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Muscle glycogen recovery after exercise measured by ^{13}C-magnetic resonance spectroscopy in humans: effect of nutritional solutions

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Abstract

The rate of glycogen resynthesis in human skeletal muscle after glycogen-depleting exercise is known to depend on carbohydrate intake and is reported to reach a plateau after an adequate amount of carbohydrate (CHO) consumption. Efforts to maximize the rate of glycogen storage by changing the type and form of CHO, as well as by adding proteins or lipids have yielded inconsistent results. The objective of this study was to assess whether isocaloric addition of proteins and arginine to a CHO diet in the first 4 h after an endurance exercise would increase the rate of glycogen synthesis. The CHO solution, given twice at a 2 h interval according to earlier optimized protocols, contained 1.7 g CHO/kg$^{body\ weight}$. The effects of this solution were compared to those of an isocaloric solution containing 1.2 g CHO/kg$^{body\ weight}$ plus 0.5 g protein/kg$^{body\ weight}$ (including 5 g arginine). Glycogen was measured in quadriceps muscle in vivo with natural abundance ^{13}C-magnetic resonance spectroscopy before exercise and twice after exercise, before and at the end of a 4-h period following the intake of one of the solutions. Eight subjects took part in a randomized cross-over trial separated by at least 1 week. Glycogen synthesis was found to be significantly increased with both regimes compared to a zero-caloric placebo diet, but no significant difference in glycogen resynthesis was found between the CHO-only diet and the one supplemented by proteins and arginine. It is estimated that significance would have been reached for an increase of 34%, while the effectively measured synthesis rates only differed by 5%. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Exercise; Glycogen; Carbohydrate; Protein; Arginine; Insulin; Magnetic resonance spectroscopy

1. Introduction

After prolonged endurance exercise, recovery of muscle glycogen is slow unless carbohydrate solutions or meals are provided to the subject [1–3]. When an adequate amount of carbohydrate is consumed immediately after exercise and at 2 h intervals thereafter, the rate of muscle glycogen storage is rapidly increased and maintained at an elevated level for up to 6 h after exercise [3–6]. However, efforts to maximize the rate of glycogen storage by increasing the amount and frequency of carbohydrate intake or by changing the type and form of the carbohydrates used yielded inconsistent results [3,5–7]. Burke et al. [8] showed that significantly greater muscle glycogen storage was associated with ingestion of foods with high rather than low glycemic index. Moreover, Zawadzki et al. [9] observed a 40% greater rate of muscle glycogen synthesis after ingestion of a nutritional mixture containing carbohydrate + protein than with carbohydrate alone, however, the two nutritional solutions were not iso-energetic. Tarnopolski et al. [10] compared the rate of glycogen synthesis after endurance exercise when the energy content of the post-exercise supplements (carbohydrate compared with carbohydrate + protein + fat) was held constant. They did not observe a significantly different effect of the two test meals on the rate of muscle glycogen synthesis, but the amount of CHO (0.75 g/kg body wt) was probably too low to maximize glycogen synthesis and, additionally, the fat content may have delayed the digestion.

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Our study aimed at maximizing the rate of muscle glycogen resynthesis following glycogen depletion by exercise, comparing the effects of the intake of two iso-energetic solutions. The first (C), containing a mixture of CHO of high glycemic index, was known to optimize glycogen synthesis. The second (CPA), containing an iso-energetic mixture of CHO of high glycemic index mixed with whey protein and arginine, was designed for its efficient insulin release properties. In two studies [3,5], it had been suggested that there is a threshold of CHO intake (about 1.2 g CHO/kg body wt) above which the rate of glycogen synthesis is maximally stimulated. Accordingly, the CHO content of solution C (1.7 g/kg body wt) compared with that of solution CPA (1.2 g/kg body wt) was expected not to lead to a different rate of muscle glycogen synthesis. It was hypothesized that, if a greater rate of glycogen synthesis was observed with the CPA solution, it could be attributed to its protein and arginine content. The rationale for this is that both protein and arginine intakes, have specific stimulating effects on insulin secretion [11–13], and insulin is known to be a strong activator of muscle glycogen synthesis [14,15]. Insulin increases the rate of muscle glucose transport and activates glycogen synthase, the rate-limiting enzyme in the glycogen synthesis pathway.

Dietary protein and arginine may also enhance glycogen synthesis by 'sparing' ingested carbohydrates for storage rather than for immediate oxidation to meet body energy requirements. Arginine could also be a potential mediator of glucose transport in skeletal muscles via the nitric oxide (NO) pathway [16] and induce a vasodilatation that is mediated, in part, by endogenously released insulin [17]. These combined mechanisms could increase muscle glucose availability and contribute to stimulate glycogen synthesis.

The first aim of this study was to test whether the addition of protein and arginine to a CHO solution significantly enhances the plasma insulin concentration compared to a solution containing CHO only. Based on positive results, the main study was designed as a cross-over study to compare muscle glycogen synthesis upon ingestion of either a CHO or an isocaloric CHO + protein + arginine solution. The rate of muscle glycogen synthesis was measured using in vivo natural abundance ^{13}C-magnetic resonance spectroscopy (^{13}C-MRS). ^{13}C-MRS techniques have made it possible to measure glycogen concentrations non-invasively in human muscle [18–23]. It is known that the ^{13}C nuclei of glycogen are almost 100% visible with MRS (Ref. [24] and references therein), hence ^{13}C-MR spectra allow a non-invasive measurement of glycogen content in a specific muscle. The possibility to measure muscle metabolites repeatedly and in relatively short intervals by ^{13}C-MRS allows the analysis of glycogen metabolism frequently before and after an exercise session, and during the recovery of glycogen stores. The potential of the specific ^{13}C-MRS techniques used to define nutritional effects on glycogen recovery rates was established in a subgroup of the investigated subjects using a zero-calorie placebo diet in comparison to the targeted C and CPA solutions.

2. Materials and methods

2.1. Preliminary study

2.1.1. Subjects

Eight healthy male subjects, aged 25–40 years, body mass index < 25 kg/m^2, took part in this study.

2.1.2. Experimental protocol

The subjects drank three nutritional solutions after an overnight fast, each solution being ingested at least 6 days apart in a random sequence. Glucose and insulin plasma levels were measured 30 min before the ingestion, at time 0 and then every 30 min during 4 h after the ingestion. Each test drink contained 50 g CHO in the form of fermented cereals. It consisted of a mixture of sucrose (94%), maltose (3%), glucose (2%) and fructose (1%). The first solution (C_1) contained 50 g of this CHO mixture only. The second solution (CP_1) was made up of 50 g of the CHO mixture plus 25 g of whey protein. The third solution (CPA_1) contained 50 g of the CHO mixture, 15 g whey protein plus 10 g L-arginine.

Blood samples were collected in cooled EDTA sample tubes, centrifuged at 3000 rpm at −4°C for 15 min and the plasma was frozen at −20°C until analysis. Glucose was determined by the hexokinase reaction [25] and insulin by the method of Herbert et al. [26].

2.2. Main study

2.2.1. Subjects

Eight healthy male subjects (25–35 years old) who were moderately trained in different sporting activities were investigated. Average height and weight (mean ± 1 SD) were 176.8 ± 5.7 cm and 69.2 ± 5.3 kg, respectively. The average maximum O_2 consumption ($VO_{2\,max}$) of these subjects was 50.3 ± 8.7 ml kg^{-1} min^{-1}. The protocol of the experiment and possible risks were fully explained to each subject before they signed an informed consent document. The protocol had been reviewed and approved by the Nestlé Research Center's Ethical committee.

2.2.2. Experimental protocol

At least one week before the trial the $VO_{2\,max}$ of each subject was determined with a gas analyzer (MMC Horizon, Gambro Ltd, Hünenberg, Switzerland) on an

electrically braked cycle ergometer (Ergo-metrics 900, Rüegge Medical Ltd, Baden, Switzerland) by means of a continuous incremental exercise test. Heart rate was monitored (Polar sportesterTM PE 3000, Polar Electro Ltd, Finland). The subjects had to maintain between 80–95 revolution/min (rpm). A respiratory exchange ratio (R) ≥ 1.15 [27] and a VO_2 plateau over the last two samples (less than 2 ml kg^{-1} min^{-1} rise in VO_2) [28] were the criteria used to ascertain $VO_{2\ max}$. In some cases, the subject was not able to maintain the prescribed pedal revolution rate. The stability criterion was replaced by the condition that the pedal rate dropped below 60 rpm.

The subjects were requested to avoid intense physical exercise and to consume a high carbohydrate diet (at least 250 g CHO) the day preceding the tests. The subjects reported to the laboratory in the morning after an overnight fast, the tests were carried out at least 1 week apart. The order of treatment administration was randomized.

The experiment started with the determination of the glycogen content of the quadriceps muscle by ^{13}C-MRS. All subjects performed bicycle ergometer exercise according to established protocols [2,7,29] to deplete their muscle glycogen stores. A 90 min exercise consisted of a 10-min warm-up at 55% $VO_{2\ max}$, followed by ten alternating work periods of 2 min at high intensity (95–100% $VO_{2\ max}$) and 4 min at low intensity (55–65% $VO_{2\ max}$), followed by 8 min at 75% $VO_{2\ max}$ and 12 min at 40–45% $VO_{2\ max}$ as part of an active recovery [29] (Fig. 1). The subjects were free to drink water ad libitum. After the exercise, the glycogen content of the quadriceps muscle was again determined by ^{13}C-MRS to measure the extent of glycogen depletion.

2.2.3. Nutritional solutions

The composition of the nutritional solutions in the present study and their timing were based on the results of previous studies [2,3,5,6]. In each of the two studies, the subjects had to ingest one portion of the following nutritional iso-caloric solutions after the exercise, followed by a second portion after a 2 h interval. Subjects were randomly assigned to drink C or CPA in their first trial and obtained the other one in the second trial.

2.2.3.1. Drink C. Carbohydrate drink, containing 600 ml water and carbohydrates (CHO, 1.7 g/kg body wt), composed of 50% of a glucose polymer solution (Glucidex 21, Roquette Frère Ltd, Lille, France) and 50% of a mixture of glucose (2%), fructose (1%), sucrose (94%) and maltose (3%).

2.2.3.2. Drink CPA. Carbohydrate composed of sugars with the same proportions as above associated with whey protein and arginine, containing 600 ml water, CHO (1.2 g/kg body wt) and a mixture of whey protein (Lacprodan 80, MD Foods Ingredients amba, Denmark) including 5 g of L-arginine (Fluka Chemie Ltd, Buchs, Switzerland) (0.5 g/kg body wt).

2.2.3.3. Drink P. Four of the eight subjects performed a third trial, where they ingested a solution without calories, (placebo consisting of 600 ml water, with Canderel™ (Aspartam, Searle Ltd, Aubonne, Switzerland)).

2.2.4. ^{13}C-MRS

Glycogen levels in the quadriceps muscle were monitored by natural abundance ^{13}C-MRS. Measurements were performed on a standard 1.5 T SIGNA scanner (General Electric, Milwaukee, WI), equipped with a SMIS 3010 ^{1}H decoupler console (s.m.i.s. Ltd, Surrey, UK). A double-tuned flexible surface coil (Medical Advance, Milwaukee, WI, ^{13}C square surface coil of 11.3 × 11.3 cm, ^{1}H Helmholtz-type coil) was used for the detection of ^{1}H decoupled ^{13}C spectra. A simple pulse and acquire scheme was applied on the carbon channel using a 2.5 ms sech/tanh adiabatic half passage excitation pulse [30] (i.e. nominally a 90° pulse). The

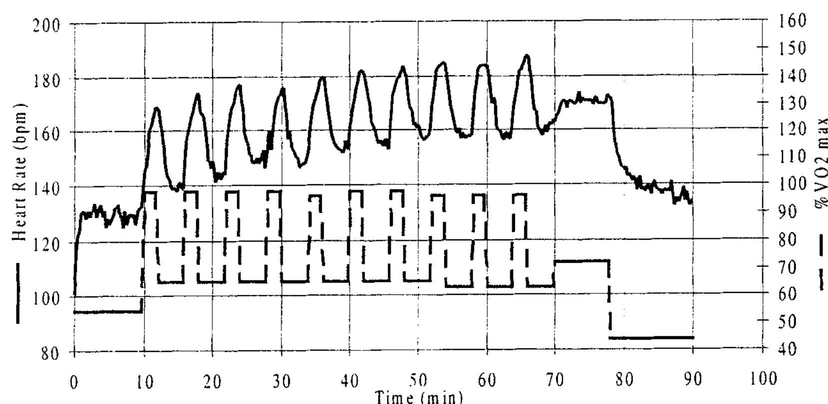

Fig. 1. Schematic representation of the glycogen-depleting exercise. Ten minutes warm-up at 55% $VO_{2\ max}$, ten alternating work periods of 2 min at high intensity 95–100% $VO_{2\ max}$ followed by 4 min at low intensity 55–65% $VO_{2\ max}$, 8 min at 75% $VO_{2\ max}$ and 12 min at 40–45% $VO_{2\ max}$ as part of an active recovery. Targeted course of VO_2 and typical corresponding heart rates are displayed.

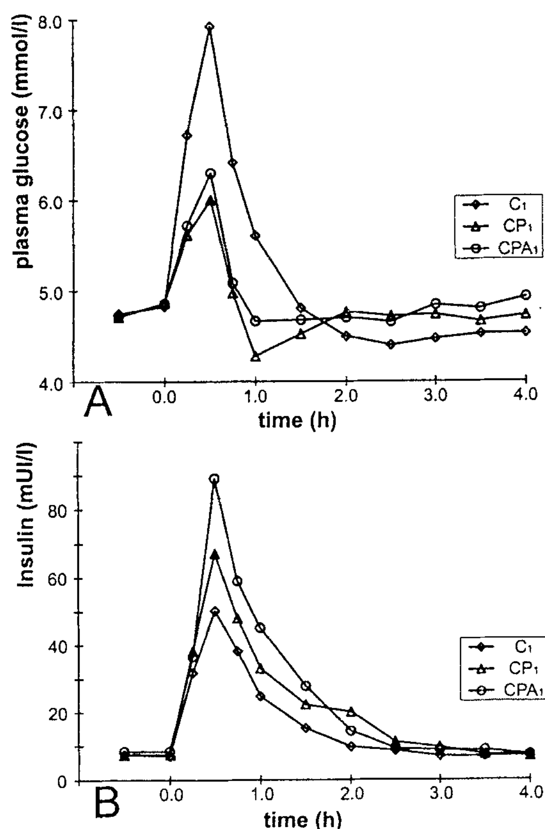

Fig. 2. Mean plasma glucose (A) and plasma insulin (B) concentrations of all eight subjects before and following ingestion of three test solutions taken at time 0. (C_1 = CHO-only, CP_1 = CHO + protein, CPA_1 = CHO + protein + arginine, as described in Section 2, part A).

excitation null at the singular point of the excitation profile of the adiabatic pulse [31] was placed 530 Hz upfield from the glycogen-C1 resonance which resulted in a considerable reduction of the large methylene resonances of fat. At the same time this pulse produced near maximum excitation at both, the resonance position of glycogen-C1 and that of the guanidino-C of creatine. The protons were continuous wave decoupled (CW decoupling at 5.4 ppm) during acquisition and irradiated at a lower level (14%) during the rest of the time to build up the Nuclear Overhauser Enhancement (NOE-Effect). The average absorbed radiofrequency power (RF-power) was locally below 5.7 W/kg (duty cycle 63%) and, therefore, within FDA recommendations of 8 W/kg for a spatial peak in any g of tissue [32]. Spectra were obtained at a sampling rate of 5 kHz with a repetition time TR of 320 ms, averaging 2000 responses. The short relaxation time of glycogen would allow a TR below 320 ms, resulting in an improved sensitivity. However, other parameters benefit from longer TR's, such as saturation of the creatine signal, reduction of the absorbed RF power, baseline distortion of the large fat signals, and technical limitations imposed by the MR-system. The spectra were processed using IDL™ and quantified in frequency domain using a recently developed fitting algorithm that allows for frequency selective fitting and implementation of prior knowledge. The glycogen C1-resonance was fitted after Lorentz–Gauss transformation (4 Hz Lorentz, 18 Hz Gauss) in two steps by a single Voigt line as described in details in Ref. [33]. This approach has proven to be robust in spite of the relatively low signal-to-noise ratio. The prior knowledge used does not include multiple components of the signal [34].

One trial consisted of three phases: the first before exercise, the second after exercise, and the third towards the end of a 4-h period following the intake of one of the isocaloric solutions or placebo given after the exercise period. During each phase, six data points corresponding to 2000 scans each were obtained. Each phase lasted about 90 min which included: positioning of the volunteer in the magnet, axial and sagittal magnetic resonance imaging (MRI) sequence to check positioning, adjustments of ^{13}C-MRS acquisition and ^{1}H–decoupling, and finally acquisition of six × 2000 scans (6 × 10.5 min). The ^{13}C surface coil was placed on the antero-lateral part of the right thigh and the subject was attached to the table to prevent movements.

The concentration of glycogen was determined relative to the area of the simultaneously recorded creatine resonance at 157 ppm, and expressed in institutional units (IU). The use of creatine as an internal standard is justified by the fact that creatine concentrations show little variation as determined in human muscle biopsies [21] and by ^{1}H-MRS of human muscle [35]. The visibility and lineshape of some of the proton MR signals of creatine (i.e. creatine plus phosphocreatine) may change during intense short time exercise [36]. However, such variations have never been observed for the ^{13}C-MR signals used as internal reference in the present study.

2.2.5. *Statistical analysis*

Significance levels for differences between the test meals were calculated by *t*-tests (paired for C vs CPA, two-sample with equal variance for P vs C and P vs CPA). Values are expressed as means ± SD or SEM as indicated.

3. Results

3.1. *Preliminary study*

Plasma glucose reached its peak concentration 30 min after ingestion of C_1, CP_1 and CPA_1 (Fig. 2A). A two way ANOVA (treatment × time) with repeated measures revealed a higher plasma glucose concentration with C_1 than with CP_1 ($p = 0.0001$) or with CPA_1 ($p = 0.003$). The areas under the curve of insulinemia after ingestion of CP_1 and CPA_1 (Fig. 2B) were greater than that of C_1 ($P = 0.015$ and 0.006 respectively).

There was a trend for higher insulin levels for CPA_1 versus CP_1 ($P = 0.09$). For the 1 h period starting with the peak insulin level (30–90 min after ingestion) this difference in insulinemia was statistically significant ($P = 0.03$).

3.2. Main study

On a full-width, undecoupled ^{13}C-MR spectrum of the human quadriceps muscle, the signals of creatine and glycogen are difficult to discern beside the large signals from subcutaneous fat (Fig. 3A). The signal-to-noise ratio is improved with continuous wave decoupling of the hydrogen nuclei bound to the carbon

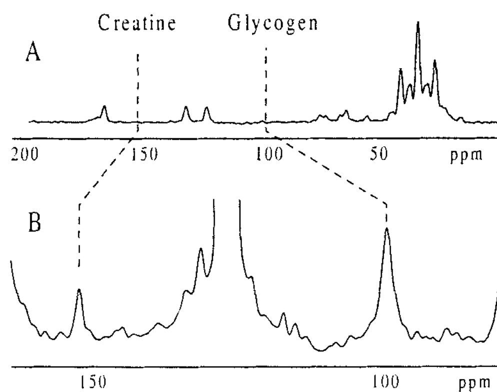

Fig. 3. *Row A*: non-decoupled full width spectrum. The signals of creatine and glycogen are almost invisible beside the large signals from subcutaneous fat. *Row B*: Magnification of the 80–170 ppm region of a ^{1}H-decoupled and NOE-enhanced spectrum. TR 320 ms, 4000 scans, after Lorentz–Gauss transformation and zero and first order (−210°) phase-correction. The decoupled singlet of the glycogen-C1 resonance at 100.5 ppm and the singlet from the guanidino-C of creatine at 157 ppm are visible.

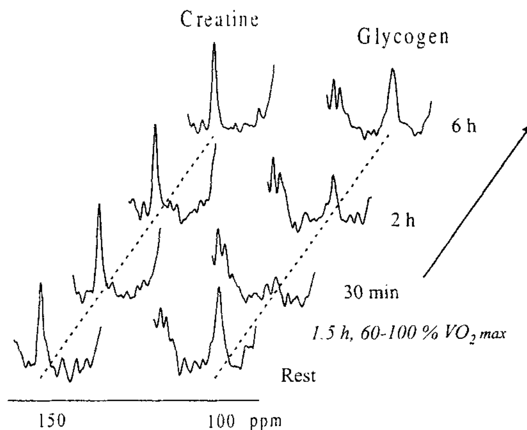

Fig. 4. ^{13}C-MR spectra with creatine and glycogen peaks in a single subject as a function time. (TR 320 ms, Lorentz–Gauss transformation and zero and first order phase-correction, see Section 2) Creatine is used as internal reference, because it remains stable in exercise and recovery. The glycogen signal is decreased after exercise and increases with time after ingestion of one of the carbohydrate drinks.

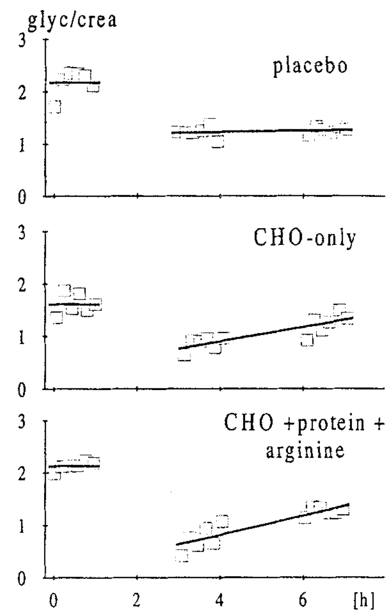

Fig. 5. Illustration of muscle glycogen levels in institutional units (IU) for one volunteer. After acquisition of six separate spectra, muscle glycogen was partially depleted by exercise and post-depletion levels were measured by six spectra after a gap of 2 h. Repletion was observed after a second gap of 2 h when another six spectra were acquired. For a placebo drink, only a minor repletion is observed, while CHO-only and CHO + protein + arginine lead to an obvious increase of muscle glycogen.

atoms, resulting in decoupling and NOE build up. Using these techniques, the resonances of the guanidino-C of creatine (157 ppm) and C1 of glycogen (100.5 ppm) become visible in a spectrum with an enlarged scale (Fig. 3B).

Two thousand acquisitions per spectrum were chosen in this dynamic study (Fig. 4) as a compromise between duration of the data acquisition and improved signal-to-noise ratio. Fig. 4 shows a series of ^{13}C-MR spectra with emphasis on the resonances of creatine and glycogen before and after exercise and after ingestion of one of the carbohydrate drinks. The glycogen resonance is decreased after exercise and increases with time after ingestion of one of the carbohydrate drinks.

Fig. 5 demonstrates the time course of the glycogen/creatine ratio for one subject in all three diets. The upmost row illustrates the slow recovery of muscle glycogen after a zero-caloric placebo drink after exercise. Pre- and post-depletion glycogen levels were measured in institutional units (IU). In the case of placebo, the rate of glycogen recovery (corresponding to the slope of the line, and indicated numerically in milli-institutional units per minute, mIU/min) is not significantly different from zero. During each MR session, six spectra with 2000 averages each were acquired and are shown in the figure. For placebo, the scattering of the data within a session, i.e. six subsequent data points, is mainly the result of the limited signal-to-noise in the

individual spectra as seen in Fig. 4. As illustrated in the lower two traces of Fig. 5, the intake of both isocaloric drinks lead to distinct, but similar recoveries of muscle glycogen in this volunteer.

Table 1 contains the results from the statistical analysis of pre- and post-depletion glycogen levels and the rates of glycogen synthesis following ingestion of the isocaloric and the placebo drinks. With the exception of pre-depletion levels during CPA diet, which were found to be significantly higher than during the placebo trial, pre- and post-depletion glycogen levels were indistinguishable for all tests. The starting glycogen levels for the different trials were comparable therefore. Recovery of glycogen synthesis was found to be significantly different for placebo compared to carbohydrates only ($P = 0.014$) and placebo compared to CPA ($P = 0.037$). Paired t-tests between the two conditions with nutrient solutions (C and CPA) did not reveal significant differences between the rates of glycogen synthesis following the intake of the solutions. Fig. 6 shows individual glycogen recovery rates for the three different diets, including the average and SEM within one diet.

Adding a uniform theoretical effect term to the measured glycogen synthesis rates in the CPA trial, it was found by variation of this hypothetical treatment effect and subsequent t-tests that an increase of 34% in the glycogen synthesis rate compared to the CHO-only diet would have been detected at the $P = 0.05$ significance level.

4. Discussion

The best nutritional solution to optimize muscle glycogen repletion after prolonged and strenuous exercise is not yet known since reported results [3,5–10] are inconsistent. Our results show that addition of whey protein and arginine to a CHO solution stimulates insulin secretion more than the stimulation due to the increase in plasma glucose. The main aim of the present study was to test whether, under isocaloric conditions, partial replacement of CHO by whey protein and arginine could improve the rate of glycogen storage following exercise-induced depletion, but no such increase was found in this study.

The carbohydrate solution (C) that was administered to the subject was based on several observations. (A) Ivy et al. [2] observed a greater rate of glycogen synthe-

Table 1
Pre- and post-depletion muscle glycogen levels and rate of glycogen synthesis during the recovery phase

Nutritional solutions[a]	n	Pre-depletion (IU)	Post-depletion (IU)	Rate of glycogen synthesis (milli IU/min)
P	4	2.03 ± 0.24	0.88 ± 0.17	0.64 ± 0.40
C	8	2.46 ± 0.24	0.97 ± 0.12	2.33 ± 0.34
CPA	8	2.77 ± 0.15	1.02 ± 0.16	2.44 ± 0.48
p (P vs. C)	4	0.296	0.655	0.014
p (P vs. CPA)	4	0.020	0.592	0.037
p (C vs. CPA)	8	0.233	0.814	0.756

[a] P: placebo, C: carbohydrate, CPA: carbohydrate + protein + arginine. Values are means ± SEM. Significance levels are calculated by t-test (paired for C vs. CPA, two-sample with equal variance for P vs. C and P vs. CPA).

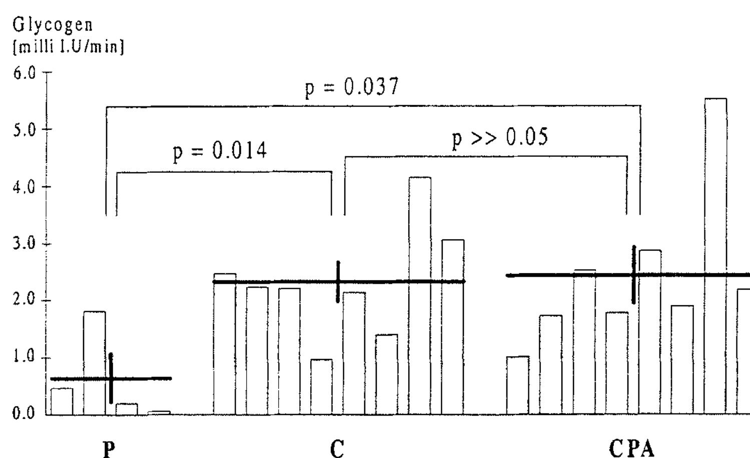

Fig. 6. Individually determined rates of glycogen synthesis [milliIU/min] for placebo (P, $n = 4$), CHO-only (C, $n = 8$), and CHO + protein + arginine (CPA, $n = 8$). Solid lines represent average ± SEM within one type of diet. The average slope of glycogen repletion is significantly lower during placebo intake than with C- or CPA-diet. Average slopes of glycogen synthesis during C- and CPA-diet are not significantly different.

sis when the carbohydrate supplement was ingested immediately after exercise compared with a 2-h delay of supplement ingestion. (B) Blom et al. [5] reported that a constant rate of muscle glycogen storage could be maintained post-exercise if a carbohydrate supplement was provided at 2-h intervals. (C) They also reported [5] that increasing carbohydrate intake from 0.7 to 1.4 g/kg body wt every 2 h did not result in an increased muscle glycogen storage rate. (D) Ivy et al. [3] reported that 1.5 g glucose/kg body wt provided immediately and 2 h after exercise, significantly enhanced muscle glycogen restoration above the basal rate. However, they reported no additional benefit of doubling the amount of glucose supplement from 1.5 to 3.0 g/kg body wt with regard to glycogen recovery during 4 h after exercise.

Zawadzki et al. [9] showed that adding 40.7 g whey protein to a solution containing 112 g carbohydrate (which corresponded to 1.5 g CHO/kg body wt), induced a further increase of the rate of muscle glycogen synthesis compared with 112 g carbohydrates alone. One of the underlying mechanisms that could explain such a result is via an increased insulin secretion due to the protein content of the solution. Our preparatory study confirmed that the plasma insulin concentration was indeed significantly higher after the ingestion of a solution of carbohydrate associated with protein and arginine compared to the ingestion of carbohydrate alone. However, the two drinks tested in Zawadzki's study were not isocaloric. Therefore it is unclear whether the added protein had a specific effect on glycogen synthesis.

According to Refs. [3,5] the higher CHO content of C (1.7 g/kg body wt) used in the present study compared to that of CPA (1.2 g/kg body wt) should not lead to a different rate of muscle glycogen synthesis. The study was designed to assess whether under isocaloric conditions, a supplement of protein and arginine could maximize the recovery rate of glycogen synthesis because the addition of protein and arginine is known to stimulate insulin secretion. While the effect of carbohydrate consumption upon glycogen recovery rates could be verified very clearly in this study, our results did not show any potentiating effect of the CHO–protein–arginine solution compared to the isocaloric CHO solution ($p = 0.76$). Error estimates indicate that an increase in glycogen synthesis rate of 34% (i.e. less than the 40% increase reported by Zawadzki et al. [9]) would have lead to a significant effect in the current experimental set-up. Given the present results, it is therefore likely that above a certain amount of carbohydrate intake (1–1.5 g/kg, every 2 h), the rate of muscle glycogen synthesis cannot substantially increase further with isocaloric nutrition — at least not by adding protein and/or arginine.

The individual results for the glycogen resynthesis rate (see Fig. 6) show a fairly large spread. This is in part due to experimental error (limited signal to noise) and inaccuracies introduced by the spectra fitting procedure (main problem: the automatically defined baseline). In addition, also interindividual differences lead to apparent scatter, which is eliminated by the paired t-test for diet comparison (C vs. CPA). One subject showed substantial glycogen resynthesis in spite of the placebo diet. This trial was repeated, but the original result was confirmed. The statistically significant difference in pre-exercise glycogen levels between P and CPA trials is not expected to have had any influence on the measured recovery rates and is thought to have been caused by individual differences and incomplete compliance with suggested diet and physical activity the day before the trials.

Acknowledgements

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