8	95.2±3.2
48 h-starved	97.0±14.6	135.1±2.3*	129.7±17.0	121.2±11.3†

cardiac Fru-2,6-P_2 concentrations were increased by almost 2 fold in hyperthyroidism. At the same time, cardiac GUI values in starved hyperthyroid rats were approx. 5.5-fold higher than in the corresponding euthyroid controls.

GLUT 4 expression

Hyperthyroidism increases GLUT 4 mRNA and protein expression in skeletal muscle in both fed and starved states (Casla *et al.*, 1990; Weinstein *et al.*, 1991). As GLUT 4 is the predominant glucose transporter isoform expressed in heart (James *et al.*, 1989; Wake *et al.*, 1991), the possibility of direct effects of hyperthyroidism on GLUT 4 expression in the heart was investigated.

Hyperthyroidism did not affect the level of immunodetectable GLUT 4 protein in the fed state, a finding consistent with the absence of its effects on glucose utilization in this condition (Table 3). Interestingly, however, the relative abundance of GLUT 4 mRNA was significantly increased by hyperthyroidism in the fed state (Table 3). The disparity between GLUT 4 mRNA relative abundance and immunodetectable GLUT 4 protein indicates post-transcriptional regulation of GLUT 4 expression in hyperthyroidism. This may involve either a decrease in the efficiency of translation of GLUT 4 mRNA or a decrease in the stability of the encoded GLUT 4 protein. Despite greatly decreased rates of glucose uptake and phosphorylation in starvation, cardiac GLUT 4 protein expression was either unchanged (control rats) or increased (hyperthyroid rats; Table 3). Hyperthyroidism again increased mRNA relative abundance in the starved state, but was without effect on GLUT 4 protein (Table 3).

GLUT 4 expression is reported to be greater in those skeletal muscles that contain relatively high proportions of oxidative (type I and IIa) fibres (Henricksen *et al.*, 1990). It is therefore pertinent to compare the regulation of GLUT 4 in heart, a highly oxidative muscle, with that in hind-limb skeletal muscle, which contains about 40% oxidative fibres (Ariano *et al.*, 1973). Immunoblotting of total membrane from a preparation of hind-limb muscle shows that fasting increases GLUT 4 protein 3-fold (Kahn & Flier, 1990); only a minor increase was observed in heart (the present work). Furthermore, whereas in the present experiments hyperthyroidism was completely without effect on GLUT 4 protein expression and evoked only a modest (approx. 40%) increase in cardiac GLUT 4 mRNA expression, Weinstein *et al.* (1991), using a dose of T_3 identical with that used in the present experiments (100 μg/100 g body wt. per day) demonstrated over a similar time scale dramatic and highly significant (2–3-fold) increases in GLUT 4 mRNA and protein abundance in (mixed) hind-limb muscles. Since the regulation of GLUT 4 gene expression has been suggested to play an important role in determining insulin sensitivity and glucose utilization (reviewed by Bell *et al.*, 1990; Kahn & Flier, 1990), the different responses of GLUT 4 expression to nutritional and hormonal signals in heart and skeletal muscle may explain, at least in part, variations in maximally stimulated glucose transport among skeletal muscles with different fibre compositions and between heart and skeletal muscle *in vivo*.

Concluding remarks

The heart has the highest rates of glucose utilization of all the muscles of the body (in the resting state), and it is characterized by the ease with which it switches from glucose to lipid as the preferred energy substrate when the lipid supply is increased. After prolonged starvation (when circulating NEFA and ketone-body concentrations are high), cardiac glucose utilization rates fall to approx. 10% of those observed in the fed state. The present results demonstrate a highly significant effect of hyperthyroidism to enhance the uptake and phosphorylation of glucose by the heart in starvation. The mechanism does not involve changes in GLUT 4 expression, but may be secondary to intracardiac regulation of fuel disposition, including changes in glucose–lipid interactions such that, despite an increased supply of lipid-derived fuels (Sugden *et al.*, 1990*a*), cardiac glucose utilization remains high. This effect may in part be attributed to the increased energy demand of the heart in the hyperthyroid state, which in starvation cannot be met by the utilization of lipid-derived fuels alone.

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