Effect of oral glucose on leucine turnover in human subjects at rest and during exercise at two levels of dietary protein

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1. The aim of this study was to determine the effect of glucose supplementation on leucine turnover during and after exercise and whether variation in the previous dietary protein content modulated this effect.

2. Postabsorptive subjects received a primed constant [1-^{13}C,^{15}N]leucine infusion for 6 h, after previous consumption of a high (1·8 g kg^{-1} day^{-1}, HP, $n = 16$) or low (0·7 g kg^{-1} day^{-1}, LP, $n = 16$) protein diet for 7 days. The subjects were studied at rest; during 2 h of exercise, during which half of the subjects from each dietary protocol received 0·75 g kg^{-1} h^{-1} glucose (HP + G, LP + G) and the other half received water (HP + W, LP + W); then again for 2 h of rest.

3. Glucose supplementation suppressed leucine oxidation ($P < 0·01$) by 20% in subjects consuming the high protein diet (58·2 ± 2·8 μmol kg^{-1} h^{-1}, HP + G; 72·4 ± 3·9 μmol kg^{-1} h^{-1}, HP + W) but not the low protein diet (51·1 ± 5·9 μmol kg^{-1} h^{-1}, LP + G; 51·7 ± 5·5 μmol kg^{-1} h^{-1}, LP + W), with no difference in skeletal muscle branched-chain 2-oxo acid dehydrogenase (BCOADH) activity between groups. Glucose supplementation did not alter the rate of whole-body protein synthesis or breakdown.

4. The sparing effect of glucose on leucine oxidation appears only to occur if previous protein intake was high. It was not mediated by a suppression of BCOADH fractional activity but may be due to reduced substrate availability.

Amino acid oxidation is increased during moderate intensity exercise (Rennie *et al.* 1981; Knapik *et al.* 1991), but does not contribute significantly to energy provision in comparison to fat and carbohydrate oxidation (Ahlborg *et al.* 1974). It has been suggested that habitual endurance exercisers may have an increased dietary protein requirement as a result of increased amino acid oxidation during exercise (Meredith *et al.* 1989). However, El-Khoury *et al.* (1997) recently demonstrated that the completion of two 90 min bouts of exercise per day at 43–54% $V_{O_2,max}$ (maximum rate of O_2 uptake) did not cause a significant deterioration in 24 h leucine homeostasis in physically active men, when consuming 1 g protein kg^{-1} day^{-1}.

Branched-chain amino acid (BCAA) oxidation involves two steps: first, reversible transamination of BCAA to form branched-chain 2-oxo acids (BCOA) with 2-oxoglutarate acting as an amino group acceptor; and second, irreversible decarboxylation of BCOA. Decarboxylation is thought to be the rate-limiting step (Harper *et al.* 1984) and is catalysed by the mitochondrial branched-chain 2-oxo acid dehydrogenase complex (BCOADH). BCOADH is present in an active dephosphorylated form and an inactive phosphorylated form, interconversion being controlled by a specific kinase–phosphatase system (for review see Harper *et al.* 1984). During exercise there is an increase in both the percentage activation of the skeletal muscle BCOADH complex (Wagenmakers *et al.* 1989; Bowtell *et al.* 1998) and amino acid oxidation (Rennie *et al.* 1981; Bowtell *et al.* 1998).

Most research on the interaction of nutrition and exercise has concentrated on the carbohydrate content of athletes' diets since, during endurance exercise, fatigue is associated with the depletion of muscle glycogen stores (Bergstrom *et al.* 1967). Several tactics are commonly employed by athletes in order to maintain glycogen stores during exercise. One of these is carbohydrate loading, which involves the consumption of a high carbohydrate diet and the tapering of training activities in the days preceding an event to ensure a high basal muscle glycogen concentration (Bosch *et al.* 1993). Another tactic involves consumption of

carbohydrate drinks during exercise in the hope of delaying fatigue (Widrick *et al.* 1993) either by sparing muscle glycogen (Yaspelkis *et al.* 1993), or by maintaining plasma glucose concentration (Bosch *et al.* 1994), which will facilitate continued glucose supply to the muscle. It has been suggested that glucose supplementation not only delays the onset of fatigue but also suppresses the exercise-induced increase in leucine oxidation (Davies *et al.* 1982). Wagenmakers *et al.* (1991) demonstrated that the combination of prior carbohydrate loading and glucose supplementation during exercise suppressed the exercise-induced increase in BCOADH activation. The first aim of this study was therefore to investigate the effect of glucose supplementation on leucine oxidation during exercise and to determine whether this could be attributed to altered skeletal muscle BCOADH activity.

Consumption of a high protein diet has been shown to stimulate amino acid oxidation both at rest and during exercise (Bowtell *et al.* 1998). This effect appears to be mediated not by increased skeletal muscle BCOADH activity, at least in the postabsorptive condition, but rather via increased flux through the oxidative pathway. There is some evidence that dietary protein may modulate insulin sensitivity.

Consumption of low protein diets stimulated increased sympathetic nervous system activation in rats and mice (Young *et al.* 1985) and high protein diets resulted in reduced noradrenaline turnover, which is indicative of reduced sympathetic nervous system activation (Johnston & Balachandran, 1987). Lembo *et al.* (1994) found that an acute noradrenergic activation caused resistance to the effects of insulin on carbohydrate metabolism. Consumption of high protein diets may therefore enhance insulin sensitivity. Indeed, Tse *et al.* (1995) found that weanling obese Zucker (fa/fa) rats fed a low protein diet for 3 weeks suffered a deterioration in glycaemic control, presumably due to increased peripheral insulin resistance. In contrast, however, Escriva *et al.* (1991) found that lean rats fed a low protein diet demonstrated increased insulin sensitivity. The second aim of the present study was, therefore, to determine whether previous dietary protein intake would influence the effect of glucose supplementation on leucine oxidation and skeletal muscle BCOADH activity.

Results from *in vitro* studies suggest that insulin inhibits protein breakdown and stimulates protein synthesis (Jefferson *et al.* 1977). However, it has proved difficult to reproduce the stimulation of protein synthesis in the hyperinsulinaemic state *in vivo* in humans (Heslin *et al.* 1992). It has been suggested that amino acid availability may be limiting for protein synthesis. During hyperinsulinaemia, the suppression of protein breakdown acts to reduce amino acid supply, thus limiting any increase in whole-body protein synthesis. The final aim of this study was, therefore, to determine whether glucose supplementation and the resultant elevation in insulin concentration modulates the endurance exercise-induced changes in whole-body protein turnover, and whether previous dietary protein intake and hence amino acid availability influences this effect.

METHODS

Subjects consumed either a high protein (1·8 g protein kg^{-1} day^{-1}, HP, $n = 16$) or low protein diet (0·7 g protein kg^{-1} day^{-1}, LP, $n = 16$) for 7 days. On day 8 of this period, whole-body leucine kinetics were traced before, during and after walking on a treadmill for 2 h at 60% $V_{O_2,max}$. During exercise, half of the subjects from each dietary protein group received water (HP + W, LP + W) and the remainder received glucose (HP + G, LP + G). Four subjects (3 male and 1 female) participated in all four trials, separated by at least 4 weeks; diets were allocated by systematic rotation. A further group of 16 subjects (14 male and 2 female) participated in one trial only, being allocated by systematic rotation to one of the protocols ($n = 4$ in each group). This second group of subjects was recruited in order to obtain muscle data since it was not ethically sound to take 12 biopsies from each subject. The subject details are given in Table 1. An incremental exercise test on a motorised treadmill, using the criteria of Taylor *et al.* (1955) was adopted for the measurement of $V_{O_2,max}$ using on-line gas analysis. The study was approved by the local Tayside Ethics Committee and was carried out in accordance with the Declaration of Helsinki. All subjects gave their written informed consent.

Diets were designed to supply each subject's normal diurnal energy requirement (by consideration of average values for subject age, weight and activity level (WHO technical report (1985). *Energy and Protein Requirements. Report of a Joint Expert Consultation.* Who technical report series no. 724. World Health Organization, Geneva), corroborated by a 1 day food diary). Diet sheets were prepared prescribing all foods to be consumed, which provided most of the energy intake (82 ± 5% of 3152 ± 178 kcal) and 0·7 g protein kg^{-1} day^{-1}; subjects marked the sheets to show compliance. Subjects allocated to the high protein diet group ($n = 16$) consumed, in addition, a whey protein supplement (Maxipro, Scientific Hospital Supplies Ltd, Liverpool, UK) supplying 1·1 g protein kg^{-1} day^{-1}; subjects allocated to the low protein diet group ($n = 16$) consumed an isoenergetic peanut oil supplement (Calogen, Scientific Hospital Supplies Ltd, Liverpool, UK). The supplements were prepared daily in liquid form and the subjects were supervised as they drank them; the diets were consumed for a period of 7 days. Subjects were instructed to maintain normal activity patterns but to refrain from exercise on day 7, the day before the exercise trial.

After 7 days of dietary equilibration, subjects ($n = 8$ in each dietary protocol) were studied during and after 2 h of treadmill exercise at 60% $V_{O_2,max}$ (Fig. 1). The subjects arrived at the laboratory after an overnight fast and cannulae were inserted into the antecubital veins of both arms. One cannula was used for withdrawal of mixed venous blood samples and one for delivery of the leucine isotope. Basal blood and breath samples were taken and the subjects then received a primed, constant (0·8 mg kg^{-1}, 1 mg kg^{-1} h^{-1}) infusion of L-[1-^{13}C,^{15}N]leucine for 6 h. Subjects rested for the first 2 h, then walked on a treadmill at a speed and gradient designed to elicit an oxygen uptake of 60% of their $V_{O_2,max}$ for 2 h and then rested for a further 2 h. Half of the subjects from each dietary protein group (high or low protein) received 5 ml water kg^{-1} h^{-1} (HP + W, LP + W, $n = 8$) and the remaining eight subjects from each dietary protein group received 0·75 g glucose kg^{-1} h^{-1} (15% w:v, dextrose monohydrate, potato starch, AVEBE Ltd, Veendam, The Netherlands; HP + G, LP + G, $n = 8$). The drinks were provided in six equal aliquots during exercise, one immediately before exercise and then five more

at 20 min intervals. The δ^{13} of the potato starch-derived glucose is low (-26 ± 2 per thousand in comparison to the International Standard Pee Dee Belemnite (PDB)) and close to that of plasma glucose (-28 ± 2 per thousand *vs.* PDB); it therefore does not interfere with the recovery of $^{13}CO_2$ derived from the infused L-[1-^{13}C,^{15}N]leucine.

Blood and breath samples were taken every 30 min for the first and last hours of the infusion and every 20 min at all other times. In half of the subjects ($n = 4$ in each dietary protocol), muscle biopsies were taken from quadriceps femoris using conchotome forceps (Dietrichson *et al.* 1987). A basal biopsy was taken in the 30 min preceding exercise and two further biopsies were taken after the first and second hours of exercise.

Blood was stored on ice until the end of the infusion period and then centrifuged at 1500 *g* for 20 min at 4 °C. The resultant plasma was stored at −70 °C until analysis could be performed. Plasma was analysed for insulin (radio-immunoassay kit, Amersham UK Ltd), glucose and urea (both kits from Sigma). To determine ^{13}C and ^{15}N labelling, plasma leucine and α-ketoisocaproate (KIC) were analysed as their tertiary butyldimethylsilyl (*t*-BDMS) (Biermann *et al.* 1986) and *t*-BDMS-quinoxalinol derivatives (Rocchicciolo *et al.* 1981), respectively, using gas-chromatograph mass spectrometry. Leucine and KIC concentrations were obtained using deuterated internal standards. Whole-body leucine oxidation, transamination, reamination, protein breakdown and protein synthesis were calculated using the model described previously (Matthews *et al.* 1981). Factors to account for the retention of ^{13}C label in the bicarbonate pool were incorporated into the calculation of leucine oxidation. These factors were determined by measuring ^{13}C recovery during a primed constant $NaH^{13}CO_3$ infusion, in conditions identical to the experimental protocol employed for the current study (Bowtell *et al.* 1994). Fractional leucine transamination, in other words the proportion of leucine nitrogen that was transaminated, was calculated as transamination divided by leucine nitrogen flux. Similarly, fractional oxidation of KIC, or the proportion of KIC formed which was oxidised, was calculated as oxidation divided by transamination.

Table 1. Subject characteristics

Dietary protocol	Age (years)	$V_{O_2,max}$ (ml kg^{-1} min^{-1})	Weight (kg)	Body fat (%)
HP + W	21·9 ± 0·6	45·8 ± 1·1	74·5 ± 3·4	12·4 ± 2·1
LP + W	22·0 ± 0·6	45·0 ± 1·2	71·9 ± 4·6	14·1 ± 2·2
HP + G	22·9 ± 0·7	44·5 ± 1·2	74·8 ± 3·6	14·4 ± 3·1
LP + G	22·8 ± 0·5	45·0 ± 1·7	72·8 ± 3·9	13·5 ± 2·4

Data are presented as means ± S.E.M. ($n = 8$).

The biopsied muscle tissue was placed in ice-cold buffer within 120 s of the cessation of exercise, to minimise any change in fractional activation, and assayed for branched-chain oxo acid dehydrogenase activity as described previously (Wagenmakers *et al.* 1989).

The breath samples were collected in 50 l Douglas bags for 4 min at rest and 2 min during exercise, and analysed for percentage content of oxygen (Taylor Servomex, Sybron Corporation) and carbon dioxide (Grubb Parsons, Newcastle, UK). Oxygen consumption, carbon dioxide production and respiratory quotient were calculated by standard methods. Following thorough mixing, small aliquots of each breath sample were transferred into 10 ml evacuated tubes and later analysed for $^{13}CO_2$ enrichment using isotope ratio mass spectrometry (IRMS) on the ANCA 20-20 (Europa Scientific Ltd, Crewe, UK).

The plasma [^{13}C]KIC enrichment, rather than plasma [^{13}C]leucine enrichment, was used to calculate whole-body leucine carbon flux and oxidation; whole-body protein synthesis was calculated as the net difference between these two variables (Matthews *et al.* 1981). KIC is produced intracellularly via the transamination of leucine and it has therefore been suggested that plasma KIC enrichment is a better predictor of intracellular leucine enrichment (Watt *et al.* 1991).

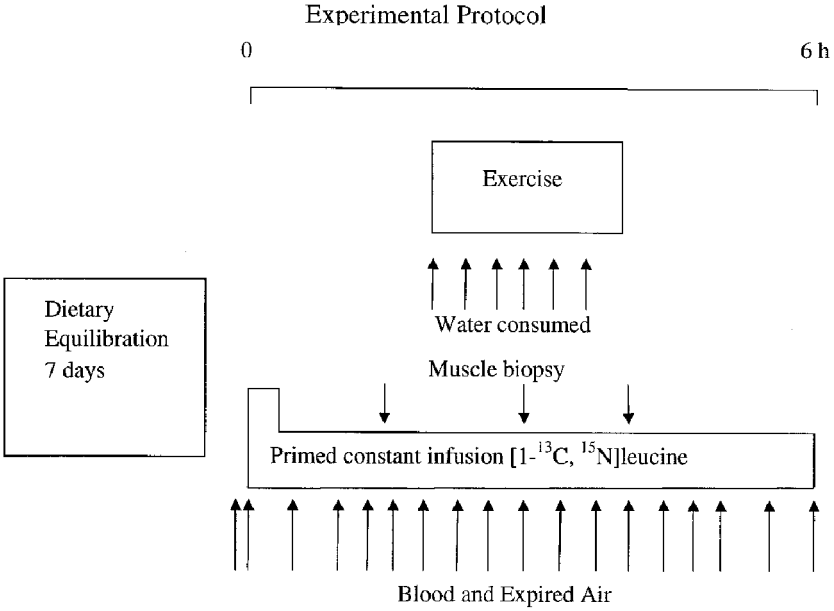

Figure 1
Schematic diagram of the study protocol.

Table 2. Whole-body leucine transamination and KIC reamination rates

Protocol	Pre-exercise	Exercise hour 1	Exercise hour 2	Post-exercise
Transamination (μmol kg^{-1} h^{-1})				
HP + W	118·0 ± 26·9	157·8 ± 20·8	127·6 ± 18·2	100·0 ± 16·1
HP + G	85·8 ± 13·3	118·4 ± 19·7	91·9 ± 8·8	81·8 ± 14·0
LP + W	74·4 ± 15·0	99·0 ± 12·9	96·8 ± 12·1	83·6 ± 11·6
LP + G	79 ± 13·1	110·3 ± 4·0	107·3 ± 2·8	84·8 ± 11·5
Reamination (μmol kg^{-1} h^{-1})				
HP + W	94·6 ± 26·4	83·0 ± 19·1	75·6 ± 17·5	74·3 ± 15·2
HP + G	66·7 ± 14·5	63·2 ± 17·2	35·6 ± 7·8	55·5 ± 15·8
LP + W	58·6 ± 13·9	50·1 ± 12·2	46·4 ± 11·5	61·8 ± 11·1
LP + G	64·6 ± 9·1	57·3 ± 5·1	56·6 ± 6·2	64·6 ± 12·3

Data are presented as means ± S.E.M. (HP + G, LP + W and LP + G, $n = 8$; HP + W, $n = 7$).

Statistics

All data are presented as means ± S.E.M. The Mann-Whitney U test was used to compare the BCOADH data, due to the small sample size. A three-way ANOVA (dietary protein (high *vs.* low) by fluid supplementation (glucose *vs.* water) by time (pre-exercise, during hour 1, during hour 2 and post-exercise)) was used to analyse the remaining variables. A dependent analysis (all factors within subjects) was carried out on the data derived from the four subjects who completed all four trials. An independent analysis (two factors between and one factor within subjects) was employed for the data derived from the subjects who completed only one trial. Provided that the direction of change in the variable was the same for the two analyses, the P values were combined using meta analysis (Fisher, 1950). A *post hoc* Tukey test was used to identify the site of the difference where appropriate and the P values combined in the same way. The effect of dietary protein manipulation and exercise upon whole-body leucine metabolism and BCOADH activation has been described in a previous paper (Bowtell *et al.* 1998). In this paper we will examine the effect of glucose supplementation during exercise upon whole-body leucine metabolism and BCOADH activation.

RESULTS

Plasma glucose concentration did not change significantly for the water control subjects throughout the 6 h protocol (Fig. 2). Plasma glucose concentration was increased by glucose supplementation for HP + G subjects relative to controls (HP + W), during and in recovery from exercise ($P < 0·01$). However, plasma glucose concentration was significantly increased, relative to water controls, only in the post-exercise period for LP subjects ($P < 0·01$).

Whole-body leucine oxidation was higher for the post-absorptive subjects consuming the high rather than the low

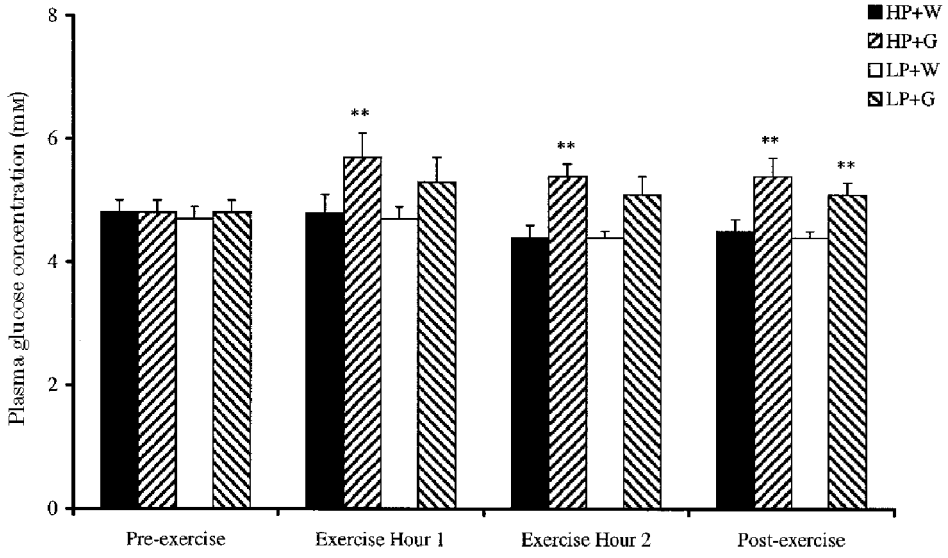

Figure 2. Effect of glucose supplementation on plasma glucose concentration at rest and during exercise, after consumption of a high or low protein diet

Values are means ± S.E.M. ($n = 8$). ** Significantly different from respective control values ($P < 0·01$).

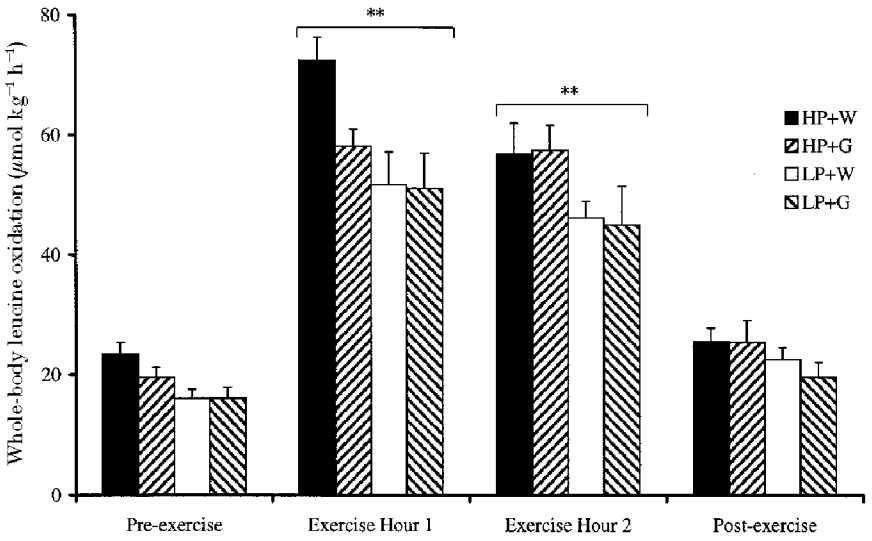

Figure 3. Effect of glucose supplementation on whole-body leucine oxidation at rest and during exercise, after consumption of a high or low protein diet

Means ± S.E.M. ($n = 8$). There was a main effect of glucose supplementation ($P < 0{\cdot}01$), and results were significantly different from Pre-exercise values (**$P < 0{\cdot}01$).

protein diet ($P < 0{\cdot}01$, Fig. 3). Leucine oxidation was increased approximately 3-fold during exercise, for all dietary protocols ($P < 0{\cdot}01$). There was a main effect of glucose supplementation upon leucine oxidation ($P < 0{\cdot}01$): leucine oxidation was reduced by 20% *vs.* controls in the first hour of exercise for HP + G subjects ($58{\cdot}2 \pm 2{\cdot}8$ *vs.* $72{\cdot}4 \pm 3{\cdot}9$ μmol kg^{-1} h^{-1}), but there was no effect in the second hour of exercise ($57{\cdot}5 \pm 4{\cdot}2$ *vs.* $56{\cdot}9 \pm 5{\cdot}1$ μmol kg^{-1} h^{-1}), and leucine oxidation was not suppressed in LP + G subjects *vs.* controls.

The tendency was for glucose supplementation to reduce transamination and reamination relative to controls in the subjects previously consuming a high protein diet, but to increase transamination and reamination relative to controls in the subjects previously consuming a low protein diet (Table 2). The suppression of transamination for glucose-supplemented subjects achieved significance for the analysis of independent data; however, due to missing data points from the dependent data set, it was not possible to test the statistical significance of the data as a whole.

Due to missing data from the dependent data set, it was also not possible to test the statistical significance of the fractional transamination data as a whole. However, there was a tendency for an exercise-induced increase in fractional

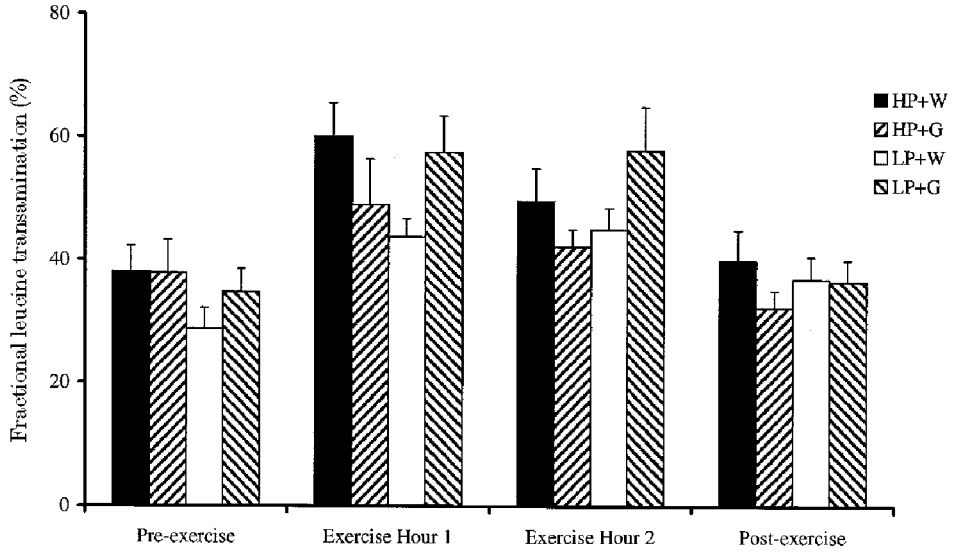

Figure 4. Effect of glucose supplementation on fractional leucine transamination at rest and during exercise, after consumption of a high or low protein diet

Means ± S.E.M. ($n = 7$).

Table 3. Skeletal muscle BCOADH activity

Protocol	Pre-exercise	Exercise hour 1	Exercise hour 2
Total activity (nmol g^{-1} min^{-1})			
HP + W	5·6 ± 0·9	5·3 ± 0·2	4·2 ± 0·3
HP + G	5·2 ± 0·2	6·8 ± 0·5	5·6 ± 0·3
LP + W	5·5 ± 0·4	4·9 ± 0·6	5·9 ± 0·4
LP + G	4·6 ± 0·3	5·0 ± 0·2	4·2 ± 0·1
Percentage activation			
HP + W	6·7 ± 2·1	10·6 ± 2·0*	14·8 ± 3·0*
HP + G	8·5 ± 1·8	14·2 ± 1·3*	16·9 ± 0·9*
LP + W	7·9 ± 0·8	21·8 ± 5·1*	20·5 ± 5·8*
LP + G	6·9 ± 0·5	10·3 ± 0·6*	18·2 ± 1·1*

Data are presented as means ± S.E.M. ($n = 4$). * Significantly different from pre-exercise values ($P < 0·05$).

transamination for all subjects, which was considerably lower for HP + G subjects (30% *vs.* 50–60% for HP + W, LP + W and LP + G subjects; Fig. 4). The fractional transamination of leucine during exercise tended to be higher for LP + G subjects than for their controls, but fractional transamination tended to be lower for HP + G subjects than in their controls. The glucose effect achieved significance for the independent data set ($P < 0·05$).

Total skeletal muscle BCOADH activity was not altered by the different dietary protocols or by exercise (Table 3). The proportion of active skeletal muscle BCOADH complex was increased 2-fold during exercise; however, there were no statistically significant differences between experimental groups at any time point, although there was a tendency for the skeletal muscle BCOADH activation state to be lower for LP + G than for control subjects during exercise.

Plasma leucine concentration changed significantly over time ($P < 0·01$, Fig. 5). Glucose supplementation tended to reduce plasma leucine concentration during the first hour of exercise for HP + G subjects and in the second hour of exercise for HP + G and LP + G subjects ($P < 0·01$). In the period following exercise, plasma leucine concentration was reduced relative to pre-exercise values (−25%, HP + G; −22%, LP + G; −8%, HP + W; and −6% LP + W, $P < 0·01$). Plasma KIC concentration data (not shown) were qualitatively identical to the plasma leucine concentration data.

At rest before exercise, plasma insulin concentration was higher for subjects on the high protein diet (18·7 ± 1·3 μunits ml^{-1}) than for those on the low protein diet (12·8 ± 1·1 μunits ml^{-1}). For HP + W subjects, the plasma insulin concentration fell during exercise to LP + W levels,

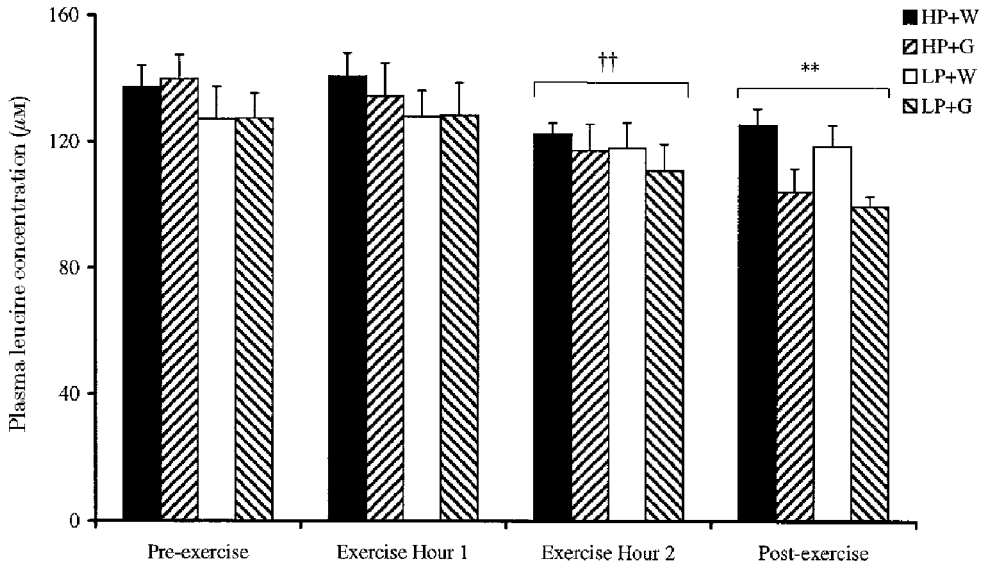

Figure 5. Effect of glucose supplementation on plasma leucine concentration at rest and during exercise, after consumption of a high or low protein diet

Means ± S.E.M. ($n = 8$). ** Significant difference from Pre-exercise values ($P < 0·01$); †† significant difference from Exercise hour 1 values ($P < 0·01$).

Table 4. Percentage difference in plasma insulin concentration between glucose-supplemented and control subjects: HP + G *vs.* HP + W; LP + G *vs.* LP + W

	Insulin HP + G (μunits ml^{-1})	Insulin HP + W (μunits ml^{-1})	Difference (%)	Insulin LP + G (μunits ml^{-1})	Insulin LP + W (μunits ml^{-1})	Difference (%)
Rest	18·4 ± 1·9	19·2 ± 2·0	−4·2	14·2 ± 2·1	11·4 ± 0·7	24·6
Exercise 40 min	20·1 ± 2·7	14·8 ± 2·6	35·8	20·4 ± 3·8	11·0 ± 0·9	85·5
Exercise 80 min	17·8 ± 2·7	14·5 ± 2·6	22·8	14·8 ± 1·6	9·9 ± 1·0	49·5
Exercise 120 min	19·0 ± 4·5	13·5 ± 2·0	40·7	15·8 ± 2·4	13·2 ± 1·8	19·7
Post-exercise	17·9 ± 4·2	14·7 ± 2·3	21·8	15·5 ± 2·3	12·5 ± 1·4	24·0

Plasma insulin concentrations are presented as means ± S.E.M. ($n = 8$).

which remained relatively constant. In the subjects previously consuming a high protein diet, glucose supplementation prevented the exercise-induced decrease in plasma insulin concentration, but there was no significant difference between HP + G and control values at any time point. There was no significant difference in plasma insulin concentration between HP + G and LP + G during or after exercise. The insulin response to the glucose load, measured as the percentage difference between plasma insulin concentration in glucose-supplemented and control subjects, was 2-fold higher during the first 80 min of exercise in the subjects previously consuming a low protein diet (Table 4). The plasma insulin concentration tended to be higher for the glucose-supplemented groups than for the controls during recovery from exercise, but this did not reach significance.

Indirect calorimetry was used to calculate the proportion of energy derived from carbohydrate and fat (Frayn, 1983), after protein oxidation had been deducted assuming leucine oxidation represented 8% of total protein oxidised. There were no statistically significant differences between experimental groups, but carbohydrate oxidation tended to be higher for the glucose-supplemented subjects relative to controls throughout exercise in the subjects who had previously consumed a high protein diet. However, carbohydrate oxidation did not appear to increase above control values until the second hour of exercise for LP + G subjects (Fig. 6).

Leucine carbon flux, which is an index of whole-body protein breakdown, did not vary between dietary protocols but there was a significant change over time ($P < 0{\cdot}05$, Table 5). The rate of protein breakdown was significantly different during the post-exercise period from values during the first hour of exercise for all subjects ($P < 0{\cdot}01$). Protein breakdown tended to decrease relative to pre-exercise values for HP + W, LP + W and HP + G subjects but increase for LP + G subjects during recovery. Non-oxidative leucine disposal, which is an index of whole-body protein synthesis, was suppressed during exercise for all subjects ($P < 0{\cdot}01$, Table 5). Although protein synthesis increased during recovery relative to values during the first hour of

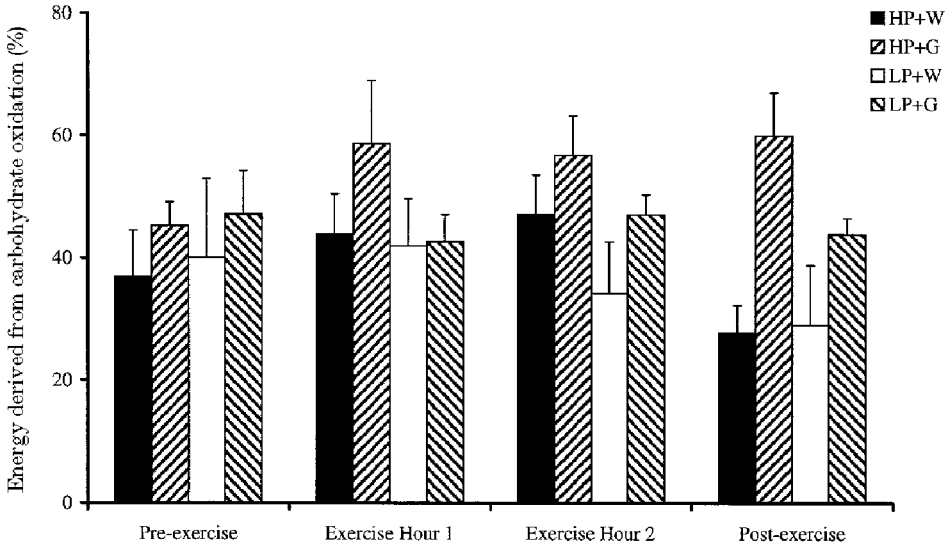

Figure 6. Effect of glucose supplementation on whole-body carbohydrate oxidation at rest and during exercise, after consumption of a high or low protein diet

Means ± S.E.M. ($n = 8$).

Table 5. Rate of whole-body protein synthesis and breakdown

Protocol	Pre-exercise	Exercise hour 1	Exercise hour 2	Post-exercise
Protein breakdown (μmol kg^{-1} h^{-1})				†
HP + W	145·6 ± 7·5	151·5 ± 8·7	141·7 ± 3·9	130·7 ± 2·7
HP + G	142·1 ± 9·7	152·2 ± 11·1	145·9 ± 9·2	132·4 ± 7·8
LP + W	144·4 ± 6·6	139·2 ± 4·0	133·1 ± 5·1	132·6 ± 5·8
LP + G	139·5 ± 8·4	140·5 ± 5·7	135·1 ± 5·4	148·2 ± 10·9
Protein synthesis (μmol kg^{-1} h^{-1})		*	*	*†
HP + W	123·8 ± 6·6	85·2 ± 24·3	84·1 ± 6·3	105·1 ± 2·8
HP + G	130·4 ± 7·6	77·8 ± 10·5	76·5 ± 9·5	110·8 ± 6·2
LP + W	128·5 ± 6·1	90·0 ± 5·7	89·3 ± 5·1	110·9 ± 6·5
LP + G	124·7 ± 7·7	82·4 ± 5·4	84·0 ± 3·8	111·5 ± 5·9

Data are presented as means ± S.E.M. ($n = 8$). There was a main effect of time: * significantly different from Pre-exercise values ($P < 0·01$); † significantly different from Exercise hour 1 values ($P < 0·01$).

exercise ($P < 0·01$), protein synthesis remained depressed relative to pre-exercise values ($P < 0·01$).

Leucine nitrogen flux was suppressed by glucose supplementation for both levels of dietary protein intake (201·5 ± 6·3 *vs.* 242·3 ± 7·9 μmol kg^{-1} h^{-1}, $P < 0·01$).

DISCUSSION

The increase in leucine oxidation observed during exercise in this and other studies (Rennie *et al.* 1981; Knapik *et al.* 1991; Bowtell *et al.* 1998) is thought to be related to the concurrent increase in the fractional activity of the skeletal muscle BCOADH complex (Wagenmakers *et al.* 1989; Bowtell *et al.* 1998). This exercise-induced increase in leucine oxidation is enhanced in glycogen-depleted subjects (Lemon & Mullin, 1980), and van Hall *et al.* (1995) found that the exercise-induced increase in BCOADH fractional activity was also enhanced during one-leg exercise in muscle with a reduced rather than a normal glycogen content. Previous work suggests that glucose supplementation during exercise reduces leucine oxidation (Davies *et al.* 1982); it seemed likely, therefore, that this may be related to changes in the fractional activity of the BCOADH complex. However, in the present study, glucose supplementation suppressed leucine oxidation but did not alter skeletal muscle BCOADH activation.

In humans, the BCOADH complex is present in many tissues, including liver, kidney, brain and adipose tissue as well as skeletal muscle; however, at rest 54% of the body's actual BCOADH activity is sited within skeletal muscle and only 13% is located in the liver (Suryawan *et al.* 1998). The predominant site of leucine oxidation in humans, therefore, is within skeletal muscle, particularly during exercise, partly as a consequence of a further activation of skeletal muscle BCOADH and also due to the increase in blood flow to the exercising muscle. Even if hepatic leucine oxidation were entirely suppressed, it is extremely unlikely that altered hepatic BCOADH activity could account for the 20% suppression in whole-body leucine oxidation that resulted from glucose supplementation in the high protein group.

The skeletal muscle BCOADH complex was activated to a similar extent by exercise for the two glucose-supplemented groups regardless of previous dietary protein intake. There were no significant differences between experimental groups in skeletal muscle BCOADH activation at any time point. However, there was a tendency for BCOADH activation to be reduced by glucose supplementation relative to controls in subjects who had previously consumed a low protein diet. Yet, in these subjects leucine oxidation was not suppressed by glucose supplementation. It appears, therefore, that the effect of glucose supplementation on leucine oxidation was independent of changes in skeletal muscle BCOADH activity. Initially this appeared surprising since Wagenmakers *et al.* (1991) found that the exercise-induced activation of skeletal muscle BCOADH was inhibited by prior carbohydrate loading and glucose supplementation during exercise when compared to BCOADH activation during exercise in glycogen-depleted muscle. Wagenmakers *et al.* (1991) concluded that there was an inverse correlation between the glycogen content of the muscle and BCOADH activation. In the present study, oral glucose was consumed during exercise but initial muscle glycogen content was not manipulated. Glucose supplementation can delay the onset of fatigue during endurance exercise (Coyle *et al.* 1983), but there is conflicting evidence in the literature as to whether this effect is mediated through a sparing of muscle glycogen (Hargreaves *et al.* 1984), or through maintenance of plasma glucose concentration and hence oxidation rates (Coyle *et al.* 1986). The absence of any effect on BCOADH activation suggests that sparing of muscle glycogen did not occur as a result of glucose supplementation in the present study. Instead, the suppression of leucine oxidation by glucose supplementation in subjects previously consuming a high

protein diet, seems to be related to a reduction in flux through the BCOADH complex via changes in substrate delivery to the skeletal muscle complex.

In rat skeletal muscle, the activity of BCAA transaminase (BCAAT) is 30-fold higher than that of BCOADH, whereas hepatic BCOADH activity is 11-fold higher than hepatic BCAAT activity (Suryawan *et al.* 1998). The skeletal muscle therefore acts as a net exporter of KIC for uptake and decarboxylation in the liver. However, in humans, BCAAT activity is 24-fold higher than BCOADH activity in skeletal muscle and 20-fold higher than BCOADH activity in the liver (Suryawan *et al.* 1998). It is perhaps unsurprising, therefore, that there is little or no KIC release from human muscle at rest (Cheng *et al.* 1985) or during exercise (Fielding *et al.* 1986). This suggests that the KIC formed by transamination and not reaminated is decarboxylated within the mitochondria of the skeletal muscle cells. Indeed, the mitochondrial KIC concentration is thought to be 20–25 μM (Hutson & Harper, 1981) and the K_m of the BCOADH complex is 15–50 μM (Harper *et al.* 1984). The complex is therefore working at or below its K_m so any change in mitochondrial KIC concentration is likely to result in an equivalent alteration in leucine oxidation. Fractional transamination tended to be higher for LP + G compared to control subjects during exercise but tended to be lower for HP + G subjects than controls. This suggests that the amount of KIC presented to the BCOADH complex was increased for LP + G relative to LP + W subjects, and one would therefore expect flux through the complex to be stimulated. However, leucine oxidation was not increased, possibly due to the tendency for lower activation of the complex. One would expect mitochondrial KIC availability to be reduced for HP + G subjects relative to controls, since both fractional transamination and plasma leucine concentration tended to be reduced. This would tend to reduce flux through the BCOADH complex resulting in the observed reduction in leucine oxidation. This is indicative of an alteration in the partitioning of leucine carbon flux between oxidation and synthesis and appears to be independent of the small changes in BCOADH activation.

In this study, it was not possible to distinguish between a direct effect of an increase in plasma glucose on leucine oxidation and an indirect effect through the increase in plasma insulin in response to the glucose load, although the suppression of leucine oxidation in the hyperinsulinaemic state is, itself, probably an indirect effect mediated through the observed fall in plasma amino acids resulting from the insulin-induced suppression of protein breakdown (Tessari *et al.* 1987). Plasma glucose was elevated by the exogenous glucose throughout exercise for HP + G subjects, but plasma glucose did not increase significantly until the recovery period for LP + G subjects. This may in part be due to the extent of the increase in plasma insulin concentration in response to the glucose load. There was no difference in the plasma insulin concentration between glucose-supplemented groups; however, the increase in plasma insulin concentration in the glucose-supplemented subjects relative to controls was approximately 2-fold greater for LP + G subjects than HP + G subjects in the first 80 min of exercise (Table 4).

In vitro experiments have shown that the provision of alternative energy substrates such as fatty acids, ketone bodies and pyruvate inhibits flux through the BCOADH complex (Wagenmakers & Veerkamp, 1984), therefore oxidation of the exogenous glucose is likely to reduce amino acid oxidation. In the present study, whole-body carbohydrate oxidation was increased for HP + G *vs.* control subjects, especially during the first hour of exercise. However, carbohydrate oxidation in the LP + G subjects did not increase above control values until the second hour of exercise; presumably the exogenous glucose was directed towards storage rather than oxidation. There is no evidence to suggest that an alteration of dietary protein content would alter the rate of gastric emptying or absorption of glucose. This apparent difference in the fate of the exogenous glucose between high and low protein dietary groups may provide one explanation for the differential effect on leucine oxidation.

Whole-body leucine carbon flux, which is indicative of whole-body protein breakdown in the postabsorptive state, was not significantly altered by glucose supplementation in subjects from either dietary protein group. However, plasma leucine concentration was decreased during recovery from exercise, suggesting that the efflux of leucine into the plasma pool was reduced. In the absence of an increase in whole-body leucine oxidation this can only be attributed to a suppression of protein breakdown. One explanation may be that the whole-body constancy masks altered protein breakdown within individual tissues; the anti-proteolytic effect of insulin has been found to be more pronounced in skeletal muscle than in other tissues of the body (Heslin *et al.* 1992).

Whole-body protein synthesis in the glucose-supplemented subjects did not differ from that of control subjects in either dietary protein group. Again, it would have been expected that the increase in insulin associated with the glucose load would stimulate protein synthesis (Jefferson *et al.* 1977); however, this interpretation is complicated by the reduction in plasma amino acid concentration which may become limiting for synthesis. Certainly, in studies conducted where hyperinsulinaemic subjects were maintained at euaminoacidaemia or hyperaminoacidaemia, a stimulation of protein synthesis was demonstrated (Tessari *et al.* 1987). More recently, Biolo *et al.* (1995) demonstrated that local hyperinsulinaemia, which did not alter leg arterial amino acid delivery from the systemic circulation, increased leg muscle protein synthesis from 0·040 to 0·068 % per hour.

In conclusion, neither whole-body protein synthesis nor whole-body protein breakdown was altered by glucose supplementation during exercise. Leucine oxidation was suppressed by the provision of exogenous glucose but only in those subjects who had previously consumed a high protein

diet. This could not be attributed to a deactivation of the skeletal muscle BCOADH complex, but rather may be due to altered substrate delivery. The oxidation of the exogenous glucose in the subjects who had previously consumed a high protein diet, rather than storage of the exogenous glucose in the subjects who had previously consumed a low protein diet, appears to conserve leucine.

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