Acta physiol. scand. 1967. 71. 129—139

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Muscle Glycogen during Prolonged Severe Exercise

By

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Received 28 March 1967

Abstract

Hermansen, L., E. Hultman and B. Saltin. *Muscle glycogen during prolonged severe exercise.* Acta physiol. scand. 1967. *71.* 129—139.

10 well trained and 10 untrained subjects worked to complete exhaustion on a bicycle ergometer with work loads averaging 77 (76—87) per cent of their individual maximal aerobic power. Determinations of glycogen used by working muscles (biopsy of lateral portion of the quadriceps femoris muscles) and of combusted carbohydrate ($\dot{V}_{O_2}$ and RQ) were performed at certain intervals from the start of work to exhaustion. At a combustion rate of about 3 g carbohydrate per minute (RQ around 0.9 or higher) and at average values for glycogen in resting muscle of 1.6 (1.1—2.5)g/100 wet muscle, the effective work time was around 85 min for the untrained and 90 min for the trained subjects. At the end of the exhaustive exercise the glycogen content averaged 0.06 g in the untrained and 0.12 g/100 g wet muscle in the trained subjects. A close relationship between utilized glycogen and combusted carbohydrate was found, and it seems highly probable that at high relative workloads primarily the glycogen stores in the exercising muscles will limit the capacity for prolonged strenuous work.

The relative role of different fuels utilized during various types and phases of muscular exercise has been widely discussed for about a century. It is generally agreed that protein plays a non-essential role (Margaria and Foa 1939). In 1896 Chauveau showed that the ventilatory exchange ratio (V.E.R.=R.Q.) increased from 0.75 during rest to 0.95 during exercise, which he considered proved an increased utilization of carbohydrates during exercise; but as the RQ was below 1.0 he concluded that fat was also used. Zuntz *et al.* (1894) demonstrated that diet could markedly influence the RQ during rest and exercise and Krogh and Lindhard (1920) confirmed that observation. The question of whether or not the RQ during exercise is a valid indicator of the relative proportions of fat and carbohydrate used by the muscle during exercise was investigated by Christensen and Hansen (1939 a). On the basis of measurements of the CO_2 and lactate content of the blood they concluded that the RQ measurements even at high but not maximal work levels after 10—15 min of exercise gave a true representation of the fuels utilized (true RQ). Christensen and Hansen (1939 d) also demonstrated that the RQ was dependent on the work level, and well-trained subjects had a lower RQ at the same external work load compared

with untrained subjects. During prolonged exercise as well as after a high fat diet, the RQ was lower indicating increased utilization of fat (Christensen and Hansen 1939 b, c). More recent studies have emphasized the role of fat as an important fuel for muscular exercise (for references see Rodahl and Issekutz 1964). During exercise the arterial concentration of FFA shows a transient decrease, followed by an increase (Friedberg *et al.* 1960 and Carlsson and Pernow 1961), which also represents an increased utilization of FFA. For example, Havel *et al.* (1963) have estimated that 41—49% of the energy output is derived from the direct oxidation of FFA during long sustained work compared to 25—26% during rest. Furthermore, Paul, Issekutz and Miller (1966) have shown, during prolonged exercise in dogs, that 90—95% of the extramuscular sources for energy are derived from fat. In view of these recent observations the concept, based on RQ-determination during exercise, that carbohydrates are the predominante fuel for heavy exercise has been questioned.

A muscle biopsy technique (Bergström 1962) permits a more precise determination of the glycogen utilization during exercise (Hultman 1967a, Ahlborg *et al.* 1967). The aim of the present study was to compare the actual amount of glycogen utilized by an exercising muscle with the calculated carbohydrate usage based on the RQ and O_2 uptake in an attempt to reinvestigate the concept that carbohydrate is the major fuel for strenous muscular exercise. As training might conceivably influence the energy utilization, the study was performed on both untrained and well-trained subjects.

Subjects

The subjects were 20 healthy males, age 20—30. Ten of the subjects had not performed regular training during the last 5 years, and their maximal oxygen uptake averaged 3.4 l/min (42—56 ml/kg min). The remaining 10 subjects trained intensively at least a couple of times per week all year round and all competed in endurance events. Their maximal oxygen uptake averaged 4.6 l/min (60—72 ml/kg min). The average height of all the subjects was 179 cm and the weight of the untrained subjects was 73 and 67 kg for the trained subjects. In an additional experiment, 8 physical education students were studied. They were all somewhat trained and their body weight averaged 71 kg, height 177 cm, and maximal oxygen uptake 4.1 l/min.

Methods and procedure

Expired air for the determination of oxygen uptake was collected in Douglas' bags and the volume measured in a spirometer (Saltin and Åstrand 1967). The gas was analyzed with a modified Haldane technique. Heart rate was determined by counting at least 30 R-R intervals from ECG recordings. Lactate and pyruvate were determined enzymatically on fingertip blood (Scholtz *et al.* 1959) and glucose by the orthototuidue method (Hultman 1959). The muscle glycogen content was determined according to the method described by Hultman (1967a). The subject's maximal oxygen uptake (Åstrand and Saltin 1961) as well as a check of their ability for prolonged heavy exercise were performed a couple of days before the actual experiment. On the experimental day, the subjects came to the laboratory in the morning after having had a cup of coffee or tea and a single sandwich. No special instructions were given for their diet and physical activities the days preceding the experiment, but no one had trained the day before the study. The subjects rested for at least 15 min in the laboratory before the basal determinations were made. These included a muscle biopsy for glycogen content, oxygen uptake, heart rate, blood lactate, pyruvate and glucose. The subjects

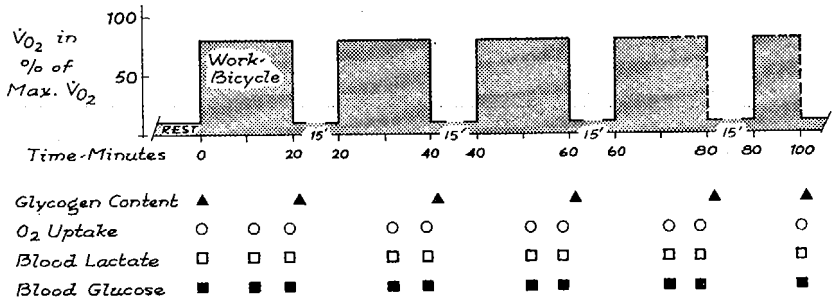

Fig. 1. The general procedure for the intermittent exercise experiments. For a more detailed description see the text.

then started to exercise on a bicycle ergometer (Krogh or Monark) with a constant pedal frequency of 50 rpm at a work load which averaged 77 (71—87) per cent of each individual's maximal oxygen uptake. Each 20 min period of exercise (I—V Table I) was followed by 15 min rest (see Fig. 1). The experimental design required that the subjects exercised to exhaustion. During the experiment the subjects had to drink a 0.15% saline solution and they kept their body weight almost constant. Around the 11th and 19th minute of the exercise periods oxygen uptake and heart rate was determined and a blood sample was drawn from a fingertip prewarmed in warm water for lactate, pyruvate and glucose determinations. Within the first couple of minutes of each rest period, the muscle biopsy was performed; the biopsy needle and the technique used have been described by Bergström (1962). After cutaneous local anesthesia an incision was made in the skin and the needle was introduced into the deeper part of the lateral portion of the quadriceps. A new incision was made for each biopsy. Usually, three biopsies were made in each thigh, starting distally.

In 4 subjects, (3 trained and 1 untrained) the prolonged exercise was repeated a few weeks later in a similar manner as described above. The only exception was that the second time no rest was permitted and the exercise was continuous until exhaustion. The incisions in the skin for the biopsies were all made before the exercise started. The biopsies were then made at 20 min intervals with the subjects sitting on the bicycle; the interruption in the continuous pedaling was limited to only 15—30 sec.

In the additional set of experiments the 8 physical education students performed three one-hour exercise periods at weekly intervals at work levels of about 25, 50 and 80 per cent of their maximal oxygen uptake.

Results

Table I summarizes the results after statistical treatment. The oxygen uptake was almost constant for the different work periods I—V thruoghout the experiment (Fig. 2). The average oxygen uptake for the untrained subjects was 2.8 l/min or 79 (73—87) per cent of their maximal oxygen uptake and the corresponding figures for the trained group were 3.4 l/min or 76 (71—80) per cent. After 11 min of exercise the heart rate was 164 for the trained and 172 for the untrained subjects. The rate inreased throughout the work period though never reaching maximal level (Fig. 2). In the untrained group the RQ during the first work period was 0.96 and dropped to 0.93 at the end of the exercise (Fig. 2). The RQ was 0.91 after 11 min of exercise in the trained group and even after 80 min of exercise it was still 0.90 but decreased to 0.88 during the last minutes of work. The carbohydrate combusted during the exercise (Fig. 2 bottom) was calculated from the average oxygen uptake and RQ in each work period. The average utilization of carbohydrates was for both groups

TABLE I. Average values, standard error of estimate, standard deviation and range for different the untrained (U) groups. At rest and during the exercise periods I—IV the number trained and 9 trained subjects

		Rest	I	II
		0.27±0.03	2.78±0.11	2.85±0.10
	U	0.08	0.36	0.32
Oxygen uptake		0.17—0.33	2.3—3.3	2.4—3.3
l/min		0.28±0.02	3.39±0.10	3.32±0.10
	T	0.06	0.31	0.32
		0.19—0.33	3.0—3.8	3.0—3.7
		0.83±0.01	0.96±0.01	0.94±0.01
	U	0.04	0.03	0.03
RQ		0.75—0.91	0.91—1.00	0.90—0.99
		0.82±0.02	0.91±0.01	0.90±0.01
	T	0.06	0.03	0.03
		0.74—0.92	0.86—0.97	0.84—0.99
		0.14	2.92±0.18	2.90±0.19
	U		0.57	0.59
Combusted		0.09—0.18	2.1—3.6	2.0—3.8
carbohydrates g/min		0.13	2.89±0.20	2.63±0.20
	T		0.63	0.62
		0.08—0.18	2.4—3.6	1.4—3.5
		1.58±0.10	0.66±0.11	0.44±0.11
	U	0.31	0.36	0.33
Glycogen content		1.25—2.21	0.23—1.48	0.04—1.09
g/100 g wet muscle		1.69±0.12	0.99±0.13	0.62±0.14
	T	0.39	0.40	0.44
		1.10—2.49	0.43—1.80	0.12—1.34
		93±2.6	83±3.4	86±3.4
	U	8.3	10.7	10.8
Blood glucose		81—110	62—97	69—107
mg/100 ml		87±2.5	82±3.3	85±4.2
	T	7.9	10.5	13.3
		78—101	63—100	69—106
		1.1±0.31	6.6±0.89	5.6±0.89
	U	0.5	2.1	2.8
Blood lactate		0.6—1.5	2.5—9.8	1.5—11.5
mM		0.9±0.28	5.1±0.79	3.7±0.60
	T	0.5	2.4	1.9
		0.5—1.3	1.7—9.2	1.5—7.2
		0.09±0.01	0.25±0.01	0.23±0.02
	U	0.02	0.04	0.05
Blood pyruvate		0.05—0.13	0.18—0.32	0.13—0.29
mM		0.08±0.01	0.21±0.02	0.19±0.02
	T	0.02	0.05	0.06
		0.05—0.12	0.15—0.30	0.10—0.26

parameters studied at rest and during each work period to exhaustion for the trained (T) and of subjects are 10 in the trained and untrained groups. In exercise period V there are 7 un-

III	IV	V	Paired t-test
2.80±0.11	2.78±0.11	2.79±0.08	
0.35	0.37	0.25	0
2.4—3.3	2.3—3.4	2.3—3.1	
3.36±0.10	3.41±0.11	3.37±0.12	
0.32	0.33	0.37	0
3.1—3.6	3.0—3.8	2.4—4.0	
0.94±0.01	0.93±0.01	0.92±0.01	
0.03	0.03	0.04	0
0.88—0.99	0.87—0.99	0.83—0.97	
0.89±0.01	0.90±0.01	0.88±0.01	
0.04	0.04	0.04	0
0.83—0.99	0.83—0.99	0.83—0.99	
2.76±0.20	2.78±0.21	2.72±0.24	
0.62	0.64	0.76	0
1.8—3.5	1.8—3.6	1.6—3.5	
2.62±0.20	2.81±0.21	2.58±0.25	
0.63	0.65	0.79	0
1.8—3.3	1.7—4.0	1.4—3.2	
0.16±0.05	0.07±0.04	0.06±0.04	R> I, II, III, IV, V
0.23	0.12	0.10	I> II, III, IV, V
0—0.67	0—0.28	0.0—0.26	II> III, IV, V; III> V
0.24±0.09	0.17±0.07	0.12±0.06	R> I, II, III, IV, V
0.27	0.21	0.18	I> II, III, IV, V, II>
0.03—0.86	0.01—0.60	0.01—0.46	III, IV, V, III,> IV, V
85±3.6	82±2.2	82±2.2	
11.3	6.8	6.2	0
71—101	72—96	72—91	
84±4.2	81±4.8	72±5.7	
13.4	15.0	17.1	0
65—108	63—102	64—104	
4.6±0.79	3.5±0.69	2.9±0.71	I> III, IV, V
2.4	2.1	2.2	II> III, IV, V
1.1—8.9	0.9—8.6	1.3—5.6	III> IV, V
3.2±0.48	2.8±0.45	2.8±0.66	I> II, III, IV, V
1.5	1.4	2.0	II> IV, V
1.3—5.8	1.1—5.5	0.8—6.2	
0.21±0.02	0.20±0.02	0.19±0.01	I> III, IV, V
0.05	0.06	0.04	II> III, IV, V
0.14—0.28	0.09—0.29	0.14—0.26	
0.19±0.02	0.18±0.02	0.19±0.02	I> II, III, IV, V
0.05	0.06	0.07	
0.13—0.25	0.12—0.26	0.09—0.24	

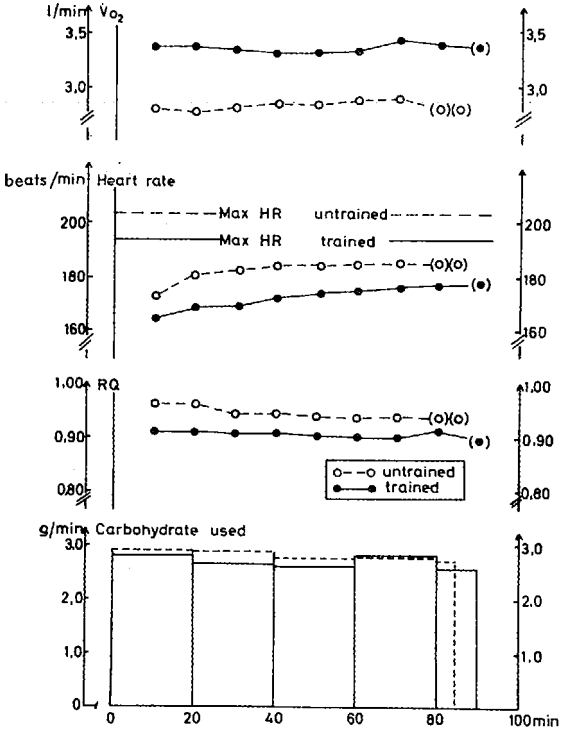

Fig. 2. Average values for the 10 trained (filled symbols) and the 10 untrained (unfilled symbols) subjects for oxygen uptake ($\dot{V}_{O_2}$), heart rate, ventilatory exchange ratio (RQ) and used carbohydrates during exercise to exhaustion. The symbols within parenthesis denotes 7 subjects (untrained) and 9 subjects (trained).

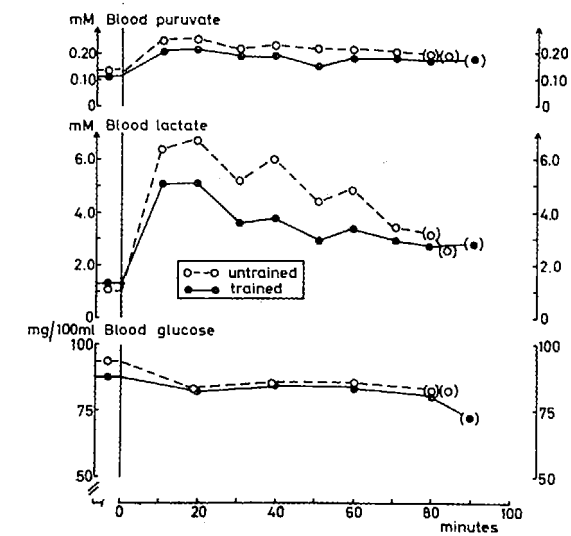

Fig. 3. Average values for blood pyruvate, lactate and glucose at rest before exercise started and during the exercise to exhaustion in the trained and untrained group.

2.8 g/min, and varied slightly during the prolonged exercise. Paired t-test of intra individual differences in RQ and combusted carbohydrates showed no significant difference between the work periods (Table I). The blood pyruvate concentration during work was slightly higher in the untrained compared to the trained and the concentration remained in both groups fairly stable throughout the whole work

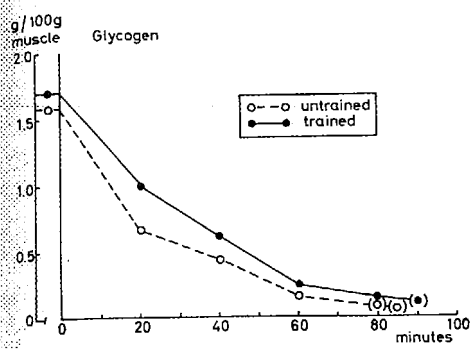

Fig. 4.

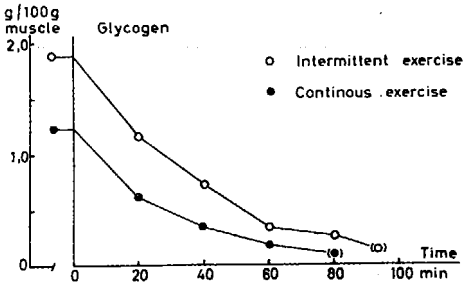

Fig. 5.

Fig. 4. Average values for glycogen content in the lateral portion of the quadriceps muscle before and during exercise until exhaustion in the trained and the untrained group.

Fig. 5. A comparison of the muscle glycogen content during prolonged exercise to exhaustion with 20 min work periods and 20 min rest (unfilled symbols) and continuous exercise (filled symbols) in 4 subjects. The symbols within parenthesis denote only two subjects.

period (Fig. 3). The blood lactate reached its highest level during the first work period and then declined (Fig. 3). The untrained subjects average peak value was 6.8 compared to 5.1 mM in the trained. In both groups, at the end of exercise before exhaustion, the blood lactate concentration approached the resting level. Before work the mean blood glucose concentration (lower part Fig. 3) was 93 in the untrained and 87 mg/100 ml blood in the trained group. At the end of the first 20 min of exercise it dropped to 83 and 82 mg/100 ml respectively, and remained at almost constant level until exhaustion.

At rest (work time 0), the average values for the muscle glycogen content were 1.58 and 1.69 g/100 g wet muscle for the untrained and the trained respectively (Fig. 4). The glycogen reduction was greatest during the first work period. Thereafter a slower removal rate was found, which was somewhat more pronounced in the untrained. Glycogen content averaged 0.06 in the untrained and 0.12 g/100 g muscle in the trained. In Fig. 5 is shown the reduction of the muscle glycogen in continuous and intermittent work on the same work load in 4 subjects. The general appearance of the curve as well as the other parameters studied (HR, blood pyruvate, lactate and glucose) were essentially the same during the intermittent and the continuous exercise even though the initial concentration of glycogen was lower in the second set of experiments; but the total work time was also shorter.

An increase in work level from 28 to 54 per cent of the subjects maximal oxygen uptake increased the utilization of glycogen in the working muscle from 0.31 to 0.83 g/100 muscle per hour (Fig. 6). When the average relative work load was increased to 78 per cent of the maximal oxygen uptake the glycogen utilization rose to 1.56 g/100 g muscle for the one hour work period. Fig. 7 demonstrates the relationship between the amount of carbohydrate combusted (estimated from $\dot{V}_{O_2}$ and RQ) during the exercise period and the amount of glycogen utilized in the exercising muscle during the same period of time.

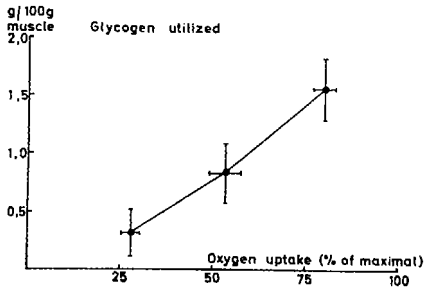

Fig. 6.

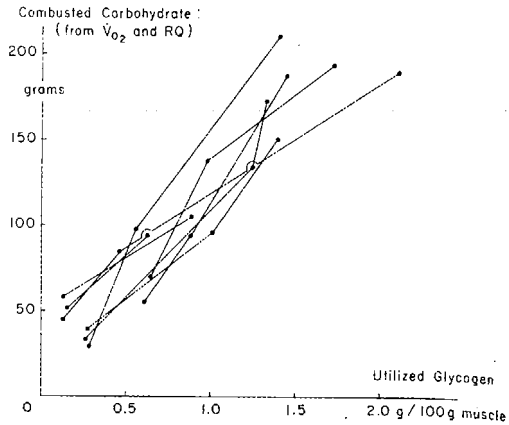

Fig. 7.

Fig. 6. Decrease of muscle glycogen after 1 hr of exercise at three different work levels, mean ± SD, n = 8.

Fig. 7. Individual values for the amount of utilized glycogen during the one hour exercise period at different work levels in relation to the calculated amount of combusted carbohydrates.

Discussion

The subjects in this study performed strenuous exercise and worked to complete exhaustion. Among the parameters studied, neither oxygen uptake nor blood concentration of pyruvate, lactate or glucose ever reached values that revealed or parallelled the subjects' fatigue or explained the exhaustion. Probably dehydration can also be excluded as an explanation of the fatigue as the subjects' body weight reduction during the experiment was kept below 1 kg (Saltin 1964 part 2). The subjects were obviously tired at the end of each work period because of the high relative work level. However, they were unable to continue pedaling only when the glycogen content reached extremely low values. The rate of reduction of glycogen in the exercising muscle was most marked at the first exercise period. Thereafter beeing lower but relatively constant in two work periods and during the last work period very small. In contrast to this, the calculated amount of carbohydrate combusted was unchanged throughout the whole work and averaged 2.8 g/min.

It is worth emphasizing that the pattern of the glycogen decrease with time was the same during continuous exercise, as when intermittent work was performed (*cf.* Fig. 5). The steeper fall in the muscle glycogen during the first work period (Fig. 4) may be explained by a greater production of lactate giving a greater accumulation of lactate in blood in the early phases of exercise. This is further shown in this series where higher blood lactate values and faster reduction in glycogen was observed in the untrained subjects, compared to the trained.

A straight line through the values for the glycogen content at 20, 40 and 60 min of exercise probably gives the best indication of the glycogen removal due to aerobic processes. As the exercise proceeded to the period when the glycogen stores in the working muscles were almost emptied, extra muscular sources must supply some additional carbohydrate if the calculated value of 2.8 g carbohydrate/min is

correct. The only possible sources for this carbohydrate are the liver glycogen and the small amount of glucose in the extra cellular fluid. Rowell *et al.* (1965) have shown that the net glucose production from the splanchnic area during 60 min of exercise averaged 0.3 g/min. There is some evidence that during prolonged severe exercise the splanchnic production of glucose is low at the beginning of exercise but increases with time (Hultman, 1967b). If so, the amount of glucose available from the liver may account for the finding that the exercising muscle at a the liver may account for the finding that the exercising muscle at a very high relative work load combusts mainly carbohydrate also at the end of exercise, even though the muscle glycogen content is close to zero. It should be remembered that glycogen is determined only in the lateral portion of quadriceps, and RQ is determined for the whole body. This may mean that other muscle groups or other parts of the same muscle richer in glycogen gradually becomes more active. This seems, however, unlikely as the oxygen uptake during work and therefore the mechanical efficiency is fairly constant throughout the exercise period.

When exhausted, the subjects were able to resume work at a lower level of activity. The inability to perform heavy exercise was not due, to a reduction in muscular strength, it could be shown in an earlier study that the maximal isometric strength was unchanged after prolonged exhausting exercise (Saltin 1964).

In the present experiments special care was taken to eliminate errors in order to get a true RQ. All subjects were very well aquainted with the experimental procedure and they had been subjects several times before. During exercise the collection of expired air started after 11 min of exercise. Frequently, two determinations of the oxygen uptake were made consecutively and the RQ's were always identical within the error of the gas analyses (Haldane). In spite of differences in blood lactate, variations in the measured RQ in each individual was very small (see Table 1). Our impression is, therefore, that the calculated values for RQ accurately estimate the true RQ. This opinion is supported by the results presented in Fig. 7 showing a close relationship between utilized muscle glycogen and combusted carbohydrate calculated from the RQ. Furthermore in a similar study, where the muscle glycogen content was varied over a wide range, a very good relationship was found between the calculated value for combusted carbohydrate and the used glycogen, determined by direct measurement in muscle biopsy specimens (Bergström *et al.* (1967).

In Christensen and Hansen's experiment (1939 b) the relative work load was around 60—70 per cent, and the RQ dropped from 0.88 to 0.80 during 180 min work. The same tendency, with a decreasing carbohydrate combustion as work proceeded, was found in Saltin and Stenberg's study (1964), in which the RQ dropped from 0.91 to 0.86 during 3 hrs exercise at a relative work level of 72 per cent.

In contrast to these results Hedman (1957) found that during cross-country skiing for 150 min at constant speed with a relative work load of 82 per cent, RQ was essentially constant from start to finish. The carbohydrate combustion was in this study close to 3 g/min, and the total consumption of carbohydrate was 445 g for the whole run. The results of the present study with relative work loads of 76 and 79

per cent are in agreement with these findings. At a relative work load of 76 per cent or higher the muscles obviously are not able to substitute carbohydrate with fat even when the available glycogen stores are emptied. At lower relative work loads exercise can be performed with a markedly lower utilization of glycogen in the active muscle (*cf.* Fig. 6). The average RQ:s were 0.87, 0.90 and 0.93 at oxygen uptakes of 1.2, 2.2 and 3.2 l/min respectively representing 29, 53 and 78 per cent of the subjects maximal oxygen uptake. When the relative work load increased from 29 to 78 per cent the fat combustion changed only from 2.5 to 3.6 kcal/min compared to a change from 3.4 to 12.3 kcal/min for carbohydrate.

It is highly probable that at high relative work loads with RQ:s around 0.9, the glycogen stores in the exercising muscles is a decisive for maximal work time. Thus the capacity for prolonged strenuous work may be limited by the initial muscle glycogen content. Whether or not lack of glycogen in the muscle at the end of the exercise is the sole physiological determinant for the physical exhaustion can, however, not be settled at present.

Supported by Swedish Medical Research Council project nr. 26X-792-01 and Förenade Liv (United Life Insurance Company) Stockholm, and the Swedish Sport Federation, Stockholm, Sweden.

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Diet, Muscle Glycogen and Physical Performance

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Received 23 February 1967

Abstract

BERGSTRÖM, J., L. HERMANSEN, E. HULTMAN and B. SALTIN. *Diet, muscle glycogen and physical performance.* Acta physiol. scand. 1967. *71.* 140—150.

The muscle glycogen content of the quadriceps femoris muscle was determined in 9 healthy subjects with the aid of the needle biopsy technique. The glycogen content could be varied in the individual subjects by instituting different diets after exhaustion of the glycogen store by hard exercise. Thus, the glycogen content after a fat + protein (P) and a carbohydrate-rich (C) diet varied maximally from 0.6 g/100 g muscle to 4.7 g. In all subjects, the glycogen content after the C diet was higher than the normal range for muscle glycogen, determined after the mixed (M) diet. After each diet period, the subjects worked on a bicycle ergometer at a work load corresponding to 75 per cent of their maximal O_2 uptake, to complete exhaustion. The average work time was 59, 126 and 189 min after diets P, M and C, and a good correlation was noted between work time and the initial muscle glycogen content. The total carbohydrate utilization during the work periods (54—798 g) was well correlated to the decrease in glycogen content. It is therefore concluded that the glycogen content of the working muscle is a determinant for the capacity to perform long-term heavy exercise. Moreover, it has been shown that the glycogen content and, consequently, the long-term work capacity can be appreciably varied by instituting different diets after glycogen depletion.

It was shown in previous studies that the muscle glycogen content decreased during work (Bergström and Hultman 1966 a), and that during exhaustive exercise the glycogen stores were almost completely emptied (Bergström and Hultman 1967). In a study by Hermansen, Hultman and Saltin (1967), it was found that the rate of combustion of carbohydrates was extremely high and constant throughout the whole work period. In this study, as well as in that of Ahlborg *et al.* (1967a), there were some indications that the initial muscle glycogen concentration was related to the ability to perform prolonged, heavy exercise (measured as the work time), provided that the subjects worked with the same relative load.

The muscle glycogen concentration in man can be considerably increased by first emptying the glycogen stores through hard work, and then giving a carbohydrate-rich diet. The enhancement of glycogen synthesis is localized to the muscles that have worked, and does not affect other muscle groups (Bergström and Hultman

TABLE I. Pertinent data in the nine subjects

Subject	Age yrs	Ht cm	B.W. kg	Max. oxygen uptake l/min
T.P.	22	179	76	4.93
B.T.	22	176	69	4.03
Å.L.	26	173	62	3.37
R.S	20	174	75	3.94
S.P.	25	179	67	3.77
S.-O.J.	23	182	72	4.96
C.F.	24	184	71	4.62
R.E.	23	173	62	3.57
K.-G.G.	24	177	73	4.46

1966 b). On the other hand, a fat + protein diet after exercise induces only a slow, incomplete resynthesis of glycogen, and if carbohydrate is given without previous exercise, only a moderate increase in muscle glycogen takes place (Hultman and Bergström 1967). Thus, by varying the type of diet after exhaustive exercise, it is possible to obtain different muscle glycogen levels in the same individual.

As early as 1939, Christensen and Hansen showed that the capacity for prolonged exercise can be markedly varied by varying the subject's diet. After 3—7 days of predominantly carbohydrate intake, the work time was 210 min on a fixed load, compared to only 80 min after an equal time on a fat diet.

The aim of the present study was to determine the extent to which the muscle glycogen content could be altered in individual subjects by varying the dietary regime after depletion of the glycogen store, and subsequently to ascertain the relation between the initial glycogen content and the capacity for prolonged hard exercise.

Material and methods

Nine physical education students participated in this study; some pertinent data regarding them are given in Table I.

The methods used are described in the previous article (Hermansen, Hultman and Saltin 1967), except for the blood glucose determination which, in this study, was made by a glucose oxidase method (Hjelm and de Verdier 1963). These experiments were performed at the Department of Physiology, Gymnastik- och Idrottshögskolan. The sequence of the measurements is shown in Fig. 1. The determination of the glycogen content in needle biopsy specimens from the lateral portion of the quadriceps femoris was made according to the method described by Hultman (1967). Muscle biopsy specimens were taken before exercise started, and immediately after the subjects were exhausted.

The week schedule for both diet and work is illustrated at the top of Fig. 1. The subjects were given a mixed, uncontrolled diet prior to the first measurements of muscle glycogen and work time. The work consisted of pedalling a bicycle to exhaustion at a work load corresponding to an oxygen uptake of 3.15 (2.4—3.7) l/min, which equals 75 (71—82) per cent of the subjects' maximal oxygen uptake. On the day of the experiment, the subjects had no breakfast before the exercise test. Six of the 9 subjects were then given a fat + protein (P) diet for 3 days before the next work period. The work again consisted of pedalling to exhaustion at the 75 per cent work load. On the next 3 days, the subjects were limited to a predominantly carbohydrate (C) diet before the last work experiment. The remaining three subjects also followed the aforementioned schedule, except that they were first given the carbohydrate diet, followed by the fat + protein diet. All the food

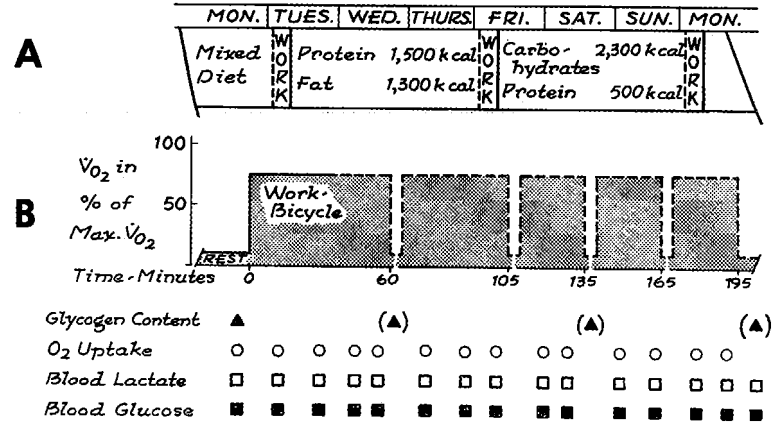

Fig. 1. A. Week schedule for diet and work programme in 6 subjects. In 3 subjects the two last diet periods were interchanged.
B. Schedule for the measurements in connexion with the exercise test.
The second biopsy for glycogen determination (▲) was made immediately after the work period, which was of different length depending on the type of diet.

eaten by the subjects when they were on the controlled diet was prepared and served at the laboratory. During work, the subjects drank water with some electrolytes, to minimize the effect of sweating. For psychological reasons, a 5-min rest period was inserted in the continuous exercise at fixed intervals (Fig. 1 b).

TABLE II. Prolonged physical exercise after three periods of different diets (mixed = M; fat + protein + carbohydrate (CH g); oxygen uptake (O_2 l/min)

Subject	After M diet					After P diet	
	Muscle glycogen		W T min	Used CH g	O_2 l/min	Muscle glycogen	
	Before	After				Before	After
T.P.	2.51	0.07	113.4	413	3.86	1.29	0.08
B.T.	1.19	0.35	148.5	375	2.99	0.60	0.32
R.S.	1.63	0.10	121.5	266	2.94	0.42	0.20
Å.L.	1.91	0.10	130.5	194	2.47	0.58	0.25
S.P.	2.20	0.53	117.3	292	2.93	0.31	0.20
S-O.J.	2.11	0.06	124.3	423	3.67	0.91	0.10
Mean	1.93	0.20	125.8	327	3.14	0.69	0.19
C.F.[1]	1.32	0.11	94.5	279	3.61	0.48	0.03
R.E.[1]	1.50	0.16	84.7	210	3.11	0.48	0.00
K-G.G.[1]	1.35	0.04	88.0	306	3.55	0.56	0.02
Total mean	1.75	0.17	113.6	306.4	3.24	0.63	0.13
± S.E.	±0.15	±0.05	±5.3	±27.4	±0.15	±0.10	±0.05

[1] In these 3 subjects the diet schedule was M, C, and P; not as in the 6 others (M, P and C).

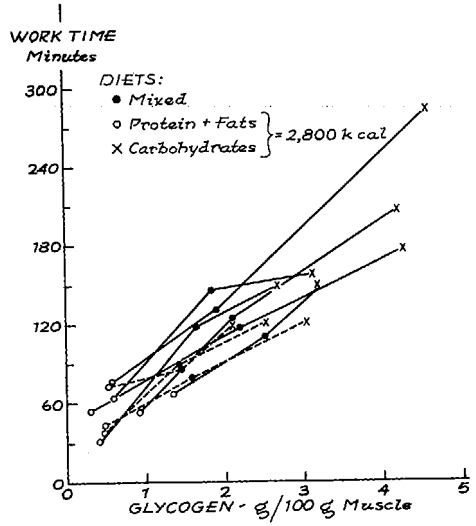

Fig. 2. Relation between initial glycogen content in quadriceps femoris and work time. Equation for regression line: $y = 41.6x + 36.8$, $r = 0.92$, $p < 0.001$. — — — the 3 subjects with carbohydrate diet prior to the fat + protein one.

Results

In Table II and Fig. 2, the individual values are given for the *muscle glycogen content* in relation to the maximal work time on a work load corresponding to 75 per cent of the maximal oxygen uptake. A good correlation is present between the

tein = P; carbohydrate = C). Muscle glycogen (g/100 g muscle); work time (W T min); used

			After C diet				
W T min	Used CH g	O_2 l/min	Muscle glycogen		W T min	Used CH g	O_2 l/min
			Before	After			
68.5	159	3.86	3.18	0.27	150.0	544	3.75
66.6	88	3.12	3.11	0.53	160.6	429	3.02
30.3	54	2.76	2.66	0.60	150.3	329	2.80
75.3	71	2.41	4.68	0.30	285.0	574	2.44
56.1	88	3.00	4.31	0.56	180.0	535	3.06
56.0	67	3.41	4.24	0.43	210.0	798	3.54
58.8	88	3.09	3.70	0.45	189.3	535	3.10
41.6	77	3.70	2.48	0.59	123.8	348	3.61
43.5	71	3.05	3.00	0.53	119.0	360	2.92
75.0	91	3.22	2.10	0.07	120.0	416	3.30
56.9	85.1	3.17	3.31	0.43	166.5	481.5	3.16
±1.7	±10.1	±0.15	±0.30	±0.06	±17.8	±47.1	±0.14

TABLE III. Statistical treatment: mean values of data (Table II and Fig. 3) obtained before, during, differences after the three diets (C, M, P)

C = carbohydrate diet
M = mixed diet
P = fat + protein diet

		n	C	M
			mean ± S.E.	mean ± S.E.
Muscle glycogen g/100 g	before work	9	3.31 ± 0.30	1.75 ± 0.15
	after work	9	0.43 ± 0.06	0.17 ± 0.05
Work time min		9	166.5 ±17.8	113.6 ± 5.3
Utilized carbohydrate g	during work	9	481.5 ±47.1	306.4 ±27.4
Oxygen uptake l/min		9	3.16 ± 0.14	3.24 ± 0.15
Blood pyruvate mM/l	at rest	6	0.163± 0.011	0.120± 0.003
	after 30 min work	6	0.238± 0.025	0.237± 0.011
	at end of work	6	0.228± 0.038	0.178± 0.017
Blood lactate mM/l	at rest	6	1.73 ± 0.17	1.02 ± 0.16
	after 30 min work	6	4.92 ± 0.74	5.22 ± 0.91
	at end of work	6	3.61 ± 0.74	2.65 ± 0.53
Respiratory quotient	at rest	6	0.943± 0.024	0.815± 0.004
	after 30 min work	6	0.942± 0.009	0.915± 0.007
	at end of work	6	0.918± 0.012	0.882± 0.020
Blood glucose mg/100 ml	at rest	6	91.7 ± 5.9	76.8 ± 4.5
	after 45 min work	5	77.3 ± 5.4	76.3 ± 8.6
	at end of work	6	63.3 ± 2.1	53.8 ± 6.2

initial muscle glycogen concentration and the maximal work time over the whole range of initial glycogen values, as well as in each subject. The muscle glycogen averaged 1.75, 0.63 and 3.31 g/100 g wet muscle after the M, P and C diet, respectively. The mean maximal work times in the corresponding situations were 114, 57 and 167 min, respectively. The mean decrease during exercise in muscle glycogen (mg/100 g tissue/min) was 14.2± 1.40, 8.78 ± 1.70 and 17.4 ± 0.85 after diets M, P and C. The differences in decrease M—P and C—M had p values of <0.05 and < 0.005, respectively (paired t test).

The three subjects (C. F., R. E. and K.-G. G.) given the C diet prior to the P one had markedly lower values for the muscle glycogen content after the C diet, compared to the other six subjects (*cf.* Table II and Fig. 2). Therefore, only six subjects following the main procedure are included in Figs. 3 and 6, where a comparison is made between the average values for blood pyruvate, blood lactate, RQ, blood glucose (Fig. 3), heart rate and oxygen uptake (Fig. 6) after the three diets. These values are also used for calculation of the statistics (*cf.* the lower part of Table III).

The mean *blood pyruvate* at rest was 0.12, 0.09 and 0.16 mmole/l after the M, P and the C diet, respectively. During the first 30 min of exercise, the pyruvate level

and after work following the three diets. Probability calculated on paired t test of intra-individual

P	C—M	C—P	M—P
mean ± S.E.	p	p	p
0.63 ± 0.10	<0.001	<0.001	<0.001
0.13 ± 0.05	<0.01	<0.01	>0.1
56.9 ± 1.7	<0.01	<0.001	<0.001
85.1 ± 10.1	<0.005	<0.001	<0.001
3.17 ± 0.15	>0.1	>0.1	>0.1
0.092 ± 0.008	<0.05	<0.01	<0.05
0.187 ± 0.017	>0.1	<0.05	<0.05
0.185 ± 0.020	>0.1	>0.1	>0.1
0.75 ± 0.17	<0.05	<0.01	>0.1
2.45 ± 0.18	>0.1	<0.01	<0.01
2.38 ± 0.24	<0.05	<0.05	>0.1
0.743 ± 0.029	<0.01	<0.01	<0.05
0.813 ± 0.009	<0.01	<0.01	<0.01
0.795 ± 0.014	<0.01	<0.01	<0.01
84.3 ± 4.0	>0.1	>0.1	>0.1
52.6 ± 2.6	>0.1	<0.05	<0.05
50.7 ± 10.8	>0.1	>0.1	>0.1

was significantly lower after the P diet than after the M and C diets (*cf.* Table III and Fig. 3).

The *blood lactate* at rest was 1.0, 0.8 and 1.7 mmole/l (*cf.* Fig. 3) after the respective diets (M. P and C). It increased during the first 30 min of exercise to 5.2, 2.5 and 4.9 mmoles/l. At the end of exercise, immediately before exhaustion, the blood lactate concentration had fallen to 3.7 mmoles/l after the C diet, and to 2.7 mmoles/l after the M diet. The last reduction was significant. After the P diet, the blood lactate was essentially unchanged during exercise. Statistical treatment of intra-individual differences after the three diets is given in Table III.

The *RQ* at rest was 0.81, 0.74 and 0.94 after the respective diets (*cf.* Fig. 3). It increased during the first 15 min of exercise to 0.93, 0.84 and 0.97, respectively. When the exercise proceeded, there was a slight reduction in the mean for the RQ with all diets, but the decrease was not significant. The probability of differences in RQ after the three diets is given in Table III.

The mean *blood glucose* values are given in Table III and Fig. 3. During exercise there was a fall in blood glucose from the beginning of exercise, except after the M diet, when a transient increase was first noted. At 45 min exercise, the concentrations after the C and the M diets were almost significantly higher than the cor-

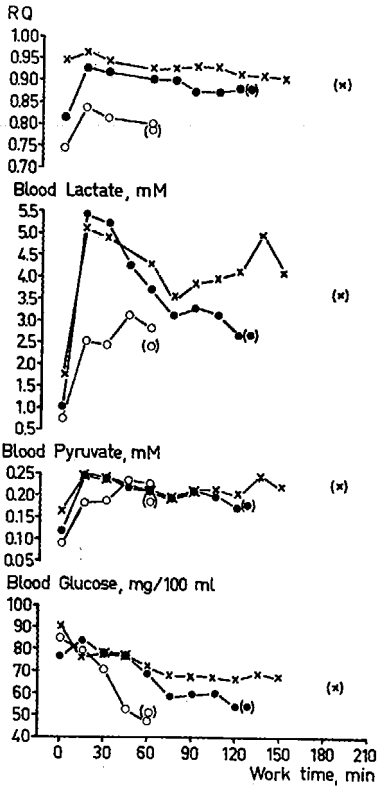

Fig. 3. Mean values of RQ, blood lactate, pyruvate and glucose in connexion with exercise after different diets in 6 subjects. × carbohydrate diet, ● mixed diet, ○ fat + protein diet, ( ) denotes the value at end of exercise.

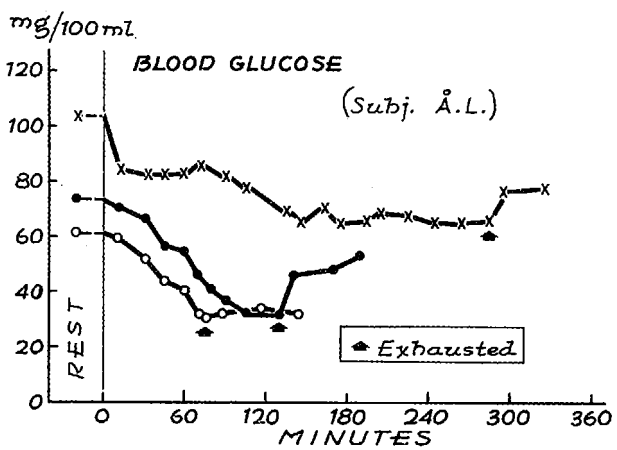

Fig. 4. Blood glucose concentration in connexion with exercise after different diets in subject Å.L. × carbohydrate diet, ● mixed diet, ○ fat + protein diet.

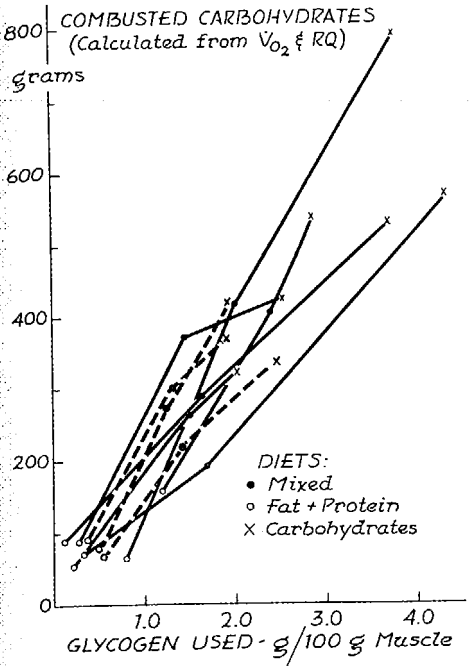

Fig. 5.

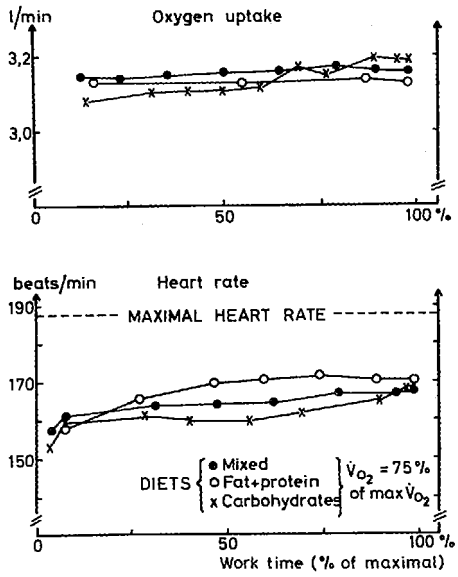

Fig. 6.

Fig. 5. Relation between used muscle glycogen and total utilization of carbohydrates during exhaustive exercise. Symbols as in Fig. 2. Equation of regression line: $y = 148.52\,x + 43.8$, $r = 0.92$, $p < 0.001$.

Fig. 6. Heart rate and oxygen uptake during exercise. Mean values in 6 subjects.

responding values after the P diet (*cf.* Table III). A slow recovery in the blood glucose concentration was marked in some subjects after the P diet. In subject Å.L. (*cf.* Fig. 4), the blood glucose concentration at exhaustion after the P diet was 32 mg/100 ml, and after 60 min recovery it was still only 34 mg/100 ml.

The *total amount of carbohydrate* combusted during the work period, calculated from the oxygen uptake and RQ, is related to the amount of glycogen utilized, *i.e.*, the difference between the muscle glycogen content before and after exercise (*cf.* Tables II and III, Fig. 5).

The calculated values for utilized carbohydrates during exercise ranged from 54 g to 798 g. In all instances there was a good correlation to the glycogen reduction in the quadriceps, which ranged from 0.11 to 4.38 g/100 g wet muscle. The mean decrease in muscle glycogen (mg/100 g tissue/g used carbohydrate) was 5.41 ± 0.65, 5.71 ± 1.02 and 6.02 ± 0.33 after the respective diets (M, P and C). The differences between the groups were not significant.

Fig. 6 illustrates the *heart rate* during exercise after the different diets. The differences were small and not significant.

The *oxygen uptake* during exercise was, on the average, 3.15 l/min, regardless of

the diet preceding the experiment. Nor were any significant changes noted in a comparison between the beginning and end of exercise.

The three subjects given the carbohydrate diet before the fat + protein diet showed similar results to those presented for the six subjects in Figs. 3 and 6, except that the differences between the M and C diet were smaller.

Discussion

The results of the present experiments demonstrate that the muscle glycogen concentration can be varied within a wide range, provided that different diets are administered after exhaustive exercise causing depletion of the local muscle glycogen stores. This is in agreement with previous studies (Bergström and Hultman 1966 b, Hultman and Bergström 1967). Thus, three days of fat + protein diet after exhaustive work did not resynthetize the muscle glycogen content to more than about 50 per cent of the initial value, whereas the carbohydrate diet raised the concentration to far above the normal range (0.95—2.0 g/100 g muscle) (Hultman 1967). Three subjects reached values above 4 g/100 g muscle, which are the highest ever reported in healthy males. It should be recalled that the three subjects given a C diet after the M diet did not reach as high muscle glycogen values as the 6 subjects given the P diet before the C one. Thus, a period of carbohydrate-free diet (*i.e.*, a period with low muscle glycogen) seems to further stimulate glycogen synthesis when carbohydrates are given (Ahlborg *et al.* 1967 b, Saltin and Hermansen 1967). It should be pointed out that the effect of the M diet on the muscle glycogen formation is not comparable to that of the P and C diet, as the M diet period was not preceded by glycogen-depleting exercise.

It is well established that the capacity for exercise is directly dependent on the individual's maximal oxygen uptake (Åstrand 1956). To minimize the inter-individual variation in work time during prolonged exercise, the work load for each subject was selected so that it represented about 75 per cent of his maximal oxygen uptake. All the subjects were somewhat trained (*cf.* maximal oxygen uptake in Table I), which implies that the exercise performed during the first part of the study should not have improved their performance in the last experiment. In subject Å.L., who had the lowest maximal oxygen uptake, measurements of his maximal uptake before and after the experimental period showed identical values (3.35 and 3.36 l/min).

The good correlation between initial glycogen concentration and work time (Fig. 2) demonstrates that the individual's ability to sustain prolonged exercise is highly dependent on the glycogen content of the muscles which, in turn, is dependent on the type of diet before exercise. It is improbable that the degree of fitness plays an essential role, either in the variation in muscle glycogen concentration or in its importance for the performance capacity, since the best-trained subject (S.-O.J.) and the least trained one (Å.L.) behaved similarly. These two subjects had the most marked increase in muscle glycogen concentration after the C diet, and they could also perform the longest on the 75 per cent work load.

Christensen and Hansen (1939) have earlier shown that the ability to perform prolonged exercise on a given work load is dependent on the type of diet before exercise. They concluded that carbohydrates were the most essential fuel during heavy muscular work. The results of the present study fully confirm this conclusion. It is of special interest that the performance time on a given load can be increased by more than 100 per cent by instituting a carbohydrate-rich diet after exhaustive exercise, and that the muscle glycogen concentration seems to be the key factor for the increase in performance capacity for prolonged work. This method of increasing the performance capacity may have practical applications in such situations as manual labour, military activities and athletics.

The output of glucose from the liver during prolonged exercise has been found to amount to 300 mg/min (Rowell, Masoro and Spencer 1965). At the end of prolonged severe exercise, a marked further increase in the glucose output was observed (Hultman, to be published). The blood glucose concentration during and after exercise fell to extremely low levels only after the P diet. In this situation the subjects experienced fatigue, not only localized to the legs but also generally. Some subjects suffered from headache and dizziness. The low blood sugar values at the end of exercise and the slow increase in blood sugar after exercise, especially after the P diet, might indicate a relative depletion of the glycogen stores in the liver in this situation.

It has been shown earlier that the diet can influence the relative role of fat and carbohydrate as fuel at rest and during exercise (Christensen and Hansen 1939). This was also manifested in the present study by a significantly higher RQ, both at rest and during exercise, after the M and C diets on the one hand, than after the P diet on the other hand. Furthermore, the calculated consumption of glycogen per time unit is lower after the P diet than after M and C diets. After the C diet both the RQ and the glycogen consumption per time unit were highest. This is in accordance with the observation that the regression line between performance time and initial muscle glycogen does not pass through zero (Fig. 2).

Blood lactate and pyruvate levels at rest and during the first part of exercise were also significantly lower after the P diet. These data suggest that the muscle cells have an ability to adapt to oxidizing more fat also at extremely high work loads, provided that the carbohydrate supply is low during the days before exercise. In contrast to this, the constantly high RQ throughout the period of prolonged heavy exercise after the carbohydrate and mixed diets indicates that the carbohydrate supply must, in fact, be limited for some time before exercise to permit the adaptation to fat combustion to take place.

As illustrated in Fig. 5, a good correlation is present between the glycogen used in the working muscles and the amount of carbohydrate utilized, calculated from the oxygen uptake and RQ. This applies over a wide range of RQ values and muscle glycogen concentrations. The glycogen decrease in relation to total carbohydrate consumption was not significantly different in the three diet groups. This indicates that the muscle glycogen store is the most important carbohydrate source during

heavy exercise. After the C diet, some subjects had available carbohydrate stores up to 700—800 g, *i.e.*, almost twice the figure presented by Hedman (1957) during cross-country skiing. His values are, however, compatible with those obtained by us after the M diet. Assuming that 20 kg of muscle are involved in the bicycle exercise, the exceedingly high figures after the carbohydrate diet noted in the present study are reasonable, since the reduction in glycogen concentration in the quadriceps femoris muscle during exercise was up to 4 g/100 g muscle.

The higher muscle glycogen concentration at exhaustion after the C diet may indicate that other factors ultimately limit the performance in this situation. Although psychological factors may have been of importance, it cannot be concluded that this is the only explanation.

This work was supported by the Swedish Medical Research Council, project no. 26X—792—01, and the City of Stockholm, Sweden.

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The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running[1, 2]

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ABSTRACT This study examined the effect of the type, amount, and the frequency of feeding of carbohydrates on muscle glycogen resynthesis after running. Trained male runners performed a 16.1 km run at 80% $\dot{V}O_2$ max to decrease gastrocnemius glycogen levels. A complex or simple carbohydrate diet (~3000 kcal) resulted in similar muscle glycogen levels 24 h after exercise. Forty-eight hours after exercise the complex carbohydrate diet resulted in significantly higher ($p < 0.05$) muscle glycogen levels. Consuming increasing amounts of carbohydrate, between 188 to 648 g carbohydrate/day, resulted in increasingly larger amounts of muscle glycogen resynthesis (24 h) after exercise. Frequent feedings of a high carbohydrate diet did not enhance muscle glycogen synthesis when compared to equal amounts of carbohydrates in two meals. It appears that muscle glycogen can be normalized between daily strenuous running activity. *Am. J. Clin. Nutr.* 34: 1831–1836, 1981.

KEY WORDS Dietary carbohydrate, glycogen, physical exertion

The importance of muscle glycogen for prolonged strenuous exercise is well documented (1–3), and various studies have examined exercise-dietary regimens to enhance muscle glycogen stores before performance (1, 3). None of these studies, however, has examined the influence of the amount or type of dietary carbohydrate consumed on glycogen resynthesis during the 24 h after strenuous running. Therefore, the purpose of this study was to determine the following: *1*) What effect does the form of carbohydrate have on glycogen resynthesis following exercise? *2*) What effect does consuming different quantities of carbohydrate have on muscle glycogen resynthesis during the 24 h after strenuous exercise? *3*) What effect does the frequency of carbohydrate feedings have on glycogen resynthesis during the 24 h after exercise? In addition, the effect of muscle glycogen levels on muscle metabolism during sprint and endurance activity was examined. Answers to these questions will provide guidelines for the management of the nutrition of athletes who engage in daily strenuous exercise.

Methods

Subjects

Six trained male runners participated in phase 1 of this study, whereas four trained male runners participated in phase 2. The physical characteristics of both groups of runners appear in **Table 1**. The subjects consumed the same diet (50% of calories derived from carbohydrate) and performed the same activity (30 min running) the 2 days preceding each trial. The subjects were fully informed of all risks associated with participation in this study before giving their written consent to participate. A flow chart of the experimental protocol for phase 1 and 2 appears in **Figure 1**.

Phase 1

To determine the effects of different forms of carbohydrate on muscle glycogen resynthesis after strenuous running, the subjects were fed isocaloric diets containing either simple sugars (glucose, sucrose, fructose) or complex carbohydrate (starches). The exercise consisted of a 16.1 km run at 80% $\dot{V}O_2$ max immediately followed by five 1-min sprint runs (3 min rest intervals) on the treadmill at speeds requiring 130% $\dot{V}O_2$ max. After this exercise, the subjects consumed one of two randomly assigned carbohydrate diets (two meals per 24 h) for the next 48 h and were restricted from any vigorous activity. During the first 24 h the subjects consumed 3700 kcal, which was calculated to meet the day's caloric expenditure; similarly, during the second 24 h the subjects consumed 2383 kcal. The carbohydrate, fat, and protein content represented 70:20:10% of the calories consumed, respectively, and totaled 648 and 415 g of carbohydrate for the 1st and 2nd days, respectively. The amount of kcal consumed was calculated to meet only the subjects'

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[2] Supported by a grant from the National Dairy Council (1979).

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energy expenditure in order to elucidate differences in glycogen resynthesis as a result of the two forms of carbohydrate. We did not wish to examine the differential effect of the two forms of carbohydrate on supercompensating muscle glycogen stores.

Muscle samples were obtained by percutaneous needle biopsy from the gastrocnemius immediately after and at 24 and 48 h after exercise. These samples, weighing 15 to 30 mg, were dissected into three samples, weighed and frozen at −180°C. The muscle sample weights were corrected for evaporative water and analyzed for muscle glycogen according to the method of Passonneau and Lauderdale (4). The average glycogen value determined from the dissected samples was used for statistical analysis and the standard error of duplicate samples was ±1.5%.

Phase 2

The influence of the frequency of feedings and the amount of carbohydrate consumed on glycogen resynthesis during the 24 h after exercise was investigated using the following dietary treatments (3000 kcal): *1*) low carbohydrate (CHO), 188 g CHO/day in two meals; *2*) mixed diet, 375 g CHO/day in two meals; *3*) high carbohydrate, 525 g CHO/day in seven feedings (high −7); *4*) high carbohydrate, 525 g CHO/day in two meals (high −2). The exercise was that described earlier and the order of the diets was randomized with at least one week separating each trial. Since the subjects could differentiate between high and low carbohydrate diets, randomization of trials eliminates any systematic effect on subsequent measurements due to the "nonblindedness" of the diet.

Muscle biopsies were obtained from the gastrocnemius immediately after exercise and 24 h later. These tissue samples were handled and analyzed for muscle glycogen as previously described. In addition, the effect of the glycogen levels (after the dietary treatments) on 300 m sprint performance was examined. Total time and split times for 100 and 200 m were recorded, and 5 min after the sprint a blood sample was obtained and analyzed for lactic acid (5). In addition, the subjects performed a 30 min treadmill run at 70% $\dot{V}O_2$ max 1 h after the 300 m sprint. At 10-min intervals samples of expired gases were collected via the semiautomated gas collection system described by Wilmore and Costill (6). From this measurement the respiratory exchange ratio was calculated. Heart rate and perceived exertion (7) were also taken at 10-min intervals. Immediately after the run, a blood sample was obtained and analyzed for lactic acid (5).

TABLE 1
Mean (±SD) characteristics of the subjects in phases 1 and 2

	Age	Ht	Wt	$\dot{V}O_2$ max	ST
	yr	*cm*	*kg*	*ml/kg min*	*%*
Phase 1 (n = 6)	24.5 (2.7)	179 (7.4)	71.8 (4.0)	56.1 (3.1)	59.6 (11.0)
Phase 2 (n = 4)	25.5 (4.4)	184 (6.1)	79.7 (6.8)	59.7 (4.6)	57.6 (3.7)

* Percentage slow twitch fibers.

Statistical analysis

Phase 1—mean values for all variables were tested for significance using the Student's *t* test for paired observations (8). Phase 2—a one-way analysis of variance was used to determine significant treatment effects. When a significant F was observed, multiple range testing was used to determine significant differences between treatments (8). The level of probability was set at $p < 0.05$.

Results

Phase 1

The strenuous running resulted in mean (± SE) muscle glycogen of 53.4 ± 7.5 and 56.1 ± 7.1 mmol/kg wet tissue before consuming the complex and simple carbohydrate diets, respectively. Glycogen restoration during the first 24 h period was similar for both the complex and simple carbohydrate diets (**Fig. 2**). The next 24 h (24 to 48 h), however, resulted in a significantly greater muscle glycogen storage ($p < 0.05$) with the complex carbohydrate diet (Fig. 2). The mean (± SE) change in muscle glycogen during this period was 22.1 ± 13.1 and 7.8 ± 11.5 for the complex and simple carbohydrate diets, respectively.

Phase 2

Mean (± SE) muscle glycogen content after the running exercise was 71.3 ± 14.3, 49.3 ± 9.4, 55.3 ± 12.0, and 46.8 ± 9.4 mmol/kg wet tissue for the low, mixed high 2 and high -7 carbohydrate diet trials, respectively. There is no significant difference ($p > 0.05$) between these mean glycogen values. Twenty-four hours later, muscle glycogen levels were 66.6 ± 7.8, 74.2 ± 3.9, 125.6

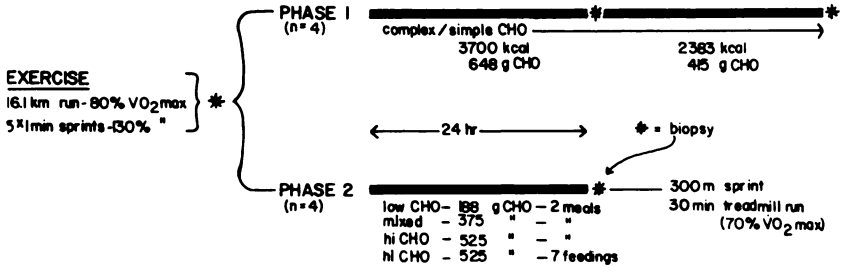

FIG. 1. Flow chart depicting the sequence of depletion and dietary intake for phase 1 and 2.

± 10.9, and 101.2 ± 20.9 mmol/kg wet tissue for the low, mixed, high-2 and high-7 carbohydrate diet trials, respectively. The change in muscle glycogen during the 24-h feeding periods for each trial is depicted in **Figure 3**. The low carbohydrate diet resulted in a significant reduction ($p < 0.05$) of muscle glycogen while the high-2 carbohydrate diet resulted in a significant gain ($p < 0.05$) in muscle glycogen when compared to the mixed diet trial.

The different initial muscle glycogen levels had no effect on either the 300 m sprint performance or the 100 and 200 m splits. In addition, there was no significant difference ($p > 0.05$) in lactate levels measured 5 min after each sprint (**Table 2**).

During the treadmill performance runs there was no significant difference ($p > 0.05$) in either the calculated oxygen uptake and total caloric cost or the measured heart rate and blood lactate accumulation between the trials (Table 2). The calculated oxidation of carbohydrate and fat, however, was affected by the diets. After the low carbohydrate diet the respiratory exchange ratio was lower ($p < 0.05$) and the grams of fat combusted were higher ($p < 0.05$) than the respiratory exchange ratio and the grams of fat combusted after the mixed diet. On the other hand, the high-7 diet resulted in a higher ($p < 0.5$) respiratory exchange ratio and larger ($p < 0.05$) gram carbohydrate oxidation than did the mixed diet trial (Table 2).

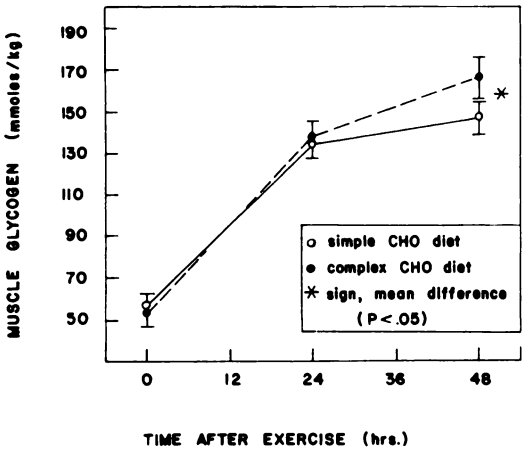

FIG. 2. Muscle glycogen levels (means ± SE) after exhaustive exercise with diets composed of 70% simple (i.e., glucose, fructose and sucrose) and complex (i.e., starch) CHO.

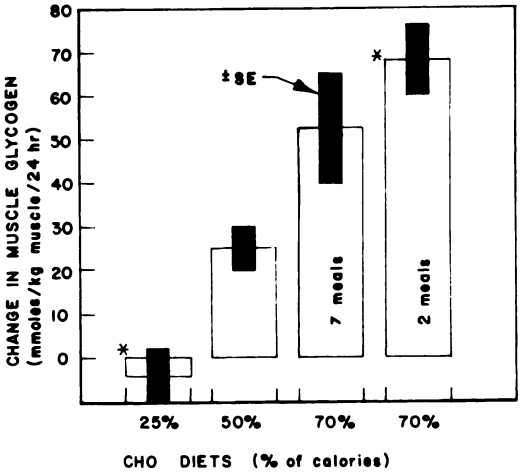

FIG. 3. Effects of varied CHO diets on the restorage of muscle glycogen. *Asterisk* denotes a significant difference between that mean and the mean change in muscle glycogen observed during the mixed diet (50% of cal from CHO).

TABLE 2
Mean (± SE) data obtained during the 300 m and 30 min treadmill runs after each of the diets*

	Low CHO	Mixed	High CHO (2 meals)	High CHO (7 meals)
300 m (s)	47.8	48.1	47.8	47.5
	(2.1)	(2.2)	(1.7)	(1.8)
HLa (m*M*)	9.6	9.1	9.0	8.6
	(0.6)	(0.4)	(0.6)	(0.5)
$\dot{V}O_2$	3.39	3.25	3.37	3.39
	(0.17)	(0.18)	(0.14)	(0.37)
R ($\dot{V}CO_2/\dot{V}O_2$)	0.801†	0.843	0.844	0.873†
	(0.006)	(0.007)	(0.011)	(0.002)
HR (beats/min)	154	156	155	156
	(1.6)	(2.3)	(2.0)	(1.4)
PE	10.6†	9.7	8.6†	9.3‡
	(0.6)	(0.5)	(0.6)	(0.6)
HLa (m*M*)	1.8	1.3	1.6	1.5
	(0.1)	(0.1)	(0.1)	(0.1)

* $\dot{V}O_2$, oxygen consumption; R, respiratory exchange ratio; HR, heart rate; PE, perceived exertion (Borg scale); HLa, blood lactate.

† Denotes significant difference ($p < 0.05$) between identified mean and the "mixed" (50% CHO) diet.

‡ Denotes significant difference ($p < 0.05$) between identified mean and the high CHO (two meals) diet.

Discussion

Phase 1 examined the effect of the type of dietary carbohydrate on muscle glycogen resynthesis during the 48-h period after strenuous running. The type of carbohydrate, simple or complex, had no differential effect on the change in muscle glycogen during the first 24 h after exercise. Reasons for the significantly ($p < 0.05$) larger change in muscle glycogen, and higher levels of muscle glycogen after the complex carbohydrate diet during the second 24 h, is presently unknown and might be explained by other factors.

The different responses in insulin and glucose as a result of the complex and simple carbohydrate diets has been reported by other investigators (11, 12). Hodges and Krehl (11) demonstrated that the insulin levels after a starch feeding were lower but remained elevated longer when compared to an equivalent g/g glucose meal. In addition, the activation of glycogen synthetase by insulin is well documented (13). Therefore, it is possible that maintained elevation of serum insulin occurring as a result of the complex carbohydrate diet is responsible for the enhanced muscle glycogen storage during the second 24-h period.

The strenuous bout of running exercise was sufficient to reduce muscle glycogen levels to an average of 55 mmol/kg wet tissue. This represents about 1 g/100 g of muscle glycogen and is slightly higher than the levels of muscle glycogen reported following cycling to exhaustion (1, 9). The recruitment pattern of muscle fibers as determined by periodic acid-Schiff staining has shown glycogen-filled fast twitch fibers after 2 h of running at 80% $\dot{V}O_2$ max (10). Thus, the glycogen remaining in the muscle sample in nonrecruited fibers following the strenuous bout of running might account for these slightly higher muscle glycogen levels.

As was anticipated, increasing amounts of carbohydrate 24 h after reduction of muscle glycogen resulted in increasing amounts of glycogen stored in the gastrocnemius (Fig. 3). This relationship was found to be significant $r = 0.84$, ($p < 0.05$) when data from phase 1 (first 24-h period) and phase 2 were combined. It is obvious that a plateauing of the change in muscle glycogen/24 h was not demonstrated up to 648 g CHO/day and that larger carbohydrate meals might maximize muscle glycogen storage during the 24 h after strenuous running. Indeed, Blom et al. (14) reported that maximal glycogen storage was attained when exercise-exhausted subjects (cycling) consumed between 1.4 to 2.0 g glucose/kg body weight every 2 h. They did not report muscle glycogen levels, but this amounts to 588 to 840 g CHO/day if a subject weighed 70 kg and consumed glucose for 12 h.

O'Dea and Puls (15) demonstrated that nibbling-fed rats incorporated more labeled glucose into glycogen and had higher muscle glycogen levels than meal-fed rats. This suggests that carbohydrate ingested at intervals after exercise might result in a greater glycogen storage than carbohydrate consumed in only two meals and indicates that an optimal glucose load might exist for glycogen synthesis during recovery from exercise. This, however, did not occur since the change in muscle glycogen during the high-7 trial was not significantly different ($p > 0.05$) from the mixed diet trial.

Bergström and Hultman (9) and Kochan et al. (16) reported normal muscle glycogen levels 24 h after exhaustive cycling exercise. Therefore, consistent with previous findings, both phase one (glucose and starch) and phase two (high-2) normalized muscle glycogen levels in 24 h. This is based on the fact that depletion levels averaged 55 mmol/kg wet tissue and the subsequent change in muscle glycogen was 81 and 70 mmol/kg wet tissue for the two diets, respectively. This would bring muscle glycogen levels to approximately 130 mmol/kg wet tissue, which is normal for rested, well-trained runners (17, 18). Thus, it appears that consuming between 525 to 648 g of carbohydrate during the 24 h after strenuous running will result in normal muscle glycogen levels.

Although muscle glycogen storage is known to play a critical role in activities lasting longer than 1 h (19), its significance in short term highly anaerobic exercise has not been examined during running. Anaerobic metabolism predominates as the energy producing system in events of high intensity lasting 0.75 to 3.0 min (20), and muscle glycogen is the substrate oxidized during such an exercise bout. Studies have demonstrated higher blood lactate levels during submaximal workloads when muscle glycogen was elevated as compared to lower levels of mus-

cle glycogen (21, 22). Therefore, higher levels of muscle glycogen preceding anaerobic activity might result in impaired performance resulting from enhanced lactate production. Table 3 illustrates that the mean performance time and blood lactate concentrations were not significantly different between any of the trials. Differences in initial muscle glycogen levels, therefore, had no effect on flux through the anaerobic energy producing systems which might impair performance.

The influence of dietary carbohydrate during exercise was first reported by Christensen and Hansen in 1939 (23). Although the mechanisms regulating a substrate shift with high and low carbohydrate diets have not been fully explained, the current data suggest that the amount of time between the final carbohydrate meal and the onset of exercise may have some bearing on the use of carbohydrate by muscle. This is supported by the fact that, although muscle glycogen was elevated after both high carbohydrate diets (two meals and seven feedings), only the seven feeding regimen produced an increase in carbohydrate oxidation during exercise. When the carbohydrate was taken in only two meals, nearly 15 h had elapsed between the second feeding and the 30-min treadmill run. In the seven feeding regimen, however, the final meal was consumed only 8 h before the exercise. Since carbohydrate availability, as measured by glucose levels (D. L. Costill, W. M. Sherman, W. J. Fink, C. Maresh, M. Witten, and J. M. Miller, unpublished data) and muscle glycogen content did not differ between the trials, the enhanced rate of carbohydrate oxidation may be related to a greater insulin sensitivity and/or activity after the seven feedings trial. This concept is supported by studies with juvenile diabetic runners who show normal muscle glycogen use, increased glucose uptake and an accelerated rate of carbohydrate oxidation when insulin is administered 3 h before exercise (24). When deprived of insulin for 24 h these men oxidized the same amount of muscle glycogen but experienced a marked reduction in glucose uptake and total carbohydrate metabolism during exercise.

The authors would like to thank Pete Watson and Rick Sharp who assisted in the data collection and the subjects for their cooperation during the study.

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