Fuel Substrate Kinetics of Carbohydrate Loading Differs From That of Carbohydrate Ingestion During Prolonged Exercise

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This study compared fuel substrate kinetics in trained cyclists who ingested a 10% carbohydrate (CHO) drink without prior CHO-loading ([NLC] n = 9) with those in cyclists who ingested a water placebo after CHO-loading ([CLP] n = 7) during 180 minutes of cycling at 70% maximum oxygen consumption ($\dot{V}O_2$ max). Muscle glycogen at the start of exercise was 194 ± 4 and 124 ± 8 mmol/kg wet weight (mean ± SEM) in CLP and NLC subjects, respectively. Total CHO oxidation was similar. Total rate of appearance of glucose from endogenous (Ra_{end}) and exogenous (Ra_{exog}) origin and plasma glucose oxidation increased significantly ($P < .05$), with NLC subjects ending significantly higher than CLP subjects (104 ± 17 *v* 79 ± 9 and 115 ± 16 *v* 74 ± 11 μmol/min/kg fat-free mass [FFM], respectively). However, Ra_{end} was lower ($P < .05$) in NLC than in CLP subjects (40 ± 10 *v* 79 ± 9 μmol/min/kg FFM), as was endogenous plasma glucose oxidation (42 ± 13 *v* 75 ± 11 μmol/min/kg FFM). Muscle glycogen disappearance was identical in the first hour, but declined thereafter in NLC subjects. Two NLC subjects with the lowest muscle glycogen content were unable to complete the trial despite CHO ingestion. We conclude that with respect to the groups studied (1) CHO loading before exercise reduces the relative contribution of plasma glucose oxidation to total CHO oxidation, but may prolong time to exhaustion as a function of higher muscle glycogen concentration; (2) CHO ingestion has a liver glycogen-sparing effect, causes a reduction in gluconeogenesis, or both, that should delay the onset of hypoglycemia; (3) the progressive increase in plasma glucose oxidation that occurs during prolonged exercise is related to muscle glycogen status and occurs irrespective of whether CHO is ingested; and (4) the effects of CHO ingestion and CHO-loading on fuel substrate kinetics are different.

IT IS NOW well established that both carbohydrate (CHO) ingestion during exercise[1-3] and CHO-loading before exercise[4] can enhance endurance performance.

The ergogenic effect of CHO ingestion during prolonged exercise was first demonstrated by Christensen and Hansen in 1939,[5] and has subsequently been repeatedly confirmed.[1,6,7] Studies such as those by Massicotte et al[8] and Pirnay et al[9] have shown that less endogenous CHO is oxidized when CHO is ingested during exercise, with data calculated from differences between total CHO and exogenous CHO oxidation. However, the effects of CHO ingestion on rates of appearance of exogenous (Ra_{exog}) and endogenous (Ra_{end}) glucose and the rates of oxidation of plasma glucose originating from exogenous and endogenous sources could not be ascertained by this approach. Using tracer techniques, Radziuk et al[10] partly answered the question as to the effects of ingesting CHO on Ra_{end}, but these studies were in nonexercising subjects and provided no information on plasma glucose oxidation rates and the influence of ingested CHO on muscle glycogen utilization. In a recent study,[11] we showed that when CHO was ingested during exercise in CHO-loaded subjects, there was a significant liver glycogen-sparing effect by a reduction in the rate of gluconeogenesis, glycogenolysis, or both; but as previously shown,[2] ingestion of CHO did not have a muscle glycogen-sparing effect. In contrast, there was no liver glycogen-sparing in another study[6] that investigated the effect of CHO-loading on splanchnic glucose appearance and associated CHO kinetics in subjects who ingested only water during exercise. All subjects in both studies who were CHO-loaded at the start of the trial were able to complete 180 minutes of cycling, but 50% of nonloaded subjects in the second study[6] were unable to complete the 180-minute trial. Thus, the individual effects on fuel substrate kinetics of CHO-loading (when only water is ingested during exercise) and of CHO versus water ingestion (in subjects who have CHO-loaded before exercise) have been determined.[6,11]

However, the options available to athletes to provide adequate CHO are either to CHO-load before the event and ingest water during the event,[6] to CHO-load before and ingest CHO during the event (the most likely regimen to provide adequate CHO substrate throughout exercise)[11] or to dispense with CHO-loading and merely ingest CHO during the event. Thus, the aim of this study was to determine what differences may exist in glucose turnover, exogenous and endogenous glucose oxidation, and muscle glycogen utilization when subjects CHO-load before exercise and ingest water during exercise, as opposed to no prior CHO-loading with ingestion of CHO during exercise.

SUBJECTS AND METHODS

Subjects

Sixteen moderately trained (~1 hour daily 5 to 6 days per week for a minimum of 6 months) male endurance cyclists took part in the study, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. Since radiolabeled tracers were infused and muscle biopsy and blood samples were taken, the procedures and risks were

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carefully explained to the subjects and their written informed consent was obtained.

The total radiation dose received by each subject was approximately 10 mrem. The radiation dose accepted as safe in this country is 500 mrem/y or 130 mrem/13 wk (information courtesy of Dr E. Hering of the Department of Medical Physics, Groote Schuur Hospital, Cape Town).

Measurement of Maximum Oxygen Consumption

Each cyclist first came to the laboratory for determination of maximum oxygen consumption ($\dot{V}O_2max$). This was measured during a progressive exercise test on an electrically braked cycle ergometer (Tunturipyora, Piisparisti, Finland) modified to the configuration of a racing bicycle. The starting work rate was 200 W, with 15-W/min increments until exhaustion. This information was used to adjust the work rate in the experimental trial so that each subject exercised at an intensity corresponding to approximately 70% of $\dot{V}O_2max$. Work rate was adjusted during the trial to maintain exercise at this intensity.

During the test, the subject wore a nose clip and inspired air via a Hans Rudolph 2700 one-way valve (Vacumed, Ventura, CA), connected to a dry-gas meter. Expired air was passed through a 15-L baffled mixing chamber and a condensation coil to an Ametek S-3A/I O_2 analyzer with an N-22M sensor and a CD-3A CO_2 analyzer with a P-61B sensor (Thermox Instruments, Pittsburgh, PA). Before each test, the gas meter was calibrated with a Hans Rudolph 3-L syringe and the analyzers were set with air and a 4% CO_2:16% O_2:80% N_2 gas mixture. Instrument outputs were processed by an on-line computer that calculated the inspired volume, oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) over each minute using conventional equations.[12]

Measurements of Fat-Free Mass

So that measurements of fuel substrate kinetics could be expressed in terms of fat-free mass (FFM), the percentage body fat of the subjects was determined from measurements of triceps, biceps, subscapular, and suprailiac skinfold thicknesses. Percentage body fat was calculated using standard formulae.[13]

Measurement of CHO Turnover During Exercise

Following the $\dot{V}O_2max$ test, cyclists were randomly assigned to a group that either would CHO-load before the trial and ingest water (placebo) during the trial ([CLP] n = 7) or would not CHO-load and ingest CHO during the experiment ([NLC] n = 9). CHO-loading was achieved by having the cyclists rest from training for 3 days and ingest approximately 600 g CHO/d, mainly in the form of a commercially available glucose polymer (200 g) in solution, bread, pasta, potato, and rice. NLC subjects also rested from training for 3 days, but continued to eat their normal diet. Separate groups were used so that exposure to radiation was kept well below accepted safe limits.

On the day of the experiment, the cyclists returned to the laboratory to start the trial 3 hours after eating a CHO-containing breakfast (70 g CHO) that they would normally ingest before a cycling race. This was to approximate the conditions under which cyclists would normally race. The breakfast consisted of 1 cup of cereal with 125 mL milk and 2 slices of toast.

An 18G Jelco (Johnson and Johnson, Halfway House, South Africa) cannula was inserted into the antecubital vein of one forearm for blood sampling every 20 to 22 minutes, and a 20G cannula was inserted into the antecubital vein of the other forearm for continuous infusion of 2-^{3}H-glucose tracer (Amersham International, Buckinghamshire, UK).

A 2-^{3}H-glucose tracer was selected because use of this tracer determines splanchnic glucose output (Ra) from exogenous sources and hepatic glycogenolysis and gluconeogenesis from 3-carbon products. Since one aspect of glucose kinetics we were interested in was total glucose Ra from all sources, this was an appropriate tracer to use. Glucose labeled with ^{3}H in the second C position is lost in the glucose-6-phosphate–fructose-6-phosphate equilibrium in the glycolytic and gluconeogenic pathways, and thus incorporation of ^{3}H-glucose into liver glycogen is minimized,[14] particularly during the preexercise infusion period. Thus, the potential incorporation and subsequent hydrolysis of ^{3}H-glucose–labeled glycosyl residues of glycogen, which would result in an underestimation of glucose Ra, is limited. However, a possible disadvantage of this tracer is the reported 10% to 20% overestimation of Ra when compared with 3-^{3}H-glucose,[10,15,16] due to substrate cycling between glucose-6-phosphate and fructose-6-phosphate in the liver and kidney. However, any overestimation of glucose Ra, if it did occur, would be the same for both groups.

Sterile, pyrogen-free 2-^{3}H-glucose (370 GBq/mmol) was infused at a constant rate of 750 kBq/h from a calibrated autosyringe (Travenol Laboratories, Hooksett, NH) for 75 minutes before and throughout the 180 minutes of exercise, after a priming dose equivalent to 45 minutes of continuous infusion. Pilot experiments showed that tracer equilibration, as evidenced by a constant plasma glucose specific activity, occurred between 60 and 75 minutes after the start of infusion.

Rates of exogenous CHO oxidation in NLC subjects were measured using a U-^{14}C-glucose label. Two hundred kilobequerels of a 7,400-kBq/mmol U-^{14}C-glucose (Amersham International) tracer was added to a 10% solution of short-chain (DP4) glucose polymer. This also enabled measurement of plasma glucose oxidation. So that plasma glucose oxidation could also be measured in CLP subjects, the tracer was also added to an artificially sweetened and colored water placebo. One hundred seventy milliliters of either solution was ingested at the start of exercise and a further 170 mL ingested every 20 minutes thereafter, so that 500 mL was ingested every hour. The ^{14}C-glucose tracer was ingested rather than infused, so that both exogenous CHO (in NLC subjects) and total plasma glucose oxidation could be determined from expired $^{14}CO_2$ (described subsequently). Since expired $^{14}CO_2$ and plasma ^{14}C-glucose specific activities were determined simultaneously (described later), the route of ^{14}C-glucose tracer administration did not affect determination of plasma glucose oxidation.

Pilot experiments in this laboratory (J.A. Hawley, unpublished observations, July 1993) conducted under conditions similar to those of the present study showed that ^{14}C-glucose tracer could successfully be used to label a glucose polymer of fivefold the glucose chain length (degree of polymerization, 22) used in the present study (degree of polymerization, 4). This is shown in Fig 1 (n = 6) which shows the relationship between plasma glucose specific activity and that of ingested glucose, maltose, and a glucose polymer of 22 glucose monomers. The line of ^{14}C-glucose as tracer with the polymer as tracee is superimposed on the lines of the "ideal" tracer/tracee combinations of ^{14}C-glucose/glucose and ^{14}C-maltose/maltose. In addition, oxidation rates of glucose, glucose polymer, and maltose measured in the validation study were identical, with peak rates being 0.9 ± 0.04, 0.9 ± 0.08, and 1.0 ± 0.04 g/min, respectively. Thus, even a polymer with five times the molecular mass of the one used in the current study can be successfully traced with a glucose tracer.

Stable-isotope techniques were not used in the present study, because it involved CHO-loading and most of the CHO-containing foods in this country are naturally enriched with ^{13}C.

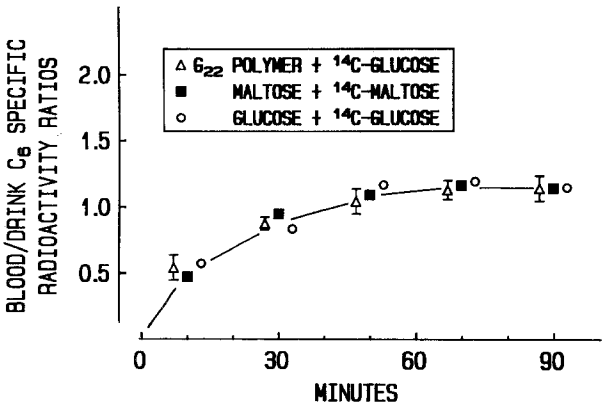

Fig 1. Plasma glucose to drink specific activity ratios of a glucose polymer of 22 glucose monomers, glucose, and maltose. The glucose polymer and glucose were labeled with [^{14}C]glucose, and maltose with [^{14}C]maltose. There were no differences in tracking between the ideal tracer/tracee combinations and [^{14}C]glucose with a fivefold longer chain polymer than that used in the present study.

$\dot{V}O_2$, $\dot{V}CO_2$, and $^{14}CO_2$ Measurements

Every 20 minutes during exercise, $\dot{V}O_2$ and $\dot{V}CO_2$ were determined as previously described and expired air was trapped for later determination of $^{14}CO_2$ specific activity. The CO_2-trapping mixture has been described previously[6,11] and consisted of 1 mL hyamine hydroxide in methanol (Packard, Meriden, CT), 1 mL ethanol, and 2 drops of 1% phenolphthalein indicator. Expired air was bubbled through the trapping mixture until the solution became clear, at which point exactly 1 mmol CO_2 had been absorbed. Liquid scintillation cocktail (10 mL Beckman Ready Gel; Beckman Instruments, Fullerton, CA) was then added, and $^{14}CO_2$ radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4640).

Plasma Glucose and Blood Lactate Concentrations

Plasma glucose concentrations were determined with a glucose analyzer (Analox LM3; Analox Instruments, London, UK) after collection of blood (5 mL) into chilled tubes containing potassium oxalate and sodium fluoride and centrifugation at 500 × *g* for 15 minutes. Blood lactate concentrations were measured by spectrophotometric (Beckman DU-62) enzymatic assays as previously described,[6,11] after collection of blood into chilled tubes containing 1 mL 0.6-mol/L $HClO_4$.

Plasma Glucose and Lactate Specific Activity

Blood samples (5 mL) were collected into prechilled tubes containing potassium oxalate and sodium fluoride and kept on ice until centrifuged at 500 × *g* for 15 minutes at 4°C. A 1-mL aliquot of the plasma was removed and 0.1 mL $HClO_4$ (3.5-mol/L) was added to deproteinize the sample and expel ^{14}C-bicarbonate as $^{14}CO_2$. The samples were then centrifuged at 4°C and the protein-free supernatant removed and kept cold. The precipitate was resuspended in 0.4 mL distilled water and recentrifuged, and the supernatant was added to that previously saved. To the combined supernatants, 0.6 mL 4-mol/L K_2CO_3 in 0.5 mol/L PIPES buffer was added slowly to the sample to return the pH to between 6.8 and 7.2. The sample was then centrifuged again to remove the precipitate. The supernatant was placed on a 5 × 1-cm column of Dowex-1 chloride (50 to 100 mesh) resin. The void volume, which contained some glucose, was collected, and the remaining glucose was eluted with distilled water (5 mL). Lactate was eluted with 0.2-mol/L $CaCl_2$ (5 mL). A complete separation of glucose and lactate was confirmed by measuring glucose and lactate concentrations in the eluted fractions. On scintillation counting, it was found that specific activity of the lactate was just above background, and therefore too low to be reliable. Thus, these data were not used.

To minimize the presence of ^3H_2O (~5%) from the metabolism of 2-^{3}H-glucose in the glycolytic pathway and reduce the water to liquid scintillation cocktail ratio during radioactivity counting, the eluates (~6 mL) were evaporated to near dryness (~0.3 mL) at 70°C over approximately 20 hours. One milliliter of distilled water was then added to the residue and mixed with 15 mL Ready Gel (Beckman) liquid scintillation cocktail for ^{3}H- and ^{14}C-radioactivity determinations (disintegrations per minute) by dual-channel counting (Packard Tri-Carb 4640). Since the 1-mL aliquot of plasma used for radiation counting was from the same plasma sample previously used for determination of glucose concentration, specific activity of glucose could be calculated after the small loss (4%) of radioactivity during the preparation of the sample was determined. Hence, whenever samples were processed, a control plasma sample was spiked with a known amount of 2-^{3}H- and U-^{14}C-glucose and evaluated concurrently so that the values (disintegrations per minute) of experimental samples could be corrected for the percentage recovery.

Glucose Turnover

Total (endogenous and exogenous) glucose Ra and glucose Ra from ingested CHO (Ra_{exog}) were calculated using Steele's equations for non–steady-state exercise.[17] These calculations have been validated by Radziuk et al[16] and are as follows: $Ra = (I - [pV \times Glu_{tot} \times dSA/dt])/SA$, $Ra_{exog} = (I - [pV \times Glu_{lab} \times dSA/dt])/SA$, and $Ra_{end} = Ra - Ra_{exog}$, where Ra, Ra_{exog}, and Ra_{end} are total (endogenous + exogenous), exogenous, and endogenous glucose Ra from liver in millimoles per minute; I is the infusion rate of 2-^{3}H-glucose in disintegrations per minute; p is the pool fraction (0.75) in which rapid changes in glucose concentration and specific activity take place[16,18]; V is the glucose distribution volume (19.6% of body mass in liters)[18]; Glu_{tot} is the mean plasma glucose concentration (^{14}C-labeled and nonlabeled) in millimoles in consecutive samples; Glu_{lab} is the mean plasma glucose concentration of ^{14}C-labeled glucose (^{14}C-glucose radioactivity (dpm) of the sample divided by SA (specific activity) of the ^{14}C-labeled ingested glucose) in millimoles in consecutive samples; dSA/dt is the change in plasma 2-^{3}H-glucose specific activities in disintegrations per minute per millimole over the sample interval in minutes; SA is the mean disintegrations per minute per millimole 2-^{3}H-glucose specific activity in successive samples.

A glucose pool fraction of 0.5 to 0.75 is usually used in this type of calculation,[15,19-22] and represents the fraction of the total glucose pool that behaves as an ideal, readily mixing pool and can be viewed as a factor to compensate for nonideal pool behavior. These values have been determined in the nonexercising state and were designed to accommodate rapid changes in blood glucose concentrations. The slower the change in blood glucose concentration, the higher the value of P becomes, until at an infinitely slow rate of change in glucose concentration the pool fraction would be 1.0.[16,23] The data of Cowan and Hetenyi[23] also suggest that P becomes larger when glucose Ra increases. During exercise, values for glucose Ra are much higher than those reported in experiments in which P was determined to be 0.65. Given these two factors, we therefore elected to use 0.75, since the change in glucose concentration in the present study occurred relatively slowly. This fraction, when multiplied by V, yields a final value for the "apparent" volume of distribution space of glucose of 147 mL/kg, a value within the range of 40 to 210 mL/kg reported by Wolfe et al[24] to be

an acceptable range for calculation of Ra data. The difference in Ra when 40 was used instead of 210 was only 10%.

Plasma Glucose Oxidation

Total (endogenous + exogenous) plasma glucose oxidation rates (R_{ox}) in grams per minute were calculated from the following equation: $R_{ox} = ([^{14}CO_2 \times 6]/SA\ glu) \times \dot{V}CO_2 \times 1.35$, where $^{14}CO_2 \times 6$ is the disintegrations per minute per millimole value multiplied by 6, since there are 6 carbon atoms per molecule of ^{14}C-glucose; SA glu is the plasma ^{14}C-glucose specific activity in disintegrations per minute per millimole; $\dot{V}CO_2$ is in liters per minute; and 1.35 is the grams of glucose oxidized to produce 1 L CO_2.

Exogenous CHO Oxidation

Rates of exogenous CHO oxidation (R_{exog}) in grams per minute were calculated from the following equation: $R_{exog} = (^{14}CO_2 \times 6/SA_{exog}) \times \dot{V}CO_2 \times 1.35$, where $^{14}CO_2 \times 6$ is the disintegrations per minute per millimole value multiplied by 6, since there are 6 carbon atoms per molecule of ^{14}C-glucose; SA_{exog} is the specific activity of the ingested CHO in disintegrations per minute per millimole; $\dot{V}CO_2$ is in liters per minute; and 1.35 is the grams of glucose oxidized to produce 1 L CO_2.

Endogenous Glucose Oxidation

Oxidation of endogenous glucose was calculated by difference between R_{ox} and R_{exog}.

These formulae do not take into account $^{14}CO_2$ retained in the bicarbonate (HCO_3^-) pool. The time required to equilibrate $^{14}CO_2$ with the plasma CO_2/HCO_3^- pool during exercise performed at 60% to 70% $\dot{V}O_2$max varies from 15 to 20 minutes,[25,26] 30 minutes,[27] 45 minutes,[19] to as long as 75 minutes.[28] However, a study by Barstow et al[26] presents strong evidence that 90% of equilibration has occurred after 16 minutes of moderate-intensity exercise. In the present study, plasma ^{14}C-glucose, which would not be affected by the HCO_3^- pool, took approximately 90 minutes to reach equilibrium, but $^{14}CO_2$ specific activity closely tracked the increase in plasma ^{14}C-glucose specific activity. It is therefore unlikely that any lag in $^{14}CO_2$ appearance was due solely to retention of $^{14}CO_2$ in the HCO_3^- pool. Our data on bicarbonate pool kinetics indicate equilibration of the pool within 45 minutes (A. Bosch and S.M. Weltan, unpublished observations, July 1993). However, any underestimation of CHO oxidation in the early part of the trial as a result of the time to equilibrate the bicarbonate pool would be similar from trial to trial.

Leg Muscle Glycogen Disappearance

The muscle biopsy technique of Bergstrom[29] as modified by Evans et al[30] was used to sample muscle tissue from the vastus lateralis before the start of exercise and hourly thereafter. Glycogen content of the biopsy samples was measured using conventional methods.[31]

Total CHO and Fat Oxidation

Total CHO and fat oxidation was calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ using the formulae of Consolazio et al[12] as updated by Frayn.[32] The nonprotein respiratory exchange ratio (RER) was calculated by correcting for urinary urea by having subjects void at the start of the experiment and again on completion of the 3-hour ride. Values (grams per minute) obtained from these formulae were converted to FFM units (micromoles per minute per kilogram) by dividing the values by the molecular weight of glucose or a hexose equivalent of glycogen (180) and by the subject's FFM.

Statistical Analyses

All results are presented as the mean ± SEM for seven NLC (two of nine NLC subjects failed to complete the trial) and seven CLP subjects. Statistical significance ($P < .05$) of between-group differences was assessed by a two-way ANOVA for repeated measures over time, and a Scheffé test was used for post hoc analysis where necessary. Student's t test was used for comparison of data between groups.

RESULTS

Subject characteristics are listed in Table 1. $\dot{V}O_2$max and peak work rate were lower than might have been expected from trained subjects, but were nevertheless consistent with the subjects' being moderately trained cyclists. All subjects who had CHO-loaded and ingested placebo (CLP) completed the prescribed 180 minutes of exercise, whereas two of nine subjects who did not CHO-load but ingested CHO during the trial were unable to complete 180 minutes, but this was not statistically significant.

Oxygen consumption (Table 2) during exercise was relatively constant (subjects were maintained at close to 70% of $\dot{V}O_2$max) and not significantly different between groups. RER also was not significantly different between groups, but decreased significantly during the trial (Table 2). Although it appears from Table 2 that CLP subjects oxidized more CHO and less fat than NLC subjects, these differences did not reach statistical significance when tested with a repeated-measures ANOVA. However, both changed significantly over time. Oxidation of CHO contributed 73% ± 8% of total energy in CLP subjects and 76% ± 8% in NLC subjects.

Plasma glucose concentrations (Fig 2) were not significantly different between groups and remained between 4.0 ± 0.2 and 5.4 ± 0.5 mmol/l throughout exercise. However, total (endogenous + exogenous) glucose Ra was significantly ($P < .05$) higher in NLC than in CLP subjects (Fig 3) and increased significantly over time. Ra_{end} was significantly lower in the NLC versus the CLP group (Fig 3), but did not change significantly over time. However, Ra_{exog} in the NLC group increased significantly ($P < .05$) until a plateau was reached after approximately 120 minutes (Fig 4), at which time 80% of Ra (Fig 5) was accounted for from exogenous CHO.

Total plasma glucose oxidation (Fig 6) also increased progressively ($P < .05$) from 13 ± 3 to 115 ± 16 μmol/min/kg FFM (0.15 ± 0.04 to 1.34 ± 0.19 g/min) in NLC subjects, and from 19 ± 4 to 74 ± 11 μmol/min/kg FFM (0.22 ± 0.05 to 0.85 ± 0.12 g/min) in CLP subjects (Fig 6).

Table 1. Characteristics of the Subjects

Characteristic	CLP (n = 7)	NLC (n = 9)
Age (yr)	25 ± 3	27 ± 3
$\dot{V}O_2$max (L/min)	3.64 ± 0.19	3.53 ± 0.26
Peak work rate (W)	332 ± 23	322 ± 22
Mass (kg)	74.5 ± 3.5	75.8 ± 4.0
FFM (kg)	63.4 ± 3.1	63.6 ± 3.0
Body fat (%)	13 ± 1.0	16 ± 2.0
Completed 180 min of exercise	7 of 7	7 of 9

NOTE. Values are the mean ± SEM.

Table 2. Steady-State Gas-Exchange Data and Total CHO Oxidation During 180 Minutes of Cycling in NLC and CLP Subjects

Parameter	Time (mins) 5	22	44	66	88	110	132	150	180
$\dot{V}O_2$ (L/min)									
NLC	2.40 ± 0.22	2.40 ± 0.22	2.40 ± 0.20	2.46 ± 0.17	2.52 ± 0.18	2.55 ± 0.17	2.54 ± 0.16	2.61 ± 0.17	2.57 ± 0.17
CLP	2.56 ± 0.20	2.56 ± 0.20	2.52 ± 0.15	2.48 ± 0.11	2.52 ± 0.15	2.51 ± 0.12	2.54 ± 0.13	2.58 ± 0.14	2.58 ± 0.15
RER*									
NLC	0.90 ± 0.01	0.90 ± 0.01	0.89 ± 0.01	0.89 ± 0.01	0.89 ± 0.02	0.88 ± 0.02	0.88 ± 0.02	0.85 ± 0.02	0.85 ± 0.02
CLP	0.94 ± 0.03	0.93 ± 0.03	0.92 ± 0.03	0.90 ± 0.03	0.90 ± 0.02	0.90 ± 0.03	0.87 ± 0.02	0.88 ± 0.03	0.88 ± 0.03
CHO_{ox} (g/min)*									
NLC	2.02 ± 0.25	2.03 ± 0.25	1.90 ± 0.23	1.91 ± 0.14	1.97 ± 0.21	1.82 ± 0.15	1.88 ± 0.16	1.55 ± 0.15	1.50 ± 0.17
CLP	2.62 ± 0.49	2.60 ± 0.49	2.39 ± 0.43	2.16 ± 0.42	2.22 ± 0.36	2.15 ± 0.36	1.82 ± 0.30	1.97 ± 0.38	1.94 ± 0.37
Fat_{ox} (g/min)*									
NLC	0.37 ± 0.05	0.37 ± 0.05	0.42 ± 0.04	0.44 ± 0.04	0.45 ± 0.08	0.52 ± 0.10	0.49 ± 0.09	0.65 ± 0.11	0.65 ± 0.12
CLP	0.23 ± 0.11	0.24 ± 0.11	0.30 ± 0.10	0.36 ± 0.11	0.36 ± 0.09	0.38 ± 0.11	0.52 ± 0.09	0.48 ± 0.11	0.49 ± 0.10

NOTE. Values are the mean ± SE.

Abbreviations: CHO_{ox}, CHO oxidation calculated from gas-exchange data; Fat_{ox}, fat oxidation from gas-exchange data.

*Significant change over time ($P < .05$). There were no significant differences between groups.

Rates of total plasma glucose oxidation were significantly higher in NLC subjects toward the end of exercise; however, oxidation of plasma glucose derived from endogenous sources was significantly lower in NLC than in CLP subjects (Fig 6). Thus, in NLC subjects, there was a large contribution from exogenous CHO (Fig 7), which reached 69 ± 7 μmol/min/kg FFM (0.80 ± 0.08 g/min) after 180 minutes. At the end of exercise, 93% ± 13% of all CHO oxidation was derived from plasma glucose in NLC subjects, and in CLP subjects, 53% ± 11% ($P < .05$; Fig 8).

Although Ra initially exceeded the rate of glucose oxidation in both groups, oxidation of plasma glucose increased until it was not significantly different from Ra (Fig 9).

Insulin levels were 4.4 ± 0.4 and 2.7 ± 0.7 μU/mL at the start of exercise in NLC and CLP groups, respectively, and decreased to 3.0 ± 0.5 and 1.2 ± 0.5 μU/mL at the end of exercise. The difference between groups at the end of exercise was significant ($P < .05$). The low initial values indicate that the breakfast ingested by the subjects was probably absorbed by the time exercise started.

Muscle glycogen content declined linearly during the trial in CLP subjects, whereas in NLC subjects, the rate of disappearance decreased after the first hour (Fig 10). Thus, at the end of the trial, mean values were identical (60 ± 10 and 60 ± 8 mmol/kg wet weight in NLC and CLP, respectively). Rates of disappearance from 0 to 60 minutes were −48 ± 2 and −55 ± 10 mmol/kg wet weight in CLP and NLC subjects, respectively (not significant); from 60 to 120 minutes, −57 ± 6 and −24 ± 5 mmol/kg wet weight ($P < .05$); and from 120 to 180 minutes, −30 ± 8 and 4 ± 5 mmol/kg wet weight, respectively ($P < .05$).

DISCUSSION

The separate effects and fuel substrate kinetics of CHO-loading (without CHO ingestion) and CHO ingestion (after CHO-loading) on CHO metabolism during exercise have been previously determined.[2,3,6,11,33] However, a direct comparison of differences that may exist in substrate kinetics between subjects who ingest water during exercise after prior CHO-loading and subjects who ingest CHO during exercise after not CHO-loading before exercise, and the relative importance of these two procedures for supplying

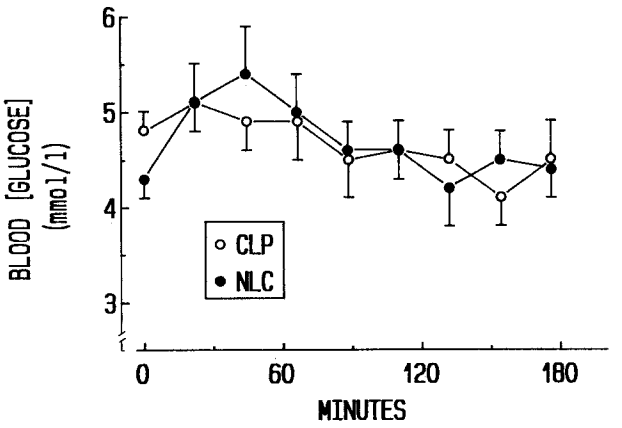

Fig 2. Plasma glucose concentrations were not significantly different between non–CHO-loaded subjects ingesting CHO (NLC) and CHO-loaded subjects ingesting water placebo (CLP). There was no significant change during the trial.

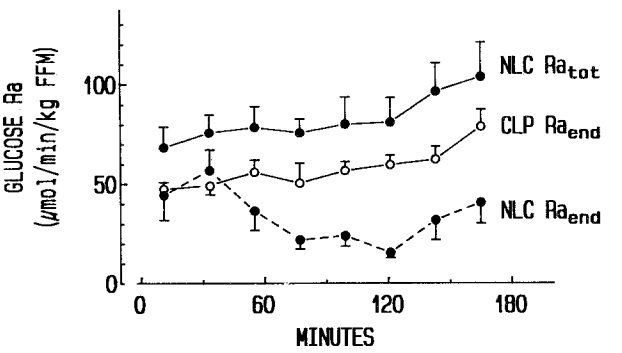

Fig 3. Glucose Ra increased significantly in both groups during the trial. Endogenous glucose Ra in NLC subjects was significantly less than in CLP subjects, although Ra from endogenous and exogenous sources together was significantly greater ($P < .05$) in NLC subjects than in CLP subjects.

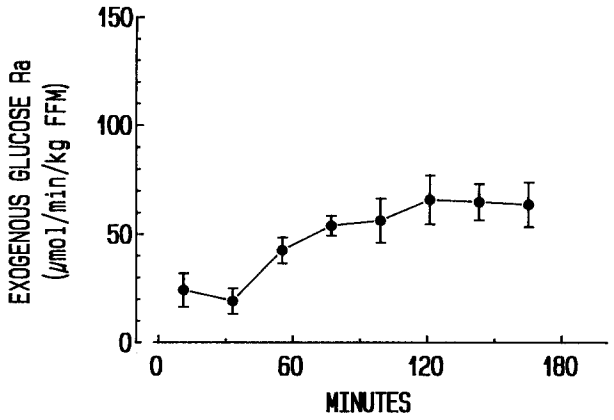

Fig 4. Ra for glucose derived from ingested (exogenous) CHO in NLC subjects.

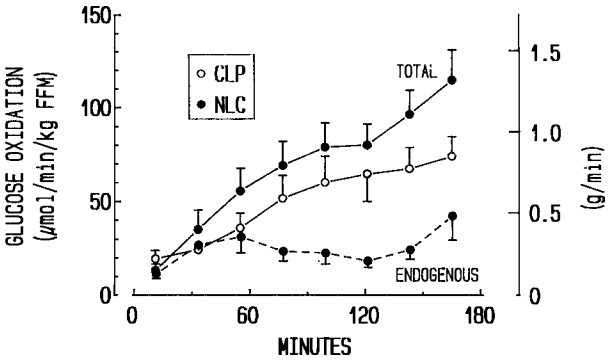

Fig 6. Plasma glucose oxidation increased significantly in both groups ($P < .05$) during the trial. Endogenous glucose oxidation in NLC subjects was significantly ($P < .05$) less than in CLP subjects, although total glucose oxidation in NLC subjects was significantly ($P < .05$) greater.

CHO to athletes participating in endurance events, have not yet been reported.

Despite CHO ingestion, two subjects in the NLC group were unable to complete the 180-minute trial. Although this was not statistically significant, these findings concur with those of a previous study[6] in which 50% of subjects who ingested only water during the trial and who, as in the present study, were not CHO-loaded at the start of exercise were unable to complete the 180-minute trial. In that study, mean muscle glycogen content of subjects who fatigued prematurely reached low levels of 22 ± 4 mmol/kg wet weight, values previously reported to be consistent with fatigue.[3] The two NLC subjects who did not complete the trial in the present study also had low (<22 mmol/kg wet weight) muscle glycogen levels when they stopped exercise. In contrast, even though CLP subjects ingested only water during exercise, all could complete the trial, suggesting that CHO-loading may result in an ergogenic effect as a consequence of higher muscle and liver glycogen levels at the start of exercise. An increased time to exhaustion is compatible with findings from other studies showing an ergogenic effect of CHO-loading.[3,4,6,34]

The rate of muscle glycogen disappearance decreased after 60 minutes in NLC subjects, but not in CLP subjects. This is most likely an effect of the lower muscle glycogen levels at the start of exercise in NLC subjects, rather than a result of CHO ingestion in CLP subjects. This is suggested from the results of a previous study,[6] in which the rate of muscle glycogen disappearance was slower in subjects who had not CHO-loaded than in subjects who had, when both groups ingested only water during exercise. Other studies[2,11] have shown that when subjects are CHO-loaded, the rate of muscle glycogen disappearance is the same irrespective of CHO or water ingestion.

Total CHO oxidation (Table 2) appeared to be higher in CLP subjects, but this was not statistically significant and declined during the trial by 26% and 25% in CLP and NLC subjects, respectively. Other studies have demonstrated similar reductions in RER and total CHO oxidation, despite CHO ingestion during exercise at approximately 70% of $\dot{V}O_2max$.[7,11] Total glucose Ra was higher in NLC subjects, and can be attributed to CHO ingestion rather than to lower muscle glycogen content in these subjects, since we have previously found that CHO-loading does not influence Ra,[6] whereas CHO ingestion does.[11] A higher Ra has also been reported by Wahren.[35] Although Ra was higher, Ra_{end} (Fig 3) was lower throughout exercise in NLC

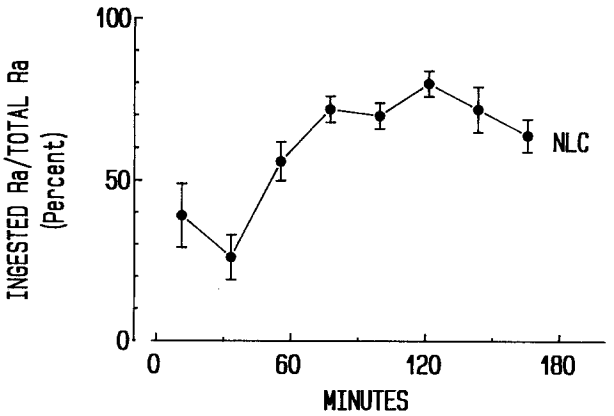

Fig 5. Percentage contribution of exogenous (ingested) CHO to total plasma glucose Ra in NLC subjects. A plateau was reached after 75 minutes.

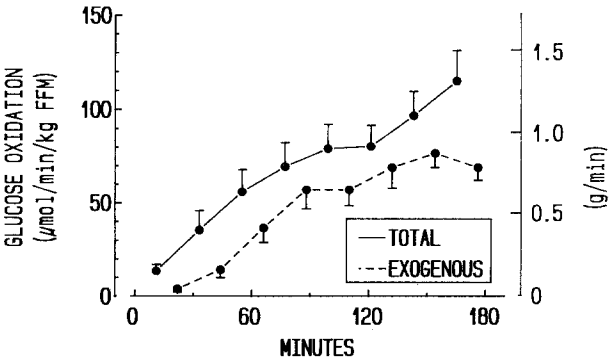

Fig 7. Contribution of exogenous (ingested) CHO to plasma glucose oxidation. Exogenous CHO contribution reached a plateau and contributed significantly to total plasma glucose oxidation, which continued to increase throughout exercise.

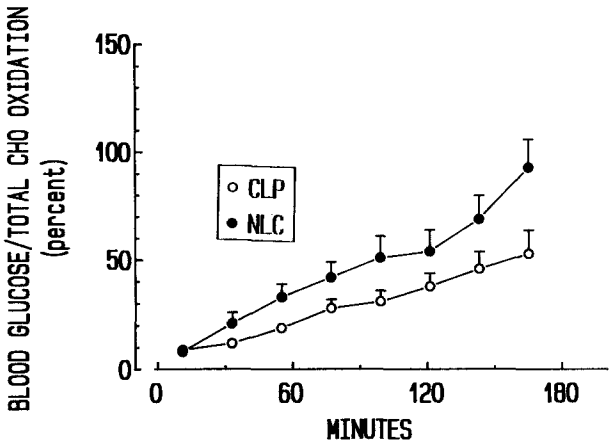

Fig 8. Percentage contribution of plasma glucose to total CHO oxidation increased progressively throughout exercise and was significantly greater in NLC subjects than in CLP subjects at the end of exercise.

subjects than in CLP subjects, as a result of a significant contribution of exogenous CHO to Ra, which increased during exercise (Fig 4) and reached a peak of approximately 80% of Ra after 120 minutes (Fig 5).

Over the duration of the trial, the mean Ra_{end} in NLC subjects was only 60% of that in CLP subjects (2.17 ± 0.53 *v* 3.63 ± 0.39 mmol/min), or a total of 70 versus 118 g. Thus, 48 g glucose was "spared" by CHO ingestion. The total Ra_{end} of 70 g in NLC subjects over the 180 minutes of exercise yields a mean of 0.4 g/min. Thus, the glucose spared (48 g) would be sufficient for an additional 120 (48 ÷ 0.4) minutes of exercise before the same total amount of endogenous glucose (118 g) had appeared as in CLP subjects. These findings are virtually identical to those in subjects in which both groups had CHO-loaded and one had ingested CHO and the other water during exercise.[11] Total Ra, the percentage contribution of exogenous CHO to Ra, and endogenous Ra in NLC subjects were the same in the present study as in subjects in the earlier study who both were CHO-loaded and ingested CHO during exercise.

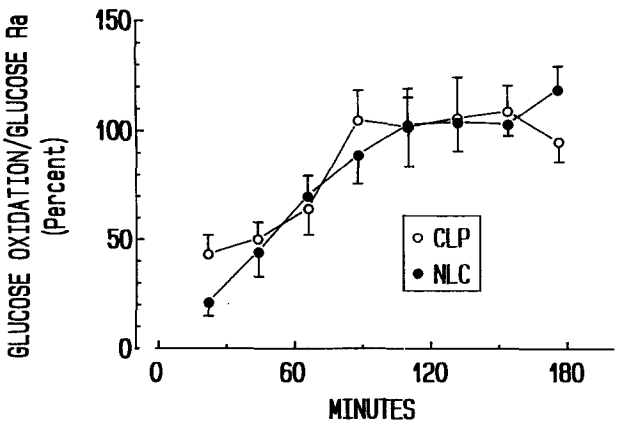

Fig 9. Plasma glucose oxidation expressed as a percentage of total plasma glucose Ra, ie, the percentage of Ra that is oxidized. Ra exceeded the rate of oxidation for the first 75 minutes of exercise, but thereafter Ra and oxidation rate were equal (100%).

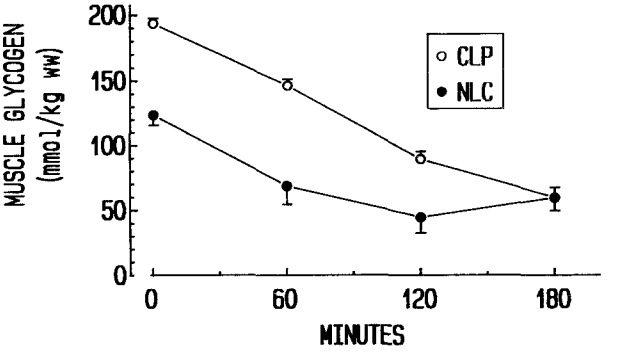

Fig 10. Rate of muscle glycogen disappearance was linear in CLP subjects throughout the trial. In NLC subjects the rate was the same as in CLP subjects over the first hour of exercise, but thereafter the rate of utilization slowed.

Oxidation of exogenous CHO increased rapidly (mean rate of increase, 0.63 μmol/min/kg FFM) until 90 minutes (Fig 7), with the rate of increase being less thereafter (0.31 μmol/min/kg FFM), thus reflecting the increase and plateau that occurred in Ra of exogenous CHO (Fig 4). Previous studies in which ^{13}C-glucose was ingested during exercise[28,33,36,37] also showed a progressive increase in the rate of oxidation of exogenous CHO. The oxidation rate for exogenous CHO at 90 minutes was 57 ± 10 μmol/min/kg FFM (0.66 ± 0.12 g/min). This value is similar to that found in another study in which subjects CHO-loaded and ingested CHO during exercise, suggesting that the rate of oxidation of exogenous CHO is independent of muscle glycogen status. It is also similar to that found in studies that have measured rates of oxidation of exogenous CHO using ^{13}C techniques, wherein rates have varied from 0.4 to 0.9 g/min, depending on exercise intensity and the amount of CHO ingested.[33,36,37]

As with Ra (Fig 3), total plasma glucose oxidation (Fig 6) also increased progressively in both groups. This was probably the result of decreasing muscle glycogen content (Fig 10), as Gollnick et al[4] have found that glucose uptake by exercising muscle increases in proportion to the number of glycogen-depleted fibers and compensates somewhat for the reduced glycogen availability. The higher rate of plasma glucose oxidation in NLC than in CLP subjects toward the end of exercise is probably attributable to the higher plasma insulin concentrations and lower muscle glycogen concentrations[4] in NLC subjects at that time.

The influence of muscle glycogen content on the rate of plasma glucose oxidation is further shown by the finding that the percentage for the plasma glucose contribution to total CHO oxidation (Fig 8) was significantly higher in NLC than in CLP subjects at the end of exercise (93% ± 13% and 53% ± 11% of total CHO oxidation, respectively). In a previous study,[11] the percentage contribution in subjects who were CHO-loaded and ingesting CHO during exercise was 57% ± 6%, which did not differ significantly from values in CLP subjects in this study. Thus, CHO-loading had a greater effect on the percentage contribution of plasma glucose to total CHO oxidation than did CHO ingestion. Further evidence for this is found when the rate

of muscle glycogen disappearance (Fig 10) is compared with the percentage contribution of plasma glucose to total CHO oxidation (Fig 8). Figure 10 shows a linear decline in muscle glycogen content and a constant rate of disappearance in CLP subjects, and Fig 8 shows a corresponding linear increase in the percentage contribution of plasma glucose to total CHO oxidation. However, in the case of NLC subjects, muscle glycogen concentration remained constant between 120 and 180 minutes after glycogen content had reached approximately 55 mmol/kg wet weight (Fig 10), and this was accompanied by a rapid increase ($P < .05$) in the percentage contribution of plasma glucose to total CHO oxidation (Fig 8). These observations agree with the conclusions of Coyle et al[2] that after 2 to 3 hours of exercise at 70% to 75% of $\dot{V}O_2max$, at which time muscle glycogen content would be very low, blood glucose represents a major source of CHO for oxidation.

The increase in plasma glucose oxidation in NLC subjects occurred mainly as a result of an increase in oxidation of plasma glucose originating from exogenous CHO (Fig 7). Thus, in NLC subjects, it appears that the higher Ra resulting from CHO ingestion resulted in an increased amount of plasma glucose available for oxidation. Similar kinetics were found in a previous study[11] in subjects who were CHO-loaded and ingested CHO during exercise, suggesting that these kinetics are independent of muscle glycogen status. Interestingly, concomitantly with Ra_{end} (Fig 3), endogenous plasma glucose oxidation (Fig 6) remained low in NLC subjects throughout the trial.

A consequence of inadequate Ra is hypoglycemia,[35,38] which would result in a reduction in exercise intensity, or termination of exercise before muscle fatigue or muscle glycogen depletion occurs. Hypoglycemia would be compounded by an increasing rate of blood glucose oxidation at the same time Ra is decreasing. Thus, the interaction between Ra and rate of plasma glucose oxidation is important (Fig 9). Oxidation was approximately the same as Ra in both groups, but it appears from mean values in Fig 9 that sometimes oxidation exceeded Ra, particularly at 154 minutes. An examination of plasma glucose concentration (Fig 2), glucose Ra (Fig 3), and glucose oxidation (Fig 6) shows that at 154 minutes, plasma glucose concentration declined significantly in CLP subjects to 4.1 ± 0.3 mmol/L. Thus, oxidation of plasma glucose at this time exceeded Ra and occurred at the expense of plasma glucose. This was followed during the period of 154 to 180 minutes by a significant increase in Ra, which provided sufficient glucose to maintain plasma glucose oxidation and simultaneously restore plasma glucose concentration to euglycemic levels. The decline in plasma glucose to approximately 4 mmol/L probably activated hormonal and nonhormonal counterregulatory mechanisms, with the resultant increase in Ra. A similar interaction was observed at approximately 90 minutes, when the mean rate of oxidation was 105% of Ra. Plasma glucose oxidation was increasing (Fig 6) with little increase in Ra (Fig 3), with a resultant decrease in plasma glucose concentration (Fig 2). However, the subsequent increase in Ra was less than at 154 minutes, probably because plasma glucose concentration remained at approximately 4.5 mmol/L and thus did not provoke a dramatic increase in Ra. Similar findings with respect to the relationship between Ra and glucose uptake have also been reported by Wolfe et al.[24] At no time was oxidation significantly greater than Ra.

In NLC subjects, a large percentage of the Ra was from exogenous CHO (Fig 4), whereas in CLP subjects the entire Ra was from endogenous sources (Fig 3). The ability of glucoregulatory mechanisms to maintain euglycemia and sufficient plasma glucose for oxidation is dependent on the availability of sufficient endogenous substrate. As described earlier, in a previous study,[6] 50% of subjects who had not CHO-loaded and who ingested only water during exercise failed to complete the 180-minute trial. The fact that CLP subjects in the present study not only completed the 180 minutes of exercise but maintained plasma glucose oxidation rates equal to those of NLC subjects indicates that CHO-loading provides sufficient endogenous CHO for exercise of this duration and intensity.

In summary, (1) CHO-loading before exercise reduces the relative contribution of plasma glucose oxidation to total CHO oxidation, but may prolong time to exhaustion as a function of higher muscle glycogen concentration; (2) CHO ingestion has a liver glycogen-sparing effect, causes a reduction in gluconeogenesis, or both, which should delay the onset of hypoglycemia; (3) the progressive increase in plasma glucose oxidation that occurs during prolonged exercise is related to muscle glycogen status and occurs irrespective of whether CHO is ingested; and (4) since the effects of CHO ingestion and CHO-loading on fuel substrate kinetics are different, both procedures may be necessary for the maintenance of optimal fuel substrate kinetics in endurance events of several hours. The benefits of these kinetics for cycling performance have been demonstrated in a recent study by Widrick et al.[39]

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