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ELEVATED MUSCLE GLYCOGEN AND ANAEROBIC ENERGY PRODUCTION DURING EXHAUSTIVE EXERCISE IN MAN

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SUMMARY

1. The effect of elevated muscle glycogen on anaerobic energy production, and glycogenolytic and glycolytic rates was examined in man by using the one-legged knee extension model, which enables evaluation of metabolism in a well-defined muscle group.

2. Six subjects performed very intense exercise to exhaustion (EX1) with one leg with normal glycogen (control) and one with a very high concentration (HG). With each leg, the exhaustive exercise was repeated after 1 h of recovery (EX2). Prior to and immediately after each exercise bout, a muscle biopsy was taken from m. vastus lateralis of the active leg for determination of glycogen, lactate, creatine phosphate (CP) and nucleotide concentrations. Measurements of leg blood flow and femoral arterial–venous differences for oxygen content, lactate, glucose, free fatty acids and potassium were performed before and regularly during the exhaustive exercises.

3. Muscle glycogen concentration prior to EX1 was 87·0 and 176·8 mmol (kg wet wt)$^{-1}$ for the control and HG leg, respectively, and the decreases during exercise were 26·3 (control) and 25·6 (HG) mmol (kg wet wt)$^{-1}$. The net glycogen utilization rate was not related to pre-exercise muscle glycogen concentration. Muscle lactate concentration at the end of EX1 was 18·8 (control) and 16·1 (HG) mmol (kg wet wt)$^{-1}$, and the net lactate production (including lactate release) was 26·5 (control) and 23·6 (HG) mmol (kg wet wt)$^{-1}$. Rate of lactate production was unrelated to initial muscle glycogen level. Time to exhaustion for EX1 was the same for the control leg (2·82 min) and HG leg (2·92 min).

4. Muscle glycogen concentration before EX2 was 14 mmol (kg wet wt)$^{-1}$ lower than prior to EX1. During EX2 the muscle glycogen decline of 19·6 mmol (kg wet wt)$^{-1}$ for the control leg was less than for the HG leg (26·2 mmol (kg wet wt)$^{-1}$). The muscle lactate concentrations at the end of EX2 were about 7–8 mmol (kg wet wt)$^{-1}$ lower compared to EX1, and the net lactate production was reduced by 40%. The exercise time during EX2 was 0·35 min shorter for the control leg, while no difference was observed for the HG leg.

5. Total reduction in ATP and CP was similar during the four exercise bouts, while a higher accumulation of inosine monophosphate (IMP) occurred during EX2 for the

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control leg (0·72 mmol (kg wet wt)$^{-1}$) compared to the HG leg (0·20 mmol (kg wet wt)$^{-1}$). There was no difference in leg oxygen uptake or leg oxygen deficit between the exercise bouts.

6. It is concluded that: (*a*) elevated muscle glycogen does not influence glycogenolytic or glycolytic rates and fatigue; (*b*) previous intense exercise reduces lactate production and time to exhaustion in spite of recovery being long enough for lactate (pH) and K^+ to return to pre-exercise levels. These findings suggest that muscle and blood lactate concentrations are not the only crucial factors for inhibiting anaerobic carbohydrate usage during intense skeletal muscle contractions and the development of fatigue.

INTRODUCTION

A consensus has been reached that dietary manipulation which elevates the carbohydrate stores of the body (muscle and liver) affects metabolism during exercise. Even though this results in an enhanced usage of carbohydrate at a given submaximal exercise intensity and an elevation in muscle and blood lactate, exercise endurance performance is improved. Such dietary manipulation was first studied by Christensen & Hansen (1939) who demonstrated that a carbohydrate-enriched diet resulted in a higher respiratory quotient (RQ) and better exercise performance in prolonged exercise when compared to exercise after days with a mixed or fat and protein diet. Some 30 years later, Bergström, Hermansen, Hultman & Saltin (1967) used a similar protocol with the addition of muscle glycogen determinations. Metabolism was affected in relation to the magnitude of glycogen storage in the exercising muscles. Exhaustion was postponed in proportion to availability of muscle glycogen and at exhaustion the active muscles were completely glycogen depleted.

In short-lasting, heavy exercise, where the rate of 'anaerobic glycolysis' peaks and its relative contribution to the energy yield is paramount, an even closer relationship between availability of glycogen in the active muscle and its utilization could be anticipated. In studies using rats, increased muscle glycogen storage was shown to induce higher glycogenolytic and glycolytic rates (Richter & Galbo, 1986). In fast twitch fibres, glycogen utilization and lactate release were linearly related to the initial muscle glycogen concentration over a 15 min exercise period. However, Spriet, Berardinucci, Marsh, Campbell & Graham (1990) were not able to confirm this, as they found no relationship between muscle glycogen storage and anaerobic glycogenolysis when the intense exercise period was shortened to 1 min.

In several studies in man, it has been concluded that the rate of anaerobic glycogenolysis is a function of muscle glycogen content, when above-normal carbohydrate storage is induced (Asmussen, Klausen, Nielsen, Egelund, Technow & Tønder, 1974; Klausen & Sjøgaard, 1980; Maughan & Poole, 1981; Greenhaff, Gleeson & Maughan, 1987, 1988). In contrast, several other investigators have failed to verify these findings (Jacobs, 1981; Symons & Jacobs, 1989; Ren, Broberg, Sahlin & Hultman, 1990; Spencer & Katz, 1991), but agree with the findings of an early study by Saltin & Hermansen (1967).

An effect on the rate of anaerobic glycogenolysis was observed in this latter study when the muscle glycogen content was below 30 mmol (kg wet wt)$^{-1}$, which is less

than half of the normal muscle glycogen stores in man after a mixed diet (see also Hultman & Sjøholm, 1983). There are no obvious explanations for the differences in results either in man or in other species. The design and protocol of the various studies, however, do vary markedly. Further, in many of the studies on man, only blood substrates and metabolites were analysed to evaluate the metabolic response. This limits direct comparisons and may explain why no firm conclusions so far have been reached.

Thus, the aim of the present study was to evaluate the effect of above-normal muscle glycogen content on anaerobic and aerobic metabolism during short-lasting, very intense exercise. To achieve variation in muscle glycogen content, dietary manipulation can be used as well as exercise prior to the test exercise. In the present study both approaches were applied. In addition, the exercise model chosen was one-legged knee extensor kicking, which allows for precise quantitative metabolic evaluation as well as relating these metabolic events directly to the work performed and to the level of exhaustion (Saltin, Kiens & Savard, 1986; Bangsbo, Gollnick, Graham, Juel, Kiens, Mizuno & Saltin, 1990).

METHODS

Subjects

Six, healthy, male subjects ranging in age from 22 to 27 years, with an average height of 182 (range: 177–190) cm, and an average weight of 76 (69–83) kg, participated in the experiment. Five of the subjects had participated in previous experiments of similar design and measurements. All subjects were habitually physical active, but none trained for competition. The subjects were fully informed of any risks and discomfort associated with these experiments before giving their consent to participate. The study was approved by the local ethical committee.

Procedures

Subjects performed one-legged exercise, in the supine position, on an ergometer that permitted the exercise to be confined to the quadriceps muscles (Andersen, Adams, Sjøgaard, Thorboe & Saltin, 1985). All subjects practiced the exercise with each leg several times on separate days before the final experiment was performed, and endurance (time to exhaustion at the same work rate used in the final experiment) for each of the legs was shown to be equal (mean: $2{\cdot}85 \pm 0{\cdot}52$) ($\pm$S.E.M) and $2{\cdot}96 \pm 0{\cdot}49$ min).

On the experimental day a catheter was placed in the femoral artery with the Seldinger technique, with the tip placed 1–2 cm proximal to the inguinal ligament. Since both legs were to be studied, two catheters were put in each of the two femoral veins. The tip of one of the catheters was positioned approximately 8 cm in the retrograde direction, i.e. 10–12 cm distal to the inguinal ligament. This catheter was used for collecting blood samples and for infusing the ice-cold saline. Another venous catheter was placed in the inguinal region with the tip about 1 cm distal to the ligament. The thermistor for measurement of venous blood temperature was inserted through this catheter and was advanced just proximal to the tip.

Protocol

In the evening 3 days prior to the experiment, the subjects exercised with one leg (high-glycogen leg; HG leg) for 2·5–3 h, consisting of 1·25 h of continuous exercise followed by intense, intermittent exercise for the next 1·25–1·75 h at work rates comparable to or higher than the work rate used in the experiment. After this, the subjects followed a carbohydrate-rich diet ($76{\cdot}7 \pm 2{\cdot}0$% carbohydrate, $12{\cdot}0 \pm 2{\cdot}0$% fat, $10{\cdot}5 \pm 0{\cdot}9$% protein; total intake (2 days): $36{\cdot}9 \pm 2{\cdot}3$ MJ ($18{\cdot}5 \pm 1{\cdot}1$ MJ day^{-1})). The evening before the experiment, the subject carried out the same exercise protocol with the other leg (control leg). After this, the subject did not ingest any food or fluid except water until finishing the experiment the following day.

On the experimental day, placement of the catheters was followed by 30 min of rest in the supine position. Then, the subject warmed up with one of the legs for 10 min at a work rate of 10 W. The choice of legs was randomized. After at least 10 min of rest, the subject exercised the leg at a mean power output of 67·0 W (range: 54·0–78·4 W; kicking frequency: 60 min^{-1}) until exhaustion (EX1). After a 1 h recovery period (REC1) the exhaustive exercise was repeated at the same power output (EX2). Following a rest period of 20 min (REC2) the subject carried out the same exercise protocol with the other leg (Fig. 1).

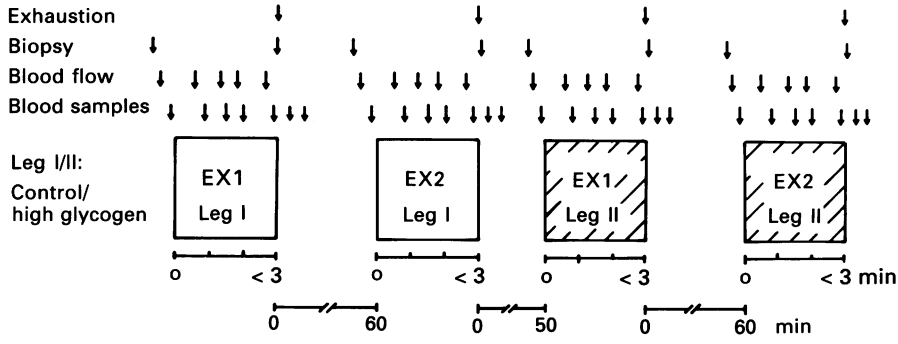

Fig. 1. Schematical representation of the experimental design.

Before and immediately after the exhaustive exercise bouts, a muscle biopsy was taken from m. vastus lateralis of the active muscle. Prior to the exercise, blood flow was determined and blood was drawn simultaneously from the femoral artery and vein. During the exercises blood flow was determined as frequently as possible followed by blood sampling from the femoral artery and vein. Figure 1 summarizes the protocol, including the sampling times during exercise. During REC1 and REC2, blood flows were measured and blood samples were collected at 1·5–2 min (reduced number of analyses) and at 10–11 min. An occlusion cuff placed just below the knee was inflated (220 mmHg) during the entire period of the intense exercise, and when measurements were performed during the recovery period. The frequency of knee extension was recorded and the mean work rate was calculated.

After the entire protocol had been completed with both legs, exercise of 8 min was performed with one leg at 20 W followed immediately by 8 min at 40 W, and, if possible, 8 min at 50 W. After a 15 min rest period, the same protocol was used for the opposite leg. Blood flow determinations and blood sample collections were performed during the last 2–3 min of each exercise.

Within 3 weeks of the main experiment, four of the subjects performed a single one-leg, exhaustive exercise at the same power output as during the main experiment, in order to evaluate the effect of a normal diet and of abstaining from long-term exercise the day before the experiment. The procedure described above for the main experiment was followed, i.e. the subject completed the protocol up to the end of EX1 for the first exercising leg.

Blood flow. Femoral venous blood flow was measured by the thermodilution technique (Andersen & Saltin, 1985). Briefly, ice-cold saline was infused at a constant rate into the femoral vein for 10–15 s to achieve a drop in femoral venous blood temperature of approximately 1 °C. At rest and in late recovery, when the blood flow was low, a 30–45 s infusion period was used (Richter, Mikines, Galbo & Kiens, 1989).

Blood analysis. Blood oxygen saturation was determined spectrophotometrically (Radiometer OSM-2 Haemoximeter). Haemoglobin concentration was determined with the Haemoximeter, which was calibrated spectrophotometrically by the cyanomethaemoglobin method (Drabkin & Austin, 1935). Haemoglobin concentrations at low oxygen saturation were adjusted with a correction factor obtained from multiple measurements of oxygen content of fully oxygenated blood samples, as determined by Van Slyke analysis (Holmgren & Pernow, 1959). Blood pH was measured with the Astrup technique (ABL 30, Radiometer, Copenhagen, Denmark). Lactate and glucose were analysed from perchloric acid-precipitated extractions of the blood samples, using a

fluorometric assay (Lowry & Passonneau, 1972). Free fatty acids (FFA) in plasma were determined according to the principles of Shimizu, Inoue, Tani & Yamada (1979). Plasma potassium concentration was measured using a flame photometer (Radiometer FLM3) with lithium as internal standard.

Muscle mass. The mass of quadriceps femoris muscles was estimated based on Simpson's rule, which included measurements of thigh length, multiple circumferences of the thigh and the skinfold thickness (Jones & Pearson, 1969). This anthropometric approach has given values similar to estimates based on multiple CAT scans (Saltin, 1985). The mean masses of the knee extensor muscles for the control and HG leg were $3{\cdot}21 \pm 0{\cdot}16$ and $3{\cdot}24 \pm 0{\cdot}18$ kg, respectively.

Muscle biopsies

Muscle samples were analysed for total water by weighing the samples before and after freeze drying, and for lactate, glycogen and creatine phosphate (CP) by fluorometric assays (Lowry & Passonneau, 1972). Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and inosine monophosphate (IMP) concentrations were determined with a HPLC technique (Schweinsberg & Loo, 1980). In addition, for both the control (C) and HG leg a piece of the biopsies taken before and after EX1 was frozen separately, and later used for serial cross-sectioning (10 μM) and stained for myofibrillar ATPase after alkanine and acid pre-incubation (Padykula & Herman, 1955; Brooke & Kaiser, 1970) for occurrence of fibre types, and the periodic acid–Schiff (PAS) method was used to determine glycogen (Andersen, 1975).

Changes in muscle variables were calculated on a dry weight basis, and normalized to the water content of resting muscle, since the muscle mass was determined at rest. This made the exchanges of substrates between femoral blood and the quadriceps muscles (expressed per kilogram active muscle mass) comparable with the muscle values.

The muscle water of about 76% was similar before the four exercise bouts and it increased during EX1-C to 77·6%, during EX1-HG to 76·5%, and during EX2 to 77·0% (EX2-C) and 77·6% (EX2-HG).

Calculations

Leg oxygen uptake and substrate and potassium exchange. Oxygen uptake ($\dot{V}_{O_2}$) and net lactate, net glucose, net FFA and net potassium exchange by the thigh were calculated by multiplying the blood flow, or for the last two variables, plasma flow, by the difference between femoral artery and venous ($a - v_{fem}$) concentrations of the variables. A continuous blood flow curve for exercise was constructed for each subject, by linear connection of the consecutive data points, to obtain time-matched values for the blood flow measurements with the blood variables. No difference between 'time-matched' and measured blood flow was larger than $0{\cdot}3$ l min^{-1} during exercise. The time-matched blood flow during recovery was determined from the linear connection between the blood flow measured immediately before and after the blood sampling.

Total oxygen uptake, and total net exchange of substrates and potassium. The total $\dot{V}_{O_2}$ (except the oxygen unloaded from haemoglobin (Hb) and myoglobin (Mb), and oxygen corresponding to the changes in femoral venous O_2) and net lactate, net glucose, net FFA and net potassium exchange are given by the time integral

$$\int_0^x f(t)\,dt,$$

where 0 is the start of exercise, x is the time for exhaustion and $f(t)$ is exchange of one of the variables at a given time (t) during exercise. In practice, the exchanges were determined as the areas under $f(t)$ curves, with time on the x-axis. The curves were produced on the assumption that there was a linear relationship between two measured values at any two consecutive time points (for possible magnitude of error introduced see Bangsbo *et al.* 1990).

The net lactate production was calculated as the sum of accumulated muscle lactate and total net lactate release during exercise (EX1 and EX2), expressed per kilogram muscle mass.

To evaluate the importance of a difference in exercise time between exercise bouts the total exchange for any variable was also calculated with a common exercise time (the shortest of the two exercise times was used as x in the above integral) for EX1-C and EX1-HG, for EX1-C and EX2-C, for EX1-HG and EX2-HG, and for EX2-C and EX2-HG.

Oxygen deficit (*Table* 1). To estimate the oxygen deficit the energy demand of the exercise

intensity used to produce exhaustion must be known. Thus, leg blood flow and a $-v_{fem}$ O_2 difference were determined at submaximal exercises (10, 20, 40 and, if possible, 50 W). The observed leg $\dot{V}_{O_2}$ for the control and HG leg at submaximal intensities were similar, being 200, 310 and 490 ml min^{-1} at 10, 20 and 40 W, respectively (Table 1). At 10 and 20 W, no net exchange of lactate was observed for the limbs, but at 40 W there was a net lactate release of 1·50 (control leg) and 2·39 (HG leg) mmol min^{-1} (Table 1). By linear extrapolation, the energy demand of the very intense exercise was then estimated from the submaximal determinations (Broun & Hollander, 1977; Bangsbo *et al.* 1990). The linear regression analysis between the exercise intensity and the leg oxygen uptake gave a mean *r* value of 0·993 (0·987–1·000). The energy demands of the exhaustive exercise bouts, estimated by extrapolation of these relationships, were 817·2±43·3 (EX1-C) and 811·8±40·3 (EX1-HG) ml O_2 equiv min^{-1}, and 800·3±37·1 (EX2-C) and 803·0±38·7 (EX2-HG) ml O_2 equiv min^{-1}.

TABLE 1. Leg blood flow, $\dot{V}_{O_2}$, and lactate release at different submaximal work loads and peak values during the first exhaustive exercise bout performed by the control leg (C) and the high-glycogen leg (HG).

	Leg blood flow (l min^{-1})		Leg $\dot{V}_{O_2}$ (ml min^{-1})		Leg lactate release (mmol min^{-1})	
Power (W)	C	HG	C	HG	C	HG
10	2·18 ±0·35	2·04 ±0·32	211·5 ±28·9	206·6 ±29·6	−0·09 ±0·05	0·00 ±0·07
20	3·04 ±0·40	3·02 ±0·41	310·7 ±29·0	304·2 ±32·5	−0·01 ±0·11	0·29 ±0·17
40	4·18 ±0·63	4·63 ±0·62	484·5 ±55·9	495·6 ±39·7	1·50 ±0·50	2·39 ±1·47
68 (peak)	5·16 ±0·62	4·74 ±0·42	671·0 ±87·5	627·8 ±52·4	11·13 ±1·62	12·34 ±1·09

Means ±S.E.M. are given.

The leg oxygen deficit for the very intense exercise was calculated as the difference between estimated energy demand and actual (measured) oxygen uptake, and used as an expression of the total anaerobic energy production (Bangsbo *et al.* 1990).

Statistics

Differences between EX1-C and EX1-HG, EX1-C and EX2-C, EX1-HG and EX2-HG, and EX2-C and EX2-HG, and differences between the obtained values and measures at rest or nil, were determined by the Wilcoxon ranking test for paired data (Pratt's modification, see Siegel, 1965). A significance level of 0·05 was chosen. Standard errors of mean values (±S.E.M.) are only given in the test where this value cannot be obtained from a figure or a table.

RESULTS

Time and rate of exercise

The mean power output was 68·7 (range: 54·0–78·4) and 67·1 (54·0–78·4) W for EX1-C and EX1-HG, respectively, with less than 1% deviation in the two other exercises (EX2-C and EX2-HG). The mean times for EX1-C and EX1-HG were 2·82±0·51 and 2·92±0·57 min, respectively. When the exercise was repeated with the same limb, the time to exhaustion for EX2-C (2·47±0·40 min) was shorter ($P < 0·05$) than that of EX1-C, while there was no difference between EX1-HG and EX2-HG (2·77±0·50 min).

TABLE 2. Muscle glycogen and lactate concentrations (mmol (kg wet wt)$^{-1}$), before (Pre) and after (Post) the exhaustive exercise bouts performed by the control leg (C) and the high-glycogen leg (HG)

	EX1				EX2			
	C		HG		C		HG	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Glycogen	87·0 ±14·4*	57·8 ±16·8*	176·8 ±22·9	149·8 ±22·3	72·6 ±14·8**†	52·0 ±15·3**	163·0 ±21·9††	124·7 ±19·1
Lactate	0·50 ±0·14*	18·75 ±2·05	1·52 ±0·88	16·12 ±2·33	1·63 ±0·51†	12·13 ±3·60†	0·87 ±0·25	8·61 ±0·95††

Means ±S.E.M. are given.
* Significant ($P < 0·05$) difference between EX1-C and EX1-HG.
** Significant ($P < 0·05$) difference between EX2-C and EX2-HG.
† Significant ($P < 0·05$) difference between EX1-C and EX2-C.
†† Significant ($P < 0·05$) difference between EX1-HG and EX2-HG.

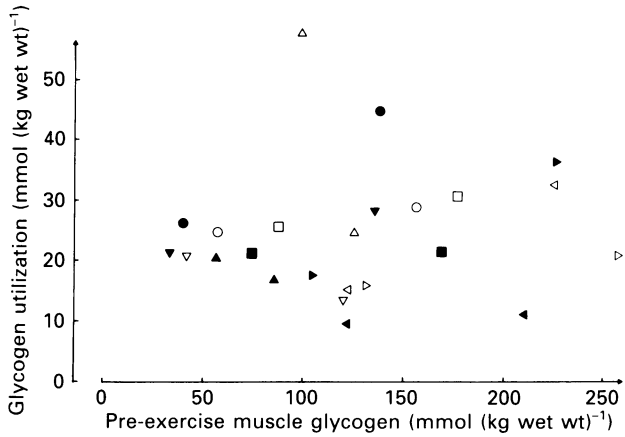

Fig. 2. Individual glycogen utilization during EX1 (open symbols) and EX2 (filled symbols) related to pre-exercise muscle glycogen concentration. There was no significant relationship for EX1 ($r = 0·04$; $P > 0·05$) or for EX2 ($r = 0·16$; $P > 0·05$).

Muscle variables

Muscle glycogen and lactate (Table 2, Figs 2–4)

The mean initial muscle glycogen concentration of the control leg was 87·0 mmol (kg wet wt)$^{-1}$, whereas the contralateral leg had a mean concentration of 176·8 mmol (kg wet wt)$^{-1}$ ($P < 0·05$). During EX1, the muscle glycogen concentration decreased to 57·8 and 149·8 mmol (kg wet wt)$^{-1}$, respectively. During the following 1 h of recovery, these concentrations became elevated to 72·6 and 163·0 mmol (kg wet wt)$^{-1}$, but they were still lower ($P < 0·05$) than prior to EX1. With EX2, the muscle glycogen concentrations were lowered to 52·0 and 124·7 mmol (kg wet wt)$^{-1}$, respectively.

The decrease in muscle glycogen was the same for EX1-C and EX1-HG (26·3 ±6·6 and 25·6 ±2·9 mmol (kg wet wt)$^{-1}$, respectively). During EX2-C, the decrease in

muscle glycogen of 19·6 ± 2·3 mmol (kg wet wt)$^{-1}$ was not different from the decrease during EX1-C, but was less ($P < 0·05$) than during EX2-HG (26·2 ± 5·5 mmol (kg wet wt)$^{-1}$). The corresponding mean glycogen utilization rates were 10·0 ± 1·7 and 10·7 ± 2·3 mmol glycosyl units (kg wet wt)$^{-1}$ min^{-1} for EX1-C and EX1-HG, respectively, and 8·6 ± 1·6 (EX2-C) and 11·6 ± 3·3 (EX2-HG) mmol (kg wet wt)$^{-1}$ min^{-1}, with a significant ($P < 0·05$) difference between the latter two values. The individual muscle glycogen concentration at rest before the exhaustive exercise bouts was unrelated to the decrease in muscle glycogen during both EX1 and EX2 (Fig. 2).

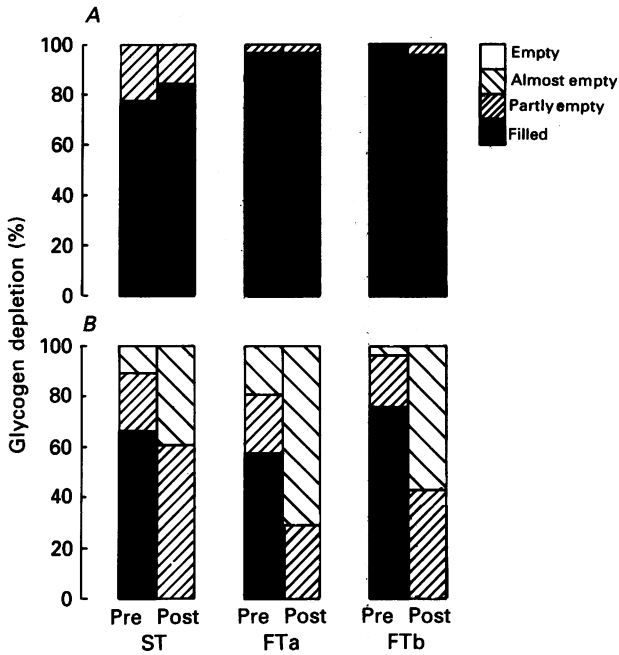

Fig. 3. Glycogen content in slow- (ST) and fast-twitch (FTa and FTb) fibres before and after the first exercise with the control leg (*B*) and the high-glycogen leg (*A*) determined by PAS staining.

The PAS staining revealed that more than 50 % of the fibres of all three types had a high glycogen level before EX1-C, and that for all fibre types a larger number of fibres were partly or almost depleted after this exercise (Fig. 3*B*). Before EX1-HG almost all fibres had a high concentration of glycogen and no changes could be detected at the end of the intense exercise (Fig. 3*A*).

At rest, before the various exercise bouts, the muscle lactate concentrations varied between 0·50 and 1·63 mmol (kg wet wt)$^{-1}$. During EX1-C and EX1-HG, muscle lactate concentration increased to 18·8 and 16·1 mmol (kg wet wt)$^{-1}$, respectively. These values were higher ($P < 0·05$) than those found after the EX2 bouts (12·1 (EX2-C) and 8·6 (EX2-HG) mmol (kg wet wt)$^{-1}$) (Table 2). The accumulation of lactate in the muscle was similar for EX1-C (19·3 mmol (kg wet wt)$^{-1}$) and EX1-HG (15·6 mmol (kg wet wt)$^{-1}$), and these values were 7–8 mmol (kg wet wt)$^{-1}$ higher

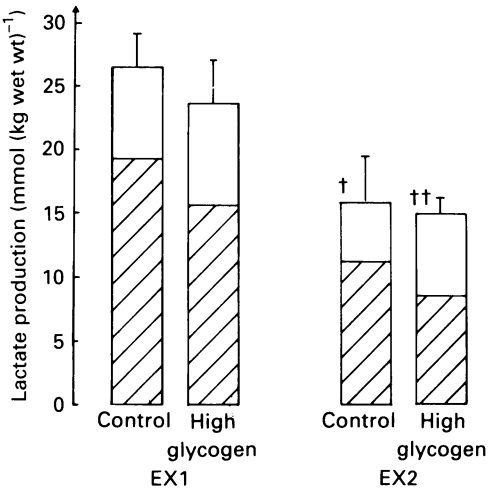

Fig. 4. Net lactate production during EX1 (left) and EX2 (right) determined from accumulation of lactate (▨) and lactate release (□) for the control leg and the high-glycogen leg. † Significant ($P < 0.05$) difference between EX1-C and EX2-C. †† Significant ($P < 0.05$) difference between EX1-HG and EX2-HG.

TABLE 3. Muscle CP, nucleotides and TAN (ATP+ADP+AMP) concentrations (mmol (kg wet wt)$^{-1}$) before (Pre) and after (Post) the exhaustive exercise bouts performed by the control leg (C) and the high-glycogen leg (HG)

	EX1				EX2			
	C		HG		C		HG	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CP	15·12	4·69	16·72	5·32	15·47	6·20	15·40	2·72
	±1·69	±1·02	±1·33	±1·98	±1·13	±2·81	±1·16	±0·37
ATP	4·74	3·71	5·11	4·03	4·52	3·78	5·01	3·59
	±0·41	±0·37	±0·71	±0·63	±0·29	±0·40	±0·19	±0·36
ADP	0·51	0·67	0·53	0·58	0·46	0·74	0·55	0·63
	±0·08	±0·14	±0·06	±0·15	±0·08	±0·19	±0·07	±0·15
AMP	0·19	0·13	0·13	0·14	0·15	0·17	0·17	0·11
	±0·01	±0·03	±0·02	±0·02	±0·01	±0·01	±0·03	±0·04
IMP	<0·01	0·68	<0·01	0·28	<0·01	0·72	<0·01	0·20
		±0·09		±0·12		±0·18**		±0·04
TAN	5·44	4·51	5·78	4·75	5·12	4·69	5·73	4·34
	±0·46	±0·43	±0·74	±0·76	±0·30	±0·23	±0·17	±0·30

Means ±S.E.M. are given.
** Significant ($P < 0.05$) difference between EX2-C and EX2-HG.

($P < 0.05$) than during the EX2 bouts (11·2 (EX2-C) and 8·5 (EX2-HG) mmol (kg wet wt)$^{-1}$) (Fig. 4).

Muscle CP and nucleotides (Table 3)

Muscle CP concentrations of 15–17 mmol (kg wet wt)$^{-1}$ before the four exhaustive exercise bouts were similar, and no differences in the CP concentrations at the end of the exercises (3–6 mmol (kg wet wt)$^{-1}$) were observed. The muscle ATP

concentration of about 5 mmol (kg wet wt)$^{-1}$ at rest was also similar in all four trials as was the decrease of 0·8–1·2 mmol (kg wet wt)$^{-1}$ after exercise. Neither the muscle ADP nor AMP concentrations changed during the exhaustive exercise bouts. At rest, the muscle IMP concentration was below 0·01 mmol (kg wet wt)$^{-1}$ and increased during exercise to 0·68 and 0·28 mmol (kg wet wt)$^{-1}$ for EX1-C and EX1-HG, respectively. The IMP concentration at the end of EX2-C (0·72 mmol (kg wet wt)$^{-1}$) was higher ($P < 0·05$) than at the end of EX2-HG (0·20 mmol (kg wet wt)$^{-1}$).

Leg blood flow, $\dot{V}_{O_2}$, and substrate and potassium exchange

Leg blood flow (Table 1)

The leg blood flow at rest of about 0·5 l min^{-1} increased gradually during exercise and reached 4·5–5·5 l min^{-1} at the end of exercise. The rate of rise in limb blood flow was similar for the four exhaustive exercise bouts, and so was the decline in blood flow in recovery, reaching 1·1 l min^{-1} (REC1-HG) or below, 10 min into recovery.

Leg $\dot{V}_{O_2}$ (Tables 1 and 4)

At rest, the thigh $\dot{V}_{O_2}$ was about 23 ml min^{-1}; during all four intense exercise bouts it increased to 460 ml min^{-1} after about 0·8 min, to 580 ml min^{-1} after about 1·5 min and at exhaustion leg $\dot{V}_{O_2}$ was 600–700 ml min^{-1} (Table 1). The total $\dot{V}_{O_2}$s of 1·51 (EX1-C) and 1·47 (EX1-HG) l were not different from the 1·26 (EX2-C) and 1·33 (EX2-HG) l. Leg $\dot{V}_{O_2}$ fell rapidly after the intense exercise to 87 and 72 ml min^{-1} after 1·6 and 1·7 min for REC1-C and REC1-HG, respectively, with a further decrease to 35 and 44 ml min^{-1} after about 11 min. Similar changes were observed for REC2-C and REC2-HG.

Lactate exchange (Figs 4 and 5)

No net leg exchange of lactate was observed before the two EX1 bouts, but an uptake of 0·05 mmol min^{-1} ($P < 0·05$) of lactate occurred prior to EX2. Lactate was released at a rate of 6–9 mmol min^{-1} after 0·8 min of exercise and at exhaustion it was 11·1 (EX1-C) and 12·3 (EX1-HG) mmol min^{-1}, and 9·6 (EX2-C) and 10·0 (EX2-HG) mmol min^{-1} (Fig. 5). The lactate efflux at the end of EX1-C was higher ($P < 0·05$) than at EX2-C.

The total net lactate releases of 23·4 ± 5·8 and 25·7 ± 4·1 mmol during EX1-C and EX1-HG, respectively, were not different, and also the releases during EX2-C (18·3 ± 3·9 mmol) and EX2-HG (20·6 ± 4·3 mmol) were similar (Fig. 4). The release during EX1-C was higher ($P < 0·05$) than during EX2-C in association with the longer exercise time of EX1-C; this difference (21·2 ± 6·0 (EX1-C) and 18·2 ± 3·9 mmol (EX2-C)) was not significant ($P > 0·05$) when a common exercise time (2·47 min) was used.

In recovery from EX1-HG, the lactate release declined to 8·27 ± 0·56 and 0·90 ± 0·41 mmol min^{-1} after 1·7 and 11 min, respectively. These values were higher ($P < 0·05$) than the corresponding values for REC1-C (6·09 ± 0·94 and 0·24 ± 0·12 mmol min^{-1}), and also higher ($P < 0·05$) than 6·02 ± 1·04 and 0·36 ± 0·15 mmol min^{-1} for REC2-HG. The lactate release for REC2-C was 4·53 ± 0·45 mmol min^{-1} at 1·7 min and 0·24 ± 0·08 mmol min^{-1} at 11 min of recovery.

Potassium efflux (Fig. 5)

Prior to exercise no net potassium exchange was observed. During exercise potassium release increased rapidly (Fig. 5). After 0·8 min the release was about 2·2 mmol min^{-1} for EX1-C and the two EX2 bouts, and 2·8 mmol min^{-1} for EX1-HG. During the remaining part of exercise the potassium release was rather constant.

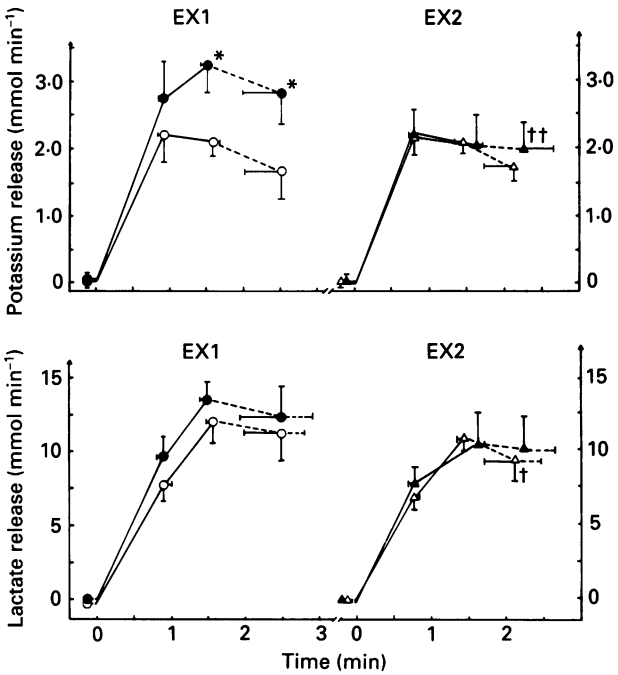

Fig. 5. Lactate (lower panel) and potassium (upper panel) release for the control leg (open symbols) and high-glycogen leg (filled symbols) during EX1 (left) and EX2 (right). * Significant ($P < 0·05$) difference between EX1-C and EX1-HG. † Significant ($P < 0·05$) difference between EX1-C and EX2-C. †† Significant ($P < 0·05$) difference between EX1-HG and EX2-HG.

At about 1·5 min and at the end of the exercise the potassium efflux during EX1-HG was higher ($P < 0·05$) than during EX1-C, and at the end of exercise the potassium release for EX1-HG was also larger ($P < 0·05$) than EX2-HG.

For EX1-C the total net release of potassium was $4·4 \pm 1·0$ mmol, which was similar to the release of $4·1 \pm 0·7$ and $4·0 \pm 0·4$ mmol for EX2-C and EX2-HG, respectively. On the other hand, the total net potassium efflux of $5·8 \pm 0·6$ mmol during EX1-HG was higher ($P < 0·05$) than during EX2-HG, even if the difference in exercise times were taken into account ($5·3 \pm 0·6$ (EX1-HG) and $3·8 \pm 0·3$ (EX2-HG) mmol; $P < 0·05$).

The potassium release during exercise changed to an uptake during recovery. After about 11 min this was higher ($P < 0·05$) for REC1-HG ($0·34 \pm 0·19$ mmol min^{-1}) than for REC1-C ($0·14 \pm 0·06$ mmol min^{-1}) and for REC2-HG ($0·12 \pm 0·05$ mmol min^{-1}). The potassium uptake was $0·07 \pm 0·03$ mmol min^{-1} after 11·4 min of REC2-C.

Exchange of glucose and FFA

At rest, before the two initial exercises (EX1-C and EX1-HG), no net glucose uptake occurred, but prior to the EX2 bouts, small uptakes ($P < 0.05$) were observed (0.30 ± 0.04 (EX2-C) and 0.24 ± 0.05 (EX2-HG) mmol min^{-1}). During the early phase of EX1-C a small net release of 1.07 ± 0.50 mmol min^{-1} ($P < 0.05$) occurred and a small net uptake of 0.93 ± 0.95 mmol min^{-1} ($P < 0.05$) was observed in EX1-HG. In the remaining part of both EX1-C and EX1-HG, a non-significant net glucose uptake ($P > 0.05$) was found with values of 0.26 ± 1.38 and 0.37 ± 1.14 mmol min^{-1}, respectively, at exhaustion. During EX2-C and EX2-HG no significant glucose exchanges were observed, with the net glucose uptake at exhaustion being 0.79 ± 0.55 and 0.00 ± 0.41 mmol min^{-1}, respectively.

The total net uptakes of glucose during EX1-HG (1.93 ± 1.32 mmol) and EX1-C (-1.50 ± 1.80 mmol) were not different from nil ($P > 0.05$). Neither were the total uptakes of glucose significant ($P > 0.05$) during the EX2 bouts, but the uptake during EX2-C (1.09 ± 0.67 mmol) was higher than for EX2-HG (-0.41 ± 0.95 mmol), which was related to the difference in exercise time (0.41 ± 0.84 and 0.12 ± 0.23 mmol, respectively, with the same exercise time; $P > 0.05$).

After about 11 min of recovery, the net uptake of glucose in REC1-C was 0.56 ± 0.31 mmol min^{-1}, which was higher ($P < 0.05$) than the 0.13 ± 0.18 mmol min^{-1} found for REC1-HG. The corresponding values for EX2-C and EX2-HG were 0.38 ± 0.07 and 0.22 ± 0.15 mmol min^{-1}, respectively.

Prior to EX1, no net exchange of FFA was observed in either leg, but before EX2-C and EX2-HG, a small net release of FFA ($P < 0.05$) was found (0.024 ± 0.009 and 0.013 ± 0.005 mmol min^{-1}, respectively). During the early part of the intense exercise, a net uptake ($P < 0.05$) of FFA occurred in all four situations. In the remaining part of the exercise the uptake of FFA increased in EX1-C and EX2-C, reaching 0.142 ± 0.071 and 0.229 ± 0.080 mmol min^{-1} by exhaustion, respectively. In contrast, the uptake of FFA decreased in the HG leg and in EX1-HG it changed to a non-significant net release of 0.032 ± 0.070 mmol min^{-1} after 1·5 min and 0.019 ± 0.061 mmol min^{-1} at exhaustion. The latter two values were significantly ($P < 0.05$) different from the corresponding values for EX1-C.

The total FFA uptake during EX1-C was 0.254 ± 0.123 mmol, which was higher ($P < 0.05$) than the uptake during EX1-HG (0.015 ± 0.102 mmol). However, the difference was eliminated ($P > 0.05$) when the different exercise time was taken into account (0.159 ± 0.051 and 0.015 ± 0.102 mmol). During EX2-C and EX2-HG, the total net uptakes were 0.235 ± 0.011 and 0.105 ± 0.157 mmol, respectively.

About 11 min after the intense exercise, the net uptake of FFA was 0.015 ± 0.011 mmol min^{-1} for REC1-C and 0.028 ± 0.21 mmol min^{-1} for REC2-C, while net releases of 0.022 ± 0.074 and 0.049 ± 0.039 mmol min^{-1} for REC1-HG and REC2-HG, respectively, were observed. None of these was different ($P > 0.05$) from nil.

Oxygen deficit and anaerobic energy production (Table 4; Figs 4, 6 and 7)

The leg oxygen deficit was 848 ml O_2 equiv for EX1-C, which was similar to the 907 ml O_2 equiv for EX1-HG (Table 4). These values were of the same magnitude as

the oxygen deficit of 745 and 891 ml O_2 equiv for EX2-C and EX2-HG, respectively. The latter two values were different ($P < 0.05$) from each other due to the longer exercise time of EX2-HG, since the corresponding data adjusted to a common exercise time were not ($P > 0.05$) different (686±81 and 715±51 ml O_2 equiv).

TABLE 4. Leg energy demand, $\dot{V}_{O_2}$, and oxygen deficit (A) and muscle lactate accumulation, release and production (B) during the exhaustive exercise bouts (EX1 and EX2) for the control leg (C) and the high-glycogen leg (HG)

	EX1		EX2	
	C	HG	C	HG
	(A) Oxygen deficit			
Energy demand (ml O_2 equiv)	2372 ±526	2378 ±508	2003 ±375	2218 ±405
Leg $\dot{V}_{O_2}$ (ml O_2)	1508 ±482	1472 ±430	1258 ±354	1328 ±353
Leg oxygen deficit (ml O_2 equiv)	868 ±136	907 ±153	745 ±62	891 ±94
	(B) Lactate production			
Lactate accumulation (mmol (kg wet wt)$^{-1}$)	19·3 ±2·3	15·6 ±3·1	11·2 ±3·6†	8·5 ±1·1††
Lactate release (mmol (kg wet wt)$^{-1}$)	7·2 ±1·6	8·0 ±1·3	4·6 ±0·4†	6·3 ±1·4
Lactate production (mmol (kg wet wt)$^{-1}$)	26·5 ±2·3	23·6 ±3·1	15·8 ±3·6 †	14·9 ±1·1††

Means ±S.E.M. are given.
† Significant ($P < 0.05$) difference between EX1-C and EX2-C.
†† Significant ($P < 0.05$) difference between EX1-HG and EX2-HG.

The changes in nucleotides corresponded to an ATP production of 1·7±0·4 (EX1-C) and 1·3±0·3 (EX1-HG) mmol (kg wet wt)$^{-1}$, and 1·9±0·5 (EX2-C) and 1·3±0·3 (EX2-HG) mmol (kg wet wt)$^{-1}$. This ATP release together with the net depletion of CP could account for 11·9 (EX1-C) and 12·4 (EX1-HG) mmol (kg wet wt)$^{-1}$, or 20·1 and 19·8% of the total anaerobic energy production estimated from the oxygen deficit (including use of Mb- and Hb-bound O_2), while the corresponding values for EX2-C and EX2-HG were 11·7 (22·5%) and 14·6 (23·8%) mmol (kg wet wt)$^{-1}$, respectively (Fig. 6).

The net lactate production was 26·5 and 23·6 mmol (kg wet wt)$^{-1}$ during EX1-C and EX1-HG, respectively. During EX2-C (15·8 mmol (kg wet wt)$^{-1}$) and EX2-HG (14·9 mmol (kg wet wt)$^{-1}$) the production was similar, but each was lower ($P < 0.05$) than the corresponding value for EX1 (Table 4; Fig. 4). For EX1-C and EX1-HG, the mean net lactate production rate was 11·1±2·0 and 9·1±1·4 mmol (kg wet wt)$^{-1}$ min^{-1}, respectively, which was higher ($P < 0.05$) than during EX2-C (7·5±2·3 mmol (kg wet wt)$^{-1}$ min^{-1}) and EX2-HG (5·9±0·9 mmol (kg wet wt)$^{-1}$ min^{-1}). Neither the total lactate production nor the mean lactate production rate were related to the pre-exercise muscle glycogen concentration (Fig. 7). In the first

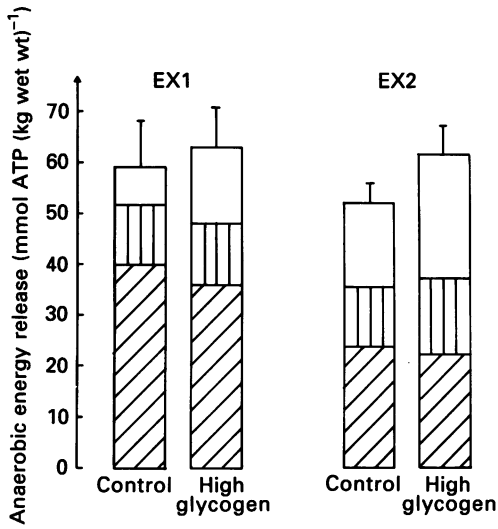

Fig. 6. Total anaerobic energy production during EX1 (left) and EX2 (right) determined from the leg oxygen deficit related to ATP release from lactate production (▨), and changes in CP and nucleotides (▥) (□, others). In the calculation is used: 1 mmol lactate = 1·5 mmol ATP ≈ 6·7 ml O_2 equiv.

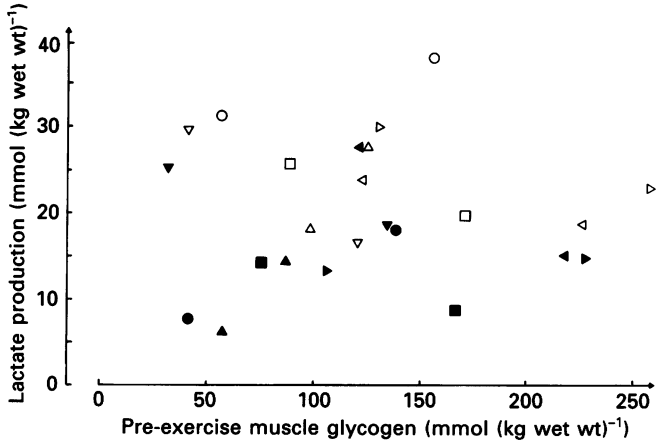

Fig. 7. Individual relationship between pre-exercise muscle glycogen concentration and lactate production during EX1 (open symbols) and EX2 (filled symbols). There was no significant relationship for EX1 ($r = 0{\cdot}06$; $P > 0{\cdot}05$) or for EX2 ($r = 0{\cdot}36$; $P > 0{\cdot}05$).

exercise bouts, the lactate production could account for 67·2 (EX1-C) and 56·3% (EX1-HG) of the total anaerobic energy release, while the corresponding values for EX2-C and EX2-HG were 45·7 and 36·4%, respectively (Fig. 6).

DISCUSSION

The present study gives a clear answer to the question of whether initial muscle glycogen content and the glycolytic rate are coupled in intense short-lasting exercise. Glycolysis, neither as a peak rate, nor as total contribution of energy during the exercise, is a function of above-normal muscle carbohydrate storage. Further, this relates both to its 'aerobic' and 'anaerobic' usage. The bulk of data in the literature on man are in line with our findings. Conflicting results, however, are also available and the question is then if these differences in results can be explained. Asmussen *et al.* (1974), and later Klausen & Sjøgaard (1980) and Maughan and colleagues (Maughan & Poole, 1981; Greenhaff *et al.* 1987, 1988), have all reported effects of dietary manipulation on blood lactate levels after short-lasting intense exercise. In the study by Klausen & Sjøgaard (1980) muscle analyses were also performed. In this study the diets produced differences in carbohydrate storage of the muscle (from approximately 60 to 140 mmol (kg wet wt)$^{-1}$). Muscle and blood lactate levels, after repeated 2 min, exhaustive exercise bouts, were reduced in relation to the lowering of the muscle glycogen content. Of very special note is their finding of muscle glycogen depletion being identical (12·5 mmol (kg wet wt)$^{-1}$) for each 2 min exercise bout (this is clearly pointed out by the authors in their discussion). This implies very similar rates of glycolysis. A possible explanation for the lower lactate concentration does not appear to be a reduced production, but rather an elevated utilization of the produced lactate in various tissues of the body when the carbohydrate storage was scarce. Muscle glycogen levels were surprisingly high after the fat and protein diet in this particular study. It is likely, however, that glycogen in the various fibres was unevenly distributed, which became further pronounced after the first exercise bout, and resulted in an elevated lactate turnover. In addition, it should be considered that several days with an extreme diet may enhance metabolic pathways for the use of lactate in oxidative metabolism (Jansson, 1980).

Several investigations are consistent with the present experiment. Jacobs and colleagues (Jacobs, 1981; Symons & Jacobs, 1989), in a series of experiments comparing a large range of muscle glycogen contents, have been unable to demonstrate an effect on the rate of glycogen depletion during short very intense exercise. Ren *et al.* (1990) made similar findings in a study using electrical stimulation of the human quadriceps muscles. In contrast, there is another study in the literature where elevated muscle glycogen content was associated with an enhanced rate of glycogenolysis and improved performance. The larger carbohydrate storage was brought about by a period of sprint training and an ordinary mixed diet. The explanation for the observed alterations is likely to be related to the conditioning rather than to the small elevation in muscle glycogen stores; a conclusion also drawn by the authors (Boobis, Williams & Wootton, 1983; Boobis, 1986).

Our conclusion, therefore, is that above-normal muscle glycogen content, and glycogenolytic and glycolytic rates in intense exercise, are not coupled. Differences in muscle and blood lactate concentrations which are sometimes found, can be attributed to the lactate being metabolized at different rates. Grisdale, Jacobs & Cafarelli (1990) have reported that exercise 24 h prior to repeated static contractions is a more potent determinant of muscle endurance than glycogen availability.

However, when our subjects were tested on a separate day without any long-term exhaustive exercise the day before, performance time and glycogenolytic and glycolytic rates were the same (Fig. 8). Thus, in the present experiments muscle glycogen content can be singled out as the sole factor studied.

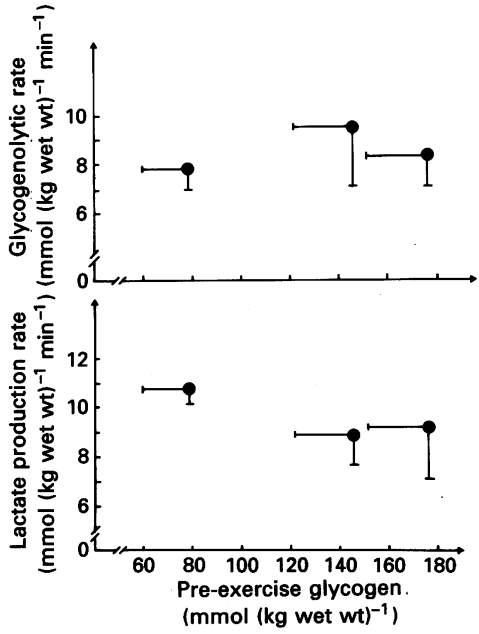

Fig. 8. Net lactate production rate (lower panel) and muscle glycogen utilization rate (upper panel) during EX1 related to pre-exercise muscle glycogen concentration for four subjects, who performed the first exhaustive exercise on a separate day.

It is more difficult to explain the findings from rat models, which in part differ from man. Richter & Galbo (1986) found, when using an isolated rat hindlimb model and electrically inducing contractions for 15 min, that a coupling existed between content of glycogen in muscle and the rate by which it was utilized, particularly in fast twitch oxidative (FO) and fast twitch oxidative and glycolytic (FOG) fibres. Spriet *et al.* (1990) raised the question as to whether this was due to the well-documented coupling of aerobic consumption to carbohydrate in more prolonged exercise. In experiments where circulation was occluded, they found a pronounced drop in tension development with intermittent stimulation of the muscle for 60 s. However, glycogen depletion and rate of lactate production were similar regardless of initial muscle glycogen content. Thus, they concluded that the findings by Richter & Galbo (1986) were compatible with their original suggestion (Spriet *et al.* 1990). Hespel and Richter re-studied this problem using their experimental model, and included muscle samples after 1 and 2 min of stimulation (personal communication). Both early and late in the stimulation period of 15 min, glycolysis was observed to be a function of the magnitude of the muscle glycogen storage. They attributed the linking of high muscle glycogen and high rates of glycolysis to a high content of phosphorylase a. Its activity at rest was positively related to the muscle glycogen content.

Electrically induced contractions mimic voluntary muscle activity; however, there are certain marked differences. One is the recruitment pattern, which with electrical stimulation favours involvement of the fast twitch (FT) fibres (Baratta, Ichie, Hurring & Solommon, 1989). This is probably not a serious concern in short-lasting experiments, where fatigue develops within 1–2 min, as the FT fibre pool in voluntary contractions also contributes the most in these circumstances. In exercise lasting 15 min, as in the case of Richter and colleagues, a 'reversed' recruitment order is obtained which means that comparisons with voluntary contractions are not valid. In the former situation, the FT pool of fibres is engaged early in the exercise for force production, but drops out quickly. For the remaining time, force production relates to slow twitch (ST) fibre tension development. Thus, the two major types of muscle fibres are studied separately, with a major impact of FT muscle fibre metabolism during the 1 and 2 min samples, whereas ST muscle response is manifested later in the exercise. This, by itself, is not an explanation for the linkage between glycogen content and its degradation. However, as a result of the supramaximal stimulation that was applied, it is likely that a high concentration of inorganic phosphate (P_i) develops, especially in fast twitch muscles. Chasiotis (1983, 1988) and later Ren & Hultman (1990) have shown that P_i is a critical activator of phosphorylase b to a, and subsequently of the rate of glycogen utilization. A likely explanation for the difference in results, comparing Richter and colleagues' findings with those of Spriet *et al.* as well as those on man, could be the difference in the activation of muscle. Supramaximal activation may cause markedly higher free P_i levels in the contracting muscle.

It has been demonstrated *in vitro* that the enzyme glycogen phosphorylase has a very low Michaelis constant ($K_m \approx 2$ mM) for glycogen (Newsholme & Leech, 1983). In the present study the lack of relationship between initial muscle glycogen and glycogenolytic rate suggests that phosphorylase is saturated with its substrate at least when muscle glycogen is higher than 30–40 mmol (kg wet wt)$^{-1}$. In several studies higher muscle IMP has been found when glycogen is low, which we also observed (Norman, Sollevi & Jansson, 1988; Broberg & Sahlin, 1989; Spencer & Katz, 1991). In addition, for the control leg muscle IMP concentration was correlated to muscle glycogen concentration at exhaustion both for EX1 and EX2 ($r = 0{\cdot}77$ and $r = 0{\cdot}83$, respectively, $P < 0{\cdot}05$), and to the relative number of almost glycogen-depleted muscle fibres after EX1-C determined by PAS staining ($r = 0{\cdot}78$, $P < 0{\cdot}05$). The elevated IMP, which may signify more of the ADP and AMP being unbound, would aid in maintaining the glycogen breakdown and formation of pyruvate when muscle glycogen and glycogen-6-phosphate are low in the muscle (Uyeda, 1979; Aragon, Tornheim & Löwenstein, 1980; Chasiotis, Sahlin & Hultman, 1982).

A reduction in muscle glycogen content was also achieved by prior exercise (i.e. EX1), followed by a long enough recovery period (1 h) for acid–base balance to become normalized. Only 26 mmol (kg wet wt)$^{-1}$ was utilized by the first exercise, and during the 1 h recovery period about 12 mmol (kg wet wt)$^{-1}$ was synthesized in part using lactate as a substrate (Bangsbo, Gollnick, Graham & Saltin, 1991). Thus, the difference in muscle glycogen prior to EX1 and EX2 was small, amounting to about 14 mmol (kg wet wt)$^{-1}$, with no subject having less than 34 mmol (kg wet wt)$^{-1}$ prior to EX2 and all fibres containing glycogen (Fig. 3). In the light of the present

results which indicate no role for muscle glycogen over the range of 30–250 mmol (kg wet wt)$^{-1}$ for anaerobic metabolism and performance, the results when exercise was performed a second time are striking. Both lactate production and performance were lowered when exercising with the control leg, but there was no decrease in performance for the high-glycogen leg (EX2-HG; Fig. 9).

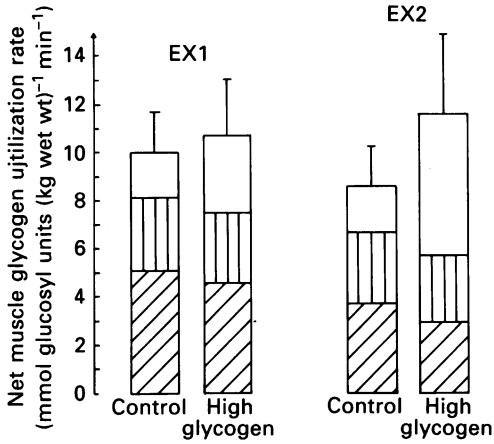

Fig. 9. Net muscle glycogen utilization rate during EX1 (left) and EX2 (right) related to lactate production rate (▨), and carbohydrate oxidation rate (▥) for the control leg (C) and the high-glycogen leg (HG) (□, others). It is assumed that leg RQ = 1 during the exercises.

What brought about the lowering of the rate of glycolysis in EX2? The IMP concentration was the same comparing EX1 and EX2. Breakdown of ATP and elevation of ADP, AMP and probably also P_i were very similar in all four exercise bouts, but it is difficult to propose a mechanism other than a change in bound to unbound ratio for these regulators acting at the level of phosphofructokinase (PFK) during the second exercise (EX2-C and EX2-HG), with this ratio changing towards less being unbound. The reason for suggesting that regulation is at the level of PFK rather than at the conversion of phosphorylase a to b, is that glycogenolysis was not lowered in EX2-HG and that glucose-6-phosphate accumulation appears to be a function of muscle glycogen content (Hultman & Sjøholm, 1983; Spencer & Katz, 1991).

The explanation for less lactate being accumulated and released from the active muscles cannot be an elevated turn-over within the muscle. Instead, the most likely explanation is that the formation of lactate is the net result of the rate of pyruvate and NADH production in the cytosol, and their uptakes by the mitochondria, the latter appearing to be unaltered in the present experiments. Lactate dehydrogenase is a near-equilibrium enzyme and so its flux is dictated by the concentrations of substrates and products. The smaller production of three-carbon skeleton via glycolysis would then result in less lactate being produced.

Muscle and blood lactate (and blood pH) at exhaustion were lower in EX2 than in EX1, which supports the notion that lactate and pH are not the only crucial 'fatigue

factors' (Edwards, 1983). In addition, nucleotide and CP metabolism were not affected. It should be emphasized that with the exercise model used in the present experiment, the site for fatigue is local. The systemic effects of one-legged dynamic knee extensor exercise are minor. Whole-body oxygen uptake is less than one-third of the subject's maximal oxygen uptake, heart activity is 120–140 beats/min and catecholamines are low (Bangsbo *et al.* 1990). Although rate of lactate production is high in the active muscle, and its release to the blood stream is high, elevations in systemic circulation are only 5–6 mM. Blood glucose is maintained above 4·5 mM. At point of exhaustion, defined as the inability to maintain the pre-set kicking rate (1 Hz), clear indications of fatigue were already present. Force recordings revealed a reduction in amplitude which was compensated for by an elongated contraction. Further, a tendency towards pushing the leg back into its vertical position was sometimes apparent the closer the exercise came to the point of exhaustion. Thus, definite objective signs of muscle fatigue were present. In this connection, it should be noted that there was no 'transfer' effect of fatigue, i.e. when the exercise was performed with leg 2 after leg 1 had exercised twice, time to exhaustion was identical when comparing EX1 with the control and HG leg.

These data speak in favour of prior exercise causing an excitation–contraction failure to develop faster when intense exercise is repeated. It is difficult to believe that the mechanism is related to a K^+/Na^+ imbalance across the sarcolemma or in the T-tubuli system as the normalization of these ions, although slow after a very fast early recovery, would have been completed by an hour of rest (Juel, 1986; Juel, Bangsbo, Graham & Saltin, 1990). Although somewhat less K^+ was released during EX2-HG, femoral venous K^+ concentrations were very similar in all exercise bouts. If this K^+ concentration reflects the interstitial concentration, it seems that exhaustion occurs at a well-defined K^+ imbalance in the muscle.

Fatigue at the level of cross-bridge interaction has also been reported (Edman, 1991), but there are no data indicating that it persists for very long. Two more possible causes should be considered, and they have in common that complete recovery after exhaustive exercise takes time. The Ca^{2+} kinetics of the SR system are affected by intense, exhaustive exercise with a marked slowing of the Ca^{2+} uptake, related to elongation of the half-relaxation time, and also a drop in maximal force development (Gollnick, Körge, Karpakka & Saltin, 1991). The time course for its normalization is more than 30 min, but exactly how long is unknown. The other possibility would be that a reduction in neural drive develops faster in EX2. It is unlikely that it is related to 'low frequency fatigue', as EX1 was very intense and short lasting. The same relates to recent findings that after prolonged exhaustive exercise the EMG activity was reduced in relation to the drop in maximal force development, which persisted long into recovery (Nicol, Komi & Marconnet, 1991). A more likely explanation may be that of a reduced nervous drive due to reflex inhibition at the spinal level as demonstrated by Garland & McComas (1990). Glycogen content may have a role, as performance was unaltered when exercising a second time with the high-glycogen leg (EX2-HG). Possible mechanisms linking the two phenomena are presently unknown.

In agreement with our previous observation (Bangsbo *et al.* 1990), the energy release from lactate production and utilization of CP and nucleotides during the first

exercise could account for 80–90 % of the total anaerobic energy production (Fig. 6). During the second exercise bout (EX2-C and EX2-HG) the alteration in these variables could only account for 60–70 % of the total anaerobic energy release. The lower glycolytic rate during EX2 indicates that the accumulation of three-carbon glycolytic intermediates is less during EX2. Similarly, it seems unlikely that the difference is related to a difference in the underestimation of the lactate production during EX2 due to lactate uptake by the knee flexor muscles, as the arterial blood lactate concentration was the same during EX1 and EX2. Another possibility could be that the energy demand during EX2 was lower compared to EX1 due to either a longer contraction time, although the power was the same (Kushmerick, 1985; di Prampero, Boutellier & Marguerat, 1988), or due to changes in factors influencing the free energy (ΔG) for ATP hydrolysis, such as increase in temperature, P_i and reduction in pH (Kawai, Guth, Winnikes, Haist & Ruegg, 1987; Cooke, Franks, Luciani & Pate, 1988). There was no indication of difference in the contraction pattern between EX1 and EX2, no difference in muscle temperature and probably none in free P_i. However, the lower muscle lactate at the end of EX2 indicated a higher muscle pH, which might have resulted in a higher energy release from ATP breakdown during EX2. If this was the case the estimated oxygen deficit could overestimate the true ATP production. Whether this factor can quantitatively account for the difference between EX1 and EX2 is, however, doubtful. Additional studies of the mechanical efficiency of *in vivo* muscle contractions, including possible variations in ΔG for the ATP hydrolysis, are needed to clarify this problem.

In conclusion we have found no coupling between above-normal muscle glycogen storage, enhanced glycogenolysis, glycolysis and performance. Prior, short, exhaustive exercise with only a small glycogen utilization followed by time for normalization of the acid–base balance of the muscle, caused a significant reduction in performance when the exercise was performed the second time. Glycogenolysis was also reduced and so was lactate production, but not the aerobic utilization of pyruvate and its contribution to ATP resynthesis.

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REFERENCES

ANDERSEN, P. (1975). Capillary density in skeletal muscle of man. *Acta Physiologica Scandinavica* **95**, 203–205.

ANDERSEN, P., ADAMS, R. P., SJØGAARD, G., THORBOE, A. & SALTIN, B. (1985). Dynamic knee extension as a model for the study of an isolated exercising muscle in man. *Journal of Applied Physiology* **59**, 1647–1653.

ANDERSEN, P. & SALTIN, B. (1985). Maximal perfusion of skeletal muscle in man. *Journal of Physiology* **366**, 233–249.

ARAGON, J. J., TORNHEIM, K. & LÖWENSTEIN, J. M. (1980). On a possible role of IMP in the regulation of phosphorylase activity in skeletal muscle. *FEBS Letters* **117**, K56–64.

ASMUSSEN, E., KLAUSEN, K., NIELSEN, L, EGELUND, L., TECHOW, O. S. A. & TØNDER, P. J. (1974). Lactate production and anaerobic work capacity after prolonged exercise. *Acta Physiologica Scandinavica* **90**, 731–742.

BANGSBO, J., GOLLNICK, P. D., GRAHAM, T. E., JUEL, C., KIENS, B., MIZUNO, M. & SALTIN, B. (1990). Anaerobic energy production and O_2 deficit – debt relationship during exhaustive exercise in humans. *Journal of Physiology* **422**, 539–559.

BANGSBO, J., GOLLNICK, P. D., GRAHAM, T. E. & SALTIN, B. (1991). Substrates for muscle glycogen synthesis in recovery from intense exercise in man. *Journal of Physiology* **434**, 423–440.

BARATTA, R. U., ICHIE, M., HURRING, S. & SOLOMMON, N. I. (1989). A method for studying muscle properties under orderly stimulated motor units with tripolar nerve cuff electrodes. *Journal of Biomedical Engineering* **11**, 141–147.

BERGSTRÖM, J., HERMANSEN, L., HULTMAN, E. & SALTIN, B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica* **71**, 140–150.

BOOBIS, L. H. (1987). Metabolic aspects of fatigue during sprinting. In '*Exercise. Benefits, Limits and Adaptations*', ed. MACLEOD, D., MAUGHAN, R., NIMMO, M., REILLY, T. & WILLIAMS, C., pp. 116–143. E & F. N. Spon, London/New York.

BOOBIS, L. H., WILLIAMS, C. & WOOTTON, S. A. (1983). Influence of sprint training on muscle metabolism during brief maximal exercise in man. *Journal of Physiology* **342**, 36–37*P*.

BROBERG, S. & SAHLIN, K, (1989). Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *Journal of Applied Physiology* **67**, 116–122.

BROOKE, M. H. & KAISER, K. K. (1970). Three "myosin adenosine trisphosphatase" systems: The nature of their pH lability and sulphydryl dependence. *Journal of Histochemistry and Cytochemistry* **18**, 670–672.

BROUN, B. W. & HOLLANDER, M. (1977). *Statistics. A Biomedical Introduction*. Wiley, New York.

CHASIOTIS, D. (1983). The regulation of glycogen phosphorylase and glycogen breakdown in human skeletal muscle. *Acta Physiologica Scandinavica Supplementum* **518**, 1–68.

CHASIOTIS, D. (1988). Role of cyclic AMP and inorganic phosphate in the regulation of muscle glycogenolysis during exercise. *Medicine and Science in Sport and Exercise* **20** (6), 545–550.

CHASIOTIS, D., SAHLIN, K. & HULTMAN, E. (1982). Regulation of glycogenolysis in human muscle at rest and during exercise. *Journal of Applied Physiology* **53** (3), 708–715.

CHRISTENSEN, E. H. & HANSEN, O. (1939). Arbeitsfahigkeit und ernahrung. *Skandinavische Archiv für Physiologie* **81**, 160–171.

COOKE, R., FRANKS, K., LUCIANI, G. B. & PATE, E. (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *Journal of Physiology* **395**, 77–97.

DI PRAMPERO, P. E., BOUTELLIER, U. & MARGUERAT, A. (1988). Efficiency of work performance and contraction velocity in isotonic tetani of frog sartorius. *Pflügers Archiv* **412**, 455–461.

DRABKIN, D. L. & AUSTIN, J. H. (1935). Spectrophotometric studies II. Preparations from washed blood cells, nitric oxide hemoglobin and sulfhemoglobin. *Journal of Biological Chemistry* **122**, 51–65.

EDMAN, K. A. P. (1991). The contractile performance of normal and fatigued skeletal muscle. In *Fatigue Mechanisms in Exercise and Training*, ed. MARCONNET, P., KOMI, P., SALTIN, B. & SEJERSTED, O. S. Karger, Basel, München, Paris, London and Sydney.

EDWARDS, R. H. T. (1983). Biochemical bases of fatigue in exercise performance: Catastrophe theory of muscular fatigue. In *Biochemistry of Exercise*, ed. KNUTTGEN, H. C., VOGEL, J. A. & POORTMANS, J., pp. 3–29. Human Kinetics, Champeign, IL, USA.

GARLAND, S. J. & MCCOMAS, A. J. (1990). Reflex inhibition of human soleus muscle during fatigue. *Journal of Physiology* **429**, 17–27.

GOLLNICK, P. D., KÖRGE, P., KARPAKKA, J. & SALTIN, B. (1991). Elongation of skeletal muscle relaxation during exercise is linked to reduced calcium uptake by the sarcoplasmic reticulum in man. *Acta Physiologica Scandinavica* **142**, 135–136.

GREENHAFF, P. L., GLEESON, M. & MAUGHAN, R. J. (1987). The effects of dietary manipulation on blood acid–base status and the performance of high intensity exercise. *European Journal of Applied Physiology* **56**, 331–337.

GREENHAFF, P. L., GLEESON, M. & MAUGHAN, R. J. (1988). The effects of a glycogen loading regimen on acid–base status and blood lactate concentration before and after a fixed period of high intensity exercise in man. *European Journal of Applied Physiology* **57**, 254–259.

GRISDALE, R. K., JACOBS, I. & CAFARELLI, E. (1990). Relative effects of glycogen depletion and previous exercise on muscle force and endurance capacity. *Journal of Applied Physiology* **69** (4), 1276–1282.

HOLMGREN, A. & PERNOW, B. (1959). Spectrophotometric determination of oxygen saturation of blood in the determination of cardiac output. *Scandinavian Journal of Clinical Laboratory Investigation* **11**, 143–149.

HULTMAN, E. & SJØHOLM, H. (1983). Substrate availability. In *Biochemistry of Exercise*, ed. KNUTTGEN, H. C., VOGEL, J. A. & POORTMANS, J. pp. 63–75. Human Kinetics, Champeign, IL, USA.

JACOBS, I. (1981). Lactate concentrations after short, maximal exercise at various glycogen levels. *Acta Physiologica Scandinavica* **111**, 465–469.

JANSSON, E. (1980). Muscle enzyme adaptation to diet in man. In *Biochemistry of Exercise* **13**, 299.

JONES, P. R. M. & PEARSON, J. (1969). Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *Journal of Physiology* **204**, 36*P*.

JUEL, C. (1986). Potassium and sodium shifts during in vitro isometric muscle contractions, and the time course of the ion-gradient recovery. *Pflügers Archiv* **406**, 458–463.

JUEL, C., BANGSBO, J., GRAHAM, T. & SALTIN, B. (1990). Lactate and potassium fluxes from skeletal muscle during intense dynamic knee-extensor exercise in man. *Acta Physiologica Scandinavica* **140**, 147–159.

KAWAI, M., GUTH, K., WINNIKES, K., HAIST, C. & RUEGG, J. C. (1987). The effect of inorganic phosphate on ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. *Pflügers Archiv* **408**, 1–9.

KLAUSEN, K. & SJØGAARD, G. (1980). Glycogen stores and lactate accumulation in skeletal muscle of man during intense bicycle exercise. *Scandinavian Journal of Sports Science* **2**, 7–12.

KUSHMERICK, M. J. (1985). Patterns in mammalian muscle energetics. *Journal of Experimental Biology* **115**, 165–177.

LOWRY, O. H. & PASSONNEAU, J. V. (1972). *A Flexible System on Enzymatic Analysis*. Academic Press, New York.

MAUGHAN, R. J. & POOLE, D. C. (1981). The effects of a glycogen loading regimen on the capacity to perform anaerobic exercise. *European Journal of Applied Physiology* **46**, 211–219.

NEWSHOLME, E. A. & LEECH, A. R. (1983). *Biochemistry for the Medical Sciences*. John Wiley and Sons, New York.

NICOL, C., KOMI, P. V. & MARCONNET, P. (1991). Fatigue effects of marathon running on neuromuscular performance. II. Changes in force, integrated electromyographic activity and endurance capacity. *Scandinavian Journal of Medicine and Science in Sports* **1** (1), 18–24.

NORMAN, B., SOLLEVI, A. & JANSSON, E. (1988). Increased IMP content in glycogen-depleted muscle fibres during submaximal exercise in man. *Acta Physiologica Scandinavica* **133**, 97–100.

PADYKULA, H. A. & HERMAN, E. (1955). The specificity of the histochemical method for adenosine triphosphate. *Journal of Histochemistry and Cytochemistry* **3**, 170–183.

REN, J. M., BROBERG, G., SAHLIN, K. & HULTMAN, E. (1990). Influence of reduced glycogen level on glycogenolysis during short-term stimulation in man. *Acta Physiologica Scandinavica* **139**, 467–474.

REN, J. M. & HULTMAN, E. (1990). Regulation of phosphorylase a activity in human skeletal muscle. *Journal of Applied Physiology* **69** (3), 919–923.

RICHTER, E. A. & GALBO, H. (1986). High glycogen levels enhance glycogen breakdown in isolated contracting skeletal muscle. *Journal of Applied Physiology* **61**, 827–831.

RICHTER, E. A., MIKINES, K. J., GALBO, H. & KIENS, B. (1989). Effect of exercise on insulin action in human skeletal muscle. *Journal of Applied Physiology* **66**, 876–885.

SALTIN, B. (1985). Hemodynamic adaptations to exercise. *American Journal of Cardiology* **55**, 42–47D.

SALTIN, B. & HERMANSEN, L. (1967). Glycogen stores and prolonged severe exercise. In *Symposium of Swedish Nutrition Foundation*, ed. BLIX, G., pp. 32–46. Almquist and Wiksell, Uppsala.

SALTIN, B., KIENS, B. & SAVARD, G. (1986). A quantitative approach to the evaluation of skeletal muscle substrate utilization in prolonged exercise. In *Biochemical Aspects of Physical Exercise*, ed. BENZI, G., PACKER, L. & SILIPRANDI, N., pp. 235–244. Elsevier Science Publisher B.V.

SCHWEINSBERG, P. D. & LOO, T. L. (1980). Simultaneous analysis of ATP, ADP, AMP and other purines in human erythrocytes by HPLC. *Journal of Chromotography* **181**, 103–107.

SHIMIZU, S., INOUE, K., TANI, Y. & YAMADA, H. (1979). Enzymatic microdetermination of serum free fatty acids. *Analytical Biochemistry* **98**, 341–345.

SIEGEL, S. (1965). *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York.

SPENCER, M. K. & KATZ, A. (1991). Role of glycogen in control of glycolysis and IMP formation in human muscle during exercise. *American Journal of Physiology* **260**, E859–864.

SPRIET, L. L., BERARDINUCCI, L., MARSH, D. R., CAMPBELL, C. B. & GRAHAM, T. (1990). Glycogen content has no effect on skeletal muscle glycogenolysis during short-term tetanic stimulation. *Journal of Applied Physiology* **68** (5), 1883–1888.

SYMONS, J. D. & JACOBS, I. (1989). High-intensity exercise performance is not impaired by low intramuscular glycogen. *Medicine and Science in Sports and Exercise* **21** (5), 550–557.

UYEDA, K. (1979). Phosphofructokinase. *Advances in Enzymology and Related Areas of Molecular Biology* **48**, 193–244.