Hyperinsulinaemia during exercise does not suppress hepatic glycogen concentrations in patients with type 1 diabetes: a magnetic resonance spectroscopy study

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Abstract

Aims/hypothesis We compared in vivo changes in liver glycogen concentration during exercise between patients with type 1 diabetes and healthy volunteers.

Methods We studied seven men with type 1 diabetes (mean $\pm$ SEM diabetes duration 10 ± 2 years, age 33 ± 3 years, BMI 24 ± 1 kg/m², HbA_{1c} $8.1 \pm 0.2\%$ and $\dot{V}O_2$ peak 43 ± 2 ml [kg lean body mass]⁻¹ min⁻¹) and five non-diabetic controls (mean $\pm$ SEM age 30 ± 3 years, BMI 22 ± 1 kg/m², HbA_{1c} $5.4 \pm 0.1\%$ and $\dot{V}O_2$ peak 52 ± 4 ml [kg lean body mass]⁻¹ min⁻¹, before and after a standardised breakfast and after three bouts (EX1, EX2, EX3) of 40 min of cycling at 60% $\dot{V}O_2$ peak. ¹³C Magnetic resonance spectroscopy of liver glycogen was acquired in a 3.0 T magnet using a surface coil. Whole-body substrate oxidation was determined using indirect calorimetry.

Results Blood glucose and serum insulin concentrations were significantly higher ($p < 0.05$) in the fasting state, during the postprandial period and during EX1 and EX2 in subjects with type 1 diabetes compared with controls. Serum insulin concentration was still different between groups during EX3 ($p < 0.05$), but blood glucose concentration was similar. There was no difference between groups in liver glycogen concentration before or after the three bouts of exercise, despite the relative hyperinsulinaemia in type 1 diabetes. There were also no differences in substrate oxidation rates between groups.

Conclusions/interpretation In patients with type 1 diabetes, hyperinsulinaemic and hyperglycaemic conditions during moderate exercise did not suppress hepatic glycogen concentrations. These findings do not support the hypothesis that exercise-induced hypoglycaemia in patients with type 1 diabetes is due to suppression of hepatic glycogen mobilisation.

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Keywords Exercise · Hyperinsulinaemia · Liver glycogen metabolism · Magnetic resonance spectroscopy · Type 1 diabetes

Abbreviations

MRS magnetic resonance spectroscopy
RER respiratory exchange ratio
 $\dot{V}O_2$ oxygen uptake

Introduction

Fear of exercise-induced hypoglycaemia poses a significant barrier to active participation in recreational sports and games in patients with type 1 diabetes [1–3]. The incidence

of hypoglycaemia during an acute bout of moderate-intensity exercise in children and adolescents with type 1 diabetes has been recently reported to be up to 30% [4]. Although there is no direct evidence, the incidence of exercise-induced hypoglycaemia can be expected to be similar in adults with type 1 diabetes. However, the physiological responses to exercise and hence the factors contributing to exercise-induced hypoglycaemia in patients with type 1 diabetes are not fully understood.

During exercise in non-diabetic individuals, either increased carbohydrate intake and/or increased hepatic glucose production is required to match the increased rate of muscle glucose uptake during exercise [5, 6]. To facilitate increased hepatic glucose output, the hormonal response to exercise performed in the postabsorptive state is characterised by reciprocal changes in plasma insulin and glucagon concentrations [5]. Increases in both hepatic glycogenolysis and gluconeogenesis contribute to augmented hepatic glucose output during exercise, usually with a greater contribution from the former [5, 7]. However, during prolonged exercise under fasting conditions a greater contribution of hepatic glucose output is derived from gluconeogenesis [8, 9].

In patients with type 1 diabetes the hormonal and metabolic milieu is disturbed, and this may affect hepatic glycogen metabolism. First, it is clearly impossible to replicate the fall in insulin concentration normally seen during exercise using exogenous insulin injections in patients with type 1 diabetes. During exercise, systemic and to a lesser extent portal insulin concentrations will be higher in patients with type 1 diabetes treated with exogenous insulin than in normal subjects. Therefore, when patients exercise typically with high insulin concentrations [3, 10], this may suppress hepatic glucose output [11] with a consequent risk of hypoglycaemia both during and after exercise [11]. Indeed, infusing insulin during exercise in normal volunteers is associated with suppressed splanchnic glucose output [12]. Second, in addition to the fall in insulin, a rise in glucagon is required for the full increment in hepatic glucose output [13]. Prevention of this physiological response results in attenuation of hepatic glucose output [14]. It has also been suggested that the increased risk of hypoglycaemia in patients with type 1 diabetes could be due to impaired stimulation of hepatic glucose output in response to glucagon [15]. Third, patients with poorly controlled diabetes have low fasting hepatic glycogen concentrations and impaired net hepatic glycogen synthesis following ingestion of a mixed meal [16, 17]. This in turn may predispose these patients to hypoglycaemia during subsequent exercise.

A large body of evidence concerning hepatic glucose metabolism during exercise in diabetes has been derived from animal experiments [18–22] but there is a lack of in

vivo human data. Human studies using magnetic resonance spectroscopy (MRS) have reported defects in mobilisation of hepatic glycogen stores in patients with type 1 diabetes [23, 24]. However, these studies were performed either under very low circulating insulin concentrations (30–60 pmol/l) or after hypoglycaemia. The impact of real-life therapeutic insulin concentrations on hepatic glycogen metabolism during exercise in patients with type 1 diabetes is not known. We have recently shown that, in patients with type 1 diabetes, exercise under peak therapeutic insulin concentrations increases exogenous glucose utilisation and that this increase is disproportionate to the relative increase in carbohydrate oxidation, suggesting an increase in glucose flux through non-oxidative pathways, perhaps towards hepatic glycogenesis [25].

The aim of this study was to compare in vivo changes in liver glycogen both before and after a meal, and during subsequent moderate exercise in patients with type 1 diabetes under therapeutic insulin concentrations, and in non-diabetic volunteers. In line with current recommendations [2, 10], we used a 50% reduction of the usual preprandial rapid-acting insulin dose. In addition, rapid-acting insulin was infused intravenously to achieve a predictable basal replacement (~0.5 U/h). Although clearly not truly real-life, we adopted this strategy to approximately replicate a real-life situation. We hypothesised that, when compared with non-diabetic controls, patients with type 1 diabetes when exercising under relative hyperinsulinaemic conditions may suppress hepatic glycogen mobilisation and thereby increase the risk of exercise-induced hypoglycaemia.

Methods

Patients and preliminary measurements We recruited seven physically active men with uncomplicated type 1 diabetes from our hospital diabetes clinics, with mean $\pm$ SEM diabetes duration 10 ± 2 years, and five non-diabetic, physically active controls. The baseline characteristics of the volunteers are outlined in Table 1. Fat-free mass was determined using a bioelectrical impedance technique (Quadscan 4000; Bodystat, Isle of Man, UK). Five patients were on a basal-bolus subcutaneous insulin regimen and two were on twice a day premixed insulin injections. All patients were screened to exclude micro- and macrovascular complications. The control subjects were medically screened and were in good health. Patients and controls were informed of all experimental procedures and the associated risks before we obtained informed consent. All procedures used in this study were performed according to the Declaration of Helsinki and approved by the Nottingham NHS Research Ethics Committee and the University of Nottingham Research Ethics Committee.

Table 1 Baseline characteristics of study participants

	Type 1 diabetes (<i>n</i> =7)	Controls (<i>n</i> =5)	<i>p</i> value
Age (years)	33±3	30±3	0.509
BMI (kg/m ²)	24±1	22±1	0.054
Lean body mass (kg)	68±3	65±5	0.548
HbA _{1c} (%)	8.1±0.2	5.4±0.1	0.000
$\dot{V}O_2$ peak (ml [kg lean body mass] ⁻¹ min ⁻¹)	43±2	52±4	0.09

Data are presented as means ± SEM

The peak oxygen uptake ($\dot{V}O_2$ peak) was determined during a preliminary visit using an incremental cycling test (Excalibur cycle ergometer; Lode, Groningen, the Netherlands). The criteria used for attainment of the $\dot{V}O_2$ peak were: heart rate response greater than 90% of predicted maximum heart rate, respiratory exchange ratio (RER) > 1.10, near maximal rating of perceived exertion (Borg scale ≥19) and a drop in pedalling rate greater than 20%.

Experimental design and protocol An outline of the study design is depicted in Fig. 1. For 3 days prior to the trials, controls and patients maintained an isoenergetic diet and patients monitored their capillary blood glucose closely to prevent episodes of hypoglycaemia. All participants were requested to avoid smoking, alcohol and exercise for 3 days prior to each study visit.

Patients (type 1 diabetes) omitted their medium-/long-acting insulin for 24–36 h prior to the study. On the evening prior to the study, all subjects consumed a standardised high-carbohydrate (2.5 g/kg fat-free mass) meal, and the patients injected their usual short-acting soluble insulin with this meal and were admitted to the hospital. An antegrade cannula was inserted into a cubital fossa vein for

a low-dose intravenous insulin infusion to maintain euglycaemia overnight. A retrograde cannula was inserted into a dorsal hand vein for blood sampling to measure glucose concentrations during the overnight stay. On the day of the study this cannula was also used for drawing arterialised blood samples for metabolite and hormone measurements, while the hand was placed in a hot air box (55°C). At 08:00 hours the following morning, subjects were transferred to the magnetic resonance (MR) centre for the study protocol. Intravenous insulin infusion was continued at a constant rate of 5 mU m⁻² min⁻¹ to cover basal insulin requirements until the end of the study.

The non-diabetic controls reported directly to the MR centre after an overnight fast from 22:00 hours and a retrograde cannula was inserted into a dorsal hand vein for drawing arterialised blood samples as above. A baseline ¹³C MRS measurement of liver glycogen was carried out for both patients and controls. All subjects were then provided with a high-glycaemic index (82.9) carbohydrate meal (2.5 g carbohydrate/kg fat-free mass). The meal consisted of Kellogg's corn flakes, skimmed milk, white bread, strawberry jam and Lucozade (Glaxo SmithKline, Brentford, UK). The diabetic subjects received a subcuta-

Fig. 1 Schematic diagram of study design. EX1, 2 and 3 represent the three bouts of exercise. Blood samples were drawn at regular intervals during the study for metabolite and hormone measurements. Indirect calorimetry was carried out during a separate visit under identical

feeding and exercise conditions, before and after breakfast and during exercise, to measure substrate oxidation rates. There was a slight variation in the insulin treatment protocol when data were acquired (see [Methods](#))

neous abdominal injection of 0.5 U of quick-acting analogue insulin (Novorapid; Novo Nordisk, Chartres, France) per 10 g of carbohydrate with breakfast (a 50% dose reduction from what these patients would have normally administered for this meal). All subjects were requested to consume the meal within 15 min and subsequently rested on a bed for 110 min to replicate a typical postprandial pre-exercise state. Further spectroscopy measurements of liver glycogen were acquired 110 min after consumption of the meal. All subjects (subjects with type 1 diabetes and controls) then carried out three bouts of cycling at 60% $\dot{V}O_2$ peak for 40 min each. The cycling sessions were interrupted for 40 min between bouts to obtain further spectroscopy measurements of liver glycogen. Hydration was maintained during cycling by ingesting water at a rate of 10 ml/kg body weight every 20 min. Heart rate and rating of perceived exertion using a Borg scale [26] were measured at 20 min intervals during exercise. Blood samples were drawn at the same intervals for metabolite and hormone measurements. Urine samples were collected throughout the experiment for glucose measurements.

On a separate day to the liver glycogen data acquisition visit, the above exercise and feeding protocol was replicated in each non-diabetic volunteer and patient with diabetes to acquire indirect calorimetry data. The presence of a strong magnetic field and working space limitations precluded us from making these measurements on the same visit as when MR scanning was taking place. During this visit there was a slight variation in the insulin treatment protocol. On the evening prior to this visit, patients had the same high-carbohydrate meal and their usual insulin treatment regime (quick-acting insulin followed by their long-acting insulin). Patients were advised to monitor their capillary glucose regularly to avoid hypoglycaemia and were not admitted overnight. Moreover, they did not receive the intravenous basal insulin infusion during the study since patients administered their usual long-acting insulin the previous night. Resting $\dot{V}CO_2$ and $\dot{V}O_2$ were measured for 20 min using a ventilated hood system (GEM; Nutren Technologies, Manchester, UK) before and 110 min after consumption of the same meal to allow calculation of energy expenditure and substrate oxidation rates [27]. Subsequently, 5 min expired gas samples ($\dot{V}max$ 29; Sensormedics, Yorba Linda, CA, USA) were collected every 20 min whilst the subject was cycling and the measured $\dot{V}CO_2$ and $\dot{V}O_2$ values were used to determine substrate oxidation rates during exercise.

Blood and urine metabolites Whole-blood and urine glucose and blood lactate were measured using a Yellow Springs analyser (YSI 2300 Stat Plus-D; Yellow Springs Instruments, Yellow Springs, OH, USA). All serum and plasma samples were stored at -80°C until analysis. Serum

insulin and glucagon were measured using commercially available radioimmunoassay kits from Diagnostic Products Corporation (Llanberis, UK). NEFA concentrations were measured using a kit from Wako Chemicals (Neuss, Germany). Whole-blood β -hydroxybutyrate was measured in blood samples treated with perchloric acid (10%) [28]. Plasma catecholamines were measured using HPLC with electrochemical detection [29].

^{13}C Magnetic resonance spectroscopy All ^{13}C MR spectra were acquired on a 3.0 T whole-body magnetic resonance scanner with a 1 m diameter bore. A surface coil was used with a carbon coil for transmission and reception, and quadrature proton coils for ^1H decoupling. This consisted of a 7 cm diameter circular ^{13}C coil and two 13 cm ^1H coils. Subjects were positioned in the scanner in the supine position with the surface coil placed over the liver region of the torso. Care was taken to reposition subjects and the coil as accurately as possible for subsequent measurements. Manual shimming was performed on the water resonance and the broadband decoupling frequency was centred on the glycogen resonance. The coils were all tuned and matched using a Network Analyser (Model 8751A; Hewlett-Packard; 5–500 kHz). ^{13}C spectra were acquired with a simple pulse-acquire (decouple) sequence. The excitation pulse was a 100 μs pulse at a peak power of 390 (10) W with Cyclops phase cycling. Broadband decoupling was achieved using three WALTZ-8 cycles during acquisition with a peak power of 50 ± 2 W. A repetition time of 360 ms was used and spectra were collected in blocks of 1000 acquisitions, giving a temporal resolution of 6 min. Two blocks were collected and summed for each time point. The sampling time was 142 μs , and 512 data points were collected during the acquisition time. RF powers were monitored throughout the acquisition period to ensure that we did not exceed the maximum values allowed according to specific absorption rate limits recommended by the National Radiological Protection Board. No adjustments to the spectrometer were made between measurements, except for tuning and matching the ^{13}C surface coil before each spectroscopic measurement.

All spectra were analysed using the Matlab version of the magnetic resonance user interface (MRUI). Spectral peaks were selected using the AMARES algorithm and were fitted to Lorentzian lineshapes. The signal of interest arises from the C1 position of glycogen at 100.5 ppm (relative to tetramethylsilane=0). The lipid peaks obscured resonances from other glycogen carbons (C2 to C6). The coefficient of variation, calculated for repeated analysis of a single spectrum, was 3.8% ($n=15$). The integral of the glycogen peak was expressed as a fraction of the formate peak derived from a phantom containing formate at the centre of the coil. Quantification was done using a liver-shaped phantom

containing a solution of 184 mmol/l oyster glycogen and 150 mmol/l KCl. Glycogen concentrations were calculated using the formula: $(R_{\text{gly(s)}} \times [\text{Glyc}]) / R_{\text{gly(ph)}}$, where $R_{\text{gly(ph)}}$ and $R_{\text{gly(s)}}$ are the ratios of glycogen to formate in the phantom and subject, respectively, and $[\text{Glyc}]$ is the concentration of glycogen in the phantom in mmol/l.

Statistics Statistical analysis was carried out using SPSS version 11.5. A mixed-factor ANOVA was used to compare data from repeated measures. A p value of <0.05 was considered significant. All data are expressed as mean $\pm$ SEM.

Results

Patients and controls cycled at a mean workload of 153 ± 12 and 169 ± 17 W. The respective mean oxygen consumptions were 26.6 ± 1.11 and 29.8 ± 2.5 ml $[\text{kg lean body mass}]^{-1} \text{ min}^{-1}$, being almost 60% of the predetermined $\dot{V}\text{O}_2$ peak. Two patients developed hypoglycaemic symptoms (blood glucose <2.5 mmol/l) at the end of the second bout of exercise and did not proceed to the third bout. A further two patients developed asymptomatic hypoglycaemia (defined as blood glucose <3.5 mmol/l) at the end of the third bout of exercise.

Blood metabolites and hormones Blood glucose concentrations were higher in patients than controls at rest and during EX1 and EX2 (Fig. 2a, $p < 0.05$) but similar at the end of EX2 and during EX3. Fasting serum insulin concentration was greater in patients than controls (Fig. 2b, $p < 0.05$). During all three bouts of exercise, serum insulin concentrations were greater in patients than controls (Fig. 2b, $p < 0.05$). Fasting plasma NEFA concentrations were comparable between groups. Plasma NEFA concentrations were suppressed after breakfast and during EX1 in both groups (Table 2). NEFA concentrations started rising during EX2 and peaked during EX3 ($p < 0.05$), with no differences between groups. There was no difference in blood β -hydroxybutyrate concentrations between groups during the entire experiment (Table 2). Fasting blood lactate concentrations were also comparable between groups (Table 2). Exercise induced a significant ($p < 0.05$) increase in blood lactate concentrations in both groups, with no significant differences between groups. Baseline plasma noradrenaline and adrenaline concentrations were comparable in the two groups (Table 2). Exercise induced a significant ($p < 0.05$) increase in both plasma adrenaline and noradrenaline concentrations in both groups during EX1, EX2 and EX3, with no difference between groups. Fasting serum glucagon concentrations were significantly ($p < 0.05$) lower in patients with type 1 diabetes compared with

Fig. 2 Blood glucose (a) and serum insulin (b) concentrations in patients with type I diabetes (circles) and controls (squares). EX1, 2 and 3 represent the first, second and third bouts of exercise, respectively. * $p < 0.05$ for differences in blood glucose and serum insulin concentrations vs controls. $n = 7$ and $n = 5$ in the patient and control groups, respectively, except $n = 5$ at time points 330 and 350 min in the patient group

controls (Table 2). Following the meal there was a significant ($p < 0.05$) increase in serum glucagon concentrations in patients with diabetes but not in controls, resulting in similar pre-exercise values in the two groups. Furthermore, there was no difference in glucagon concentrations during exercise between groups.

Whole-body substrate metabolism There were no differences in either fasting or postprandial RER values between patients and controls (Table 3). A substantial increase in whole body carbohydrate oxidation was observed with the onset of exercise but there was no difference between groups (Table 3). Similarly, a substantial increase in whole-body fat oxidation was observed during exercise, again with no differences between groups (Table 3).

Liver glycogen There were no significant differences in fasting liver glycogen between groups (type 1 diabetes,

Table 2 Blood metabolite and hormone concentrations before and after breakfast and at the end of each exercise session

	Fasting		After meal		End of EX1		End of EX2		End of EX3	
	Type 1 diabetes	Controls	Type 1 diabetes	Controls	Type 1 diabetes	Controls	Type 1 diabetes	Controls	Type 1 diabetes	Controls
Plasma NEFA (mmol/l)	0.31±0.05	0.32±0.07	0.05±0.02*	0.06±0.01*	0.05±0.02*	0.06±0.02*	0.45±0.17	0.48±0.10	0.87±0.13*	1.29±0.18*
Blood β -hydroxybutyrate (mmol/l)	0.32±0.19	0.05±0.01	0.06±0.01	0.02±0.01	0.10±0.01	0.09±0.01	0.06±0.01	0.08±0.02	0.46±0.12	0.42±0.18
Blood lactate (mmol/l)	1.0±0.2	0.9±0.1	1.2±0.1	1.0±0.1	2.5±0.6*	1.6±0.3*	1.9±0.3*	1.2±0.2*	1.6±0.3*	1.2±0.2*
Plasma noradrenaline (nmol/l)	3.4±0.2	2.8±0.3	2.0±0.1	2.3±0.2	9.4±0.6*	8.5±1.1*	7.8±1.6*	8.9±1.1*	10.5±1.1*	8.0±1.6*
Plasma adrenaline (nmol/l)	0.2±0.1	0.1±0.03	0.2±0.04	0.2±0.03	1.1±0.2*	1.4±0.5*	2.2±0.8*	2.2±1.0*	5.7±1.6*	2.1±0.5*
Serum glucagon (pg/ml)	50.3±3.1	70.9±9.4**	78.6±5.0	77.4±17.5	77.5±6.6*	90.2±14.1*	79.1±5.0*	87.4±12.9*	78.3±3.9*	92.0±11.5*

Data are presented as means $\pm$ SEM

Type 1 diabetes $n=7$ and controls $n=5$ for all metabolites and hormones except β -hydroxybutyrate ($n=4$ both groups) and for all metabolites and hormones during EX3 ($n=5$ in the patient group)

* $p<0.05$ for difference from fasting value within the same trial

** $p<0.05$ compared with type 1 diabetes

178±54 mmol/l; controls, 212±63 mmol/l; Fig. 3). Following the high-carbohydrate breakfast, there was a two- to threefold increase in hepatic glycogen concentration in both patients and controls but there was no difference between groups (type 1 diabetes, 589±138 mmol/l; controls, 463±77 mmol/l; Fig. 3). Hepatic glycogen concentrations decreased after EX1 (type 1 diabetes, 298±67 mmol/l; controls, 303±59 mmol/l), EX2 (type 1 diabetes, 267±41 mmol/l; controls, 210±51 mmol/l) and EX3 (type 1 diabetes, 195±80 mmol/l; controls, 145±63 mmol/l) in both groups (Fig. 3). There was no difference in hepatic

glycogen concentrations during exercise between patients and controls.

Discussion

There were three main findings of this study. First, following a standardised evening meal and overnight control of glycaemia, fasting hepatic glycogen concentrations in patients with type 1 diabetes were comparable with

Table 3 RER and substrate oxidation rates before and after breakfast and during exercise

	Carbohydrate oxidation (mg min ⁻¹ [kg lean body mass] ⁻¹)		Fat oxidation (mg min ⁻¹ [kg lean body mass] ⁻¹)		RER	
	Type 1 diabetes	Controls	Type 1 diabetes	Controls	Type 1 diabetes	Controls
Fasting	0.17±0.06	0.28±0.04	0.14±0.02	0.09±0.02	0.79±0.03	0.87±0.03
1 h after breakfast	0.44±0.07	0.49±0.10	0.06±0.02	0.06±0.05	0.91±0.03	0.94±0.05
2 h after breakfast	0.46±0.03	0.55±0.06	0.06±0.01	0.02±0.03	0.93±0.02	0.98±0.03
EX1	3.47±0.16	3.59±0.23	0.49±0.04	0.50±0.06	0.92±0.01	0.92±0.01
EX2	3.11±0.20	3.78±0.28	0.56±0.06	0.43±0.12	0.90±0.01	0.93±0.02
EX3	2.92±0.31	3.21±0.17	0.72±0.13	0.73±0.10	0.88±0.02	0.89±0.01

Data are presented as means±SEM

EX1, 2 and 3 represent the first, second and third bouts of exercise, respectively

Values for EX1, EX2 and EX3 represent the average during each bout of exercise

$n=7$ and $n=5$ in the patient and control groups, respectively, except $n=6$ during EX3 in the patient group

Fig. 3 Liver glycogen concentrations in patients with type 1 diabetes (circles) and controls (squares) before and after a standardised breakfast, and after three bouts of 40 min of submaximal exercise. EX1, 2 and 3 represent the first, second and third bouts of exercise, respectively. $n=7$ and $n=5$ in the patient and control groups, respectively, except $n=5$ after EX3 in the patient group

those in non-diabetic controls. Second, similar postprandial liver glycogen deposition occurred after consumption of a high-glycaemic index breakfast and injection of a lower than normal dose of subcutaneously administered rapid-acting insulin analogue. Third, changes in liver glycogen content during exercise were similar in patients with type 1 diabetes and in controls despite the higher relative systemic hyperinsulinaemia (threefold) present in the former compared with the latter group.

Insulin, glucagon and catecholamines respond in a hierarchical fashion to regulate hepatic glucose output and prevent exercise-induced hypoglycaemia [14, 30–32]. In non-diabetic individuals a fall in serum insulin concentration and an increase in glucagon occur during exercise. This allows increased hepatic glucose output to match the increased muscle glucose uptake which occurs during exercise [33]. In patients with type 1 diabetes managed with subcutaneous insulin injections, a fall in serum insulin concentration cannot occur during exercise. Hence, if the physiological augmentation of hepatic glucose output is thereby inhibited, the blood glucose concentration may fall. However, the present study shows that the change in liver glycogen content during exercise in patients with type 1 diabetes was comparable to that of the non-diabetic controls despite the prevailing hyperinsulinaemic, hyperglycaemic conditions. This is in contrast to previous studies in healthy humans [24] and some [34] but not all animal studies [22, 35], which demonstrated insulin-induced suppression of hepatic glucose output. It could be argued that, despite the prevailing hyperinsulinaemic conditions, a preserved glucagon response to exercise could have accounted for the lack of differences in exercise-induced changes in liver glycogen content between groups. Indeed, a rise in glucagon is required during postabsorptive exercise for the full increment in hepatic glucose output [8, 13]. On the contrary, it has been shown in patients with type 1 diabetes that, during exercise under fasting conditions, hepatic glucose output is comparable to that of controls, and this is despite higher circulating insulin concentrations and lower glucagon responses in the patient group [36]. The

role of glucagon in the regulation of hepatic glucose output during exercise performed in the postprandial state is not clear. We have recently shown that moderate intensity exercise performed during a hyperinsulinaemic clamp does not induce a significant increase in glucagon concentrations in patients with type 1 diabetes [25]. Furthermore, in the present study there was no difference in serum glucagon concentrations during exercise performed in the postprandial state between patients with type 1 diabetes and controls. This resulted in a lower ratio of glucagon to insulin in the patient group when compared with the control group. Therefore, the parallel reduction in hepatic glycogen content between groups despite the obvious differences in the glucagon/insulin ratio suggests that other factors are important for the regulation of hepatic glucose output during exercise performed in the postprandial state in patients with type 1 diabetes. It is plausible that a preserved exercise-induced increase in circulating catecholamines rather than the glucagon response enabled continuous substrate mobilisation from the liver in this patient cohort [14, 30, 36–38]. It is known that pre-exercise hypoglycaemia is associated with blunted counter-regulation and impaired hepatic glycogenolysis [23, 39]. Since in the present study, patients were admitted overnight and hypoglycaemia was avoided prior to the experiment, counter-regulatory mechanisms were likely to have been preserved. Hence, counter-regulatory mechanisms may play a more prominent role in hepatic glycogen metabolism during exercise than the suppressive effect of relative hyperinsulinaemia in patients with type 1 diabetes.

Hepatic carbohydrate metabolism has been reported to be defective in patients with poorly controlled type 1 diabetes [16, 17]. It is likely that portal hypoinsulinaemia due to poorly controlled type 1 diabetes contributed to the lower fasting hepatic glycogen concentrations observed in these studies [21]. Since in the present study the patients were infused with intravenous insulin overnight, portal hypoinsulinaemia would have been alleviated, which may explain why we did not observe any difference in fasting glycogen concentrations. Patients with poorly controlled type 1 diabetes have impaired net hepatic glycogen synthesis following ingestion of a mixed meal and show an increase in hepatic gluconeogenesis [17]. However, these defects can be corrected after combined long-term and overnight control of glycaemia with intravenous insulin [40], and our results support this observation. Postprandial, pre-exercise liver glycogen concentration in type 1 diabetes was comparable to that in control subjects despite the relative lower estimated portal insulin concentration (~150–200 pmol/l) when compared with controls (~600 pmol/l). This is most likely to be the result of hyperglycaemic conditions in the postprandial period. Combined hyperglycaemic and hyperinsulinaemic conditions result in

increased hepatic glycogen synthesis as a result of the stimulatory effect on glycogen synthase flux and inhibition of glycogen phosphorylase [41].

Previous studies demonstrated a preferential utilisation of NEFAs over carbohydrate in patients with type 1 diabetes during moderate exercise in the fasting state [42, 43]. However, these experiments were performed after insulin withdrawal for 24 h. When moderate exercise was performed with insulin replacement (~ 65 pmol/l), patients with type 1 diabetes had comparable carbohydrate but higher fat oxidation rates when compared with controls [11, 36]. It is likely that, in the present study, the higher circulating systemic insulin concentrations (~ 150 pmol/l) corrected the previously reported differences in substrate oxidation between patients with type 1 diabetes and controls.

A typical carbohydrate-rich pre-exercise meal and a reduced subcutaneous insulin dose were used in this study. The patients were hyperglycaemic at the start of exercise and, despite the 50% insulin dose reduction, patients were still hyperinsulinaemic (>200 pmol/l). Routine clinical care would recommend that patients do not carry out exercise at these levels of hyperglycaemia for fear of inducing diabetic ketoacidosis. Our data demonstrate that as long as adequate insulinisation is maintained the risk of inducing significant ketosis is minimal. However, there is a dearth of clinical evidence as to the ideal glycaemic level at which patients should exercise to derive both health benefits and prevent exercise-induced hypoglycaemia. We observed a greater fall in blood glucose concentration (from 13 to 8 mmol/l) during EX1, whereas only a modest fall (from 8 to 5 mmol/l) was observed during EX2 and little or no change during EX3. It is likely that this was due to the combined effect of hyperglycaemia and a muscle contraction-induced increase in glucose disposal.

We have recently shown that exercise under differing insulin concentrations (~ 150 vs ~ 500 pmol/l) is associated with similar energy expenditure and muscle glycogen utilisation despite substantial increase in exogenous glucose utilisation [25]. Hence, we suggested that glucose was possibly being diverted during exercise to non-oxidative pathways in the liver and/or non-contracting muscle. However, in the present study, contrary to our hypothesis, we did not observe any sparing of hepatic glycogen utilisation in patients with type 1 diabetes under hyperinsulinaemic conditions when compared with controls. Interestingly, four patients developed hypoglycaemia at the end of the second and third bouts of exercise despite the lack of suppression of hepatic glycogen utilisation. This was possibly related to either the duration of exercise [44], the suppression of gluconeogenesis at these high insulin concentrations or differences in the relative contributions of blood glucose and muscle glycogen to muscle carbohydrate oxidation during the second and third bouts of exercise.

In conclusion, in patients with type 1 diabetes, hyperinsulinaemic and hyperglycaemic conditions during moderate exercise did not suppress changes in hepatic glycogen concentration when compared with non-diabetic controls. These findings do not support the hypothesis that exercise-induced hypoglycaemia in patients with type 1 diabetes is due to suppression of changes in hepatic glycogen content. It is possible that diminished gluconeogenesis and/or increased peripheral glucose uptake might be important factors contributing to exercise-induced hypoglycaemia.

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