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Carbohydrate feeding and glycogen synthesis during exercise in man

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Abstract. In 7 male cyclists glycogen synthesis during exercise and rest was studied. Each subject did two exercise trials (A and B), in random order. In both trials, after determining the maximal workload (W_{max}), intermittent exercise was given to exhaustion. After the exhaustive exercise and taking a muscle biopsy the subjects either exercised at 40% W_{max} for 3 h (trial A) or rested for 3 h (trial B), during which they consumed approximately 2 l of a 25% malto-dextrine drink in both trials. After 3 h rest (trial A) or 3 h of mild exercise (trial B) a second muscle biopsy was taken for total glycogen and histochemistry (ATPase and PAS). Blood glucose and insulin levels were elevated during the first 2 h of exercise ($p < 0.05$). Glycogen depletion was most pronounced in type I and to a less extent in type IIA fibers. In trial A muscle glycogen increased from 136 ± 66 to 199 ± 71 mmol/kg DW, and in trial B from 145 ± 56 to 257 ± 79 mmol/kg DW. During exercise glycogen repletion was restricted to type IIA and IIB fibers, whereas during rest glycogen synthesis occurred both in type I and type II fibers. The present study demonstrates that oral carbohydrate administered during exercise may not only provide substrate for energy metabolism, but can also be utilized for glycogen synthesis in the non-active muscle fibers.

Key words: Carbohydrate – Glycogen synthesis – Exercise – Man – Muscle fiber type

Introduction

Exercise capacity for endurance activities is related to pre-exercise glycogen levels [17, 18]. Consequently, in athletic activities such as bike racing on successive days quick repletion of glycogen stores seems to be of paramount importance. Although no data are available about glycogen resynthesis in cyclists during successive days of competition, Costill et al. [5] reported incomplete glycogen repletion in runners who ran 10 miles on 3 successive days. In cyclists glycogen repletion after exercise may be compromized as well since the recovery periods are often less than 20 h while appetite may be absent for some hours after exhaustive exercise [1]. Glycogen resynthesis in glycogen depleted muscle, however, seems to attain its highest rates during the first hours after exercise [11], provided that the carbohydrate supplementation is sufficient [21]. Therefore, glycogen repletion may be incomplete at the start of the race. Still, in cycling events like the Tour de France it can be observed that the cyclists are able to exercise vigorously for 4–8 h on successive days for 3 weeks and are still able to sprint after many hours of work. This suggests that even after many hours of exercise those athletes are not fully glycogen depleted. Sparing of endogenous carbohydrate may result from oxidation of the exogenous carbohydrate, consumed during exercise [23, 24, 27]. In addition, glycogen synthesis during exercise has to be considered as well. When exogenous supplementation exceeds breakdown of endogenous carbohydrate net synthesis may occur. It has been demonstrated that glycogen synthesis in skeletal muscle may occur during light exercise in a glycogen depleted state [3, 4, 13, 20].

The present investigation was designed to study muscle glycogen synthesis rate in cyclists who ingested carbohydrate during exercise as well as in the resting state, both after prior glycogen depletion.

Methods

Subjects. Seven well trained male cyclists participated in the study. Their mean age was 32 years (range 23–42), mean body weight 71.8 kg (range 60.3–89.0 kg), the mean $\dot{V}O_{2max}$ per kilogram body weight 69 ml/kg/min (range 62–74). Before giving written consent the subjects were fully informed about the purpose of the study and the stresses and risks associated with it.

Procedure. One week prior to the actual experiment the subjects reported to the laboratory for instruction and for determination of the maximal workload (in watt) attained (W_{max}) on an electro-magnetically braked cycle ergometer (Lode, The Netherlands). The exercise started at 100 W for 5 min whereupon each 2.5 min the workload was increased by 50 W, but from a heart rate of 150 beats per minute with 25 W. The subjects maintained the revolution rate between 75 and 90 rpm. In each individual W_{max} was calculated from

$$W_{max} = W_{out} + (t/150) \times \Delta W$$

in which W_{out} is the highest workload which the subject completed, t the number of seconds the final uncompleted workload was sustained and ΔW the load increment. The subjects stopped the exercise when the revolution rate dropped below 50 rpm, in spite of encouragement from the experimentator. Oxygen uptake was measured continuously using a computerized automatic system (Ergoscreen, Fenyves & Gut, Switzerland).

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For the actual experiment all volunteers were subjected to two trials A and B of which the order was randomized in each subject. At least 2 weeks separated the two trials.

In order to deplete muscle glycogen the subjects were instructed to make a 2–3 h ride in the late afternoon on the day before each trial. After this exercise they maintained a carbohydrate-poor, but fat- and protein-rich diet in order to prevent high pre-exercise muscle glycogen levels [12]. In keeping with this, breakfast before starting both trials was at 6.30 a.m. and consisted of ham and eggs with artificially sweetened tea, milk or yogurt. The subjects reported to the laboratory at 8 a.m. After dressing in sportswear the subjects mounted the cycle ergometer. In both trials A and B glycogen depletion was induced as follows: measuring W_{max} as described above, 5 min recovery, followed by intermittent exercise. The intermittent exercise consisted of 2 min bouts at 90% W_{max}, interspersed with 2 min at 50% W_{max}. If the subject was unable to complete the 2 min intensive exercise at 90% W_{max}, the workload was lowered, subsequently to 80% and 70% W_{max}. The exercise was stopped when the 2 min at 70% W_{max} could not be completed. On the average 14 bouts (range 8–32) of heavy exercise were performed. During exercise the subjects were allowed to consume water. In both trials, after the intermittent exercise a percutaneous needle biopsy was taken from the vastus lateralis muscle [9].

In trial A the subject mounted the bike again after taking the biopsy and exercised for 3 h at a fixed workload of 40% W_{max}. From the beginning of this exercise the subjects started to drink a 25% maltodextrine-fructose solution (Perform, Wander, Bern, Switzerland; 85% maltodextrine, 15% fructose). The temperature of the drink was kept between 4 and 7°C, because of its enhancing effect on gastric emptying [6]. The subjects were encouraged to consume at least 2 l of this drink divided over 2 h and 45 min of exercise. The subjects stopped drinking 15 min before stopping the exercise in order to keep the eventual gastric residue low. Each 30 min oxygen uptake ($\dot{V}O_2$) and respiratory exchange ratio (R) were determined to calculate the amount of carbohydrate used during exercise [22]. Heart rate was displayed on a EKG monitor (Statscope, Electrodyne) and recorded during the measurement of the respiratory variables. Just before starting the 3 h exercise and after 1, 2 and 3 h of exercise blood was drawn from a forearm vein for measuring haematocrit and for spectro-photometric analysis of glucose (Kit 263826, Boehringer, Mannheim, FRG) and determination of insulin by radio immuno assay (Medgenix, Belgium). Intra assay coefficients of variation for insulin measurement, as calculated from the duplicates ranged from 5.5% ($\bar{x}$ 10.6 mU/l) to 4.2% ($\bar{x}$ 22.6 mU/l).

In trial B, after determining W_{max}, the intermittent exercise and taking the first biopsy, the subjects rested, sitting in a comfortable chair for 3 h. They were asked to consume at least 2 l of the aforementioned maltodextrine-fructose drink during the 3 h of rest. In trial B no blood samples were taken.

After the 3 h rest or exercise a second muscle biopsy was taken from the contralateral leg.

Biochemical and histochemical analysis. The muscle biopsy sample was divided into two portions. One portion for histochemistry was mounted on a cork and frozen in freon, cooled in liquid nitrogen. The second portion was frozen in liquid nitrogen for analysis of glycogen. After freeze-drying (GT2, Leybold-Heraeus, FRG) glycogen was determined fluorimetrically after HCl hydrolysis [25] and expressed as mmol of glycosyl units per kilogram dry weight (DW).

From the portion selected for histochemistry 8 μm thick sections were cut in a cryostat at −20°C and mounted on cover glasses for histochemical staining. Serial sections were stained for ATPase [29] and classified into type I, IIA and IIB, and PAS stained [8] in order to visualize glycogen depletion and/or repletion in different fiber types. For identification and counting of muscle fibers color pictures were made of the serial sections of the PAS and ATPase stains, using a photo microscope (Zeiss). The PAS stain was classified as negative, light, moderate, or dark.

Statistical analysis. The data were analyzed with analysis of variance (ANOVA) for repeated measures. The level of significance was set at $p < 0.05$.

Table 1. The individual muscle glycogen values (mmol/kg DW) in trial A (exhaustion, followed by 3 h exercise at 40% W_{max}), and trial B (exhaustion, followed by 3 h rest)

Subj.	Trial A		Trial B	
	Exhaust.	3 h cycl.	Exhaust.	3 h rest
1	270	286	174	233
2	57	132	224	357
3	166	270	80	189
4	101	200	97	245
5	103	160	95	179
6	165	248	198	379
7	105	102	150	222
$\bar{x} \pm$ s.d.	136 ± 66	199 ± 71	145 ± 56	257 ± 79

Results

The mean W_{max} from the test prior to the actual experiment and in trials A and B was 362, 357 and 365 W, respectively ($p < 0.10$).

Muscle glycogen at exhaustion (mean ± sd) after the interval depletion rides in protocols A and B were 136 ± 66 mmol/kg DW and 145 ± 56 mmol/kg DW, respectively ($p > 0.10$; Table 1). From the PAS stain (Fig. 1) it was observed that at exhaustion on the average 98% (range 95–99%) of type I fibers stained negative and 2% light. On the average 5% of type IIA fibers stained dark, 5% moderate, 82% light and 8% negative. All type IIB fibers stained dark.

In trial A muscle glycogen increased in all subjects during mild exercise (Table 1). The mean (± sd) muscle glycogen content increased significantly from 136 ± 66 to 199 ± 71 mmol/kg DW ($p < 0.01$). From the PAS stain (Fig. 1) it was observed that all type IIB fibers stained dark while 97% of IIA fibers stained moderate and 3% light. On the average 97% of type I fibers stained negative and 3% light.

In trial B, in which the subject consumed the carbohydrate drink during 3 h rest, muscle glycogen increased significantly from 145 ± 56 mmol/kg DW to 257 ± 79 mmol/kg DW ($p < 0.01$; Table 1). From the histochemical stains (Fig. 1) it was observed that on the average 8% of type I fibers stained light, 92% moderate while 100% of type II fibers stained dark.

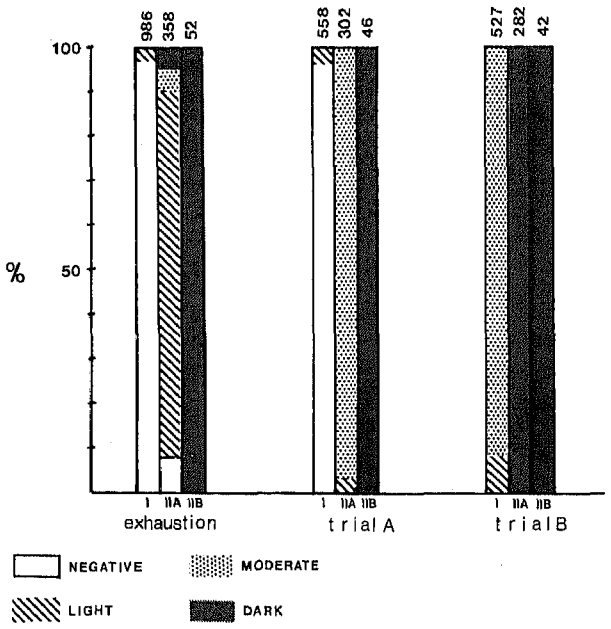

Fig. 1. The relationship between relative number of fibers and PAS stain in the different fiber types at exhaustion, after 3 h of exercise at 40% W_{max} (*Trial A*), and after 3 h of rest (*Trial B*)

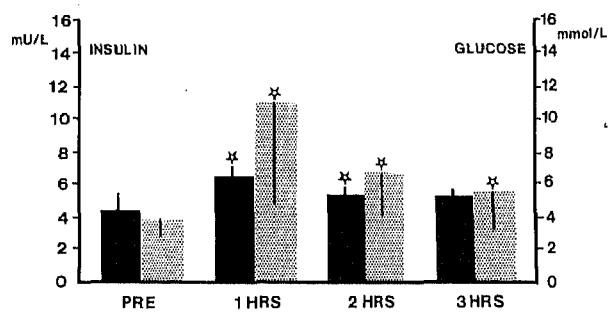

Fig. 2. Mean plasma glucose (■) and insulin (▩) concentration just before (pre) exercise, and at different intervals during 3 h of exercise (trial A). *Solid bars* represent sd. * $p < 0.05$ relative to pre-values

The average oxygen uptake during the exercise at 40% W_{max} (trial A) was 1.78 l/min (range 1.5–2.2 l/min). In all subjects the *R*-value remained stable throughout the exercise and was on the average 0.85 (range 0.81–0.88). Using the *R*-values and oxygen uptake it was calculated that the mean carbohydrate combustion over 3 h of mild exercise was 196 g (range 122–290).

The amount of carbohydrate ingested during the 3 h of exercise in trial A was on the average 491 g (range 370–568). One subject was not able to consume the 2 l because of sensations of filled stomach. None of the subjects encountered gastro-intestinal problems which interfered with the exercise. Two subjects reported transient flatulence and mild abdominal cramps during the early evening following the experiments. In trial B the average amount of carbohydrate ingested was 401 g (range 197–568). Three of the subjects were not able to consume 2 l of the solution, because of sensations of an overfilled stomach. None of the subjects reported gastro-intestinal problems during the experiment. One subject reported loose stools during the early evening.

Blood glucose levels after 1 and 2 h of exercise were significantly elevated relative to the value just before starting the 3 h of exercise ($p < 0.01$) (Fig. 2).

Insulin levels after 1, 2 and 3 h of exercise were significantly elevated relative to the value just before starting the exercise (Fig. 2).

Discussion

The present study demonstrates that supplementation of carbohydrate during mild exercise after prior glycogen depletion promotes glycogen synthesis. Although the exercise intensity of 40% W_{max} may seem to be low for athletic activities, it was observed in two professional cyclists who participated in the Tour de France that, based on heart rate registration (Sport tester PE 3000) the work intensity during the first 3 h of exercise varied between 40 and 50% W_{max} (unpublished data). In addition, the cyclists in the present study had been exhausted before cycling at 40% W_{max}, which lowered their ability to perform exercise at maximal and submaximal aerobic power since at the end of the depletion ride they were unable to complete the 2 min bouts at 70% W_{max} and had more difficulty to sustain the 50 W_{max} as well. Thus, the relative load of 40% of the pre-exhaustive W_{max} may be higher in the glycogen depleted state. Another important observation from the practical point of view is that cyclists consume carbohydrate rich foodstuffs and drinks during "easy" periods of long lasting events resulting in an average carbohydrate intake of 100 g [31]. Since no gastro-intestinal disorders occurred during exercise, blood glucose levels rose substantially, and no hemoconcentration occurred, it is suggested that the carbohydrate solution passed the stomach and was absorbed adequately. In terms of gastric emptying this means that on the average 2 l of the drink passed the stomach, resulting in a stomach emptying rate of 11 ml/min which is in line with the findings of Costill and co-workers [6], taking the osmolality (470 mOsmol) into account. Although preliminary results of a gastric emptying study indicate that the average calculated emptying rate of 11 ml/min are in keeping with the actual measurements, it cannot be ruled out that a portion of the drink may still have been present as gastric residue.

The average increase in muscle glycogen during exercise was 21 mmol/kg DW/h (range 0–35) and 37 mmol/kg DW/h (range 27–52) in the resting state. The glycogen synthesis rate during resting conditions is comparable to the maximal synthesis rates after exercise as calculated from the data reported by other investigators [19, 21, 26]. In each subject carbohydrate oxidation was estimated, using $\dot{V}O_2$ and *R*-value. Assuming that about 8 kg of muscle is actively involved in cycling at mild intensity [14] and using the muscle glycogen values before and after exercise, on the average 196 g of carbohydrate has been oxidized and 20 g has been incorporated into glycogen. This leaves about 275 g of the ingested feeding which has neither been oxidized nor incorporated into muscle glycogen. The value of the carbohydrate incorporated into muscle glycogen is probably higher since the muscle mass actively involved during the previous maximal exercise may have exceeded 8 kg. Hence, glycogen depletion and resynthesis may have occurred in more than 8 kg of muscle tissue. The data of the present study do not allow

conclusions about the fate of the amount which has neither been incorporated into muscle glycogen nor has been oxidized. Animal studies suggest that the contribution of the liver in clearing glucose during exercise is of minor importance [20]. However, the surplus of carbohydrate, the slightly elevated blood glucose levels and the increased insulin levels as well as the fructose content of the drink are considered to favor glycogen synthesis in the liver [7, 28]. The average glycogen synthesis rate during rest in the present study is lower than the value calculated from the data, reported by Bonen et al. [3]. Moreover, from those data it was calculated that during one-legged exercise at 20% $\dot{V}O_{2\,max}$ the average net glycogen synthesis rate dropped to 30% of resting values [3], whereas in the present study the average net glycogen synthesis rate during exercise was 57% of that in the resting state. Since no perchloric acid pre-treatment was applied the method for glycogen determination in the present study measures total glycogen [16] as in the study by Bonen et al. [3]. The different results may be explained by the different training status of the subjects. In contrast to the subjects in Bonen's study [3], the subjects in the present study were endurance trained competitive cyclists. It is likely that the endurance training elicited adaptations in their carbohydrate metabolism [15, 30]. The specificity of training adaptations may also explain why one subject failed to increase muscle glycogen during exercise (Table 1). Although this particular subject attained the highest absolute and relative workload, in the period of the experiments he did not make training or competitive rides over 1 h and never really challanged his carbohydrate stores. It is speculated that adaptations which enhance glycogen synthesis during exercise failed to develop in this particular subject.

From the histochemical stain (Fig. 1) it was demonstrated that glycogen depletion from intensive cycling occurred preferentially in type I and IIA fibres which is in agreement with data reported by Gollnick et al. [10]. Repletion during exercise with carbohydrate feeding occurred mainly in type II fibers. This may be explained by a selective recruitment of type I fibers, whereas type II fibers are probably less actively involved in this moderate exercise [10]. Moreover, the high blood glucose and the elevated insulin levels are favorable for glycogen synthesis [2]. In the resting state glycogen synthesis was found to occur in both type I and type II fibers. Although the data of the present study do not provide conclusive evidence it is suggested that glycogen synthesis during exercise occurs preferentially in the non-active fibers. Taking into account that on the average 39% of the muscle samples consisted of type II fibers and that the fiber area of type I and type II was generally equal, on the average 39% of vastus lateralis volume was inactive. This would implicate that the average measured net glycogen synthesis rate of 21 mmol/kg DW/h underestimates the real synthesis rate in type II fibers.

In summary, the present study demonstrates that oral carbohydrate administered during exercise may not only provide substrate for energy metabolism, but can also be utilized for glycogen synthesis in the non-active muscle fibers, especially the fast twitch fibers, which may enable athletes to sprint after long lasting exercise.

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