

Muscle glycogen storage following prolonged exercise: effect of timing of ingestion of high glycemic index food

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ABSTRACT

PARKIN, J. A. M., M. F. CAREY, I. K. MARTIN, L. STOJANOVSKA, and M. A. FEBBRAIO. Muscle glycogen storage following prolonged exercise: effect of timing of ingestion of high glycemic index food. *Med. Sci. Sports Exerc.*, Vol. 29, No. 2, pp. 220–224, 1997. This study examined the effect of delaying the ingestion of carbohydrate on muscle glycogen storage following prolonged exhaustive exercise. Six endurance trained men cycled on two separate occasions at a workload corresponding to 70% $\text{VO}_{2\text{max}}$ for 2 h followed by four “all-out” 30-s sprints. Following exercise, subjects were fed five high glycemic index (HGI) meals over a 24-h period, with the first three being fed either at 0–4 h (IT) or 2–6 h (DT) at 2-h intervals. Muscle biopsies were taken immediately after exercise and at 8 and 24 h post-exercise and analyzed for glycogen and glucose-6-phosphate. Blood samples were obtained prior to and at 30, 60, and 90 min after each meal and analyzed for glucose and insulin. No differences were observed in the incremental glucose and insulin areas after each meal when IT and DT were compared. In addition, no differences were observed in muscle glycogen or glucose-6-phosphate any time in the two trials. These data indicate that delayed feeding of a HGI meal by 2 h has no effect on the rate of muscle glycogen resynthesis at 8 and 24 h post-exercise, providing that sufficient carbohydrate is ingested during the recovery period.

RECOVERY, RESYNTHESIS, GLUCOSE, INSULIN, GLYCEMIC INDEX

Fatigue during prolonged exercise is often associated with muscle glycogen depletion (6,7,10), and sufficient intramuscular stores of this metabolite are essential for optimal endurance performance (2). Maximal glycogen concentrations are desirable for both athletic competition and quality training. Many endurance athletes compete or train repeatedly on the same day or on consecutive days, and hence the rapid restoration of muscle glycogen is essential. Muscle glycogen resynthesis following prolonged exercise has, accordingly, been

well investigated with respect to the amount (3,9,16), type (5,9,19,26), physical form (18,24) and timing (15,21) of the ingested carbohydrate.

Muscle glycogen storage has been observed to be greater 4 h post-exercise following the immediate ingestion of a glucose polymer compared with ingestion after a 2-h delay (15), probably a result of the combined effects of insulin and the insulin-like effects of muscle contractions. It is possible, however, that muscle glycogen content can influence the rate of muscle glucose uptake. Although the relationship between muscle glycogen content and glucose uptake in human studies *in vivo* has produced conflicting results (4,14,28,29), the inconsistency within the literature may be a result of the difficulty in altering muscle glycogen content while maintaining circulating hormones and substrates at similar levels. Studies that have adopted the perfused rat hindlimb model, in which it is possible to examine the direct effects of muscle glycogen content on glucose uptake, have demonstrated that an increased muscle glycogen content is associated with decreased muscle glucose uptake (11,13,25). Therefore, it is possible that the rate of muscle glycogen resynthesis is greater in humans after 4 h post-exercise with delayed carbohydrate ingestion compared with immediate feeding. This may eventually result in a similar level of glycogen storage after this time. No studies, however, have examined the effect of delayed feeding on muscle glycogen storage beyond 4 h post-exercise. This is significant from an athlete's perspective since the delay between races or training sessions within the 1 d is often longer than 4 h. In addition, athletes need to eat food, rather than single nutrients, following racing and training for nutritional requirements as well as for practical reasons (5). Hence, this study was undertaken to examine the effect of delaying the ingestion of high glycemic index (HGI) food by 2 h on muscle glycogen storage at 8 and 24 h following prolonged exhaustive exercise.

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METHODS

Subjects. Six endurance trained men (25 ± 4 yr; 180 ± 9 cm; 73.0 ± 9.1 kg; $\dot{V}O_{2\max} = 60.5 \pm 3.2$ ml·kg⁻¹·min⁻¹ (mean \pm SD)), volunteered as subjects for this study after being informed of the risks associated with the procedures and signing a letter of informed consent. The study was approved by the Victoria University of Technology Human Research Ethics Committee.

Maximal oxygen uptake. Maximal oxygen uptake ($\dot{V}O_{2\max}$) was determined during incremental cycling exercise to volitional fatigue on an air braked cycle ergometer integrated into a computer to display power output. Expired air was directed by a Hans-Rudolph valve through a ventilometer (Pneumoscan S30, KL Engineering, CA) into a mixing chamber and analyzed for oxygen and carbon dioxide by gas analyzers (Applied Electrochemistry S-3A O₂ and CD-3A CO₂, Amtek, Pittsburgh, PA) which were calibrated prior to each test with commercially prepared gas mixtures. The criterion used to determine the attainment of $\dot{V}O_{2\max}$ was the achievement of a plateau in $\dot{V}O_2$ (<2 ml·kg⁻¹·min⁻¹ increase) with an increase in workrate. All subjects fulfilled this criterion.

Experimental trials. On two occasions 1 wk apart subjects cycled for 2 h at a workload estimated to require approximately 70% $\dot{V}O_{2\max}$ before completing four "all-out" 30-s sprints with 2-min recovery between sprints. This protocol was adopted to deplete muscle glycogen stores in both type I and II muscle fibers. These exercise trials were conducted using the cycle ergometer employed during the $\dot{V}O_{2\max}$ test. Heart rate was recorded during each trial at 15, 30, 60, and 90 min of exercise using a monitor (Sports Tester PE3000, Polar, Finland). Oxygen consumption and RER were also measured at these times using Douglas bags. Expired gas composition was determined on the same analyzers used for the $\dot{V}O_{2\max}$ test. Volumes were determined using a gas meter (Parkinson-Cowan, Manchester, U.K.). To minimize the occurrence of thermal stress during exercise, a fan was used to circulate air and subjects were provided with 250 ml of water at 30, 60, and 90 min of exercise. Before each submaximal exercise trial, subjects arrived at the laboratory after an overnight fast, having refrained from exercise, alcohol, tobacco, and caffeine for 24 h. To minimize differences in resting muscle glycogen concentration, subjects completed an activity and diet recall log in which they recorded diet and activity patterns 48 h prior to the first trial. This was copied and returned to the subjects who were instructed to follow the same patterns prior to the second trial. Subjects were weighed nude prior to each exercise trial and wore cycling shorts and shoes during exercise.

Carbohydrate feeding protocol. Following the exercise subjects were removed from the cycle ergometer.

TABLE 1. Diets fed to subjects during 24 h of recovery after prolonged exercise.

	Food	Amount	GI
Meal 1 (t = 0 h IT and 2 h DT) and 5 (t = 22 h)	Lucozade	300 ml	95
	Cornflakes	65 g	121
	Milk	150 ml	44
	Bread	80 g	100
	Honey	20 g	87
Nutrient intake (kJ)			
Total meal 3264			
CHO 2802			
Fat 150			
Protein 313			
Meal 2 (t = 2 h IT and 4 h DT) and 4 (t = 8 h)	Lucozade	450 ml	95
	Rice	150 g	72
	Ryvita biscuits	40 g	70
	Honey	25 g	87
	Nutrient intake (kJ)		
Total meal 2972			
CHO 2800			
Fat 52			
Protein 120			
Meal 3 (t = 4 h IT and 6 h DT)	Lucozade	300 ml	95
	Instant Potato	80 g	120
	Bread	80 g	100
	Honey	25 g	87
	Nutrient intake (kJ)		
Total meal 3312			
CHO 2813			
Fat 266			
Protein 233			
Nutrient intake (24 h)			
CHO 875 g			
Fat 24 g			
Protein 71 g			
Total 16.1 MJ			

GI indicates glycemic index from refs. 16 Ivy, J. L., M. C. Lee, J. T. Brozinick, Jr., and M. J. Reed. Muscle glycogen storage after different amounts of carbohydrate ingestion. *J. Appl. Physiol.* 65:2018-2023, 1988; and 27 Wolever, T. M. S. The glycemic index. *World. Rev. Nutr. Diet.* 62:120-185, 1990.

A muscle sample was obtained from the vastus lateralis 2-5 min post-exercise and an intravenous saline drip was then placed into a vein in the antecubital space. Three subjects completed the immediate feeding trial (IT) first, while the remaining subjects completed the delayed feeding trial (DT) first. During the IT, a blood sample was obtained immediately after exercise, and subjects were fed the first meal following the biopsy. Subjects were subsequently fed at 2, 4, 8, and 22 h post-exercise. During the DT, the first blood sample was collected 2 h post-exercise, and this was immediately followed with the consumption of the first meal. During DT subjects were subsequently fed at 4, 6, 8, and 22 h post-exercise. To provide variety and to keep the protocol as practical as possible, three different meals were designed, each of which provided 2.5 g of carbohydrate per kg of body weight. Each meal was matched for fat, protein, and carbohydrate content. The foods were all HGI (17,27), since their ingestion post-exercise results in a more rapid storage of muscle glycogen compared with ingestion of low glycemic index carbohydrate (5). Subjects were fed the meals in the same order for each trial (Table 1). The subjects only ate the meals provided during the 24-h

TABLE 2. Mean oxygen uptake ($\dot{V}O_2$) and respiratory exchange ratio (RER) during 2 h of exercise in the immediate (IT) and delayed (DT) feeding trials ($N = 6$).

	IT	DT
$\dot{V}O_2$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	41.1 ± 2.2	41.3 ± 2.0
RER	0.84 ± 0.03	0.84 ± 0.03

recovery period, but they were permitted to drink water overnight. The volume of water consumed overnight during the first trial was recorded and an equivalent volume was consumed overnight during the second trial.

Tissue collection and analysis. Muscle biopsy samples were obtained from the vastus lateralis, using the percutaneous needle biopsy technique modified to include suction, at the completion of the exercise and at 8 and 24 h post-exercise in both IT and DT. Three separate incisions at least 3 cm apart were made at each time, and samples were taken distal to the previous incision (8). Contralateral limbs were used for the two trials. Samples were immediately frozen in liquid nitrogen. Each sample was freeze dried, dissected of any blood and connective tissue, powdered, and divided into two aliquots. One aliquot was extracted according to the method of Harris et al. (12) and analyzed for glucose-6-phosphate (G-6-P) according to the method of Lowry and Passonneau (20). The second powdered aliquot was hydrolyzed, neutralized, and analyzed for glycogen according to the procedure of Passonneau and Lauderdale (22).

Blood samples were obtained from a catheter (20G Jelco, Tampa, FL) inserted into a vein in the antecubital space, prior to and at 30, 60, and 90 min following each meal. The catheter was kept patent using a saline drip. Samples were placed in a tube containing fluoride heparin stored on ice and later centrifuged. The resultant plasma was stored at -80°C until analysis for glucose and insulin. Glucose was analyzed by an automated glucose oxidase method (YSI 23AM glucose analyzer) and insulin was analyzed by radioimmunoassay (Incstar, Stillwater, MN). The incremental area under the curve for glucose and insulin for each meal was calculated using the trapezoid rule, taking the immediate premeal value as baseline and including all time points until the 90 min reading (5).

Statistical analysis. Two-way (time and treatment) analysis of variance (ANOVA) with repeated measures was used to compare the data from the two trials. When these analyses revealed significant differences, a Newman Kuels *post-hoc* test was used to locate the difference. When two-way ANOVA revealed a significant interaction, simple main effects analysis was used to locate differences. A biomedical data processing (BMDP) computer software package was used to compute these statistics. The level of probability to reject the null hypothesis was set at $P < 0.05$. All data are reported as means \pm SE of the mean.

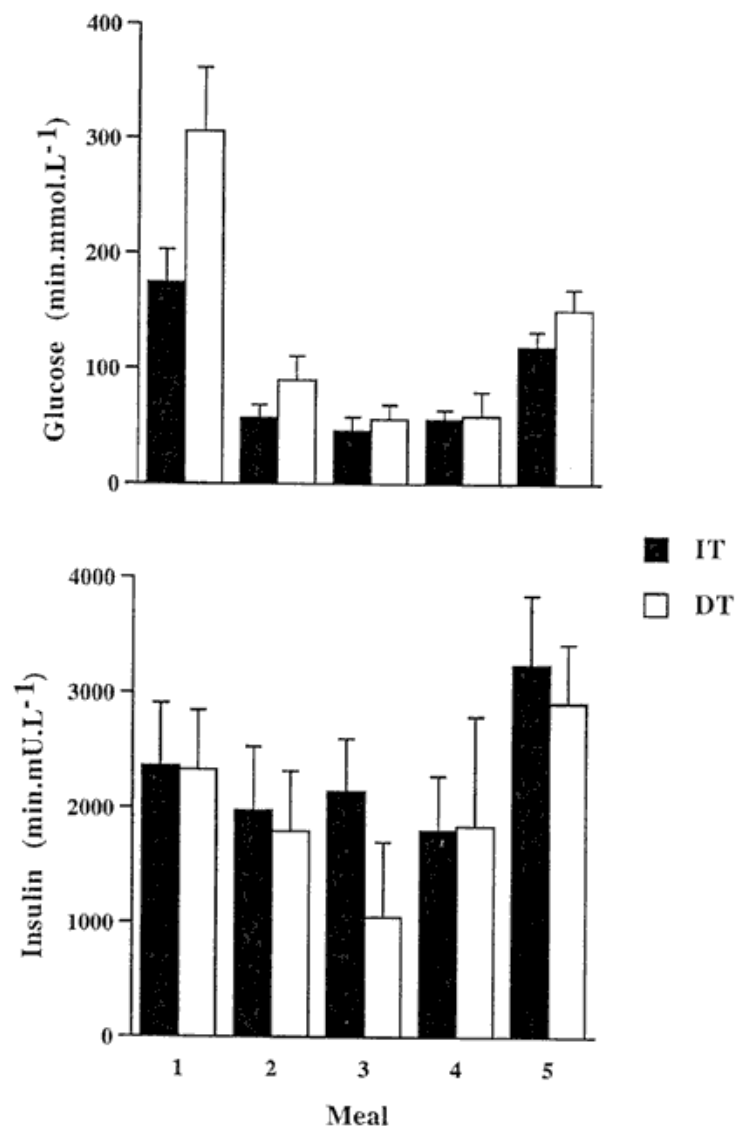


Figure 1—Incremental area (time multiplied by concentration) for plasma glucose (top) and insulin (bottom) after each meal when meal 1 was fed either immediately (IT) or after a 2-h delay (DT). ($N = 6$).

RESULTS

Subjects exercised at $68 \pm 3\%$ $\dot{V}O_{2\text{max}}$. $\dot{V}O_2$ and RER were not different when comparing IT with DT (Table 2). The incremental plasma glucose and insulin areas were not different when comparing IT with DT (Fig. 1).

Muscle glycogen concentration was lower ($P < 0.01$) immediately following exercise compared with 8 and 24 h post-exercise (Fig. 2). In addition, concentrations of this metabolite were lower ($P < 0.01$) when comparing 8 with 24 h post-exercise in both IT and DT. There were no differences in muscle glycogen content at any point when comparing IT with DT. Muscle G-6-P concentration was higher ($P < 0.01$) immediately following exercise compared with 8 and 24 h post-exercise. Concentrations of this metabolite were not different when comparing 8 with 24 h post-exercise in both IT and DT. In addition, there were no differences in G-6-P content at any point when comparing IT with DT (Fig. 3).

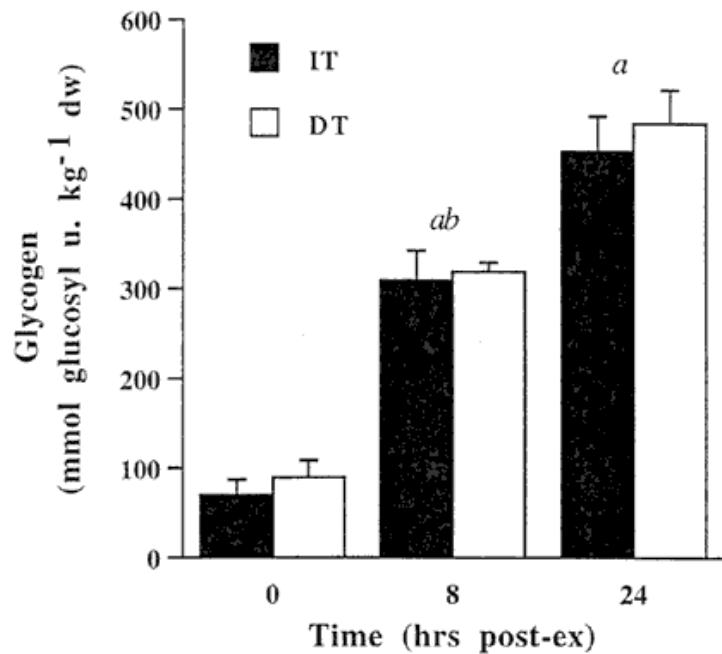


Figure 2—Muscle glycogen concentration immediately after (0), 8 and 24 h post-exercise with immediate feeding (IT) or following a 2-h delay (DT) ($N = 6$). ^a indicates difference ($P < 0.01$) from 0 h; ^b indicates difference ($P < 0.01$) from 24 h.

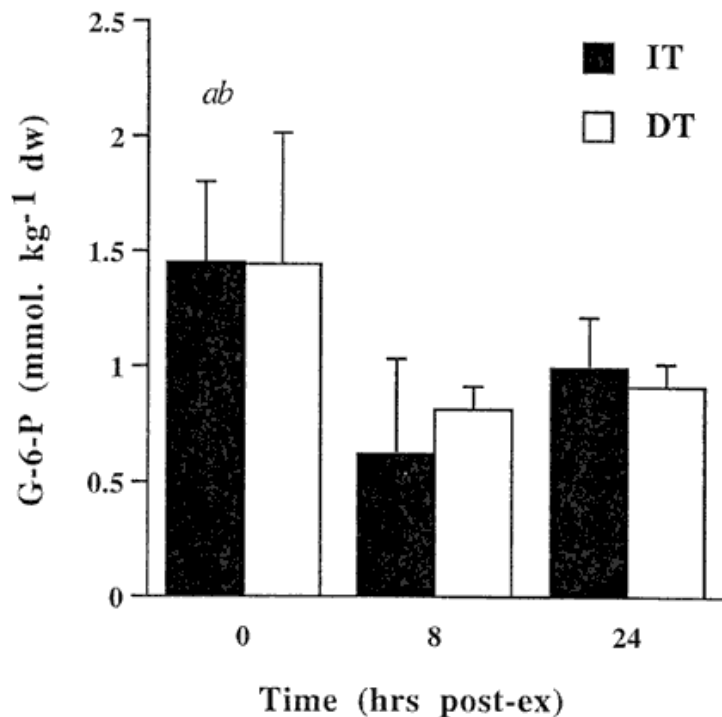


Figure 3—Muscle glucose-6-phosphate (G-6-P) concentration immediately after 0, 8, and 24 h post-exercise with immediate feeding (IT) or following a 2-h delay (DT) ($N = 6$). ^a indicates difference ($P < 0.01$) from 0 h; ^b indicates difference ($P < 0.01$) from 24 h.

DISCUSSION

The major finding from this study is that delaying the ingestion of HGI foods by 2 h has no effect on muscle glycogen storage at 8 and 24 h post-exercise respectively, providing that sufficient carbohydrate is ingested during the recovery period. This finding indicates that, despite glycogen storage being most rapid immediately follow-

ing exercise (15), athletes need not consume carbohydrates immediately after training or competition to restore glycogen to high levels providing that they ingest sufficient carbohydrates in the 6 h post-exercise and are not required to exercise again within 8 h.

During this study, only foods classified as HGI were chosen since ingestion of these foods following exhaustive exercise results in the most rapid rate of glycogen storage (5). In addition, subjects consumed the same meals in the same sequence during each trial (Table 1). This resulted in a similar glucose and insulin response following the ingestion of each meal (Fig. 1). The persistent effect of contraction is the most potent stimulus for muscle glucose uptake (23); hence we anticipated a significantly lower area under the glucose curve in meal 1 during IT compared with DT. Exercise results, however, in a greater hepatic escape of an oral glucose load (21). Taken together, these effects may account for the similar glucose response to meal 1. Although the mean area under the glucose curve was slightly higher in subsequent meals, this was not significant ($P > 0.30$). Three subjects demonstrated higher incremental areas in IT for meals 2–4, and two subjects demonstrated higher incremental areas in IT for meal 5. Hence, we are confident that our plasma glucose data are valid despite the low subject number.

The similar rates of muscle glycogen storage at 8 and 24 h post-exercise when comparing IT with DT appear to contradict previous observations (15). However, during the study of Ivy et al. (15), muscle glycogen was only examined 4 h following exercise and subjects were only fed one meal. An increased muscle glycogen content is associated with decreased muscle glucose uptake (13,25) and glycogen synthesis (11) in rats. It is possible in the present study, therefore, that the relatively higher muscle glycogen content in the initial post-exercise period with immediate ingestion may lead to a comparatively reduced rate of glycogen synthesis after this time. Of note, during the study of Ivy et al. (15) the fraction of glycogen synthase in the active form was higher 4 h post-exercise when feeding was delayed compared with immediate ingestion. It is possible that in the present study glycogen content was higher in IT prior to 8 h post-exercise but had leveled off by this time.

Other factors may influence the glycogen storage in the present study. In an attempt to deplete all fibers of glycogen, the subjects performed 4 all-out sprints at the completion of 2 h exercise. This would have resulted in a significant flux through the glycolytic pathway, as evidenced by the elevated G-6-P in the post-exercise sample (Fig. 3). This is likely to have elevated intramuscular lactate concentrations. It has been estimated that between 13 and 27% of lactate accumulated during intense exercise is converted to glycogen during recovery

(1). Hence, glycogenesis may have contributed to the glycogen synthesis in both IT and DT in the initial post-exercise period.

In conclusion, these data suggest that delayed ingestion of HGI carbohydrate by up to 2 h does not affect glycogen storage 8 h post exercise or beyond if the initial meal is followed by further carbohydrate intake. It is likely that the initial contraction mediated increase in glycogen syn-

these activity is subsequently regulated by the intramuscular glycogen concentration.

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