FATIGUE DURING PROLONGED EXERCISE is associated with muscle glycogen depletion and/or hypoglycemia (3, 4, 18). It has been suggested that carbohydrate depletion results in an inability of muscle to resynthesize ATP at a rate that matches the rate of ATP degradation (4, 18). It is thought that insufficient carbohydrate substrate causes falls in muscle pyruvate, a substrate for both acetyl CoA formation and for reactions that provide tricarboxylic acid cycle intermediates (4, 18). A fall in the pyruvate level may increase endurance capacity, at least in part, by improving muscle energy balance.

exercise; inosine monophosphate; fatigue

METHODOLOGY

Eight well-trained men [22 ± 1 yr (SE), 71.8 ± 1.6 kg] took part in this study after being informed of all risks and stresses associated with participation and providing informed, written consent. The study was approved by the Monash University Standing Committee for Research in Humans and by the University of Melbourne Human Research Ethics Committee. Peak pulmonary oxygen uptake ($V_{\text{O}}_{2}^{\text{peak}}$) was measured for each subject during incremental cycling exercise to volitional fatigue on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands) and averaged 6.80 ± 0.11 l/min (66.9 ± 1.3 ml·min⁻¹·kg⁻¹). The study comprised two exercise bouts to volitional fatigue, on the electrically braked cycle ergometer, at a workload requiring 69 ± 1% $V_{\text{O}}_{2}^{\text{peak}}$. On the day before a trial, subjects reported to the laboratory and performed a standard training session consisting of 45 min of cycling exercise at 70% $V_{\text{O}}_{2}^{\text{peak}}$. For the next 24 h, subjects were provided with carbohydrate-rich foods for all meals (14.0 MJ, 82% CHO, 6% fat, 12% protein) and refrained from strenuous exercise, alcohol, and caffeine. Subjects reported to the laboratory in the morning after an overnight fast. A catheter was positioned in a forearm vein, and a resting blood sample was obtained. A muscle sample was then obtained from vastus lateralis, by using the
percutaneous needle-biopsy technique with suction, and quickly frozen (within 10–15 s) in liquid nitrogen for later metabolite analysis. Subjects then commenced exercise and continued until volitional fatigue. Subjects were instructed to ride at 80–90 rpm, and fatigue was defined as the point when they were unable to maintain a pedaling rate of 60 rpm, despite strong verbal encouragement from one of the investigators. On one occasion, subjects ingested 250 ml of an 8% carbohydrate solution immediately before exercise and then every 15 min during exercise (CHO trial), whereas on the other occasion they ingested 250 ml of an artificially sweetened and flavored placebo (Con trial). The trials were conducted as double blind and in counterbalanced order at least 1 wk apart. Venous blood samples were obtained at rest, at 30-min intervals during exercise, and at the point of fatigue for plasma glucose measurement. Plasma samples obtained at rest, every 60 min during exercise, and at fatigue were also analyzed for insulin and lactate, whereas plasma samples obtained at rest, after 120 min, and at the point of fatigue were analyzed for nonesterified fatty acids (NEFA). Expired gas samples were collected into Douglas bags at 20-min intervals during exercise and approaching the point of fatigue for the measurement of oxygen uptake and respiratory exchange ratio. Heart rate was monitored continuously. A second muscle sample was obtained as soon as the subject stopped exercising, still on the ergometer, and quickly frozen (within 15–20 s of cessation of exercise) in liquid nitrogen. This sample, together with the one obtained at rest, was analyzed for glycogen, lactate, ATP, phosphocreatine (PCr), creatine, and IMP.

Analytical techniques. Plasma glucose was analyzed by using an automated glucose oxidase method (YSI 23AM analyzer, Yellow Springs, OH), plasma insulin by radioimmunoassay (Incstar, Stillwater, MN), and lactate on deproteinized plasma by using an enzymatic, spectrophotometric technique (11). Plasma NEFA levels were measured by using an enzymatic, colorimetric procedure (NEFA-C test, Wako, Osaka, Japan). Freeze-dried muscle was weighed and divided into two portions. For glycogen analysis, ~1 mg was powdered, incubated in 2 N HCl for 2 h, neutralized with 0.67 N NaOH, and analyzed for glucose by using an enzymatic, fluorometric technique (16). The remaining muscle (~2 mg) was powdered and extracted according to the procedure of Harris et al. (11). Muscle lactate, ATP, PCr, and creatine were analyzed by using enzymatic, fluorometric techniques (11), whereas IMP was measured by high-performance liquid chromatography (21). The concentrations of ATP, PCr, creatine, and IMP were corrected for the peak total creatine (PCr + creatine) concentration in each subject to account for any nonmuscle contamination of the muscle samples. Glycogen (glucosyl units) and lactate concentrations were not corrected. Because of technical difficulties, muscle glycogen measurements were only obtained in six subjects and the other muscle metabolites in seven subjects. Oxygen and carbon dioxide contents of the Douglas bags were measured by Applied Electrochemistry (Ametek, Pittsburgh, PA) S-3A/II and CD-3A analyzers, respectively. These analyzers were calibrated before and during each trial by using commercial gases of known composition. Volume was determined by using a Parkinson-Cowan gas meter, calibrated against a Tissot spirometer. The data from the two trials were compared by using analysis of variance for repeated measures. Specific differences were located by using Student-Newman-Keuls post hoc test. Where appropriate, paired comparisons were made by t-test. Significance was set at the $P < 0.05$ level, and all data are reported as means ± SE.

### RESULTS

No differences in oxygen uptake, respiratory exchange ratio, and heart rate were observed between the two trials at any time point during exercise. Mean values over the exercise period are summarized in Table 1. Carbohydrate oxidation during the latter stages of exercise, estimated from respiratory exchange data collected 10–15 min before the point of fatigue, was similar ($P = 0.44$) in the two trials (Con: 2.77 ± 0.11 g/min; CHO: 2.88 ± 0.19 g/min, $n = 7$). Exercise time to fatigue was increased by 47 min (30%) when subjects ingested carbohydrate (Table 1). Plasma glucose levels were similar at rest in the two trials (Fig. 1), but were higher ($P < 0.05$) throughout exercise when carbohydrate was ingested (Fig. 1). At the point of fatigue in the CHO trial, plasma glucose was not different from the preexercise value (Fig. 1); in contrast, plasma glucose levels fell in the Con trial (Fig. 1). Plasma insulin, NEFA, and lactate levels were similar before exercise in the two trials (Table 2). Plasma lactate levels increased to a similar extent during exercise in the two trials (Table 2). Plasma insulin levels were higher, and plasma NEFA levels lower,
throughout exercise when carbohydrate was ingested, except at the point of fatigue when plasma insulin levels were similar in the two trials (Table 2). Preexercise muscle metabolite concentrations were similar in the two trials (Table 3, Fig. 2). Although exercise decreased the levels of glycogen and PCr and increased lactate, no differences were observed between trials (Table 3). Neither exercise nor carbohydrate ingestion had an effect on muscle ATP levels (Table 3). Muscle IMP concentration increased \((P < 0.05)\) with exercise in both trials, with a tendency \((P = 0.06)\) for an exercise-by-time interaction (Fig. 2). Six of the seven subjects analyzed had higher muscle IMP levels at the end of exercise in the Con trial compared with the CHO trial. A two-tailed paired \(t\)-test revealed that muscle IMP was significantly higher \((P < 0.05)\) at the end of exercise in the Con compared with CHO trials (Con: 0.23 \(\pm\) 0.09, CHO: 0.16 \(\pm\) 0.08 mmol/kg dry muscle).

**DISCUSSION**

The results of this study demonstrate that ingestion of carbohydrate increases endurance performance during prolonged, strenuous exercise. The 30% increase in exercise time to fatigue observed in the present study (Table 1) was similar in magnitude to that seen in previous studies in which similar exercise protocols were used (3, 5–7). Despite the subjects cycling 30% longer in the CHO ingestion trial, muscle IMP levels at the point of fatigue were lower than at the point of fatigue in the Con trial. These results imply that carbohydrate ingestion may increase work capacity during prolonged exercise, at least in part, by improving metabolic energy supply within contracting skeletal muscle.

Spencer et al. (19) found that muscle IMP levels rose less during prolonged exercise when carbohydrate was ingested, such that at the same time point that fatigue occurred in a control trial muscle IMP levels were lower when carbohydrate was ingested. We have extended these results by showing that muscle IMP levels remain lower even at the point of fatigue when carbohydrate is ingested, despite the subjects being able to exercise 47 min longer than in the Con trial (Fig. 2). Other evidence that carbohydrate ingestion during exercise improves energy balance at the point of fatigue is provided by a study that examined the effect of carbohydrate ingestion on muscle metabolism during prolonged running (20). These authors found that carbohydrate ingestion increased exercise time from 102 min in the control trial to 132 min in the carbohydrate ingestion trial. At the point of fatigue, they found that muscle ATP and PCr were lower than the resting value in the control trial but unchanged from rest in the carbohydrate ingestion trial (20).

Although muscle IMP levels increased significantly with exercise in both trials (Fig. 2), the absolute levels were relatively low compared with previous studies examining muscle IMP during prolonged exercise (15, 18, 19). It is possible that the higher levels of muscle IMP in the studies by Norman et al. (15) and Sahlin et al. (18) were as a result of the lower fitness levels of the subjects in these studies (mean maximal oxygen uptake \(= 47\) and 45 ml·kg\(^{-1}\)·min\(^{-1}\), respectively). It has been observed that exercise training reduces the extent of muscle IMP accumulation during exercise (10) and that trained individuals incur lower transient increases in ADP and AMP and have lower muscle IMP levels at fatigue than do untrained individuals (1). It
is also likely that the relatively untrained subjects in the studies by Norman et al. (15) and Sahlin et al. (18) possessed higher levels of type II muscle fibers than the endurance-trained subjects in the present study (8). Type II muscle fibers demonstrate higher rates of IMP formation during exercise than do type I fibers (17).

Our conclusions are dependent on the validity of muscle IMP accumulation as a marker of energy imbalance within skeletal muscle. Whereas this is believed to be so (15, 18, 19), any IMP reamination that may occur within fatigued, noncontracting muscle fibers late in exercise (9) influences the IMP levels at fatigue and perhaps invalidates its use as a marker of an imbalance between the rates of ATP utilization and resynthesis during exercise. In addition, a muscle biopsy sample is likely to contain glycogen-depleted and -nondepleted fibers. It is possible that large increases in IMP occurred in the depleted fibers (14), but the whole muscle IMP level was diluted by the nondepleted fibers.

Carbohydrate oxidation, as calculated from pulmonary oxygen uptake and respiratory exchange ratio, was not different between the two trials, even at the point of fatigue. Similar observations have been made by other investigators (2, 7), and several studies have shown no fall in CHO oxidation late in prolonged exercise to exhaustion at ~70% maximal oxygen uptake (13, 18–20). Thus the often-quoted fall in carbohydrate oxidation late in prolonged exercise to exhaustion at 70% maximal oxygen uptake (5) is not always observed. The lower muscle IMP levels at the point of fatigue when carbohydrate was ingested suggest that carbohydrate ingestion increases endurance by improving metabolic regulation, but it is possible that nonmetabolic factors also play a role. Although this issue is controversial and speculative, it has been suggested that carbohydrate ingestion enhances endurance exercise performance in some subjects via alterations in central nervous system function (Davis et al. [7]).

In conclusion, the results of the present study suggest that carbohydrate ingestion may increase exercise endurance capacity at least in part by improving muscle energy balance, since muscle IMP levels at the point of fatigue were lower when carbohydrate was ingested compared with the placebo trial, despite the subjects exercising 30% longer.

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