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Insulin and exercise differentially regulate PI3-kinase and glycogen synthase in human skeletal muscle

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[1]*Noll Physiological Research Center and the General Clinical Research Center, Pennsylvania State University, University Park, Pennsylvania 16802; and Departments of* [2]*Reproductive Biology and*
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O'Gorman, Donal J., Luis F. del Aguila, David L. Williamson, Raj K. Krishnan, and John P. Kirwan. Insulin and exercise differentially regulate PI3-kinase and glycogen synthase in human skeletal muscle. *J Appl Physiol* 89: 1412–1419, 2000.—The purpose of this study was to determine the separate and combined effects of exercise and insulin on the activation of phosphatidylinositol 3-kinase (PI3-kinase) and glycogen synthase in human skeletal muscle in vivo. Seven healthy men performed three trials in random order. The trials included *1*) ingestion of 2 g/kg body wt carbohydrate in a 10% solution (CHO); *2*) 75 min of semirecumbent cycling exercise at 75% of peak O_2 consumption; followed by 5×1-min maximal sprints (Ex); and *3*) Ex, immediately followed by ingestion of the carbohydrate solution (ExCHO). Plasma glucose and insulin were increased ($P < 0.05$) at 15 and 30 (Post-15 and Post-30) min after the trial during CHO and ExCHO, although insulin was lower for ExCHO. Hyperinsulinemia during recovery in CHO and ExCHO led to an increase ($P < 0.001$) in PI3-kinase activity at Post-30 compared with basal, although the increase was lower ($P < 0.004$) for ExCHO. Furthermore, PI3-kinase activity was suppressed ($P < 0.02$) immediately after exercise (Post-0) during Ex and ExCHO. Area under the insulin response curve for all trials was positively associated with PI3-kinase activity ($r = 0.66, P < 0.001$). Glycogen synthase activity did not increase during CHO but was increased ($P < 0.05$) at Post-0 and Post-30 during Ex and ExCHO. Ingestion of the drink increased ($P < 0.05$) carbohydrate oxidation during CHO and ExCHO, although the increase after ExCHO was lower ($P < 0.05$) than CHO. Carbohydrate oxidation was directly correlated with PI3-kinase activity for all trials ($r = 0.63, P < 0.001$). In conclusion, under resting conditions, ingestion of a carbohydrate solution led to activation of the PI3-kinase pathway and oxidation of the carbohydrate. However, when carbohydrate was ingested after intense exercise, the PI3-kinase response was attenuated and glycogen synthase activity was augmented, thus facilitating nonoxidative metabolism or storage of the carbohydrate. Activation of glycogen synthase was independent of PI3-kinase.

insulin action; muscle enzymes

RESTORATION OF MUSCLE GLYCOGEN after intense exercise is an important determinant of recovery between suc-cessive days of exercise training (7, 17). Rates of gly-cogen resynthesis are highest during the initial 2 h after exercise, and people who exercise on a regular basis are encouraged to maximize carbohydrate intake during this period (9, 16). However, ingestion of carbo-hydrate after exercise may create an unusual dynamic between the effects of exercise and insulin on glucose uptake and metabolism. Both exercise and insulin are reported to have independent and synergistic effects on skeletal muscle glucose uptake and transport (3, 10, 28, 36). In addition, exercise and insulin can facilitate increased glycogen storage in skeletal muscle (2, 8, 11). In contrast, there is also evidence of glucose intoler-ance for a period immediately after exercise (25, 27). These combined observations suggest the potential for an interesting juxtaposition between the relative roles of exercise and/or insulin in postexercise muscle glyco-gen resynthesis and carbohydrate oxidation.

Under normal physiological conditions, carbohy-drate ingestion stimulates a rise in plasma insulin, which in turn helps regulate glucose entry into muscle cells for storage and/or oxidation. The process is facil-itated by a signaling cascade that includes insulin-receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI3-kinase). An acute bout of exercise can also stimulate glucose metabolism, although studies examining the effects of exercise on insulin signaling are equivocal (19, 38). In one study, insulin-stimulated IRS-1 phosphorylation and IRS-1-associated PI3-kinase activity were unchanged after 30 min of mod-erate exercise (19). In another report, insulin-activated PI3-kinase was decreased for up to 5 h after exercise (38). Therefore, it seems that postexercise glucose me-tabolism is mediated by an exercise/contraction-stim-ulated mechanism rather than by insulin. However, there is no clear evidence in humans that this contin-ues to be the case when insulin is elevated during carbohydrate ingestion in the postexercise recovery period.

After uptake by the muscle, glucose is phosphory-lated and, in a series of enzyme-regulated steps, un-

dergoes glycolysis and oxidation or is converted to glycogen in a reaction catalyzed by glycogen synthase (21). Although exercise and insulin have both been shown to activate glycogen synthase, the role of PI3-kinase in the activation of glycogen synthase in human skeletal muscle during the postexercise recovery period is not clear. There are reports suggesting that insulin-stimulated PI3-kinase may be involved in the regulation of glycogen synthase activity in cultured cell lines (34). However, results on in vivo human skeletal muscle under physiological conditions are not available to support these findings. Therefore, the purpose of the present study was to investigate the effects of insulin and exercise on human skeletal muscle IRS-1-associated PI3-kinase and glycogen synthase, key regulators of glucose uptake and glycogen synthesis, respectively. We also evaluated whether insulin signaling via PI3-kinase is involved in the regulation of glycogen synthase activity when carbohydrate is ingested after intense exercise in humans.

METHODS

Subjects

Seven healthy active men volunteered to participate in the study. Subject characteristics are presented in Table 1. Written informed consent was obtained from each subject in accordance with the guidelines for the protection of human subjects at the Pennsylvania State University. Initial screening included a physical examination, a blood and urine chemistry, and a resting electrocardiogram. Each subject completed a semirecumbent, incremental cycle ergometer test to determine peak oxygen consumption ($\dot{V}O_{2 \, peak}$). Body density was determined by using a modification of the technique described by Akers and Buskirk (1). Residual lung volume was determined by nitrogen dilution, and percent body fat was estimated by using the Siri equation (33). Height was measured to the nearest 1.0 cm, without shoes. Body mass was measured to the nearest 0.1 kg with the subject wearing shorts.

Experimental Design

Three trials were performed, in random order, and separated by at least 10 days (Fig. 1). For 2 days before each trial, subjects were provided with all of their meals from the General Clinical Research Center (GCRC) metabolic kitchen. The macronutrient composition of the diet provided 50% carbohydrate, 35% fat, and 15% protein. Total caloric intake (Table 1) was based on height, weight, age, and activity according to the Harris-Benedict equation (12). Exercise training for the 2 days before each trial was controlled and consisted of 45 min of semirecumbent bicycle exercise at 60% of $\dot{V}O_{2 \, peak}$. Subjects resided in the GCRC the night preceding

Table 1. *Descriptive characteristics of the subjects*

Age, yr	24.9 ± 0.2
Weight, kg	75.8 ± 2.3
Body fat, %	9.6 ± 0.6
BMI, kg/m²	24 ± 1
$\dot{V}O_{2peak}$, ml·kg⁻¹·min⁻¹	49.8 ± 1.5
Caloric intake, kcal/day	$3,427 \pm 256$

Values are means $\pm$ SE for 7 subjects. BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen consumption.

each trial and the following morning were awoken at 7:00 AM to have an intravenous catheter inserted into an antecubital vein. The three trials were as follows (Fig. 1).

Carbohydrate trial (CHO trial). After the baseline blood and muscle biopsy samples were obtained, subjects lay in a semirecumbent position for 85 min, equivalent to the length of the exercise session. They were then provided with a drink containing 2 g/kg body wt (BW) carbohydrate in a 10% aqueous solution to stimulate pancreatic insulin secretion. This drink was consumed within 5 min, and the subjects remained supine for a further 30 min when final blood [immediately (Post-0), 15 min (Post-15), and 30 min (Post-30) after carbohydrate ingestion], muscle (Post-0 and Post-30), and breath (Post-0 to Post-30) measurements were made.

Exercise trial (Ex trial). After the baseline blood and muscle biopsy samples were obtained, subjects cycled for 75 min at 75% $\dot{V}O_{2 \, peak}$ on a semirecumbent cycle ergometer at a pedaling rate of 70 revolutions/min. This intensity has been shown to equally deplete muscle glycogen in fast- and slow-twitch muscle fibers (2). To maximize glycogen depletion, subjects were allowed to rest for 1 min after the 75 min of continuous exercise and then performed 5×1-min maximal sprints with 1-min rest intervals. The exercise intensity for the sprints was 125% of the resistance used for the 75-min continuous exercise. During the exercise bout, subjects were allowed to drink water ad libitum. Immediately after the last sprint, a muscle biopsy was performed. Subjects lay on a bed for 30 min after exercise, and then final blood, muscle, and breath measurements were collected.

Exercise and carbohydrate trial (ExCHO trial). Baseline blood and a muscle biopsy were performed before exercise. The subjects then performed the same exercise bout as described for the Ex trial. Immediately after the last sprint, a muscle biopsy was taken, and then a drink containing 2 g/kg BW carbohydrate in a 10% solution was consumed within 5 min to stimulate pancreatic insulin secretion. Subjects were monitored for a further 30 min similar to previous trials.

Blood Samples

Baseline blood samples were taken for measurement of fasting plasma glucose and insulin concentrations (basal). During steady-state exercise, blood samples were taken every 15 min, and a final sample was taken at the end of the 5×1-min maximal effort sprints. Additional blood samples were taken at 15 and 30 min after exercise (Post-15 and Post-30, respectively). For the CHO trial, a blood sample was taken just before carbohydrate ingestion and again at Post-15 and Post-30. Plasma glucose was measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin was measured by using a commercially available double-antibody radioimmunoassay (Linco Research, St. Charles, MO).

Muscle Samples

A baseline biopsy was taken from the vastus lateralis muscle before each trial (basal). During the exercise trials, the biopsy was obtained within 2 min after the last sprint (Post-0). The final biopsy was taken 30 min after carbohydrate ingestion or rest (Post-30). The biopsies were obtained under local anesthesia, and the samples were immediately frozen in liquid nitrogen and stored at $-80°C$ for subsequent analysis of muscle enzyme activity.

IRS-1-associated PI3-kinase activity. Skeletal muscle protein was isolated and PI3-kinase activity determined as previously described (18). Briefly, 1 mg of cell lysate was immunoprecipitated with an IRS-1 polyclonal antibody (Upstate

Fig. 1. Schematic representation of the study design. Carbohydrate trial (CHO), ingestion of 2 g/kg body wt carbohydrate in a 10% solution; exercise trial (Ex), 75 min of semirecumbent cycling exercise at 75% of peak oxygen consumption ($\dot{V}_{O_{2\,peak}}$), followed by 5 × 1-min maximal sprints; exercise and carbohydrate trial (ExCHO), Ex, immediately followed by ingestion of the carbohydrate solution. Basal, before the trial. Post 0, immediately after exercise; Post 15, 15 min after exercise and/or carbohydrate ingestion; Post 30, 30 min after exercise and/or carbohydrate ingestion.
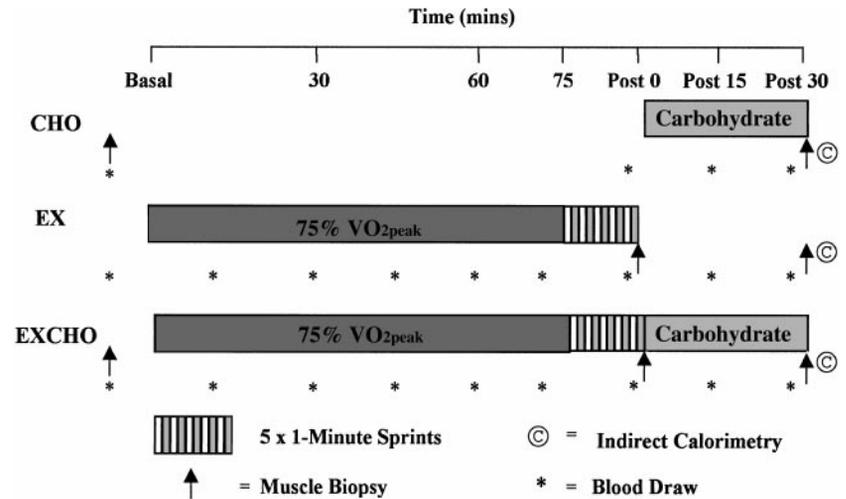
Biotechnology, Lake Placid, NY) and an immunocomplex formed with protein A-Sepharose. A 2 µg/µl phosphatidylinositol mixture was prepared, and a reaction was started with unlabeled and labeled γ-^{32}P-labeled ATP. Phosphatidylinositol 3-phosphate was extracted with chloroform-methanol (1:1) and centrifuged, and the organic phase was separated by thin-layer chromatography. The radioactivity incorporated into PI3-phosphate was determined by PhosphoImaging (Molecular Dynamics, Sunnyvale, CA).

Glycogen synthase activity. Glycogen synthase activity was determined as previously described (26). Briefly, 5 mg of freeze-dried muscle tissue were homogenized and incubated in the presence (D form) or absence (I form) of 10 mM glucose 6-phosphate. An enzymatic reaction increased the NADH concentration, which was measured by fluorometry. Glycogen synthase activity was determined as the active fraction of the total enzyme (I/I+D).

Substrate Oxidation

During exercise inspired air volumes were measured from pressure changes detected with a pneumotach (Hans Rudolph). Concentrations of O_2 and CO_2 were measured on an electrochemical O_2 analyzer (model S-3A, Applied Electrochemistry) and infrared CO_2 analyzer (model LB-2, Beckman), respectively. Oxidation rates were calculated from oxygen uptake ($\dot{V}_{O_2}$) and respiratory exchange ratio (RER) data.

For 30 min after carbohydrate ingestion the subjects lay on a bed with a Plexiglas flow-through hood placed over their head. Airflow was controlled by a mass flowmeter (model SXCI-1300, National Instruments) at a rate of 50 l/min while maintaining a slight negative pressure. Continuous open-circuit exhaled breath samples were analyzed by using Hartmann-Braun (Frankfurt, Germany) differential paramagnetic oxygen (model 4G, Magnos) and nondispersive infrared carbon dioxide (Uras 4) analyzers set at a 1% scale. Nonprotein substrate oxidation rates were calculated from $\dot{V}_{O_2}$, carbon dioxide production, and RER data during the last 5 min of the collection period (22).

Statistical Analysis

All values are expressed as means ± SE. A repeated-measures analysis of variance for a crossover study was used to determine significant main effects and interactions. A Newman-Keuls post hoc test was used to identify specific mean differences. A minimum level of statistical significance

was accepted at the $P < 0.05$ level of confidence for all variables.

RESULTS

Exercise

Mean exercise data are presented in Table 2. $\dot{V}_{O_2}$, measured at 30 and 60 min during exercise, was 75.8 ± 1.2 and 76.5 ± 0.9% $\dot{V}_{O_2\,peak}$ for the Ex and ExCHO trials, respectively. RER decreased significantly ($P < 0.05$) during steady-state exercise but was not different between trials. Heart rates, measured by radiotelemetry throughout exercise, were not significantly different between trials.

Glucose Response

Fasting plasma glucose levels were similar for all trials (Table 3). Plasma glucose levels were significantly elevated ($P < 0.05$) at Post-0 for both exercise trials. Ingestion of the carbohydrate solution induced the expected increases in plasma glucose concentrations ($P < 0.05$) at Post-15 and Post-30 during the CHO and ExCHO trials. When carbohydrate was not ingested after exercise, plasma glucose returned to baseline during the 30-min recovery period.

Insulin Response

Fasting plasma insulin concentrations (Fig. 2) were similar for all trials (43 ± 3, 45 ± 3, and 48 ± 4 pM for

Table 2. *Exercise performance data for the Ex and ExCHO trials*

Trial	30 min		60 min	
	$\dot{V}_{O_2}$, ml· $kg^{-1} \cdot min^{-1}$	RER	$\dot{V}_{O_2}$, ml· $kg^{-1} \cdot min^{-1}$	RER
Ex	37.4 ± 1.2	0.88 ± 0.01	37.1 ± 1.6	0.85 ± 0.01*
ExCHO	37.4 ± 1.2	0.89 ± 0.02	37.8 ± 1.0	0.86 ± 0.01*

Values are means ± SE for 7 subjects. Ex, exercise trial, no glucose; ExCHO, exercise followed by carbohydrate ingestion; $\dot{V}_{O_2}$, oxygen consumption; RER, respiratory exchange ratio. *Significantly lower than 30 min, $P < 0.05$.

Table 3. *Plasma glucose concentrations at rest, during exercise, and during recovery after glucose ingestion*

Trial	Basal	Ex-15	Ex-30	Ex-45	Ex-60	Ex-75	Post-0	Post-15	Post-30
CHO	4.9 ± 0.2						5.1 ± 0.1	7.4 ± 0.4*†	8.8 ± 0.6*†
Ex	5.2 ± 0.2	5.2 ± 0.2	5.2 ± 0.2	5.1 ± 0.2	4.7 ± 0.1	4.8 ± 0.2	6.1 ± 0.4*	5.7 ± 0.4	4.9 ± 0.2
ExCHO	5.2 ± 0.2	5.0 ± 0.2	5.1 ± 0.1	5.0 ± 0.2	4.9 ± 0.1	4.7 ± 0.3	6.0 ± 0.3*	7.0 ± 0.4*†	9.3 ± 0.5*†

Values are means $\pm$ SE given in mmol/l for 7 subjects. CHO, carbohydrate ingestion at rest; Basal, before trial; Ex-15–Ex-75, 15–75 min of exercise, respectively; Post-0, immediately after exercise; Post-15 and Post-30, 15 and 30 min after exercise and/or carbohydrate ingestion, respectively. *Significantly higher than basal, $P < 0.05$. †Significantly higher than Ex, $P < 0.05$.

the CHO, Ex, and ExCHO trials, respectively). When carbohydrate was ingested at rest and after exercise, plasma insulin was increased at Post-15 (235 ± 43 and 93 ± 23 pM for the CHO and ExCHO trials, respectively) and Post-30 (424 ± 71 and 270 ± 41 pM for the CHO and ExCHO trials, respectively). The absolute insulin response and the area under the insulin curve (AUC) were both significantly greater ($P < 0.05$) for the CHO ($6,156 \pm 857$ pM/min) vs. ExCHO trial ($2,401 \pm 481$ pM/min). Plasma insulin during the Ex trial (55 ± 5 and 38 ± 1 pM for Post-15 and Post-30, respectively) was not different from the respective baseline value. Insulin AUC was lower ($P < 0.05$) during the Ex trial (245 ± 70 pM/min) compared with both the CHO and ExCHO trials. During exercise plasma insulin levels declined to 29 ± 2 pM (Ex trial) and 34 ± 3 pM (ExCHO trial) after 75 min of continuous cycling.

Substrate Oxidation

After ingestion of the drink at rest, carbohydrate was the primary fuel oxidized (6.39 ± 0.32 mg·kg^{-1}·min^{-1}), whereas the relative contribution of fat was difficult to detect by using indirect calorimetry. Conversely, after exercise and in the absence of carbohydrate ingestion and hyperinsulinemia, carbohydrate oxidation was negligible and fat oxidation provided the majority of the oxidized energy (2.87 ± 0.16 mg·kg^{-1}·min^{-1}). When carbohydrate was ingested after exercise, carbohydrate oxidation (2.25 ± 0.43 mg·kg^{-1}·min^{-1}) was blunted ($P < 0.05$)

compared with the CHO trial, whereas fat oxidation (2.37 ± 0.31 mg·kg^{-1}·min^{-1}) was similar to the Ex trial. Thus, when carbohydrate was ingested after exercise, most of the carbohydrate appeared to be directed toward nonoxidative pathways. However, when carbohydrate was ingested at rest, the glucose flux favored oxidative pathways.

IRS-1-Associated PI3-Kinase Activity

IRS-1-associated PI3-kinase was expressed as multiples of increase in activity above basal (Fig. 3). With hyperinsulinemia at rest and after exercise, there was an increase ($P < 0.001$) in PI3-kinase activity at Post-30 (3.21 ± 0.8- and 1.58 ± 0.15-fold increase for the CHO and ExCHO trial, respectively). The increase in PI3-kinase after the ExCHO trial was lower ($P < 0.05$) than in the CHO trial. In contrast, exercise caused a decrease ($P < 0.02$) in PI3-kinase activity in muscle samples obtained at Post-0 (0.46 ± 0.10- and 0.44 ± 0.05-fold for the Ex and ExCHO trials, respectively). Correlation data showed a positive relationship between PI3-kinase after all trials and the insulin AUC ($r = 0.66$, $P < 0.001$). These data suggest that the
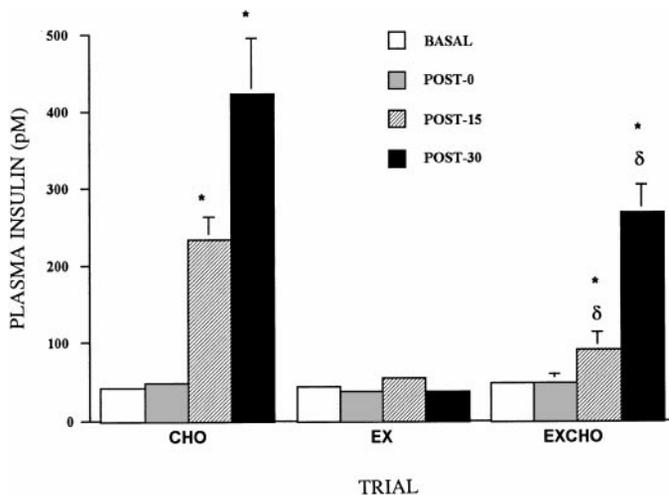
Fig. 2. Plasma insulin concentrations in response to carbohydrate ingestion and exercise. Values are means $\pm$ SE for 7 subjects. *Significantly higher than basal, $P < 0.05$. δSignificantly lower than the corresponding time point during the CHO trial, $P < 0.05$.
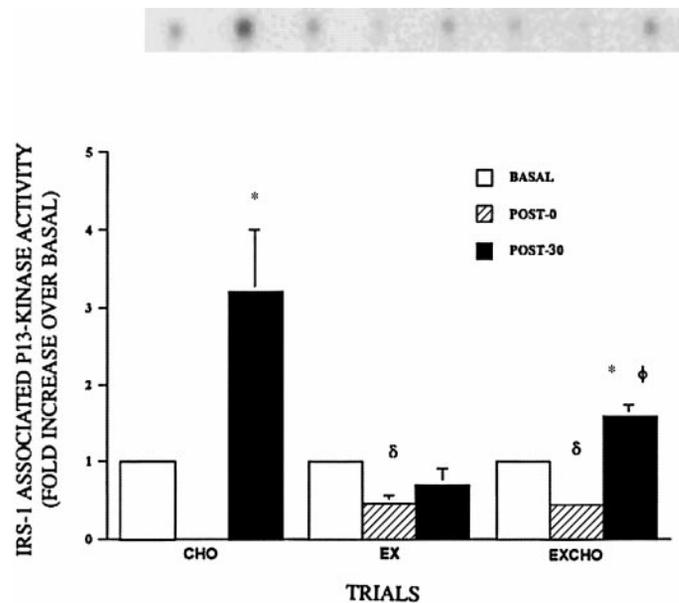

Fig. 3. Fold increase in insulin-receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase (PI3-kinase) activity in response to carbohydrate ingestion and exercise. Values are means $\pm$ SE for 7 subjects. *Significantly higher than basal, $P < 0.001$. δSignificantly lower than basal, $P < 0.02$. φSignificantly lower than the corresponding time point during the CHO trial, $P < 0.05$.

exercise-induced reduction in the PI3-kinase signal may have been due to a weaker insulin stimulus arising from the lower circulating insulin compared with the resting trial. A direct association was also noted between PI3-kinase and carbohydrate oxidation ($r = 0.63$, $P < 0.001$).

Glycogen Synthase Activity

Glycogen synthase activity (I/I+D) for all trials is illustrated in Fig. 4. Basal glycogen synthase activity was not significantly different between trials. When carbohydrate was ingested at rest, the elevated insulin did not lead to a significant increase in glycogen synthase activity (0.22 ± 0.02 vs. 0.28 ± 0.04 unit). Conversely, glycogen synthase activity was increased ($P < 0.05$) immediately after both the Ex (0.21 ± 0.02 vs. 0.39 ± 0.03 unit) and ExCHO (0.20 ± 0.04 vs. 0.37 ± 0.03 unit) trials. Glycogen synthase activity remained elevated for 30 min after exercise and was not affected by the rise in insulin that occurred with carbohydrate ingestion (0.39 ± 0.04 vs. 0.39 ± 0.05 unit for the Ex and ExCHO trials, respectively). There was no correlation between glycogen synthase activity and PI3-kinase, insulin AUC, or carbohydrate oxidation.

DISCUSSION

During prolonged moderate- to high-intensity exercise, muscle and hepatic glycogen stores are utilized to fuel the energy requirements of the muscle cells. Immediately after exercise there is a need to replace glycogen and the body shifts into a replenishment/storage mode. Individuals who perform intense exercise are encouraged to ingest carbohydrate as soon as possible afterward to restore muscle glycogen (9, 16, 17). The ingestion of carbohydrate stimulates insulin secretion, which in turn facilitates glucose uptake by the body. A greater knowledge of the interaction between exercise and insulin in the postexercise period may provide important information concerning optimal strategies for recovery from exercise. Furthermore, an understanding of the cellular and molecular mechanisms that regulate the pathways of glucose

Fig. 4. Glycogen synthase activity in response to carbohydrate ingestion and exercise. Values are means $\pm$ SE for 7 subjects. *Significantly higher than basal, $P < 0.05$.

uptake and glycogen synthesis may help in developing appropriate nutritional guidelines for exercise recovery. The observation that PI3-kinase is a key protein in the signaling cascade that facilitates glucose uptake by skeletal muscle prompted us to examine how exercise and insulin might influence activation of PI3-kinase in the immediate postexercise recovery period (31).

The role of PI3-kinase in insulin-stimulated glucose transport was first demonstrated in cultured cell lines and rat skeletal muscle under resting conditions (4, 6, 20, 39). It was subsequently shown that PI3-kinase activity was also directly involved in insulin stimulated glucose disposal in human skeletal muscle at rest (13, 18). Data from the present study extend these findings by showing that PI3-kinase activity in human skeletal muscle can be stimulated under more physiological conditions of hyperinsulinemia associated with carbohydrate ingestion. In contrast, PI3-kinase activity was decreased immediately after exercise and was only partially reactivated 30 min after the exercise bout. Two previous studies have reported that insulin-stimulated PI3-kinase is increased in muscle after exercise (5, 40). Zhou and Dohm (40) showed an increase in insulin-stimulated PI3-kinase activity in rat skeletal muscle immediately after exercise. However, they used an anti-phosphotyrosine antibody, which precipitated both IRS-1- and IRS-2-associated PI3-kinase. It is possible that only IRS-1-associated PI3-kinase activity is downregulated immediately after exercise. Chibalin et al. (5) reported an increase in both IRS-1- and IRS-2-associated PI3-kinase activity in rat skeletal muscle 16 h after an acute bout of exercise. However, there may be a time course for reactivation of the enzyme after exercise and 16 h may be well within this range for rat muscle. Although Chibalin et al. found that both IRS-1- and IRS-2-associated PI3-kinase was increased 1 day after exercise, only IRS-1-associated PI3-kinase was increased 16 h after the last exercise bout when animals had exercised on 5 consecutive days. These data suggest that exercise induces a differential effect on insulin stimulated IRS-1 and IRS-2 associated PI3-kinase activation. Whether exercise has a similar effect immediately after exercise remains to be demonstrated. The decrease in IRS-1-associated PI3-kinase activity after exercise that we report in the present study is consistent with data reported by Wojtaszewski et al. (37, 38), who also found a reduction in PI3-kinase activity, and, more recently, insulin receptor tyrosine kinase activity, after intense exercise. From these data, it appears that the effects of exercise on insulin-signaling molecules may be time dependent. Immediately after and for up to 4 h, PI3-kinase activation may be suppressed by exercise, whereas 1 day later and thereafter, PI3-kinase activation may be enhanced. Exercise training data in humans tend to support this observation (14, 18).

Carbohydrate ingestion after exercise resulted in an attenuated PI3-kinase response compared with the carbohydrate control trial. Data from the present study suggest that the decrease in PI3-kinase activity may be a function of the circulating insulin after exercise. The
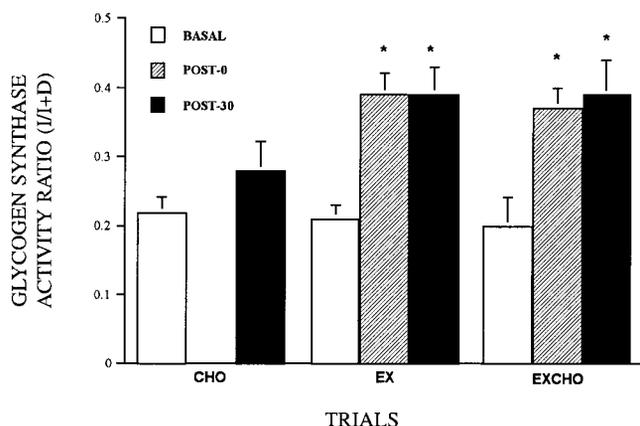
observation of a direct association between the insulin response and PI3-kinase activity is consistent with a decrease due to the lower circulating insulin during recovery. Our laboratory previously observed glucose intolerance and a reduced insulin response when athletes ingested carbohydrate immediately after prolonged exercise (25). Other investigators have shown that endogenous insulin secretion is reduced and insulin clearance is enhanced after exercise in healthy men (27, 35). Both mechanisms may have contributed to the lower insulin response in the present study. Taken together, our data support the idea that the circulating insulin response to a similar carbohydrate load is lower when preceded by exercise and that this lower insulin response produces a weaker insulin signal and less activation of PI3-kinase. This does not preclude the possibility that inhibition of insulin signaling at some point proximal to the insulin receptor may have been responsible for the blunted PI3-kinase response. It is possible that an increase in tyrosine phosphatase activity may cause dephosphorylation of the insulin receptor or IRS-1, leading to attenuated IRS-1-associated PI3-kinase activity (24).

In addition to stimulating PI3-kinase activity and regulating glucose uptake, insulin is also directly involved in the dephosphorylation and catalytic activity of glycogen synthase, a key protein that controls glycogen synthesis (34). In the present study, we examined the effects of insulin on glycogen synthase at rest and during recovery from exercise by using a normal physiological stimulus, i.e., ingestion of glucose to induce hyperinsulinemia. We did not see a significant increase in glycogen synthase with hyperinsulinemia or any correlation between the insulin AUC and glycogen synthase. It appears that the ability of insulin to activate glycogen synthase at rest after oral glucose ingestion is less than during glucose and insulin infusion (37). In general, muscle glycogen stores are adequately filled under normal resting conditions in vivo, and most of the ingested glucose gets oxidized in the mitochondria. We observed that carbohydrate oxidation rates were almost 2.5-fold higher when carbohydrate was ingested under resting conditions compared with after exercise. Furthermore, glycogen synthase activity is inversely related to glycogen concentration, and trained individuals have greater glycogen stores (23, 32). The subjects who participated in the present study were physically active and were provided with a balanced diet for 2 days preceding each trial. Therefore, the absence of any significant change in glycogen synthase activity after carbohydrate ingestion and hyperinsulinemia at rest may have been a function of the prevailing high muscle glycogen stores, training, and nutritional status of the subjects.

In contrast to the observations with hyperinsulinemia at rest, glycogen synthase activity was approximately twofold greater than baseline immediately after exercise and remained elevated for at least 30 min. The increase in insulin when carbohydrate was ingested after exercise did not have any additional effect on glycogen synthase activity, and this further sup-

ports the idea that insulin does not play a major role in stimulating postexercise glycogen synthesis. These observations are consistent with the idea that glucose metabolism after prolonged exercise may be regulated by contraction-mediated recruitment of glucose transporters or blood flow rather than by insulin (15, 30).

Data from other investigators are consistent with the concept that carbohydrate metabolism is independent of plasma insulin in the postexercise period (29, 30). Price et al. (29) reported that when muscle glycogen levels were decreased below 30 mM by exercise, glycogen synthesis was not affected by insulin. Koval et al. (19) reported a decrease in insulin-receptor tyrosine kinase activity, no change in IRS-1 phosphorylation, or p85 association with IRS-1, but an increase in glycogen synthase after exercise. These data and the observations from our study do not support a relationship between PI3-kinase and glycogen synthase activity after exercise. In fact, these enzymes appear to be differentially regulated during early recovery from exercise.

The positive correlation between carbohydrate oxidation and insulin-stimulated PI3-kinase activity is an important observation and suggests some unidentified link between insulin signaling in the cytoplasm and glucose oxidation in the mitochondria. Because the association is on the basis of a correlation, no cause and effect can be attributed to these data. Nevertheless, it raises the possibility that PI3-kinase may be directing glucose toward oxidation when circulating glucose levels are elevated and glycogen stores are adequate. This is consistent with the overall variation in carbohydrate oxidation between trials. After carbohydrate ingestion at rest, most of the carbohydrate entering the muscle was oxidized and PI3-kinase activity was high. Conversely, carbohydrate oxidation was negligible during recovery from exercise when no carbohydrate was ingested, and this was accompanied by a suppression in PI3-kinase activity. When carbohydrate was ingested after exercise, carbohydrate oxidation was elevated but the rate was lower then the resting trial, as was PI3-kinase. Because the plasma glucose concentration was similar during recovery for the two feeding trials, the balance of the glucose must have been disposed via nonoxidative pathways in the muscle, or as glycogen in the liver. The elevation of glycogen synthase is taken to support the idea that, at least in muscle, a nonoxidative mechanism of glycogenesis was active and that most of the glucose entering the cell was being stored as glycogen. We did not see any association between PI3-kinase and glycogen synthase, which may rule out a link between these two steps in the insulin signaling and glucose storage pathways in humans under physiological conditions. Indeed, the correlation between PI3-kinase activity and rates of carbohydrate oxidation suggests there may be some direct link between insulin signaling and glucose oxidation in skeletal muscle.

In summary, the present study was designed to determine the individual and combined effects of exercise and insulin on the activation of IRS-1-associated PI3-kinase and glycogen synthase within normal physiological conditions in human skeletal muscle in vivo.

When carbohydrate was ingested under resting conditions, PI3-kinase was directly activated by the resulting increase in plasma insulin. In contrast, exercise alone caused a decrease in PI3-kinase activity that remained suppressed during the first 30 min of recovery. When carbohydrate was ingested after exercise, PI3-kinase activity was decreased by exercise, but it increased after 30 min. The increase was less than when carbohydrate was ingested at rest. Carbohydrate ingestion, with the concomitant rise in insulin at rest, did not significantly increase glycogen synthase activity, whereas exercise caused an approximately twofold increase, with no additional effect of insulin when carbohydrate was ingested after exercise. There was no correlation between IRS-1-associated PI3-kinase and glycogen synthase activity. The present results indicate that insulin and acute exercise differentially regulate PI3-kinase and glycogen synthase activity. Thus the insulin-stimulated IRS-1-associated PI3-kinase pathway is not a primary player in human skeletal muscle when carbohydrate is ingested in the early postexercise recovery period.

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