Eccentric exercise decreases maximal insulin action in humans: muscle and systemic effects

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1. Unaccustomed eccentric exercise decreases whole-body insulin action in humans. To study the effects of one-legged eccentric exercise on insulin action in muscle and systemically, the euglycaemic clamp technique combined with arterial and bilateral femoral venous catheterization was used. Seven subjects participated in two euglycaemic clamps, performed in random order. One clamp was preceded 2 days earlier by one-legged eccentric exercise (post-eccentric exercise clamp (PEC)) and one was without the prior exercise (control clamp (CC)).

2. During PEC the maximal insulin-stimulated glucose uptake over the eccentric thigh was marginally lower when compared with the control thigh, (11·9%, 64·6 ± 10·3 *vs.* 73·3 ± 10·2 μmol kg^{-1} min^{-1}, $P = 0{\cdot}08$), whereas no inter-thigh difference was observed at a submaximal insulin concentration. The glycogen concentration was lower in the eccentric thigh for all three clamp steps used ($P < 0{\cdot}05$). The glucose transporter GLUT4 protein content was on average 39% lower ($P < 0{\cdot}05$) in the eccentric thigh in the basal state, whereas the maximal activity of glycogen synthase was identical in the two thighs for all clamp steps.

3. The glucose infusion rate (GIR) necessary to maintain euglycaemia during maximal insulin stimulation was lower during PEC compared with CC (15·7%, 81·3 ± 3·2 *vs.* 96·4 ± 8·8 μmol kg^{-1} min^{-1}, $P < 0{\cdot}05$).

4. Our data show that 2 days after unaccustomed eccentric exercise, muscle and whole-body insulin action is impaired at maximal but not submaximal concentrations. The local effect cannot account for the whole-body effect, suggesting the release of a factor which decreases insulin responsiveness systemically.

Eccentric exercise is dynamic exercise which involves forced lengthening of active muscle. A previous study in humans has shown that an unaccustomed bout of eccentric exercise leads to insulin resistance at the whole-body level (Kirwan, Hickner, Yarasheski, Kohrt, Wiethop & Holloszy, 1992), whereas a single bout of concentric exercise (which involves shortening of active muscle) is a recognised enhancer of insulin action in muscle (Richter, Garetto, Goodman & Ruderman, 1982; Bogardus, Thuillez, Ravussin, Vasquez, Narimiga & Azhar, 1983; Mikines, Sonne, Farrell, Tronier & Galbo, 1988; Richter, Mikines, Galbo & Kiens, 1989). Furthermore, eccentric exercise transiently decreases the skeletal muscle glucose transporter GLUT4 protein content (Asp, Daugaard & Richter, 1995*a*; Asp, Kristiansen & Richter, 1995*b*) and impairs muscle glycogen resynthesis after exercise (O'Reilly, Warhol, Fielding, Frontera, Meredith & Evans, 1987; Costill, Pascoe, Fink, Robergs, Barr & Pearson, 1990; Widrick, Costill, McConell, Anderson, Pearson & Zachwieja, 1992; Doyle, Sherman & Strauss, 1993; Asp *et al.* 1995*a*, *b*). Since insulin-induced glucose uptake has been found to correlate with muscle GLUT4 protein content (Andersen, Lund, Vestergaard, Junker, Kahn & Pedersen, 1993; Dela, Handberg, Mikines, Vinten & Galbo, 1993), the purpose of the present study was to elucidate if a local decrease in the GLUT4 content following eccentric exercise is accompanied by a local as well as a whole-body change in insulin action. A previous study from our laboratory (Asp *et al.* 1995*a*) revealed that the most pronounced effect of eccentric exercise on skeletal muscle GLUT4 content is found 2 days after the bout. Thus, to measure insulin action locally in the eccentric and control thigh, euglycaemic hyperinsulinaemic clamps combined with leg catheterization were performed 2 days after one-legged eccentric exercise. Furthermore, to detect any systemic effect of the bout, each subject also participated in a control clamp which was performed without the prior one-legged eccentric exercise.

METHODS

Subjects

Seven young healthy, habitually physically active male university students aged 20–30 years with no medical records and especially no history of cardiovascular disease, clotting disorders, diabetes or other endocrine diseases served as subjects. Subjects were recruited by advertisement at the University and were fully informed of any risks and discomfort associated with these experiments before giving their informed consent to participate. All were paid a small honorarium for the time and discomfort involved. Their mean weight and height were 75 kg (range 66–83 kg) and 181 cm (range 175–186 cm). None participated in competitive sports, but all used a bicycle for local transportation. The mean per cent body fat determined by the skin-fold method was 12% (range 5–17%), and the average maximal O_2 consumption, determined on a bicycle ergometer at least 12 days before the start of the experiment, was 50 ml kg^{-1} min^{-1} (range 42–55 ml kg^{-1} min^{-1}). The study was approved by the Copenhagen Ethics Committee and conforms with the code of ethics of the World Medical Association (Declaration of Helsinki). Subjects were covered by state medical insurance and in addition by the same insurance as hospitalized patients are covered by in case of complications.

Diet

Four days before each clamp the subjects commenced a standard weight-maintaining diet, containing 5 g carbohydrate per kilogram body weight. The energy content of the diet was estimated for each individual by having the subjects record their food intake for 3 days. The standard diet was calculated to have an energy distribution of approximately 50% from carbohydrate, 35% from fat and 15% from protein. In case the subjects needed additional calories these were provided by adding more protein so that the carbohydrate content was constant at 5 g (kg body weight)$^{-1}$. This diet was maintained until the clamp was performed, and the subjects were instructed to avoid alcohol, smoking and drugs during this period. The subjects kept a constant activity level, where slow walking and bicycling was allowed, but it was forbidden to participate in any hard exercise.

Eccentric exercise

The seated subjects performed maximal voluntary eccentric contractions with one thigh by resisting either the backward or forward motion of one of the lower legs, which was created by a rod connected to the ankle and attached to a motor-driven device as described (Asp *et al.* 1995*a*). The bout consisted of eight 5 min sessions with a motor velocity of 30 r.p.m. interrupted by 2 min rest periods. The first four sessions were aimed at exercising the quadriceps muscle. The fixed hip angle (which was dependent on the angle of the chair back) and the knee angle around which the lower leg moved equidistantly (dependent on the distance between the chair and the motor-driven device) varied in the different exercise sessions in order to vary the strain on the muscle. The hip/knee angles during the different sessions were 90/90 deg, 120/90 deg, 90/120 deg or 120/120 deg. In order also to exercise the hamstrings of the thigh, four more 5 min sessions were added, where the subjects were instructed to resist the forward motion, and where the fixed hip angle (chair back angle) and the knee angle (determined by the distance from the chair to the motor-driven device) around which the lower leg moved equidistantly were both 90 deg during all sessions.

Experimental design

Each subject participated in one post-eccentric exercise clamp (PEC) and one control clamp (CC) session, which were performed in random order at least 6 weeks apart. PEC was preceded 2 days earlier by one-legged eccentric exercise, whereas CC was without the prior exercise. Blood samples were taken from a forearm vein after an overnight fast, prior to the exercise bout and also on the clamp days. On the days where euglycaemic hyperinsulinaemic clamps were performed the subjects arrived in the morning at the laboratory after a 10–12 h fast. After resting 30 min in the supine position in a room specially arranged for invasive procedures, Teflon catheters were placed in both femoral veins and in one femoral artery under local anaesthesia using aseptic techniques, and the tips were advanced centrally to approximately 2 cm below and above the inguinal ligament, respectively. A thermistor probe (Edslab probe 94-030-2·5-F, Baxter) for measuring venous blood temperature was inserted through each venous catheter and advanced 8–10 cm proximal to the catheter tip. Additional catheters were placed in two cubital veins for infusion of insulin and glucose and in a retrograde direction in a dorsal hand vein for sampling of arterialized blood. These invasive procedures have been performed in many previous studies and all catheters were removed after about 6 h (Andersen & Saltin, 1985; Richter *et al.* 1989). The procedures were performed by a medical doctor with more than 10 years' experience in catherization and in obtaining muscle biopsies, and who was present throughout the entire experiment. After insertion of the catheters the subjects were moved to the experimental room and rested in the supine position for 1 h. Blood was then obtained twice (10 min apart) from the three femoral catheters simultaneously. Before and after each blood sampling, femoral venous blood flow in both legs was measured by the thermo-dilution procedure, modified for resting conditions (Richter *et al.* 1989). Briefly, ice-cold saline was infused for 30–40 s through each venous catheter, and the temperature in the femoral venous blood was recorded during the last 10 s. Whenever blood was sampled or flow measured, pneumatic cuffs below the knees were inflated to 230 mmHg to exclude circulation below the knee. A muscle biopsy was obtained from each vastus lateralis muscle, and a two-step sequential euglycaemic hyperinsulinaemic clamp procedure (DeFronzo, Tobin & Andres, 1979) was begun. Human insulin (Actrapid, Novo, Copenhagen, Denmark) was infused at 0·8 and 13 mU kg^{-1} min^{-1} after a priming dose, reaching an approximate concentration of 350 and 13 000 pmol l^{-1}, respectively, at steady state. Each clamp step was run for 110 min, and blood samples were collected after 100 and 110 min from the three femoral catheters, and femoral venous blood flow was also measured in duplicate in each leg. The average glucose infusion rate (GIR) necessary to maintain euglycaemia from 85 to 110 min was used as the steady-state GIR.

At the end of the first and second clamp step, muscle biopsies were obtained from each thigh through new incisions spaced at least 4 cm from the preceding incision. During insulin infusion, arterialized blood from a heated hand vein was obtained every 5–10 min for determination of plasma glucose concentration. Arterialized blood was found to give glucose values very close to arterial values, and arterialized blood from a hand vein was used because of the ease of obtaining frequent samples. Before each clamp step, 30 mequiv K^+ (as slowly released KCl; Kalinorm, Alfred Benzon, Copenhagen, Denmark) were administered by mouth to prevent a decrease in plasma K^+ concentration. Blood pressure was continuously recorded by a Gould pressure transducer (Gould Electronics, Rilthofen, The Netherlands) connected to the arterial catheter, and heart rate was calculated from the pressure tracings recorded on an Elema Mingo graph (Siemens, Stockholm, Sweden). After the clamps all catheters were removed and pressure was applied for 15 min to avoid bleeding and haematoma. The subjects were offered

Table 1. Glycogen concentration and glycogen synthase fractional velocity and maximal activity in control and exercise thigh at each step during the two clamp sessions

	Glycogen mmol (kg dry wt)$^{-1}$	GS FV (%)	MA (nmol (mg dry wt)$^{-1}$ min^{-1})
	Post-eccentric clamp (PEC)		
Control thigh			
Basal state	361 ± 26	32·4 ± 2·2	17·1 ± 1·6
Step 1	401 ± 34†	48·3 ± 4·5‡	17·9 ± 1·6
Step 2	416 ± 28†	60·8 ± 2·8‡	19·0 ± 1·8
'Eccentric thigh'			
Basal state	297 ± 27*	32·0 ± 2·9	17·6 ± 1·4
Step 1	315 ± 36*	35·5 ± 3·6*	17·8 ± 1·1
Step 2	350 ± 30*†	60·4 ± 5·4‡	17·6 ± 1·8
	Control clamp (CC)		
Control thigh			
Basal state	380 ± 31	30·2 ± 3·1	17·2 ± 1·6
Step 1	410 ± 37†	45·3 ± 5·2‡	16·1 ± 2·3
Step 2	449 ± 31†	56·7 ± 6·4‡	17·4 ± 2·1
'Eccentric thigh'			
Basal state	366 ± 25	33·9 ± 4·1	17·2 ± 2·0
Step 1	388 ± 25	47·1 ± 4·4‡	17·3 ± 1·9
Step 2	436 ± 38†	68·9 ± 3·7‡	15·9 ± 1·9

Values are means ± S.E.M. of 7 observations in each group. 'Eccentric thigh', the thigh which performed the eccentric exercise during PEC, but acted as control thigh during CC. GS FV, glycogen synthase fractional velocity; MA, maximal activity. * Significantly different from the control thigh ($P < 0·05$). † Significantly different from the basal value ($P < 0·05$). ‡ Significantly different from the value on the previous clamp step ($P < 0·05$).

food and drink and throughout the next hour they remained in the bed and were continuously monitored on the cardial scope. Plasma glucose was measured every 5–15 min until glucose infusion was no longer necessary for maintenance of euglycaemia.

Analytical procedures

Blood and plasma glucose and blood lactate were measured with glucose and lactate analysers, respectively (Yellow Springs, OH, USA). Insulin in plasma was determined by a commercially available radioimmunoassay kit (kindly donated by Novo-Nordisk, Copenhagen, Denmark). Haemoglobin in blood and O_2 saturation of haemoglobin were determined by an OSM 3 Hemoxymeter (Radiometer, Copenhagen, Denmark), and K^+ in plasma by flame photometry. Catecholamines in plasma were assayed using a single-isotope radioenzymatic assay (Christensen, Vestergaard, Sørensen & Rafaelsen, 1980). Plasma free fatty acids (FFAs) were determined fluorometrically as previously described (Kiens, Essen-Gustavsson, Christensen & Saltin, 1993). Creatine kinase was measured at 37 °C using a commercially available kit (Boehringer Mannheim, Germany). Muscle biopsies were freeze-dried and dissected free of blood and connective tissue before analysis. Glycogen was measured by a hexokinase method after acid hydrolysis (Lowry & Passonneau, 1972). Glycogen synthase activity was measured with a modification of the filter paper method of Thomas, Schlender & Larner (1968). Assay conditions were 37 °C, uridine 5′-diphosphoglucose at 1·5 mM (saturating), and glucose 6-phosphate (G-6-P) at 0·17 and 8·0 mM, the latter concentration being saturating. Maximal activity was measured at saturating (8 mM) G-6-P concentration. Percentage fractional velocity was calculated as activity at the submaximal G-6-P concentration (0·17 mM) as a percentage of maximal activity. The GLUT4 protein content was quantified by Western blotting using a mouse monoclonal primary antibody directed against the thirteen C-terminal amino acids of GLUT4 and a horseradish peroxidase-labelled goat anti-mouse antibody as described previously (Asp *et al.* 1995*a*). Muscle soreness was subjectively rated by palpation of the proximal, medial and distal areas of the vastus lateralis and the hamstring muscles, using a rating scale from 0 (no soreness) to 4 (extreme soreness). Palpation and recording was always done by the same person. Soreness scores (proximal, medial and distal) were averaged each day for statistical comparison.

Statistics

To compare mean values in muscle or plasma at the different clamp steps a one-way analysis of variance for repeated measures was used. Student's paired t test was used as *post hoc* test. Thigh values during each clamp (control thigh *vs.* exercise thigh) and interclamp plasma values (control clamp *vs.* post-eccentric clamp) were compared paired on each clamp step by Student's paired t test. Since the creatine kinase (CK) data were not distributed normally, a non-parametric test was used (Wilcoxon matched pairs test) for these data. The level of significance was set at $P < 0·05$ during all tests. All figures display means ± S.E.M., and $n = 7$ for all groups.

Calculations

The local glucose exchange over the two thighs was calculated by multiplying arterial–venous differences in blood by the blood flow in each thigh divided by the thigh mass. The thigh mass was estimated from the thigh volume as described by Bangsbo, Gollnick, Graham & Saltin (1991), with the mass density being taken as 1 g ml^{-1}.

The total one-legged eccentric work (kJ) was calculated by multiplying the average velocity (m s^{-1}) of the ankle during the resistance with the area under the force × time (N s) curve.

Table 2. Thigh arterial–venous glucose difference, blood flow, glucose uptake, lactate venous–arterial difference and lactate release at each clamp step during the post-eccentric and control clamp

	Glucose A–V diff. (mM)	Blood flow (ml (kg thigh)$^{-1}$ min^{-1})	Glucose uptake (μmol kg^{-1} min^{-1})	Lactate V–A diff. (mM)	Lactate release (μmol kg^{-1} min^{-1})
			Post-eccentric clamp (PEC)		
Control thigh					
Basal state	0·08 ± 0·02	33·5 ± 5·3	2·1 ± 0·5	0·01 ± 0·03	0·73 ± 0·78
Step 1	0·75 ± 0·13	44·8 ± 5·1	32·0 ± 5·4	−0·01 ± 0·00	−0·24 ± 0·15
Step 2	1·22 ± 0·14	60·0 ± 5·2	73·3 ± 10·2	0·12 ± 0·04	7·56 ± 3·27
Eccentric thigh					
Basal state	0·08 ± 0·03	30·6 ± 3·8	1·8 ± 0·8	0·05 ± 0·03 *	1·53 ± 0·77 †^{1}
Step 1	0·72 ± 0·13	41·4 ± 4·2	30·1 ± 6·2	0·02 ± 0·01 †^{3}	0·70 ± 0·36 †^{1}
Step 2	1·10 ± 0·10 †^{2}	59·1 ± 7·9	64·6 ± 10·3 †^{3}	0·16 ± 0·03	9·66 ± 2·20
			Control clamp (CC)		
Control thigh					
Basal state	0·11 ± 0·05	36·0 ± 4·1	3·06 ± 1·44	0·04 ± 0·03	1·73 ± 1·15
Step 1	0·89 ± 0·26	47·5 ± 5·0	41·6 ± 11·44	0·02 ± 0·02	1·00 ± 0·86
Step 2	1·03 ± 0·18	74·7 ± 11·2	66·5 ± 6·33	0·16 ± 0·03	12·44 ± 3·12
'Eccentric thigh'					
Basal state	0·14 ± 0·05	38·9 ± 6·5	4·33 ± 1·22	0·04 ± 0·02	1·88 ± 1·05
Step 1	0·81 ± 0·22	45·9 ± 5·9	34·8 ± 8·61	0·01 ± 0·02	0·89 ± 1·07
Step 2	1·14 ± 0·18	73·0 ± 10·8	73·72 ± 6·05	0·14 ± 0·03	10·54 ± 2·40

Values are means ± S.E.M. of 7 observations in each group. Glucose A–V diff., the difference in blood glucose between the femoral artery and vein; Lactate V–A diff., the difference in blood lactate between the femoral vein and artery. * Significantly different from control thigh ($P < 0·05$). † Marginally different from control thigh; †^{1}, $P = 0·06$; †^{2}, $P = 0·07$; †^{3}, $P = 0·08$.

RESULTS

Post-eccentric exercise clamp (muscle effects)

The total work absorbed by the quadriceps muscle in the four sessions averaged 29 ± 4, 23 ± 3, 22 ± 2 and 19 ± 2 kJ, respectively, whereas the hamstrings absorbed 21 ± 4, 23 ± 3, 21 ± 2 and 22 ± 2 kJ, respectively. The mean power during the quadriceps sessions was 97 ± 12, 76 ± 10, 72 ± 7 and 65 ± 8 W, respectively, whereas the mean power during the hamstring sessions was 71 ± 13, 76 ± 11, 71 ± 8 and 74 ± 7 W, respectively.

The venous blood CK concentration increased significantly from 123 ± 21 U l^{-1} before to 5266 ± 3179 U l^{-1} (range 261–19 200 U l^{-1}) ($P < 0·05$) 2 days after the one-legged eccentric exercise, and at this stage the mean soreness was 2·6 ± 0·3 arbitrary units in the eccentric thigh and 1·2 ± 0·4 arbitrary units in the eccentric hamstrings. The control thigh and hamstrings were not sore.

The muscle glycogen concentration was lower in the eccentric thigh than in the control thigh during all steps of the clamp (Table 1). After clamp step 1, muscle glycogen had increased significantly in the control thigh, but not in the eccentric thigh whereas increases from basal levels to the end of step 2 were identical in the two thighs (Table 1). There were no differences in the maximal activity of glycogen synthase between the two thighs for any of the clamp steps (Table 1). The fractional velocity of glycogen synthase increased from basal level to step 1 and from step 1 to step 2 in the control thigh during the PEC. In the

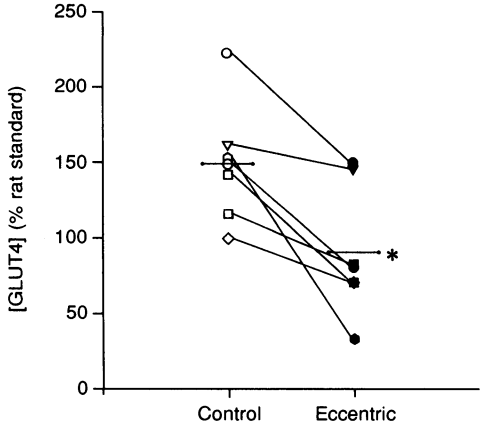

Figure 1. Individual skeletal muscle GLUT4 protein content in the basal state during the post-eccentric clamp

GLUT4 protein content per microgram sample protein is expressed relative to a rat heart standard (5 μg protein) run on the same gel. Each pair of symbols represents a subject, where the open symbols are the values from the control thigh in the basal state during the post-eccentric clamp, and the filled symbols are the values in the eccentric thigh. The horizontal lines represent the average in each thigh. Equal amounts of protein were loaded for every determination. * Significantly different from the control thigh ($P < 0·05$).

Table 3. Concentrations of glucose and insulin in arterial blood and plasma, respectively, during the post-eccentric and control clamp

	Glucose (mM)	Insulin (pmol l^{-1})
	Post-eccentric clamp (PEC)	
Basal state	4·3 ± 0·2	48 ± 11
Step 1	4·5 ± 0·2	372 ± 21
Step 2	4·5 ± 0·1	12712 ± 685
	Control clamp (CC)	
Basal state	4·5 ± 0·2	43 ± 5
Step 1	4·5 ± 0·3	341 ± 25
Step 2	4·5 ± 0·3	13535 ± 959

Values are means ± S.E.M. of 7 observations in each group.

eccentric thigh the increase from basal level to step 1 was not significant, and on step 1 the value was significantly lower ($P < 0·05$) than in the control thigh. There was a significant increase in the eccentric thigh from step 1 to step 2 ($P < 0·05$).

In the basal state and at step 1 of the clamp there were no differences in femoral arterial–venous glucose difference or blood flow between the two thighs (Table 2). During maximal insulin stimulation the arterial–venous glucose difference was marginally lower (10·0%, $P = 0·07$) over the eccentric thigh compared with the control thigh whereas there was no difference in blood flow (Table 2). Also the maximal glucose uptake over the eccentric thigh was marginally lower (11·9%, $P = 0·08$) when compared with the control thigh (Table 2). The GLUT4 content in the basal state was 39% ($P < 0·05$) lower in the eccentric thigh compared with the control thigh (Fig. 1). Furthermore a correlation was observed between the GLUT4 content and the maximal thigh glucose uptake when both thighs were analysed together ($r = 0·716$, $P < 0·05$) (Fig. 2). This correlation was also observed in the control ($r = 0·796$, $P < 0·05$) and the eccentric thigh ($r = 0·774$, $P < 0·05$) separately (Fig. 2), and the regression lines for these two correlations were not significantly different. At submaximal insulin stimulation the correlation between the muscle GLUT4 protein content and thigh glucose uptake was insignificant in the control and in the eccentric thigh. Also there was no correlation between muscle soreness or CK release and the reduction in the GLUT4 content.

The lactate release was marginally higher in the eccentric thigh in the basal state ($P = 0·06$) and during step 1 of the clamp ($P = 0·06$) compared with the control thigh, whereas there were no differences during step 2 of the clamp (Table 2).

Post-eccentric exercise *vs.* control clamp (systemic effects)

Blood glucose concentration was stable throughout the clamp steps and did not differ between PEC and CC (Table 3). Also, plasma insulin concentration increased similarly during PEC and CC at the two clamp steps.

The glucose infusion rate (GIR) was not different on the two clamp occasions on the first step of the clamp, but it was higher during CC than during PEC (96·44 ± 8·83 *vs.* 81·28 ± 3·17 μmol kg^{-1} min^{-1}, $P < 0·05$) with maximum insulin concentrations (Fig. 3).

There were no differences in the arterial plasma concentrations of lactate, potassium, FFA or catecholamines on either clamp step on the two clamp occasions (Table 4).

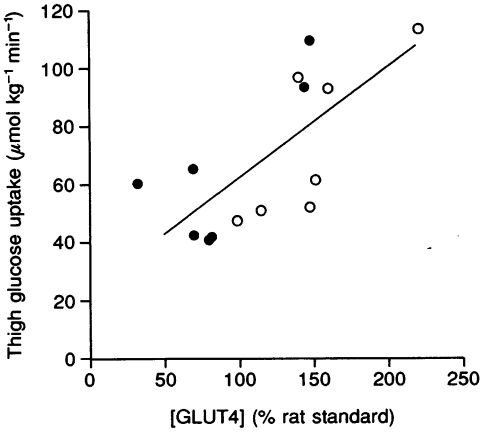

Figure 2. Correlation between the total GLUT4 content and the thigh glucose uptake during step 2 of the post-eccentric hyperinsulinaemic clamp

The open symbols are the values from control thighs during step 2 of the post-eccentric clamp, and the filled symbols are the values in eccentric thighs. The continuous line represents the correlation between the GLUT4 content and the thigh glucose uptake in both control and eccentric thigh ($Y = 23·5 + 0·383X$, $r = 0·716$, $P < 0·05$). Also the correlations in the control ($r = 0·796$, $P < 0·05$) and eccentric thigh ($r = 0·774$, $P < 0·05$) were both significant and not different.

Table 4. Concentrations of lactate in arterial blood and of noradrenaline, adrenaline, potassium and free fatty acids in arterial plasma during the post-eccentric and control clamp

	Lactate (mM)	Noradrenaline (nM)	Adrenaline (nM)	Potassium (mM)	Free fatty acids (μM)
			Post-eccentric clamp (PEC)		
Basal state	0·54 ± 0·10	1·80 ± 0·47	0·58 ± 0·16	3·96 ± 0·12	636 ± 91
Step 1	0·69 ± 0·05	2·08 ± 0·28	0·70 ± 0·10	3·75 ± 0·03	170 ± 39†
Step 2	1·17 ± 0·06*†	2·34 ± 0·32	0·74 ± 0·12	3·44 ± 0·10*†	105 ± 34*†
			Control clamp (CC)		
Basal state	0·47 ± 0·05	1·51 ± 0·22	0·48 ± 0·07	3·89 ± 0·10	812 ± 97
Step 1	0·70 ± 0·06†	1·65 ± 0·19	0·65 ± 0·09†	3·61 ± 0·06†	165 ± 29†
Step 2	1·24 ± 0·10*†	1·95 ± 0·26*	0·73 ± 0·11*	3·47 ± 0·11*	112 ± 31*†

Values are means ± S.E.M. of 7 observations in each group. * Significantly different from the basal value ($P < 0·05$). † Significantly different from the value on the previous clamp step ($P < 0·05$).

Also there were no differences in haematocrit, heart rate or blood pressure (data not shown) during the two clamps. The plasma CK concentration on the control clamp day (137 ± 28 U l^{-1}) was not different from the values obtained before the eccentric exercise (123 ± 21 U l^{-1}).

DISCUSSION

In the present study we characterized insulin action in muscle and on a whole-body basis 2 days after a bout of unaccustomed one-legged eccentric exercise. Whole-body and, less significantly, muscle glucose uptake was impaired during maximum insulin stimulation after eccentric exercise (decreased responsiveness), whereas there was no effect of eccentric exercise at submaximal insulin concentrations. On the other hand, activation of glycogen synthase was impaired after eccentric exercise at a submaximal but not at a maximal insulin concentration. Although the magnitude of these differences was not large, the findings suggest that muscle subjected to prior unaccustomed eccentric exercise becomes resistant to various aspects of insulin action.

Previous studies have indicated that a bout of unaccustomed eccentric exercise impairs submaximal insulin action on a whole-body basis (Kirwan, Bourey, Kohrt, Staten & Holloszy, 1991; Kirwan *et al.* 1992) and decreases the skeletal muscle GLUT4 protein content in man and rat (Asp *et al.* 1995*a*, *b*). Therefore in the present study it was of interest to explore whether the previously observed decrease in muscle GLUT4 protein content after eccentric exercise could be linked with decreased insulin-stimulated muscle glucose uptake. The total GLUT4 protein content was on average 39% lower in the eccentric than in the control thigh muscle during the PEC (Fig. 1). In agreement with others (Andersen *et al.* 1993; Dela *et al.* 1993), we also found a significant correlation between muscle GLUT4 protein content and muscle glucose uptake at maximal insulin stimulation (Fig. 2). Still, the average 39% lower GLUT4 content was accompanied by an only marginally lower thigh glucose uptake of 11·9% at maximal insulin stimulation. This suggests that muscle GLUT4 protein is not the only determinant of muscle glucose uptake at maximal insulin stimulation in agreement with the only moderately high r value in the correlation between GLUT4 and thigh glucose uptake (Fig. 2). On the other hand, the fact that the correlation lines for both thighs were similar suggests that eccentric exercise does not markedly affect maximal insulin-stimulated glucose uptake in any other way than by affecting muscle GLUT4 content. If this interpretation is true a more marked reduction in muscle GLUT4 should lead to a marked reduction in muscle glucose transport. In our

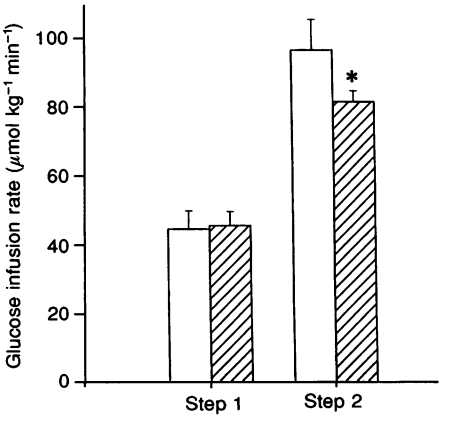

Figure 3. The glucose infusion rate (GIR) necessary to maintain euglycaemia during the control and post-eccentric clamp

Values are means ± S.E.M. of 7 observations in each group. The open bars represent the control clamp and the hatched bars represent the post-eccentric clamp. * Significantly different from the control clamp ($P < 0·05$).

rat model of eccentric muscle damage (Asp *et al.* 1995*b*) a reduction in GLUT4 protein of ~65% is obtainable in fast-twitch white fibres and such a marked reduction is accompanied by a clear reduction in insulin-stimulated muscle glucose transport (S. Asp & E. A. Richter, unpublished observations).

At high insulin concentrations glycogen synthase activation as well as glycogen synthesis seemed to be unimpaired in the eccentric muscle compared with the control muscle (Table 1) suggesting that glucose disposal was not impaired. In contrast, glycogen synthase activation at the first clamp step was impaired in the eccentric muscle and glycogen synthesis was also low (Table 1). This observation may suggest that the insulin signalling mechanism activating glycogen synthase has reduced insulin sensitivity after eccentric exercise. Our findings are in contrast to a study by Doyle and co-workers (Doyle *et al.* 1993) in which glycogen synthase activity after eccentric exercise was stimulated normally when plasma insulin increased during food intake. In that study, however, there was a tendency towards lower synthase activity in the eccentric muscle.

The whole-body glucose infusion rate was 15·7% lower ($P < 0{\cdot}05$) during PEC compared with CC (Fig. 2), and considering the thigh mass (~8 kg) and subject weight (~75 kg), the local thigh effect can only account for approximately 8% of the whole-body effect. This suggests that as a result of eccentric exercise a systemic factor is released which causes insulin resistance. A likely candidate may be the cytokine tumour necrosis factor (Lang, Dobrescu & Bagby, 1992), which is released by inflammatory cells that may accumulate after eccentric muscle damage. Our earlier studies using this model of eccentric exercise did not show significant accumulation of inflammatory cells when muscle sections were treated with standard stains such as Haematoxylin and Eosin (Asp *et al.* 1995*a*), but recent observations using sensitive immunohistochemical techniques show some infiltration (Y. Hellsten, U. Frandsen & E. A. Richter, unpublished observations). If a factor which causes insulin resistance is released after the eccentric exercise bout, it would be expected that the higher whole-body glucose infusion rate during CC compared with PEC at maximal insulin concentrations should be reflected in a higher control thigh glucose uptake during CC than during PEC. However, this was not the case but probably just reflects the greater accuracy with which GIR is measured compared with measurement of thigh glucose uptake.

It has also been suggested that competition between inflammatory cells and muscle fibres for available plasma glucose actually causes the impaired glycogen resynthesis after eccentric exercise (Widrick *et al.* 1992). Furthermore, phagocytic cells have been shown to produce a factor stimulating glycolytic flux in muscle (Shearer, Amaral & Caldwell, 1988; Forster *et al.* 1989), thus possibly diverting glucose away from muscle glycogen synthesis. However, if inflammatory cells were consuming glucose at a high rate, thigh glucose uptake should be expected to be higher in the eccentric thigh than in the control thigh and this was not the case at either insulin concentration in the present study.

Kirwan *et al.* (1992) previously reported that 30 min of running at 60% of maximum O_2 uptake ($V_{O_2,max}$) on a treadmill with a negative incline of 17 deg is followed by whole-body insulin resistance 2 days later. Muscle GLUT4 protein content or glucose uptake were not measured. In that study whole-body glucose disposal was decreased by 37% at insulin levels around 35 μU ml^{-1}. This is in contrast to our data where we did not find any change in GIR or thigh glucose uptake at a slightly higher (50–60 μU ml^{-1}) submaximal insulin concentration. However, there are differences in insulin concentrations and the muscle mass involved in the two exercise protocols, and it should also be noted that Kirwan *et al.* made no attempt to control the diet before the clamps. Our data are consistent with recent findings by King, Feltmeyer, Baldus, Sharp & Nespor (1993), who found no difference in GIR during a hyperglycaemic clamp at insulin levels around 40 μU ml^{-1} approximately 36 h after two-legged eccentric exercise.

In conclusion, we have shown that 2 days after one-legged eccentric exercise, maximal insulin-mediated glucose uptake in muscle and on a whole-body basis is impaired. The local effect cannot account for the effect on the whole-body basis, suggesting the release of a factor which induces systemic insulin resistance. Decreased insulin action was accompanied by decreased GLUT4 protein and glycogen concentration as well as decreased glycogen synthase activation at the first clamp step in muscle subjected to prior eccentric exercise. The lack of decrease in glucose uptake at submaximal insulin concentrations makes muscle insulin-resistant glucose transport a mechanism that alone can hardly explain the sustained decrease in muscle glycogen after eccentric exercise. Rather, decreased activation of glycogen synthase or increased glycogenolysis may be of importance. The latter assumption is supported by the higher lactate release in the eccentric thigh than in the control thigh at the two first clamp steps.

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