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# Muscle glycogen recovery after exercise during glucose and fructose intake monitored by $^{13}\text{C}$ -NMR

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**Van Den Bergh, Adrianus J., Sibrand Houtman, Arend Heerschap, Nancy J. Rehrer, Hendrikus J. Van Den Boogert, Berend Oeseburg, and Maria T. E. Hopman.** Muscle glycogen recovery after exercise during glucose and fructose intake monitored by  $^{13}\text{C}$ -NMR. *J. Appl. Physiol.* 81(4): 1495–1500, 1996.—The purpose of this study was to examine muscle glycogen recovery with glucose feeding (GF) compared with fructose feeding (FF) during the first 8 h after partial glycogen depletion by using  $^{13}\text{C}$ -nuclear magnetic resonance (NMR) on a clinical 1.5-T NMR system. After measurement of the glycogen concentration of the vastus lateralis (VL) muscle in seven male subjects, glycogen stores of the VL were depleted by bicycle exercise. During 8 h after completion of exercise, subjects were orally given either GF or FF while the glycogen content of the VL was monitored by  $^{13}\text{C}$ -NMR spectroscopy every second hour. The muscular glycogen concentration was expressed as a percentage of the glycogen concentration measured before exercise. The glycogen recovery rate during GF ( $4.2 \pm 0.2\%/h$ ) was significantly higher ( $P < 0.05$ ) compared with values during FF ( $2.2 \pm 0.3\%/h$ ). This study shows that 1) muscle glycogen levels are perceptible by  $^{13}\text{C}$ -NMR spectroscopy at 1.5 T and 2) the glycogen restoration rate is higher after GF compared with after FF.

glycogen depletion; vastus lateralis muscle; bicycle exercise; 1.5 tesla; carbon-13 nuclear magnetic resonance spectroscopy

ONE OF THE MOST IMPORTANT limiting factors in long-term muscular performance is the depletion of glycogen in the working muscle (3, 6, 12). It has been suggested that some of the feelings of tiredness associated with overtraining are related to lowered glycogen reserves. The resynthesis of muscle glycogen during the recovery period is therefore an important metabolic process that is highly dependent on adequate carbohydrate (CHO) intake (8, 11). Biopsy studies of the vastus lateralis (VL) muscle have shown that glucose feeding (GF) results in a faster recovery of muscle glycogen levels than fructose feeding (FF) (5).

However, the performance of biopsies is difficult at short time intervals from the same muscle, and it may affect metabolic processes.  $^{13}\text{C}$ -nuclear magnetic resonance (NMR) spectroscopy has emerged as an appropriate noninvasive alternative for the assessment of muscle glycogen metabolism. Taylor et al. (20) showed that the C-1 resonance from glycogen, which is well resolved from other spectral components, can be used to quantify muscle glycogen levels. Price et al. (18, 19) studied muscle glycogen resynthesis employing  $^{13}\text{C}$ -NMR spectroscopy. After depletion of up to 25% of its original amount, glycogen repletion was fast in the first hour

and slower in the following hours without any feeding. Recently, Moriarty et al. (16) also used  $^{13}\text{C}$ -NMR spectroscopy to study glycogen utilization and restoration in liver and skeletal muscle. In their study, the effect of the consumption of glucose and sucrose drinks on glycogen repletion was compared. No difference was detected between glycogen recovery rates (GRRs) as a result of glucose and sucrose intake. The above-mentioned studies (16, 19) were performed at magnet field strengths of 4.7 and 3.0 T. It has been demonstrated that glycogen depletion and recovery can also be observed at the more commonly available field strength of 1.5 T (1, 10).

So far, no  $^{13}\text{C}$ -NMR data are available comparing glycogen restoration during GF and during FF after glycogen depletion. In the present study, muscle glycogen recovery was examined for the first 8 h after depletion during GF and FF with  $^{13}\text{C}$ -NMR spectroscopy at 1.5 T.

## METHODS

**Subjects.** Seven healthy well-trained male subjects participated in this study after an informed consent was obtained. Mean age of the subjects was 24 yr (range 23–27 yr), body mass 78 kg (71–90 kg), height 1.87 m (1.79–1.93 m), and fat percentage 10% (7.0–12.0%). The Medical Faculty Ethics Committee approved this study.

**Preparation protocol.** At least 1 wk before the actual experiments were performed, the individual maximum power output ( $W_{\max}$ ) was determined on an electromagnetically braked bicycle ergometer (Lode) by using a continuous incremental exercise test. The exercise started at 20 W and increased at 20 W/min. The subjects had to maintain the revolution rate between 60 and 80 revolutions/min (rpm). The individual  $W_{\max}$  was equal to the last power output completed for 1 min with a pedaling rate above 60 rpm.

All subjects performed two glycogen “depletion-restoration” experiments with at least 5 days in between. Subjects were asked not to participate in exhaustive exercise 2 days in advance of both experiments and to fast (except for water) 10 h before the start of the experiments. The actual experiment started with the determination of the glycogen content of the VL muscle by using  $^{13}\text{C}$ -NMR spectroscopy.

**Glycogen-depletion protocol.** All subjects performed bicycle exercise according to a protocol described by Kuipers et al. (15) to deplete the VL of glycogen. Bicycle exercise was performed on an electromagnetically braked bicycle ergometer. The glycogen-depletion protocol consisted of alternating 2-min intervals of 90 and 50%  $W_{\max}$ . When the subject was unable to perform at 90%  $W_{\max}$ , the workload was subsequently lowered to 80, 70, and 60%  $W_{\max}$ . When 60%  $W_{\max}$  could not be maintained, the exercise was stopped, and the glycogen content of the VL was determined again by  $^{13}\text{C}$ -NMR spectroscopy to measure the extent of the glycogen depletion.



The exercise was performed at  $\pm 18^\circ\text{C}$ , and subjects were free to drink water.

**CHO feeding protocol.** During 8 h after the depletion exercise and the second NMR measurement, CHO ingestion was alternated with glycogen measurements every 1 h, according to Fig. 1.

During the CHO feeding hours, subjects drank 500 ml of a CHO solution at 0 min, 250 ml at 30 min, and 250 ml at 60 min. CHO feeding was given in counterbalanced order: in one experiment GF, in the other experiment FF. CHO solutions consisted of 80 g of CHO (Amylum) and 1.15 g of NaCl (Merck) dissolved in 950 ml of tap water. The CHO solutions were stored at  $\pm 6^\circ\text{C}$  for an optimal fast resorption.

Blood and urine samples were obtained to verify possible loss of glucose. Blood glucose was determined in a capillary blood sample from the finger by using a photometer apparatus (Hemocue). Urine was checked for glucose by using glucosticks (Bayer).

During CHO intake, the actual  $^{13}\text{C}$ -NMR measurement started always exactly 2 h after the start of the previous  $^{13}\text{C}$ -NMR measurement.

**$^{13}\text{C}$ -NMR spectroscopy.** VL glycogen levels were monitored by natural abundance  $^{13}\text{C}$ -NMR spectroscopy at 1.5 T on a model SP63/84 Siemens Magnetom (Siemens) equipped with a second radio frequency channel. The radio frequency probe consisted of two surface coils: a butterfly-shaped ( $0.11 \times 0.24$  m)  $^1\text{H}$ -coil and a circular  $^{13}\text{C}$ -coil (0.10-m diam). The proton coil was used for shimming, imaging, and decoupling. The line width at one-half the height of the water resonance was shimmed to below 30 Hz.

The pulse sequence for acquisition of  $^{13}\text{C}$ -NMR spectra consisted of an adiabatic excitation pulse of 2.56 ms length preceded by a low-angle hard pulse and a dephasing gradient to reduce signals from superficial fat. During the first 65 ms of the acquisition period, broadband Waltz-4 proton decoupling was used. A chemical shift imaging-based calibration program was used to compute an optimum for repetition time and decoupling power within the guidelines for specific absorption rate recommended by the Food and Drug Administration (2). Average power dissipated at the body surface was 7.5 and 0.9 W/kg averaged over the tissue sampled by the coil, calculated according to Heerschap et al. (10). The repetition time was 0.7 s, the sample width was 8,000 Hz, and the number of data points was 1,024. Each  $^{13}\text{C}$ -NMR measurement consisted of 3,000 acquisitions, which equals 35 min of acquisition time. The total measurement time was 50 min,

including imaging and shimming. Possible nuclear Overhauser enhancement (NOE) and saturation of the creatine C-3 resonance under the present measurement conditions were determined for three volunteers by acquiring spectra without decoupling and at a repetition time of 1.4 s. For the glycogen C-1 resonance, these were determined in a 110 mM (glucose units) glycogen solution.

**Positioning.** The anterolateral part of the right leg was placed on the  $^{13}\text{C}$ -surface coil with the center of the coil at 65% of the distance from the spina iliaca anterosuperior to the medial femoral epicondyle. By means of an NMR image, the position of the VL was checked. Once the first glycogen measurement was done at a certain position, this position was reproduced as closely as possible for the subsequent measurements.

**Spectroscopic analysis.** The spectroscopic data were analyzed with the software package Luise (Siemens). Free induction decays (FIDs) were zero filled to 4,096, multiplied by a 10-Hz gauss filter, Fourier transformed, and phase and baseline corrected. The C-1 resonance of glycogen and the C-3 resonance of creatine were fitted to a Gaussian line shape. The natural line width at one-half maximum of the C-1 resonance of glycogen before exercise ranged from 55 to 89 Hz. The fit program was allowed to vary the line width between 50 and 100 Hz in all evaluations. These line width limits for the glycogen C-1 resonance were the only prior knowledge constraints used in the fitting procedure. Integrated areas of the fits were taken for further evaluation. Signal-to-noise ratios (SNRs) of signals were calculated by  $2.5 \times \text{signal height/peak-to-peak noise}$ .

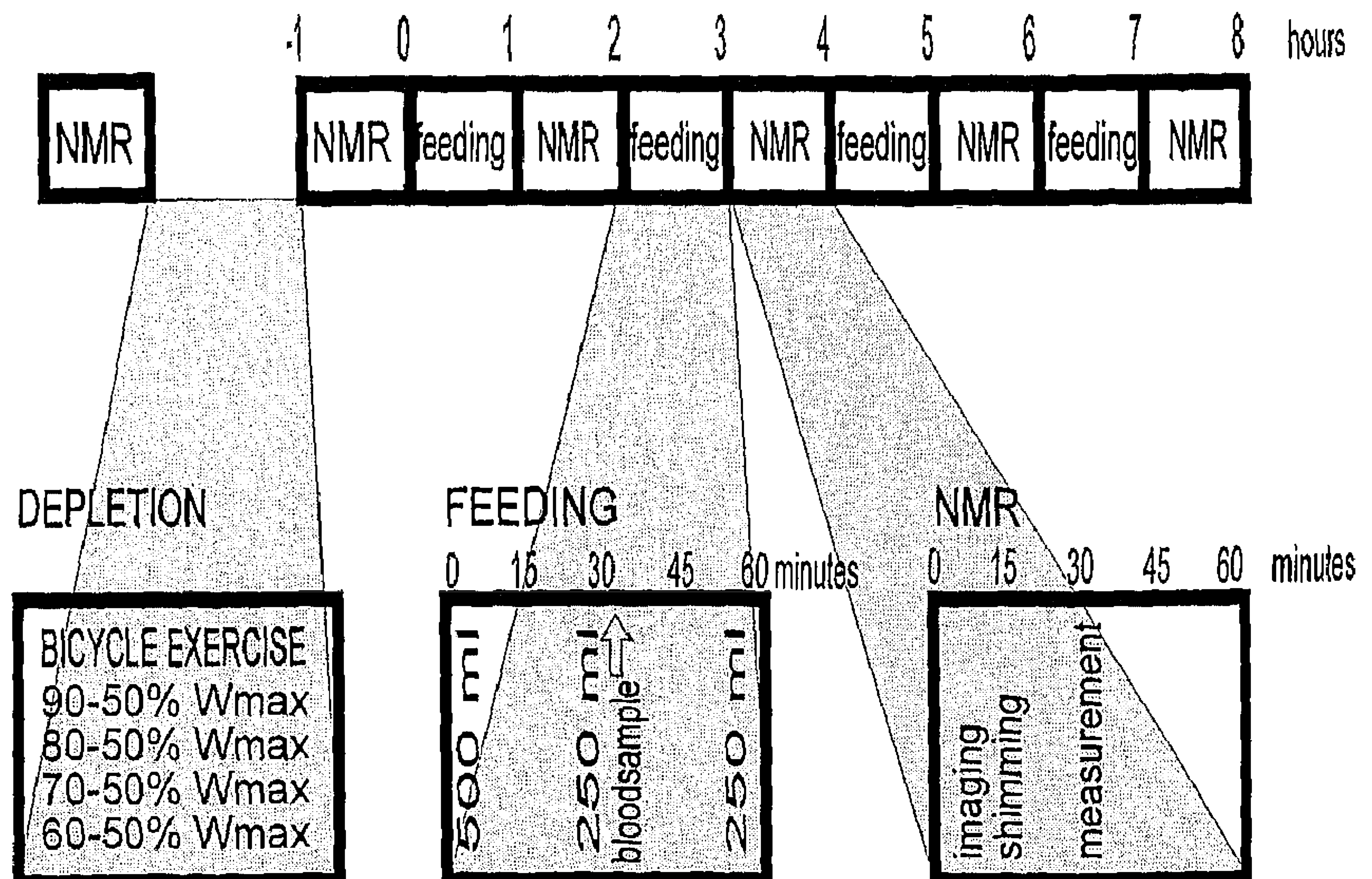
Glycogen-restoration data are presented as relative glycogen content (RGC), with RGC at time  $t = (\text{peak integral at } t / \text{peak integral before depletion}) \times 100$ .

**Statistical analysis.** Least squares linear regression lines were fitted through the averaged RGC values ( $n = 7$ ) of each time point. Slopes and intercepts of both monosaccharides were tested by analysis of variance (ANOVA) for significant differences. All statistical analyses were two sided, except when mentioned otherwise, with  $P < 0.05$  considered statistically significant.

## RESULTS

The mean exercise time of the glycogen-depletion protocol was 96 min (range 54–130 min). The mean

Fig. 1. Schematic representation of experiments 1 and 2. During 8 h after glycogen depletion,  $^{13}\text{C}$ -nuclear magnetic resonance (NMR) measurements and feeding hours were alternated. Feeding and NMR hours were structured as shown in enlarged sections.  $W_{\text{max}}$ , individual maximum power output.



absolute difference within subjects between the first and second glycogen depletion was 7 min in duration, which was not statistically significant.

Blood glucose during GF [ $6.3 \pm 1.4$  (SD) mM] was significantly different ( $P < 0.05$ ) from blood glucose during FF ( $4.6 \pm 0.6$  mM). The blood glucose level remained between 3.1 and 10.0 mM throughout both feeding periods. Glucosuria was not detected in any subject (loss of  $<5.5$  mM).

$^{13}\text{C}$ -NMR spectroscopy. Figure 2 shows two  $^{13}\text{C}$ -NMR spectra obtained at 1.5 T from the VL muscle of a volunteer during a depletion-repletion experiment with GF. The bottom spectrum was recorded preexercise. Glycogen signals can be observed at 100.5 parts/million (ppm) (C-1) and also at 70–74 ppm (C-2 to C-5), close to the glycerol backbone C-2 resonance. The top spectrum was obtained shortly after exercise. Depletion of muscle glycogen is clearly visible in the top spectrum for the resonance at 100.5 ppm and also in the region 70–74 ppm despite the overlapping signals from the glycerol backbone. Notice the good reproducibility of the spectra for signals of other muscle compounds, i.e., creatine at 157 ppm.

The reproducibility of the  $^{13}\text{C}$ -NMR experiment concerning positioning and instrument performance was examined in three volunteers. Glycogen signal levels were measured in the nondepleted state under the

same conditions during two separate examinations, one performed immediately after the other. The mean coefficient of variation was  $5.0 \pm 4.0\%$ .

The SNR of the glycogen C-1 resonance in the resting state was found to vary between 4 and 13 with an average of  $8 \pm 1$  (SE). A paired  $t$ -test of these SNR values obtained from all control spectra before GF and before FF revealed an effective pairing (1-tailed ANOVA;  $P = 0.039$ ) and a nonsignificant difference between these groups ( $P = 0.99$ ).

From each control spectrum, obtained at rest before the start of a GF or FF experiment, the glycogen-to-creatine signal integral ratio was also determined. This ratio showed, as with the SNR values above, a quite large intersubject variability, i.e., it ranged from 0.6 to 2.2 with an average of  $1.36 \pm 0.13$  (SE). However, for all individuals this ratio was almost the same in both experiments. A paired  $t$ -test of the data sets revealed a nonsignificant difference ( $P = 0.394$ ); the pairing in this test was very effective (1-tailed ANOVA;  $P = 0.0019$ ).

To estimate the average muscular glycogen content in the VL from the glycogen-to-creatine signal integral ratios, any NOE and saturation of the C-1 glycogen and C-3 creatine resonances imposed by the present experimental conditions (decoupling and scan repetition time) were evaluated. For the C-1 resonance of glycogen in

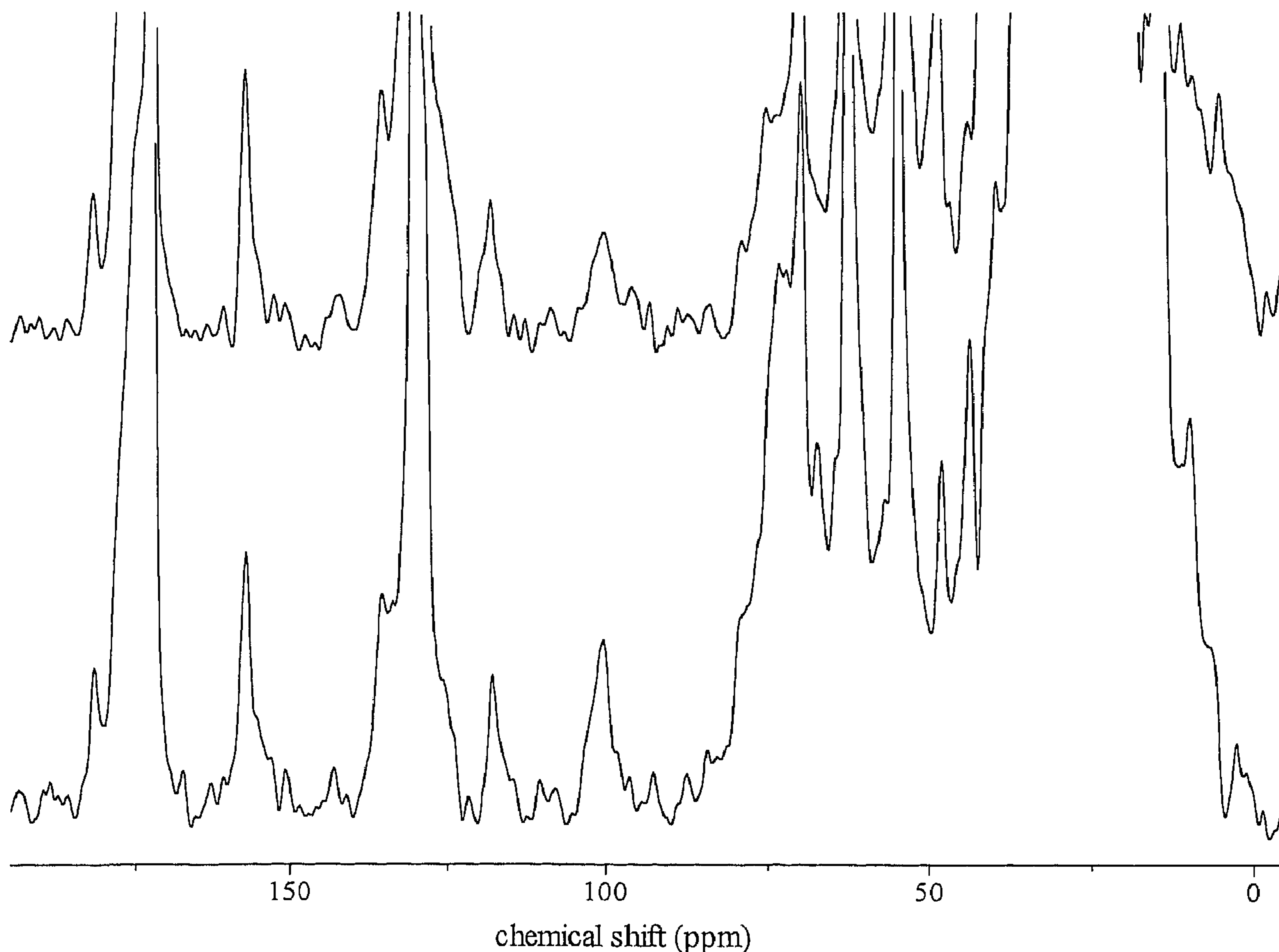


Fig. 2.  $^{13}\text{C}$ -NMR spectra recorded preexercise (*bottom*) and postexercise (*top*) of vastus lateralis muscle of volunteer obtained during 1 experiment with glucose intake. Muscle was depleted by up to 62% of initial glycogen content. ppm, Parts/million.



Table 1. Mean relative glycogen content pre- and postexercise

Feeding	n	Preexercise	Postexercise				
			0 h	2 h	4 h	6 h	8 h
Glucose	7	100	48 ± 4	58 ± 7	64 ± 7	72 ± 5	82 ± 5
Fructose	7	100	43 ± 4	52 ± 7	54 ± 6	56 ± 7	63 ± 5

Postexercise values are mean relative glycogen content ± SE in %; n = no. of subjects.

solution, no NOE and signal saturation were detected; this is also expected to be so in vivo (22). For the C-3 resonance of creatine, signal saturation was not significant, but an enhancement factor of  $1.6 \pm 0.17$  (SE) was observed. If we assume the muscular creatine content to be 42.7 mM (9) and take a correction factor of 1.6 into account, the average muscular glycogen content in the nondepleted state in our volunteers is estimated to be  $93 \pm 8.8$  (SE) mM.

**Glycogen restoration.** Table 1 shows the mean RGC values for each point in time during GF and FF. During the time course of the CHO intake, a linear relationship between glycogen content and time was assumed. Figure 3 shows the linear regression lines for the glucose ( $r^2 = 0.993$ ) and fructose ( $r^2 = 0.946$ ) experiments; lines are drawn with their 95% confidence intervals. The intercepts, representing the glycogen left after depletion, were  $48 \pm 1.0$  (SE) and  $45 \pm 1.5\%$  of the initial glycogen content for GF and FF, respectively, which is not significantly different. The slope,

representing GRR, was significantly higher ( $P < 0.05$ ) during GF ( $4.2 \pm 0.2\%/h$ ) than during FF ( $2.2 \pm 0.3\%/h$ ).

## DISCUSSION

Although biopsy studies have shown a faster glycogen restoration rate after  $\geq 10$  h with GF compared with FF, only a few studies have focused on GRR in humans during the first hours after depletion.

Taylor et al. (20) demonstrated that in vivo  $^{13}\text{C}$ -NMR measurements of muscular glycogen levels can be performed accurately and with a higher precision than in biopsy assessment. For the setup in the present study, the precision to measure glycogen in the nondepleted state appears to be comparable. Employing the C-3 signal of creatine as an internal reference, the estimated average glycogen content in the VL of the volunteers is 93 mM, which is similar to the average glycogen content measured in the human gastrocnemius by  $^{13}\text{C}$ -NMR employing external standardization.

All subjects refrained from exercise 48 h before the experiments and performed both experiments at the same time of the day. It is therefore reasonable to assume that the initial glycogen content in individuals before exercise was the same in both CHO intake experiments and that the GRR could be compared based on RGC values. This assumption is supported by the nonsignificant difference and the effective pairing between the control spectra before GF and FF for the

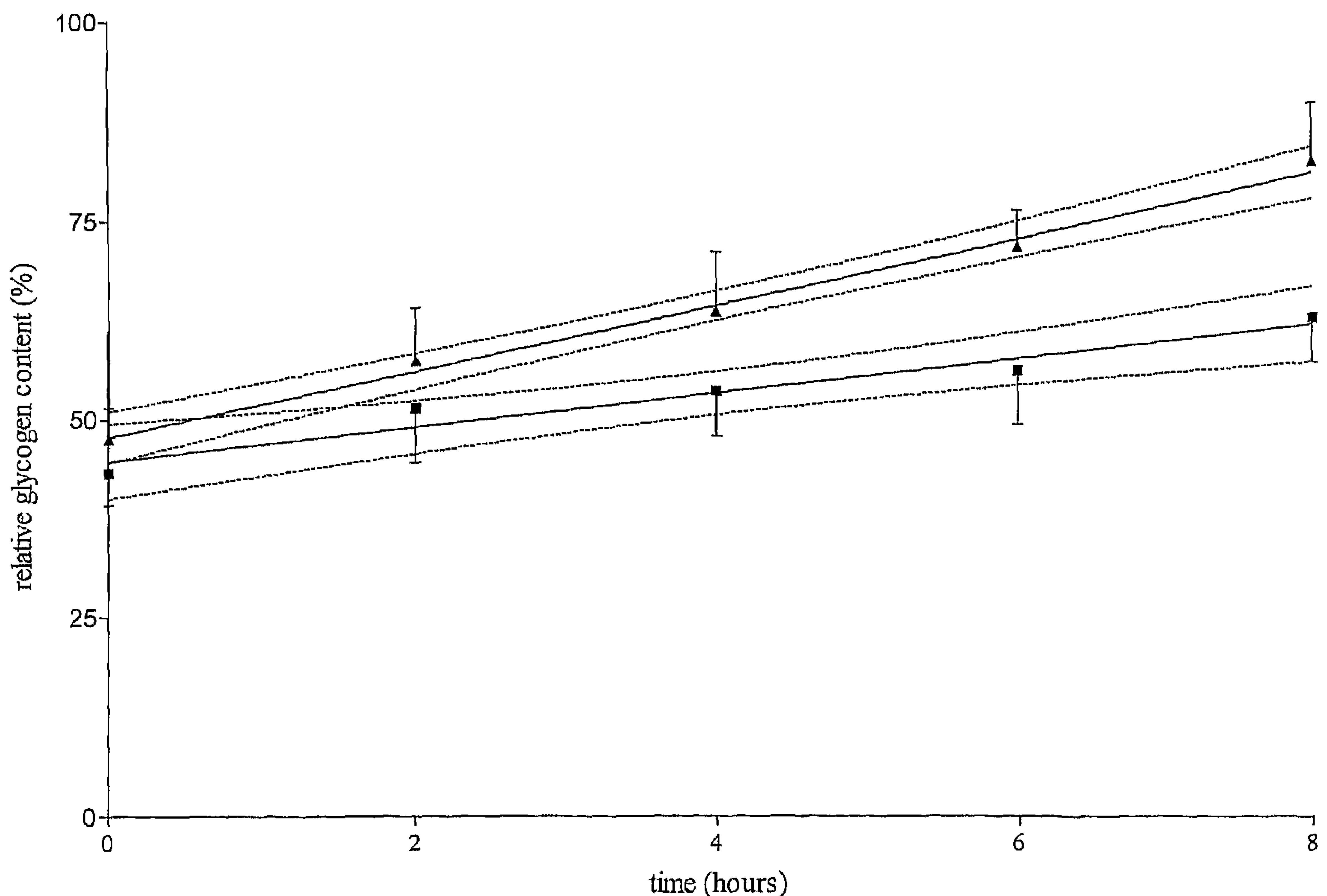


Fig. 3. Muscular glycogen levels during fructose (■) and glucose (▲) feeding. Data are means ± SE. Linear regression curves (solid lines) are shown with 95% confidence intervals (dashed lines).

glycogen-to-creatine signal ratios as well as the SNR of the C-1 resonance of glycogen.

Because the number of intervals and the total exercise time within subjects was about equal in both experiments, it is likely that glycogen was depleted to the same level in both experiments. This is confirmed by the fact that the intercepts of the restoration curves were not significantly different between the two sets of experiments.

In this study, the first RGC value measured is high compared with values of ~20% reported in other studies (5, 6). This can partly be explained by the glycogen restoration that occurs during the  $\pm 40$  min between cessation of cycling and the actual <sup>13</sup>C-NMR measurement.

The amount of monosaccharides administered in this study (i.e., 80 g every 2 h) should be sufficient to achieve a maximal glycogen resynthesis rate with GF or FF (4, 12). Significant loss of glucose or fructose in the urine or feces could lead to an underestimation of GRR. If CHO was not absorbed in the bowel, this would lead to osmotic diarrhea, which was not observed in this study. Because glucose was not detected in the urine of any subject and because fructose in the urine is very rare, loss of glucose or fructose may be considered minimal.

Price et al. (19) found a linear and single-phased relationship between glycogen concentration and time after glycogen depletion to 50%. Therefore, linear regression lines were fitted to RGC vs. time to obtain GRR. The finding that glucose results in a faster muscle glycogen recovery confirms earlier biopsy studies by Blom et al. (5); however, the recovery rates we found were lower, i.e., 2.2 vs. 3.0%/h for fructose and 4.2 vs. 5.8%/h for glucose. A possible explanation for this difference is that glycogen depletion at the start of our recovery experiments was less than in the experiments performed by Blom et al. At depletion levels down to 20%, the glycogen recovery has been reported to be biphasic (19). A stage of fast recovery is followed by a much slower recovery rate when a level of 30 mM is reached. Another explanation for the slower recovery rate is the 1-h interval between cessation of exercise and the start of the CHO intake. GRR is reported to be lower if CHO ingestion after exercise is delayed (13, 14).

To our knowledge, there have been no comparable <sup>13</sup>C-NMR studies on glycogen restoration with GF or FF with which to compare the results of this study. However, it has generally been found in biopsy studies that GRR is higher after GF compared with FF (5, 6, 8). Although fructose probably can be transformed into glycogen in the skeletal muscle cell (4), several factors may contribute to a slower muscle glycogen restoration with FF compared with GF. It may be because of a slower absorption of fructose from the intestine. Because the blood glucose level during GF is significantly higher than during FF, a higher plasma insulin level is expected to be present (21) and thus an increased glucose uptake will occur. In addition, fructose gives rise to more liver glycogen than glucose (7, 8, 17), thus

leaving less CHO directly available for muscle glycogen resynthesis.

**Conclusion.** This study demonstrates that GF restores muscle glycogen faster than FF during the first 8 h after depletion to a level of  $\pm 40\%$  of the initial glycogen content. In general, it appears that <sup>13</sup>C-NMR spectroscopy at 1.5 T is suitable to monitor physiological changes in muscle glycogen levels.

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