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## Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training

D. C. Nieman,<sup>1</sup> J. M. Davis,<sup>2</sup> V. A. Brown,<sup>1</sup> D. A. Henson,<sup>1</sup> C. L. Dumke,<sup>1</sup> A. C. Utter,<sup>1</sup> D. M. Vinci,<sup>1</sup> M. F. Downs,<sup>1</sup> J. C. Smith,<sup>1</sup> J. Carson,<sup>2</sup> A. Brown,<sup>2</sup> S. R. McAnulty,<sup>1</sup> and L. S. McAnulty<sup>1</sup>

<sup>1</sup>Departments of Health, Leisure, and Exercise Science, Biology, Family and Consumer Sciences, Fischer Hamilton/Nycom Biochemistry Laboratory, Appalachian State University, Boone, North Carolina 28608; and <sup>2</sup>Department of Exercise Science, University of South Carolina, Columbia, South Carolina 29208

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**Nieman, D. C., J. M. Davis, V. A. Brown, D. A. Henson, C. L. Dumke, A. C. Utter, D. M. Vinci, M. F. Downs, J. C. Smith, J. Carson, A. Brown, S. R. McAnulty, and L. S. McAnulty.** Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. *J Appl Physiol* 96: 1292–1298, 2004. First published December 12, 2003; 10.1152/jappphysiol.01064.2003.—Thirty strength-trained subjects were randomized to carbohydrate (CHO) or placebo (Pla) groups and lifted weights for 2 h (10 exercises, 4 sets each, 10 repetitions, with 2- to 3-min rest intervals). Subjects received 10 ml·kg<sup>-1</sup>·h<sup>-1</sup> CHO (6%) or Pla beverages during the weight training bout. Blood, saliva, and vastus lateralis muscle biopsy samples were collected before and after exercise. Blood cell counts were determined, and plasma was analyzed for IL-6, IL-10, IL-1 receptor antagonist (IL-1ra), IL-8, and cortisol. Muscle was analyzed for glycogen content and relative gene expression of 13 cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN- $\gamma$ , TNF- $\alpha$ ) by use of real-time quantitative RT-PCR. Significant but modest increases were measured for plasma IL-6, IL-10, IL-1ra, and IL-8, but the pattern of increase did not differ between CHO and Pla groups. The rate of decrease in muscle glycogen content did not differ between CHO and Pla ( $P = 0.463$ ). Muscle cytokine mRNA was detected preexercise for IL-1 $\beta$ , IL-6, IL-15, IL-8, and TNF- $\alpha$ , and of these, IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  were significantly increased after the 2-h weight training bout. The increase in mRNA (fold difference from preexercise) did not differ between CHO and Pla groups. In summary, CHO vs. Pla ingestion did not alter modest increases measured for plasma IL-6, IL-10, IL-1ra, and IL-8, and muscle gene expression for IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  in strength-trained subjects lifting weights intensively for 2 h.

cytokines; gene expression; muscle glycogen; real-time quantitative reverse transcription polymerase chain reaction

IN CONTRAST TO MODERATE PHYSICAL activity, prolonged and intensive exertion such as marathon running causes numerous changes in immunity that reflect physiological stress and immunosuppression (19, 20, 22, 25). Carbohydrate ingestion has emerged as an effective countermeasure, and data from several studies of endurance athletes suggest that carbohydrate compared with placebo ingestion during sustained and prolonged exertion is associated with an attenuated cortisol, growth hormone, and epinephrine response, fewer perturbations in blood immune cell counts, lower granulocyte and monocyte phagocytosis and oxidative burst activity, and a diminished pro- and anti-inflammatory cytokine response (20, 22, 25).

Recently, we showed that carbohydrate compared with placebo ingestion attenuated increases in plasma levels of IL-1 receptor antagonist (IL-1ra), IL-6, and IL-10 and muscle gene expression for IL-6 and IL-8 in 16 experienced marathoners running on treadmills for 3 h at ~70% maximal oxygen uptake (20). Change in muscle glycogen content did not differ between carbohydrate and placebo conditions, and we speculated that IL-6 mRNA and IL-8 mRNA expression in our subjects was diminished in part through a blood glucose-sympathoadrenal pathway during carbohydrate ingestion (20). This finding is not consistent with results from other studies, but differences in exercise mode and workloads make comparisons between studies difficult. Starkie et al. (29) reported that skeletal muscle IL-6 mRNA expression and the rate of decrease in muscle glycogen content were unaffected by carbohydrate ingestion in seven men who ran or cycled for 60 min. In this study, the plasma IL-6 response was blunted by carbohydrate ingestion (29). Febbraio et al. (9) showed that carbohydrate compared with placebo ingestion attenuated arterial IL-6 levels and net leg IL-6 release but had no influence on intramuscular expression of IL-6 mRNA in seven subjects cycling in a semirecumbent position for 2 h. In another study, carbohydrate compared with placebo ingestion reduced both plasma IL-6 and adipose IL-6 mRNA levels in eight male subjects performing 3 h of bicycling (13). Thus a consistent finding has been that carbohydrate compared with placebo ingestion attenuates postexercise increases in plasma IL-6. Still unresolved is the influence of carbohydrate ingestion on muscle IL-6 mRNA expression during differing exercise modes and workloads.

Febbraio and Pedersen (8) have hypothesized that muscle glycogen availability may influence key signaling molecules to enhance IL-6 gene transcription within skeletal muscle. Pedersen et al. (27) concluded that muscle glycogen content is a determining factor in the production of IL-6 across contracting limbs. Recent data indicate that leg IL-6 release is greater when exercise is performed in the glycogen-depleted compared with the glycogen-loaded state (17, 30). Intensive resistance training induces significant depletion of muscle glycogen stores (28) and may therefore induce IL-6 mRNA expression and release. Little is known about the acute immune response to resistance exercise. In general, most studies have reported that immune changes after resistance exercise are modest compared with those measured after intensive and sustained cardiorespiratory endurance exercise (6, 15, 21). Koch et al. (15) reported small

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perturbations in immunity and no influence of carbohydrate ingestion on the lymphocyte response to a high-intensity, short-rest-interval squat workout in 10 resistance-trained men. In these resistance training studies, the total duration of the exercise bout was under 60 min.

We designed a study to test the hypothesis that carbohydrate blunts plasma levels of cytokines and muscle cytokine gene expression during an intensive and prolonged bout of resistance training. Resistance-trained men lifted weights for 2 h with 2- to 3-min rest intervals under carbohydrate and placebo conditions, with muscle and blood samples collected pre- and postexercise. The muscle samples were tested for muscle glycogen and gene expression of 13 different cytokines. We hypothesized that carbohydrate compared with placebo ingestion would attenuate decreases in muscle glycogen during resistance training and that group differences in the net change in muscle glycogen levels would be related to both muscle gene expression for several cytokines and plasma cytokine levels.

## MATERIALS AND METHODS

**Subjects.** Thirty strength-trained athletes were recruited through mass advertising. Male subjects ranging in age from 19 to 27 yr were accepted into the study if they had a minimum of 6 mo of total body resistance training experience, were able to back squat to midhigh parallel at least 1.25 times their body mass, and were willing to adhere to all aspects of the research design. Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements of the Institutional Review Board of Appalachian State University.

**Research design.** Three to 6 wk before the resistance training session, subjects reported to the Appalachian State University Human Performance Lab for orientation and measurement of body mass, body composition, and strength. Body composition was assessed from a three-site skinfold test by use of a Lange skinfold caliper (Cambridge Scientific Industries, Cambridge, MD). One-repetition maximum (1-RM) strength was assessed over two testing sessions. After a two-set warm-up, subjects were allowed three attempts at a given weight to attain 1-RM. Flat bench press, back squat, military press, bent-over row, and biceps curl were assessed during the first 1-RM testing session. Incline bench press, front squat, deadlift, upright row, and French curl were assessed during the second 1-RM testing session. A standard Olympic barbell was used for all exercises (York Barbell, York, PA). Basic demographic and training data were obtained through a questionnaire.

After orientation and baseline testing, subjects were randomized to carbohydrate and placebo groups. A standardized liquid meal (Boost Plus, Mead Johnson Nutritionals, Evansville, IN) was ingested at an energy level of 12 kcal/kg body mass by all subjects before 10:00 AM on the day of the resistance training session. Boost Plus is a nutritionally complete, high-energy oral supplement with an energy density of 1.52 kcal/ml and 16% of energy as protein, 34% as fat, and 50% as carbohydrate. In quantities of 1,000 ml, Boost Plus exceeds daily value recommendations for all major vitamins and minerals. Subjects next reported to the lab at 3:00 PM not having ingested energy in any form for at least 5 h and received carbohydrate (6% or 60 g/l) or placebo beverages 15–30 min preexercise (8 ml/kg) and during the 2-h resistance training bout ( $10 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). The beverages were supplied by the Gatorade Sports Science Institute (Barrington, IL) as in earlier studies (20–22, 25). The carbohydrate and placebo beverages were identical in appearance and taste and in sodium ( $\sim 19.0 \text{ meq/l}$ ) and potassium ( $\sim 3.0 \text{ meq/l}$ ) concentration and pH ( $\sim 3.0$ ). No other beverages or food were ingested during the 2-h resistance training bout or for 1 h postexercise. Blood, saliva, and

skeletal muscle biopsy samples were collected  $\sim 30$  min preexercise and immediately postexercise, with additional blood and saliva samples collected 1 h postexercise.

Subjects agreed to avoid the use of large-dose vitamin and mineral supplements (above 100% of recommended dietary allowances), nutritional supplements, ergogenic aids, herbs, and medications known to affect immune function for 1 wk before test sessions. During orientation, a dietitian instructed the subjects to follow a diet moderate in carbohydrate during the 3 days before the test session and to record intake in a food record. The food records were analyzed by use of a computerized dietary assessment program (Food Processor, ESHA Research, Salem, OR).

**Resistance training protocol.** Subjects were requested to avoid exercise the day of the test session. Subjects were free of systemic illness the day of testing. The exercise session consisted of 10 different resistance exercises: flat bench press, incline bench press, military press, upright row, bent-over row, French curl, biceps curl, back squat, front squat, and deadlift.

Subjects performed four sets of 10 repetitions for each resistance exercise, with the first set at 40% of the subject's 1-RM and the subsequent sets at 60% 1-RM. Two-minute rest intervals separated sets for the bench press, incline bench press, military press, upright row, bent-over row, French curl, and biceps curl. Three-minute rest intervals were given after the completion of each exercise and between sets for the back squat, front squat, and deadlift. Ratings of perceived exertion were taken at the end of every set by use of the 6–20 scale.

**Skeletal muscle biopsies.** Skeletal muscle biopsy samples were acquired before and after exercise after blood and saliva sample collection. The same procedures were utilized pre- and postexercise, with incisions made in the same thigh  $\sim 3$  cm apart. Local anesthesia (1% Xylocaine) was injected subcutaneously and into the vastus lateralis. A muscle biopsy sample was then obtained by using the percutaneous needle biopsy procedure modified to include suction (1, 7). Muscle biopsy samples were divided into two pieces and immediately frozen in liquid nitrogen. Samples were stored at  $-80^\circ\text{C}$  until subsequent analysis.

**Muscle glycogen analysis.** Frozen muscle samples ( $\sim 15$  mg) were weighed before glass-on-glass homogenization in 0.3 M perchloric acid. Muscle homogenate samples were then digested by amyloglucosidase to release glucose. Hexokinase and glucose-6-phosphate dehydrogenase digestion result in NADH production, which was determined spectrophotometrically (26).

**Total RNA isolation and cDNA synthesis.** Procedures for RNA isolation were adapted from Carson and Booth (3). Briefly, skeletal muscle tissue was homogenized under liquid nitrogen with a polytron, and total RNA was extracted using the guanidine thiocyanate method of Chomczynski and Sacchi (4) with TRIzol reagent (Life Technologies, GIBCO BRL). The extracted RNA ( $2.5 \mu\text{l}$  of sample) was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically at 260-nm wavelength. RNA was reverse transcribed into cDNA in a  $100\text{-}\mu\text{l}$  reaction volume containing  $34.75 \mu\text{l}$  RNA ( $1.5 \mu\text{g}$ ) in RNase-free water,  $10 \mu\text{l}$  of  $10\times$  RT buffer,  $22 \mu\text{l}$  of  $25 \text{ mM MgCl}_2$ ,  $20 \mu\text{l}$  deoxyNTPs mixture,  $5 \mu\text{l}$  random hexamers,  $2 \mu\text{l}$  RNase inhibitor, and  $6.25 \mu\text{l}$  multiscrite reverse transcriptase ( $50 \text{ U}/\mu\text{l}$ ). Reverse transcription was performed at  $25^\circ\text{C}$  for 10 min,  $37^\circ\text{C}$  for 60 min, and  $95^\circ\text{C}$  for 5 min, followed by quick chilling on ice and stored at  $-20^\circ\text{C}$  until subsequent amplification.

**Quantitative real-time RT-PCR analysis.** Quantitative real-time RT-PCR analysis was done as per manufacturer's instructions (Applied Biosystems) with the use of predeveloped assay reagents (IL-6) and Taqman cytokine gene expression plate 1 (IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IFN- $\gamma$ , IL-15, and TNF- $\alpha$ ). DNA amplification was carried out in  $25 \mu\text{l}$  of Taqman Universal PCR master mix (AmpliQ Gold DNA polymerase, passive reference 1, buffer, dNTPs, AmpErase UNG),  $2 \mu\text{l}$  of cDNA,  $18 \mu\text{l}$  of RNase-free water, and  $2.5 \mu\text{l}$  of 18S primer (VIC) and  $2.5 \mu\text{l}$  of

Table 1. *Subject characteristics*

Variable	Carbohydrate (n = 15)	Placebo (n = 15)	P Value
Age, yr	21.6±0.5	21.3±0.5	0.627
Stature, m	1.79±0.02	1.82±0.01	0.117
Body mass, kg	78.7±2.4	83.1±2.6	0.226
Body composition, %fat	8.9±1.1	10.5±1.1	0.314
Upper body training, yr	5.2±0.7	5.3±0.5	0.922
Lower body training, yr	4.9±0.8	4.6±0.6	0.761
1-RM flat bench press, kg	106.2±5.5	104.0±4.3	0.759
1-RM incline bench press, kg	89.8±6.0	87.8±4.3	0.787
1-RM military press, kg	70.0±4.1	69.2±3.4	0.827
1-RM upright row, kg	60.7±4.2	58.3±2.6	0.640
1-RM bent-over row, kg	82.2±5.4	85.7±6.5	0.682
1-RM French curl, kg	49.2±2.5	49.2±3.0	1.000
1-RM biceps curl, kg	53.8±3.2	53.2±2.5	0.872
1-RM back squat, kg	136.2±8.7	134.7±8.3	0.902
1-RM front squat, kg	95.8±5.4	102.7±6.3	0.419
1-RM deadlift, kg	136.2±9.8	139.0±8.4	0.828

Values are means ± SE; n = 30 subjects. 1-RM, 1-repetition maximum.

primer (FAM) (for endogenous reference and target cytokine) in a final volume of 50 µl/well. Human control RNA (calibrator RNA) was also used and served as a calibrator for each plate. Samples were loaded in a MicroAmp 96-well reaction plate. For the Taqman cytokine gene expression *plate 1*, all 12 cytokine primers and probes were dried and preloaded in a MicroAmp 96-well reaction plate by the manufacturer. Two dye layers were used for the quantification of the target cytokine mRNA (FAM) and the 18S ribosomal RNA endogenous control (VIC). Plates were run by using the ABI sequence detection system. After 2 min at 50°C and 10 min at 95°C, plates were coamplified by 50 repeated cycles of which one cycle consisted of a 15-s denaturing step at 95°C and a 1-min annealing and extending step at 60°C. Data were analyzed by ABI software using the cycle threshold ( $C_T$ ), which is the value calculated and based on the time (measured by PCR cycle number) at which the reporter fluorescent emission increases beyond a threshold level (based on the background fluorescence of the system) (32). Samples were initially run in duplicate on three cytokine plates and one IL-6 plate to determine intra-assay coefficient of variation (CV) as well as interassay CV. Intra-assay CVs were 1.65, 2.40, and 2.09% for the FAM, VIC, and  $C_{TS}$ , respectively. Interassay CVs were 3.95 and 5.73% for the cytokine plate and IL-6, respectively. On the basis of this initial data, the remaining samples were run in singles.

**Calculations for relative quantification.** Quantification of cytokine gene expression was calculated by using the  $C_T$  method as described by Livak and Schmittgen (16). This method uses a single sample, the calibrator sample, for comparison of every unknown sample's gene expression. This method of analysis and quantification has been shown to give similar results as the standard curve method (25). Briefly,  $\Delta C_T [C_T(\text{FAM}) - C_T(\text{VIC})]$  was calculated for each sample and calibrator.  $\Delta\Delta C_T [\Delta C_T(\text{calibrator}) - \Delta C_T(\text{sample})]$  was then calculated for each sample, and relative quantification was calculated as  $2^{\Delta\Delta C_T}$ . Initial exclusion criteria consisted of FAM  $C_T \geq 40$  and VIC  $C_T \geq 23$ . All samples had FAM  $C_T$  values between 25 and 38. A one-unit change in  $C_T$  reflects a twofold change in mRNA content. Lower  $C_T$  values reflect higher mRNA levels. Detectable cytokine gene expression (mRNA) was defined as a  $\Delta C_T$  value of  $\leq 26$ . This reflects a FAM  $C_T$  value of  $\leq 36$  with an average VIC  $C_T$  value that was very consistent at  $\sim 10.2$ . The unusually high levels of IL-10 and TNF- $\alpha$  mRNA in the human calibrator mRNA resulted in a very low  $\Delta\Delta C_T$  (and resulting relative fold difference from calibrator) value for these cytokines in the muscle samples, which does not necessarily indicate a low level of IL-10 and TNF- $\alpha$  mRNA in the samples.

**Blood cell counts, hormone.** Blood samples were drawn from an antecubital vein with subjects in the seated position. Routine complete

blood counts were performed by our clinical hematology laboratory and provided leukocyte subset counts, hemoglobin, and hematocrit. Other blood samples were centrifuged in sodium heparin tubes, and plasma was aliquoted and then stored at  $-80^\circ\text{C}$ . Plasma cortisol was assayed in duplicate by using a competitive enzyme immunoassay kit provided by R&D Systems (Minneapolis, MN) with a minimum detectable cortisol concentration of 1.6 nmol/l. Plasma volume changes were estimated using the method of Dill and Costill (5).

**Plasma cytokine measurements.** Total plasma concentrations of IL-1ra, IL-6, IL-8, and IL-10 were determined by using quantitative sandwich ELISA kits provided by R&D Systems. All samples and provided standards were analyzed in duplicate. A high-sensitivity kit was used to analyze IL-6 in the preexercise plasma samples. A standard curve was constructed by using standards provided in the kits, and the cytokine concentrations were determined from the standard curves by use of linear regression analysis. The assays were a two-step "sandwich" enzyme immunoassay in which samples and standards were incubated in a 96-well microtiter plate coated with polyclonal antibodies for the test cytokine as the capture antibody. After the appropriate incubation time, the wells were washed and a second detection antibody conjugated to either alkaline phosphatase (IL-6 high sensitivity) or horseradish peroxidase (IL-1ra, IL-8, IL-10) was added. The plates were incubated and washed, and the amount of bound enzyme-labeled detection antibody was measured by adding a chromogenic substrate. The plates were then read at the appropriate wavelength (450 minus 570 nm for IL-1ra, IL-6, IL-8, IL-10; 490 minus 650 nm for IL-6 high sensitivity). The minimum detectable concentration of IL-1ra was  $<22$  pg/ml, IL-6  $<0.70$  pg/ml, IL-6 high sensitivity  $<0.039$  pg/ml, IL-8  $<10$  pg/ml, and IL-10  $<3.9$  pg/ml.

**Salivary samples.** Unstimulated saliva was collected by expectoration into 15-ml sterilized plastic vials for 4 min. Participants were urged to pass as much saliva as possible into the vials during the 4-min timed session. The saliva samples were frozen at  $-80^\circ\text{C}$  until analysis. Saliva volume was measured to the nearest 0.1 ml, and saliva total protein was quantified by use of the Coomassie protein assay reagent. Salivary IgA was measured by ELISA according to the procedures adapted from the Hunter Immunology Unit (Royal Newcastle Hospital, Newcastle, NSW, Australia). The data were expressed as concentration of salivary IgA relative to total protein concentration ( $\mu\text{g}/\text{mg}$ ).

**Statistical analysis.** Statistical significance was set at the  $P < 0.05$  level, and values were expressed as means ± SE. Performance measures were compared under carbohydrate and placebo conditions by using Student's *t*-tests. Other data were analyzed using a 2 (carbohydrate and placebo conditions)  $\times$  2 or 3 (times of measurement) repeated-measures ANOVA. If the condition  $\times$  time interaction  $P$  value was  $\leq 0.05$ , the change from pre- to postexercise values was calculated and compared between conditions using Student's *t*-tests.

Table 2. *Volume load*

Variable	Carbohydrate (n = 15)	Placebo (n = 15)	P Value
Flat bench press, kg	2,347±122	2,288±96	0.710
Incline bench press, kg	1,941±128	1,902±97	0.808
Military press, kg	1,500±87	1,452±63	0.658
Upright row, kg	1,320±82	1,287±58	0.740
Bent-over row, kg	1,794±124	1,883±142	0.638
French curl, kg	1,105±51	1,098±64	0.934
Biceps curl, kg	1,185±70	1,172±51	0.888
Back squat, kg	3,000±194	2,957±186	0.873
Front squat, kg	2,059±94	2,159±119	0.512
Deadlift, kg	3,000±212	2,852±149	0.563
Total volume load, kg	19,255±1022	19,052±846	0.879

Values are means ± SE.

Table 3. Change in blood leukocyte subset counts, plasma cortisol, plasma cytokines, and salivary IgA output during a 2-h intense, resistance training bout in 30 strength-trained subjects randomized to carbohydrate and placebo ingestion

Variable	Carbohydrate			Placebo			P Values: Interaction; Time
	Preexercise	Postexercise	1-h Postexercise	Preexercise	Postexercise	1-h Postexercise	
Leukocytes, 10 <sup>9</sup> /l	7.51±0.50	9.82±0.55*	8.21±0.42*	7.12±0.45	11.38±1.08	9.41±0.60	0.018; <0.001
Neutrophils, 10 <sup>9</sup> /l	5.02±0.52	6.96±0.55	5.90±0.44	4.39±0.35	7.99±0.88	6.90±0.59	0.017; <0.001
Lymphocytes, 10 <sup>9</sup> /l	1.84±0.18	2.15±0.15	1.73±0.13	2.13±0.09	2.55±0.17	1.85±0.09	0.443; <0.001
Monocytes, 10 <sup>9</sup> /l	0.47±0.03	0.49±0.05	0.40±0.03	0.33±0.03	0.47±0.04	0.46±0.08	0.061; 0.050
Cortisol, nmol/l	123±13	197±32	203±24	127±20	193±42	230±47	0.851; 0.006
IL-6, pg/ml	1.75±0.92	6.55±1.96	7.72±2.19	0.98±0.16	6.43±1.12	6.61±1.48	0.781; <0.001
IL-10, pg/ml	2.18±0.44	5.31±0.84	5.87±0.80	1.86±0.68	6.33±1.04	8.49±1.72	0.338; <0.001
IL-8, pg/ml	2.88±0.62	4.67±1.34	7.72±1.42	2.22±0.27	4.27±0.65	8.99±0.92	0.348; <0.001
IL-1ra, pg/ml	228±48	257±59	233±52	197±32	226±22	209±21	0.712; 0.002
sIgA, µg/min	346±27	252±43	318±49	360±49	172±22	264±31	0.212; <0.001

Values are means ± SE. IL-1ra, IL-1 receptor antagonist; sIgA, salivary IgA. \*Significant difference in change from preexercise between groups,  $P < 0.025$ .

Pearson product-moment correlations were used to test the relationship between changes in plasma and muscle measures.

## RESULTS

Table 1 summarizes characteristics by group status for the 30 subjects completing all phases of the study. Subjects in the carbohydrate and placebo groups did not differ significantly for age, body mass and composition, years of resistance training, or 1-RM for 10 different exercises. Subjects were able to lift  $1.7 \pm 0.1$  times their body weight in the back squat. Carbohydrate intake during the 3 days before the 2-h resistance training test session did not differ significantly between subjects in the carbohydrate and placebo groups ( $53.9 \pm 2.9$  and  $50.6 \pm 2.2\%$  of total energy intake, respectively,  $P = 0.371$ ).

Volume loads (weight lifted multiplied by total repetitions across all sets) for each of the 10 exercises and the total volume load are summarized in Table 2, and no significant differences were found between the carbohydrate and placebo groups. The ending ratings of perceived exertion for the carbohydrate and placebo groups were  $17.2 \pm 0.6$  and  $17.5 \pm 0.5$  ( $P = 0.673$ ), indicating "very hard" exertion. Plasma volume decreased  $<1.5\%$  for both groups immediately after the 2-h resistance training bout, and a slight weight gain was measured ( $0.9 \pm 0.1$  and  $0.6 \pm 0.2$  kg in the carbohydrate and placebo groups, respectively,  $P = 0.149$ ).

The increase in blood cell counts for total leukocytes, neutrophils, and monocytes was attenuated in the carbohydrate compared with placebo group (Table 3). Mean neutrocytosis was 39 and 82%, and monocytosis 4 and 42% in the carbohydrate and placebo conditions, respectively (interaction effects,  $P < 0.05$ ). The pattern of change in blood lymphocyte counts did not differ between groups (interaction effect,  $P = 0.443$ ).

Muscle glycogen decreased significantly for both carbohydrate (38%) and placebo (44%) groups at a similar rate (interaction effect,  $P = 0.463$ ) (Fig. 1). Plasma cortisol increased 65 and 81% at 1-h postexercise in the carbohydrate and placebo groups, respectively (interaction effect,  $P = 0.851$ ) (Table 3). The pattern of increase in plasma IL-6, IL-10, IL-8, and IL-1ra did not differ significantly between groups (Table 3). The pattern of decrease in salivary IgA secretion rate did not differ significantly between groups (Table 3).

Muscle cytokine mRNA was detected preexercise for IL-6, IL-8, IL-15, IL-1 $\beta$ , and TNF- $\alpha$  (Table 4), and of these significant increases were measured postexercise for IL-6, IL-8,

IL-1 $\beta$ , and TNF- $\alpha$ , with no differences measured between groups (Figs. 2 and 3).

Change in muscle glycogen content did not correlate significantly with muscle IL-6 ( $r = 0.08$ ,  $P = 0.664$ ), IL-1 $\beta$  ( $r = -0.21$ ,  $P = 0.272$ ), or IL-8 ( $r = 0.13$ ,  $P = 0.509$ ) gene expression. Change in plasma cortisol levels correlated significantly with change in plasma IL-6 ( $r = 0.46$ ,  $P = 0.016$ ), IL-8 ( $r = 0.48$ ,  $P = 0.012$ ), and IL-10 ( $r = 0.47$ ,  $P = 0.014$ ), but not with muscle IL-6 and IL-8 gene expression. Muscle IL-6 and IL-8 gene expression were significantly correlated ( $r = 0.54$ ,  $P = 0.002$ ), but no other significant correlations were seen with IL-1 $\beta$  or TNF- $\alpha$ .

## DISCUSSION

The major finding of this study is that carbohydrate compared with placebo ingestion did not alter modest increases measured for plasma IL-6, IL-10, IL-1ra, and IL-8 and for muscle gene expression for IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  in strength-trained subjects lifting weights intensively for 2 h. In contrast, we recently reported that carbohydrate compared with placebo ingestion attenuated increases in plasma IL-6, IL-1ra, and IL-10 and muscle gene expression for IL-6 and IL-8, but not IL-1 $\beta$  or TNF- $\alpha$ , in subjects running 3 h at 70% maximal oxygen uptake on treadmills (20).

In the present study, increases in blood leukocyte subset counts were similar to those measured after 3 h of treadmill

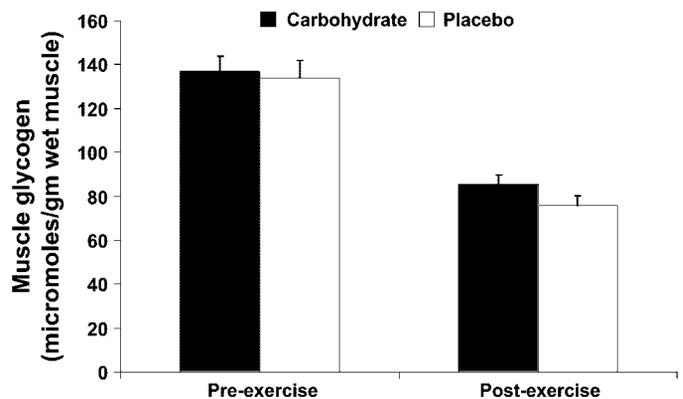


Fig. 1. Decrease in skeletal muscle glycogen content did not differ between carbohydrate and placebo groups after a 2-h resistance training bout (time effect,  $P < 0.001$ ; interaction effect,  $P = 0.463$ ).

Table 4. Change in skeletal muscle cytokine mRNA content during a 2-h intense, resistance training bout in 30 strength-trained subjects randomized to carbohydrate and placebo ingestion

Variable (Fold Difference From Calibrator)	Carbohydrate		Placebo		P Values: Interaction; Time
	Preexercise	Postexercise	Preexercise	Postexercise	
IL-1 $\alpha$	0.63 $\pm$ 0.11	0.66 $\pm$ 0.12	0.45 $\pm$ 0.06	0.71 $\pm$ 0.27	0.417; 0.322
IL-2	1.25 $\pm$ 0.15	1.23 $\pm$ 0.11	1.34 $\pm$ 0.22	1.33 $\pm$ 0.24	0.963; 0.913
IL-4	0.31 $\pm$ 0.05	0.28 $\pm$ 0.05	0.23 $\pm$ 0.05	0.28 $\pm$ 0.08	0.301; 0.761
IL-5	0.57 $\pm$ 0.11	0.46 $\pm$ 0.07	0.43 $\pm$ 0.07	0.33 $\pm$ 0.04	0.916; 0.071
IL-10	0.001 $\pm$ 0.0001	0.002 $\pm$ 0.001	0.002 $\pm$ 0.001	0.003 $\pm$ 0.001	0.699; 0.147
IL-12p35	1.69 $\pm$ 0.31	2.10 $\pm$ 0.46	1.41 $\pm$ 0.21	1.69 $\pm$ 0.31	0.738; 0.091
IL-12p40	0.24 $\pm$ 0.07	0.15 $\pm$ 0.03	0.13 $\pm$ 0.03	0.23 $\pm$ 0.10	0.115; 0.960
IL-15	84.1 $\pm$ 11.8	89.4 $\pm$ 15.5	82.4 $\pm$ 15.7	80.4 $\pm$ 14.1	0.670; 0.847
IFN- $\gamma$	0.70 $\pm$ 0.27	0.38 $\pm$ 0.08	0.60 $\pm$ 0.24	0.47 $\pm$ 0.14	0.631; 0.262

Values are means  $\pm$  SE.

running, whereas increases in plasma cytokines and muscle cytokine mRNA were considerably smaller (20). For example, plasma IL-6 reached 6–7 pg/ml and muscle IL-6 mRNA  $\sim$ 80-fold difference from calibrator after 2 h of resistance

training compared with 10–15 pg/ml and 200- to 800-fold difference from calibrator, respectively, after 3 h of treadmill running (20). Other studies have also shown that postexercise plasma cytokine concentrations are relatively low when rest intervals are inserted within an exercise regimen including

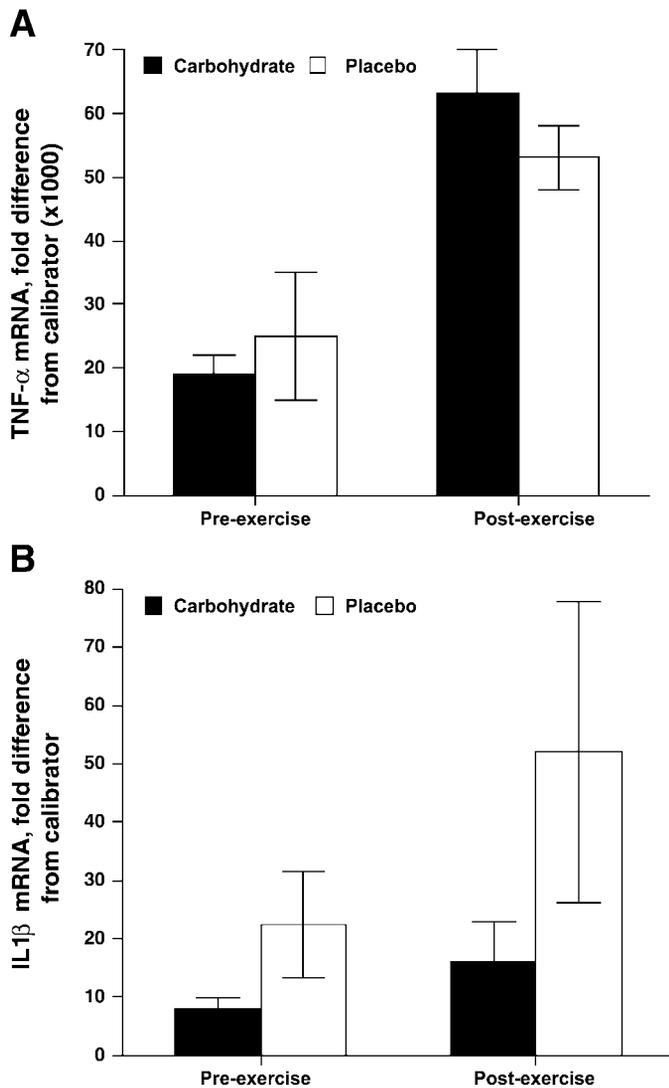


Fig. 2. Pattern of increase in muscle TNF- $\alpha$  (A) and IL-1 $\beta$  (B) mRNA expression after 2 h of resistance training did not differ between groups (time effects,  $P = 0.029$  and  $P < 0.001$ , respectively; interaction effects,  $P = 0.247$  and  $P = 0.330$ , respectively).

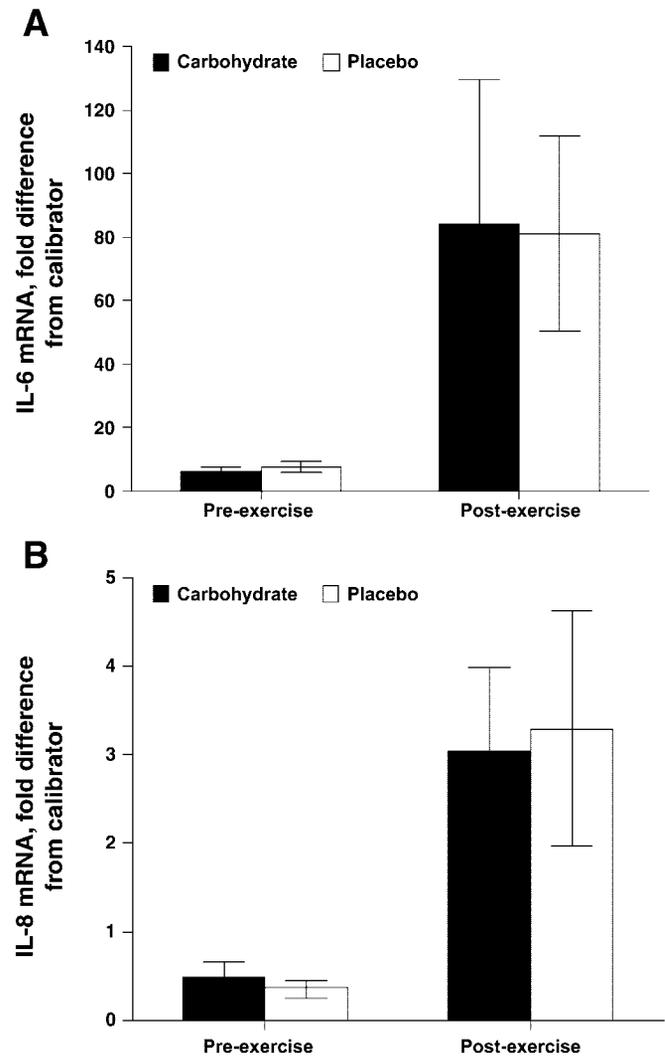


Fig. 3. Pattern of increase in muscle IL-6 (A) and IL-8 (B) mRNA expression after 2 h of resistance training did not differ between groups (time effects,  $P = 0.010$  and  $P = 0.002$ , respectively; interaction effects,  $P = 0.936$  and  $P = 0.819$ , respectively).

intense intervals extended over 90–120 min (2, 12, 23, 24). Within this context (low plasma cytokine levels after long-term exercise with rest intervals), no difference in the patterns of change in plasma cytokines has been reported when groups ingesting carbohydrate or placebo were compared (2, 24). Data from the present study also indicate that carbohydrate compared with placebo ingestion does not alter the fairly small increases in muscle cytokine gene expression measured after a 2-h bout of resistance training utilizing 2- to 3-min rest intervals.

Low preexercise muscle glycogen content has been reported to be an important trigger for muscle IL-6 mRNA expression (14, 30). Steensberg et al. (30) had subjects engage in two-legged, knee extensor exercise, with one leg before exercise containing 40% less glycogen than the other. In the postexercise samples, subjects with the lowest glycogen content expressed the highest levels of IL-6 mRNA (27, 30). Our study did not test the effect of low preexercise muscle glycogen levels on IL-6 mRNA expression during exercise, but examined the potential effect of carbohydrate compared with placebo ingestion on the rate of decrease in muscle glycogen and muscle cytokine gene expression. In our previous study, the increase in skeletal muscle IL-6 mRNA was considerably diminished in the carbohydrate compared with placebo condition despite no group difference in post-3-h-run muscle glycogen levels (20). In that study, muscle glycogen decreased by 24–28% after 3 h of running, substantially less than the 38–43% decrease measured in the present study after 2 h of intensive resistance training, and yet muscle IL-6 mRNA expression was considerably higher after the 3-h run. The key signaling molecules for IL-6 gene expression during exercise await further research, but we suggest that focus should be directed toward factors generated during unrelenting, high-intensity exercise workloads but not during exercise regimens with rest intervals (10, 11, 21, 30).

In our strength-trained subjects, muscle cytokine mRNA was detected preexercise for IL-6, IL-8, IL-15, IL-1 $\beta$ , and TNF- $\alpha$ , and significant increases were measured postexercise for all these except IL-15. In marathon runners, muscle cytokine mRNA was detected preexercise for IL-1 $\beta$ , IL-2, IL-6, IL-10, IL-12p35, IL-15, IFN- $\gamma$ , and TNF- $\alpha$ , with IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$  significantly increased above preexercise levels after the 3-h run (20). In both studies, the largest postexercise increases in muscle cytokine mRNA (fold difference from preexercise) were measured for IL-6, IL-8, and IL-1 $\beta$ . Prior investigations have focused on exercise-induced changes in muscle gene expression for IL-6, and little is presently known about the signaling agents for IL-8 and IL-1 $\beta$ . Neutrophils invade active muscle tissues within several hours after intensive exercise, and it is probable that muscle IL-8 and IL-1 $\beta$  expression play a role (18).

In the present study, carbohydrate compared with placebo ingestion had a small but significant effect in attenuating postexercise increases in blood neutrophil and monocyte counts but no effect on the postexercise decrement in salivary IgA output, similar to previous reports (19, 20, 22, 25). Koch et al. (15) also studied the influence of carbohydrate and placebo ingestion on immune responses to resistance exercise (high-intensity squats with short rest intervals) and reported little or no significant effects on postexercise changes in blood leukocyte subset counts or lymphocyte proliferation.

In summary, changes in plasma cytokine levels and muscle cytokine mRNA expression after an intensive 2-h resistance training bout were fairly small. Carbohydrate compared with placebo ingestion had a small but significant attenuating effect on increases in blood leukocyte subset counts, but no other group differences were measured for all other variables including muscle glycogen content, plasma cytokine levels, muscle cytokine mRNA expression, plasma cortisol, and salivary IgA output. Resistance training induced gene expression within the muscle for two primary proinflammatory cytokines, IL-1 $\beta$  and TNF- $\alpha$ , and two components of the secondary proinflammatory cascade, IL-6 and IL-8. Further research is warranted to determine the signaling agents for gene expression of these cytokines, their roles during and after resistance exercise, and why prolonged and intensive running causes a much greater gene expression.

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