

Smoking impairs muscle recovery from exercise

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Price, Thomas B., Suchitra Krishnan-Sarin, and Douglas L. Rothman. Smoking impairs muscle recovery from exercise. *Am J Physiol Endocrinol Metab* 285: E116–E122, 2003. First published March 11, 2003; 10.1152/ajpendo.00543.2002.—Cigarette smoking is a leading cause of many adverse health consequences. Chronic nicotine exposure leads to insulin resistance and may increase the risk of developing non-insulin-dependent diabetes mellitus in young otherwise healthy smokers. To evaluate smoking-induced effects on carbohydrate metabolism, we studied muscle glycogen recovery from exercise in a young healthy population of smokers. The study used ³¹P-¹³C NMR spectroscopy to compare muscle glycogen and glucose 6-phosphate levels during recovery in exercised gastrocnemius muscles of randomized cohorts of healthy male smokers (S) and controls (C). Data for the two groups were as follows: S, ≥20 cigarettes/day ($n = 8$), 24 ± 2 yr, 173 ± 3 cm, 70 ± 4 kg and age- and weight-matched nonsmoking C ($n = 10$), 23 ± 1 yr, 175 ± 3 cm, 67 ± 3 kg. Subjects performed single-leg toe raises to deplete glycogen to ~20 mmol/l, and glycogen resynthesis was measured during the first 4 h of recovery. Plasma samples were assayed for glucose and insulin at rest and during recovery. Test subjects were recruited from the general community surrounding Yale University. Glycogen was depleted to similar levels in the two groups [23.5 ± 1.2 (S) and 19.1 ± 1.3 (C) mmol/l]. During the 1st h of recovery, glycogen synthesis rates were similar [13.8 ± 1.1 (S) and 15.3 ± 1.3 (C) $\text{mmol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$]. Between hours 1 and 4, glycogen synthesis was impaired in smokers [0.8 ± 0.2 (S) and 4.5 ± 0.5 (C) $\text{mmol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$, $P = 0.0002$] compared with controls. Glucose 6-phosphate was reduced in smokers during hours 1–4 [0.105 ± 0.006 (S) and 0.217 ± 0.019 (C) mmol/l, $P = 0.0212$]. We conclude that cigarette smoking impairs the insulin-independent portion of muscle recovery from glycogen-depleting exercise. This impairment likely results from a reduction in glucose uptake.

carbon-13; nuclear magnetic resonance; metabolism; glycogen

EACH DAY 3,000 children and adolescents become regular tobacco users (42). Smoking habits acquired in adolescence are likely to carry into adulthood and can lead to smoking-related health problems. Although statistics indicate many adult smokers are trying to quit, smoking rates among adolescents remain relatively high (5). Habitual smoking is associated with insulin resistance (29) and impaired glucose tolerance in intravenous glucose tolerance tests (12). Cigarette

smoking alters metabolism at a number of different points that may impact insulin resistance. These include free fatty acid and glycerol mobilization (10), muscle glycogen utilization (9, 10), circulating catecholamines (9), and blood lactate (10, 28) levels, as well as insulin-dependent glucose metabolism (12, 22). Insulin-resistant glucose metabolism is a well-established risk factor in the development of adult-onset diabetes (12, 41). People who smoke >15 cigarettes/day carry a twofold greater risk of diabetes compared with those who have never smoked (38). Adolescent smokers may represent a population at risk for developing adult-onset diabetes in early adulthood resulting from chronic smoking-induced insulin resistance. Furthermore, the use of tobacco products may further increase the risk of developing adult-onset diabetes in people with a family history of diabetes. Reduced insulin sensitivity from smoking may also impair the effectiveness of treatments for diabetes.

Muscle glycogen is the primary depot for ingested carbohydrates, and glycogen metabolism is strongly associated with insulin-dependent glucose uptake (46). After a meal, muscle glycogen synthesis accounts for the majority of glucose uptake (43, 46). Impaired insulin-stimulated muscle glycogen synthesis is an early defect in the pathogenesis of diabetes and is present in individuals at high risk of diabetes before the development of impaired glucose tolerance (41). Exercise stimulates glucose uptake and muscle glycogen synthesis independent of insulin (45); however, for an exercised muscle to fully recover its glycogen stores, insulin-stimulated glucose uptake is also required (36). Fasting postexercise muscle glycogen recovery has been shown to occur in two distinct phases (24, 34, 36). An initial phase, which occurs during the 1st h after exercise, is rapid, glycogen concentration dependent, and insulin independent (15, 34, 36). This is followed by a subsequent phase that proceeds steadily at a slower rate, depends on the presence of insulin (24, 36), and requires that the exercised muscle is not insulin resistant (24, 34). Postexercise muscle metabolism plays a major role in systemic carbohydrate balance (46) and may be influenced by smoking. Although the association of cigarette smoking with insulin resistance and impaired glucose tolerance is established (12, 29), the question of whether this smoking effect carries through

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to its logical end point, impaired muscle glycogen storage, has yet to be addressed. The current study was undertaken to determine whether the insulin-dependent phase of postexercise muscle glycogen synthesis is impaired in a fasting population of young healthy cigarette smokers.

METHODS

Study subjects. Eighteen male subjects were studied (8 healthy smokers, 10 healthy age- and weight-matched controls). All subjects were within 10% of ideal body weight according to the 1959 Metropolitan Life Insurance tables ($104 \pm 2\%$ smokers and $102 \pm 3\%$ controls). The mean \pm SE weight in the smokers and control subjects was 69 ± 4 and 67 ± 3 kg, respectively; mean heights were 173 ± 1 and 175 ± 3 cm; and mean ages were 24 ± 2 and 23 ± 1 yr. The smoking group smoked 23 ± 2 cigarettes/day over 8 ± 3 yr (9.2 ± 3.2 pack years). In both groups, physical examination and medical history were obtained, followed by screening for exercise and dietary habits. Subjects with a family history of diabetes and those who trained aerobically >5 days/wk (34, 36) were excluded from the study. Informed consent was obtained from all subjects after an explanation of the purpose, nature, and possible consequences of the study. The protocol was reviewed and approved by the Human Investigation Committee of the Yale University School of Medicine.

Experimental protocol. Subjects ate breakfast between 6:00 AM and 7:00 AM and were allowed only water thereafter. Study subjects arrived at the General Clinical Research Center (GCRC) at 3:00 PM on the day of the study (8-h fast). Smokers were asked to abstain from smoking from their arrival at the GCRC, 1 h before the study, until the study was completed (6 h of total abstinence). Subjects started the exercise protocol at 1.5 h after arrival at the GCRC, and the recovery period began immediately after exercise (30 min of exercise), ending 4 h later. Subjects reported having their last cigarette immediately before arrival at the GCRC (1.5 h before exercise, 2 h before the measured recovery period began). Six smokers were able to abstain throughout the study; the other two were allowed to smoke one cigarette after the 2-h NMR measurement during the recovery period (4 h of total abstinence). Two hours were chosen because by 2 h into the recovery period muscle glycogen resynthesis had already ceased in these two subjects, and their overall rates were not different from the other six subjects who did not smoke. At least 30 min before blood sampling, a venous catheter was inserted in the antecubital vein. Subjects were then taken to the magnetic resonance (MR) Center, and two interleaved ^{13}C and ^{31}P NMR spectra were obtained. During this period, baseline blood samples were obtained. After baseline measurements, subjects performed an exercise protocol of single-leg toe raises from an erect standing position (knee fully extended) to isolate the gastrocnemius (34, 36). The standing toe-raise protocol has been shown to effectively isolate the gastrocnemius, thereby minimizing any significant systemic hormonal response to the exercise (34, 36). To minimize lactate accumulation in the muscle, subjects exercised by alternating 1 min of toe raises (~ 35 raises/min) with 1 min of rest throughout the exercise period (34, 36). Interleaved ^{13}C and ^{31}P NMR spectra and blood samples were obtained during and immediately after exercise (time = 0 min). The exercise protocol ended when the subject had depleted his/her muscle glycogen concentration by $45\text{--}50$ mmol/kg ($\sim 75\%$ depletion). Exercise duration was not significantly different between smokers and controls (20 ± 2 min smokers; 24 ± 3 min controls), nor was the total work

performed (29.4 ± 2.3 kJ smokers; 30.6 ± 3.3 kJ controls; Table 1). Exercise-induced glycogen depletion rates and total depletion were also similar (Table 1). When exercise was completed, gastrocnemius recovery was monitored over 4 h with interleaved ^{13}C and ^{31}P NMR spectroscopy and blood sampling. NMR spectra were obtained continuously over the 1st h of recovery (5.4 min/spectrum) and every 30 min from hours 1–4 (36). Throughout the protocol, dietary intake was restricted to water only.

NMR spectroscopic measurements. Interleaved natural abundance ^{13}C and ^{31}P NMR spectroscopy was performed at 4.7 T on a Bruker Biospec spectrometer with a 30-cm-diameter magnet bore, as described previously (34, 36). During the measurements, subjects remained supine with one leg positioned within the homogeneous volume of the magnet and with the lower portion of that leg resting on the stage of a surface coil radiofrequency (RF) probe. A 5.1-cm-diameter circular ^{13}C - ^{31}P double-tuned surface coil RF probe was used for interleaved acquisitions (7). Shimming, imaging, and ^1H decoupling at 200.4 MHz were performed with a 9 cm \times 9 cm series butterfly coil. Proton linewidths were shimmed to <50 Hz. A microsphere containing ^{13}C and ^{31}P reference standards was fixed at the center of the RF coil for calibration of RF pulse widths. The subject's lower legs were positioned using imaging so that the isocenter of the magnetic field was ~ 1 cm in the medial head of the gastrocnemius. The interleaved ^1H decoupled ^{13}C - ^{31}P RF pulse sequence was designed so that 72 ^{31}P transients were acquired during the same period that 2,736 ^{13}C transients were obtained (38 ^{13}C scans/ ^{31}P relaxation period), and free induction decays were saved separately in two blocks (7). Because the repetition time for ^{31}P acquisition was 4.6 s, there were no significant saturation effects, nor were there signal differences between the two ^{31}P metabolites studied because of relative saturation differences. The measured compound [glucose 6-phosphate (G-6-P)] was compared with an internal standard (ATP). The NMR behavior of these two compounds is similar, as established by Rothman et al. (41) in 1992. The total scan time was 5.5 min. Power deposition, assessed by magnetic vector potential specific absorption rate calculation, was <4 W/kg (4).

Intramuscular glycogen concentrations were determined by comparison with an external standard solution (150 mmol/kg glycogen + 50 mmol/kg KCl) in a leg cast that loaded the RF coil the same as the subject legs (34, 47). The ^{13}C NMR technique for assessing intramuscular glycogen concentrations has been validated *in situ* in frozen rabbit muscle (16) and by comparison with biopsied human gastrocnemius muscle tissue samples (47). Concentrations of inorganic phosphate (P_i) and creatine phosphate (PCr) were also calculated by comparison with β -ATP (30, 41). Values of pH were calculated according to the chemical shift difference between the P_i peak and the PCr peak. Intramuscular G-6-P

Table 1. Muscle glycogen depletion data

	Smokers	Controls
Baseline glycogen, mmol/l	69.0 ± 1.9	71.8 ± 4.2
Exercise time, min	20 ± 2	24 ± 3
Total work, kJ	29.4 ± 2.3	30.6 ± 3.3
Total glycogen depleted, mmol/l	45.6 ± 1.4	53.5 ± 4.5
Total glycogen resynthesized, mmol/l	$14.3 \pm 1.4^*$	26.8 ± 1.8

Values are means \pm SE. Gastrocnemius glycogen in smokers and controls immediately before exercise, time and work required to deplete 75% of stored glycogen, total glycogen depleted during exercise, and total glycogen resynthesized during the 4-h measurement period after exercise. * $P = 0.0001$, smokers vs. controls.

was quantified by comparison with the β -ATP resonance as an internal reference standard (34, 41, 39). Measurement of G-6-P by ^{31}P NMR has been validated in an animal model by comparison with chemical assay of G-6-P done on rat muscle frozen *in situ* (3).

Analytical procedures. Venous blood samples were assayed for glucose and insulin. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA; see Ref. 23). Plasma insulin was assayed by a double-antibody RIA technique (Diagnostic Systems Laboratories, Webster, TX; see Ref. 44).

Statistical analysis. Blood data and NMR-determined metabolite concentrations are presented as means \pm SE. NMR precision was calculated by pooled variance analysis (18, 35). Paired two-tailed *t*-tests were used for comparison of data within individual subjects. Between-group comparisons were performed using ANOVA with Bonferroni correction factor. Glycogen repletion rates for each subject were determined by least squares linear regression analysis.

RESULTS

Muscle glycogen content and glycogen synthesis rate after exercise. Baseline gastrocnemius glycogen concentrations were similar in smokers and controls (Table 1). Exercise-induced glycogen depletion data (Table 1) indicate that both groups depleted similar amounts of glycogen over a similar period of time while performing a similar amount of work. Figure 1 shows the pattern of muscle glycogen depletion and recovery in smokers and controls. After exercise, there was steady glycogen resynthesis for ~ 1 h that was similar in both groups ($P = 0.2524$; Fig. 1). During the subsequent period, hours 1–4 of recovery, steady glycogen synthesis continued at a reduced rate in the control group; however, glycogen synthesis ceased in the smokers (Fig. 1). During this subsequent 1- to 4-h period, the glycogen synthesis rate in the smoking group was 74% lower than in the control group ($P < 0.0001$; Fig. 2). Total glycogen recovery over the 4-h measurement period was significantly greater (1.9-fold) in controls than in smokers ($P = 0.0001$; Table 1).

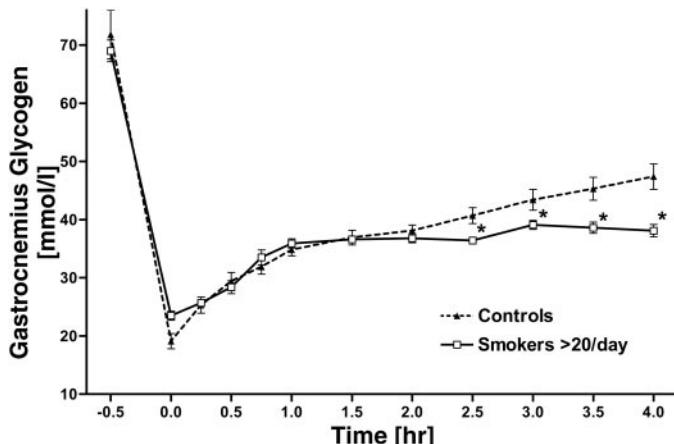


Fig. 1. Time course of glycogen recovery in smokers and controls: NMR-determined gastrocnemius glycogen concentrations at rest and during the 4-h period of fasting recovery by smokers (□) and healthy controls (▲). Concentrations (mmol/l) are shown as means \pm SE. * $P < 0.05$ vs. control.

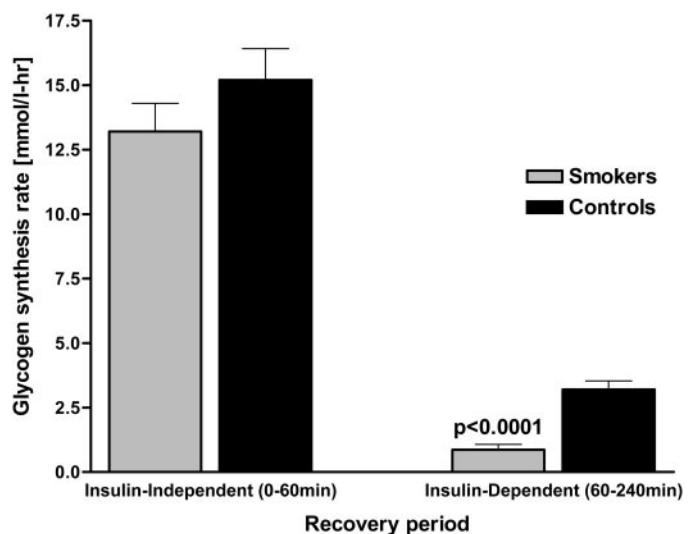


Fig. 2. Glycogen synthesis rates: gastrocnemius glycogen synthesis rates ($\text{mmol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) in smokers and controls during insulin-independent (0–60 min) and insulin-dependent (60–240 min) periods of recovery. Rates are given as means \pm SE.

Intracellular G-6-P. Baseline concentrations of G-6-P, P_i , PCr, and the intracellular pH were similar in the two groups. Throughout the 4-h postexercise measurement period, P_i and PCr concentrations and intracellular pH were similar between the two groups. During the 1st h of recovery, G-6-P levels were not significantly different between the two groups ($P = 0.2180$; Fig. 3). However, over the subsequent 3 h of recovery, mean G-6-P concentrations were significantly lower in the smoking group (52% lower) compared with the control group ($P = 0.0214$; Fig. 3).

Plasma glucose and insulin. Baseline plasma glucose concentrations were not significantly different between groups [smokers ($6.1 \pm 0.5 \text{ mmol/l}$) and controls ($5.1 \pm 0.2 \text{ mmol/l}$; $P = 0.0616$)], nor were they different after exercise [5.1 ± 0.1 and $4.9 \pm 0.1 \text{ mmol/l}$ ($P = 0.1819$), 1st h, and 5.0 ± 0.1 and $4.9 \pm 0.1 \text{ mmol/l}$ ($P = 0.1943$),

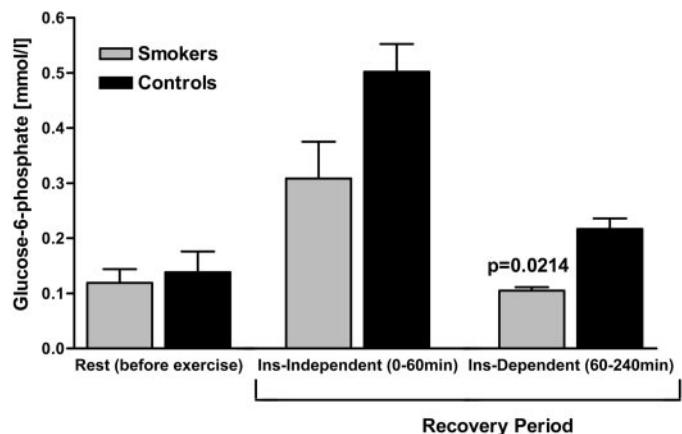


Fig. 3. Glucose 6-phosphate concentrations: NMR-determined intracellular glucose 6-phosphate concentrations (mmol/l) at rest and during insulin-independent (0–60 min) and insulin-dependent (60–240 min) periods of recovery. Concentrations are shown as means \pm SE of all data points collected over the entire time period.

hours 1–4]. Baseline insulin levels were also similar between the smokers ($18.5 \pm 4.0 \mu\text{U}/\text{ml}$) and controls ($14.9 \pm 1.1 \mu\text{U}/\text{ml}$; $P = 0.3115$). Plasma insulin levels were not significantly different between smokers and controls during the 1st h of recovery [$12.6 \pm 1.5 \mu\text{U}/\text{ml}$ smokers and $14.2 \pm 0.8 \mu\text{U}/\text{ml}$ controls ($P = 0.3402$)] or during the insulin-dependent (hours 1–4) period [$11.3 \pm 1.3 \mu\text{U}/\text{ml}$ smokers and $12.7 \pm 0.6 \mu\text{U}/\text{ml}$ controls ($P = 0.3044$)].

DISCUSSION

Habitual nicotine exposure has been linked to insulin resistance (1) and is a major risk factor for coronary and peripheral vascular disease (12, 26, 44), as well as hypertension (13, 37). Habitual nicotine exposure also impairs insulin-dependent glucose metabolism (12, 22, 29). Insulin resistance is known to be a major risk factor in the development of adult-onset diabetes (12, 27). Furthermore, there may be a dose-response relationship between smoking and the risk of diabetes (27). Exercise is known to increase skeletal muscle insulin sensitivity and glycogen synthesis in both healthy controls and insulin-resistant subjects (11, 14, 20, 29, 31); however, the mechanisms by which exercise enhances and/or nicotine impairs muscle insulin sensitivity are not well characterized.

After glycogen-depleting exercise, local factors that control glycogen resynthesis rates are 1) glucose transport, 2) phosphorylation, and 3) glycogen synthase activity, all of which are influenced by circulating insulin. Muscle recovery from glycogen-depleting exercise occurs in two phases. Immediately after glycogen-depleting exercise, when glycogen levels are low, glycogen synthesis is insulin independent (24, 34, 36). Later, as glycogen concentrations rise, synthesis becomes insulin dependent (24, 34, 36). This insulin-dependent phase may play a major role in the enhancement of insulin sensitivity by exercise and has been shown to be impaired in fasting prediabetics (34) and in insulin-deprived juvenile diabetics (24). In this study, we report that insulin-dependent muscle glycogen synthesis is also impaired in a healthy population of young smokers, much like that observed in prediabetic subjects (34). This is, to our knowledge, the first report of smoking-induced alteration of skeletal muscle glycogen synthesis.

An important systemic factor in postexercise muscle glycogen recovery is the efficiency with which plasma glucose is delivered to the exercised muscle. Although cigarette smoking has been shown to inhibit nitric oxide (NO) bioactivity, thereby enhancing vasoconstriction (6, 25), insulin induces NO-mediated vasodilation (19). In this study, the association between insulin and NO in the vasodilatory response and the impact of cigarette smoking on vasodilation could have exerted an effect on the level of postexercise perfusion of the observed gastrocnemius muscle. A significant reduction in postexercise muscle perfusion could therefore have affected the rate of plasma glucose delivery. However, if glucose delivery had been impaired signif-

icantly, we might have expected to observe a significantly reduced rate of insulin-independent muscle glycogen synthesis immediately after exercise. Although the initial insulin-independent glycogen synthesis rate was $2.0 \text{ mmol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ greater in the control population, this was not significant. However, it is possible that a reduction in flow could have contributed to the slower rate seen in the smokers. It is also possible that early insulin-independent postexercise muscle glycogen synthesis has both an insulin-independent component and an insulin-dependent component. Data from the current study can neither confirm nor refute the contribution of impaired blood flow to the observed impairment of postexercise insulin-dependent glycogen synthesis. However, it is clear that the end result is reduced muscle glycogen in smokers after 4 h of fasting recovery from glycogen-depleting exercise.

This study was designed so that the smoking population would be screened according to age, size, and number of cigarettes smoked per day. Although we did not biochemically verify nicotine/cotinine levels, or obtain carbon monoxide data, our subjects reported smoking approximately a pack of cigarettes per day for an average of 11 ± 3 yr, which was sufficient to establish dependence and maintain addiction (2). The consistency of data observed in this study suggests that the effect of smoking on insulin-mediated postexercise glycogen synthesis may be an all-or-none physiological response that occurs when smokers smoke a certain amount and achieve a certain threshold of nicotine/cotinine levels. Clearly, the next study should include measurement of the nicotine/cotinine dose-response relationships. Additionally, the design of this study precludes any definitive statement regarding whether the reported result represents an acute or a chronic effect of smoking. Subjects in this study were allowed to smoke up to 1 h before beginning exercise. Therefore, by the time they reached the insulin-dependent stage of recovery (~1 h postexercise), they had been abstinent from cigarettes for ~2.5 h. Two of the subjects were unable to continue to refrain from smoking over the entire recovery period. These two (vs. 6 successful abstainers) were allowed to have one cigarette after the 2-h muscle glycogen measurement (2.25 h into the recovery period). Results from these two were examined for possible exclusion from the study, and it was determined that the results were not affected by these two having been allowed to smoke (i.e., the results were the same either with or without these two subjects). We therefore determined that the study was best served by allowing their data to remain and simply reporting that they had been allowed to smoke during the recovery period. Nicotine has a half-life of ~2 h; therefore, it is possible that all of the smokers in this study still had biologically active levels of nicotine in their system that could have mediated the effect. Levels of nicotine in smokers who start smoking in the morning are known to rise over the first 6–8 h and plateau during the remainder of the day while they are regularly smoking (2). Furthermore, early morning nicotine in smokers who have not smoked overnight

may still be at biologically active levels. Regular smokers, such as those who participated in this study, constantly have nicotine in their systems. Therefore, the issue of whether or not the results of this study reflect acute or chronic effects could only be addressed with a protocol that required smokers to go through a significant period of withdrawal, which then would not reflect the impact of regular smoking on postexercise muscle glycogen recovery.

Fasting before the study is a component of the design that could potentially have an effect on the results. Furthermore, fasting during the recovery period is likely to have had an effect on postexercise glycogen synthesis. If the prestudy fast were long enough to substantially deplete liver glycogen reserves, postexercise muscle glycogen recovery might have been blunted in both study groups, as the body attempted to spare glucose for neurological function. Postprandial liver glycogen levels increase during the first 3–6 h of a fast, reaching a peak 5–6 h into the fasting period (32). Petersen et al. (32) reported a peak liver glycogen concentration of 406 mmol/l that steadily declined over the next 6–9 h to 325 mmol/l (net depletion rate = 0.225 mmol·l⁻¹·min⁻¹). Rothman et al. (40) reported a mean liver glycogen concentration of 396 mmol/l at 4 h of fasting that fell to 331 mmol/l at 10 h and 251 mmol/l at 15 h (net depletion rate = 0.22 mmol·l⁻¹·min⁻¹; see Ref. 40). In addition, Petersen et al. (32) reported that, during the first 12 h of fasting, liver glycogenolysis contributed <50% (45%) to total body glucose production, whereas gluconeogenesis contributed 55%. Exercise would be expected to increase levels of catabolically derived 3-C compounds (such as lactate) that would likely provide additional fuel for gluconeogenesis during the recovery period of this study. Elevated plasma lactate was, in fact, reported by our laboratory (34, 36) in 1994 and 1996.

Because this study was performed during the initial 15 h of fasting, it is unlikely that a substantial reduction of liver glycogen played a significant role in the reported muscle glycogen depletion and recovery pattern. However, if the period of extended fasting had been increased beyond 15 h, continued decreases in liver glycogen might have played a significant role in reduced muscle glucose uptake, favoring glucose sparing for neurological function. In addition, it is likely that, if subjects had been fed a meal immediately after exercise, muscle glycogen synthesis during the observed recovery period would have been affected. Resting glycogen concentrations were not significantly different between controls and smokers, indicating that smokers are capable of insulin-mediated muscle glycogen synthesis. It is likely that, if the smokers ingested a postexercise meal, elevated postprandial plasma glucose levels would have driven insulin-mediated glucose uptake forward. It is of great interest to determine what drives postexercise muscle glycogen synthesis forward in smokers and whether or not postexercise glycogen recovery in the fed state is enhanced or impaired relative to controls. This is an important area

that needs to be addressed so that the effects of smoking can be more clearly understood.

During the initial insulin-independent recovery period, G-6-P concentrations were increased in both smokers and controls. However, G-6-P was significantly lower in smokers during the subsequent insulin-dependent recovery period. This result was not observed by Price et al. (34) in their 1996 study of prediabetics. That study hypothesized that, because G-6-P levels were not significantly lower during insulin-dependent recovery, it was likely that both glucose transport and glycogen synthase activity were impaired in the prediabetic population (34). In the current study, the significant reduction in G-6-P observed in smokers could have resulted from an impairment of glucose uptake that was not coupled to a reduction in glycogen synthase activity. In that scenario, glycogen synthase activity would be reduced and glycogen synthesis would stop when G-6-P levels dropped below the threshold required for the continuation of synthesis. The implication of this observed reduction of G-6-P in smokers during insulin-dependent recovery is that there are different mechanisms of impaired glycogen recovery in smokers and prediabetics.

It is unclear whether the alterations in muscle metabolism with smoking are the result of a direct effect of nicotine on the muscle or through the action of nicotine on insulin secretion. Postexercise plasma insulin levels were not significantly different in the smoking group compared with the controls, suggesting that there was insulin available to bind insulin receptors on the membrane. Animal studies report that one of the effects of chronic nicotine exposure is a reduction in pancreatic insulin secretion (17); however, that was not measured in the current study. It is possible that the localized exercise protocol did not produce a systemic effect that was of sufficient magnitude to elicit measurable between-group differences in insulin secretion.

This study looked at both the insulin-independent portion of muscle recovery from glycogen depletion that occurs immediately after exercise ceases and continues for ~1 h and the insulin-dependent portion that begins after ~1 h and continues until recovery is complete (34, 36). Exercise and insulin are both known to stimulate muscle glucose uptake and glycogen synthesis in an independent and additive manner, suggesting the existence of two separate pools of glucose transporters (15, 45, 48). Although the mechanisms that underlie glycogen synthesis after exercise have not been fully elucidated, elevated concentrations of G-6-P have been observed immediately after exercise using ³¹P NMR in both healthy humans (34) and animals (3). Although these results suggest that glucose transport/phosphorylation is rate controlling during early recovery from heavy exercise, the animal study also found a 2.5-fold increase in the active G-6-P-independent (I)-form of glycogen synthase, suggesting a role for both glucose transport and enzyme activation in early recovery from exercise (3). A more recent animal study has shown that blood glucose transported in the muscle cell is

immediately phosphorylated to G-6-P by hexokinase, supporting the contention that measured G-6-P concentrations after exercise are a reasonable indicator of exercise-induced glucose transport (8). Goodyear et al. (15) reported a fourfold increase in glucose transport by rat gastrocnemius muscle immediately after exercise that declined to 1.8-fold after 30 min of recovery. Both transporter number and intrinsic activity had returned to basal levels by 2 h into the recovery period (15). The time course of the increase in membrane-bound GLUT4 number and activity reported by Goodyear et al. is similar to the time course of the increase in G-6-P seen in the human control population of a 1996 study conducted in this laboratory [4.2-fold (immediately after exercise), 1.9-fold (30 min after exercise), and 1.2-fold (2 h after exercise); see Ref. 34]. Based on these similar observations, we might hypothesize that an increase in membrane-bound GLUT4 transporter number and activity could be responsible for the rise in G-6-P immediately after exercise. Furthermore, the return of membrane-bound GLUT4 to basal levels 2 h after exercise (15) suggests that exercise-induced translocation of GLUT4 from an exercise-stimulated intracellular pool is transient, lasting <2 h. Therefore, any prolonged exercise effect would have to come from an enhanced translocation of insulin-stimulated GLUT4 transporters, and, if this mechanism were impaired, glucose uptake by the exercised muscle would cease. The current results are consistent with this hypothesis [3.8-fold (immediately after exercise), 2.9-fold (30 min after exercise), 0.7-fold (2 h after exercise)], suggesting that, in young and healthy smokers, the translocation of the insulin-stimulated pool of GLUT4 transporters may be affected after exercise.

The exercise protocol that was employed in this study is a well-established, "local" protocol that was designed to exert minimal systemic effect (34, 36). Because the gastrocnemius (medial and lateral) represents <2% of the body's total musculature, the localized exercise protocols used in this study would not be expected to produce large systemic metabolic changes. To confirm this isolation of the gastrocnemius, in previous studies using this paradigm, we have tracked changes in blood metabolites and changes in muscle pH (34, 36), reporting that, although plasma lactate rises approximately twofold after exercise, plasma insulin and glucose remain stable during exercise and recovery (33, 34, 36). The insulin-dependent response of isolated fasting muscle to exercise has been well established in these earlier studies of insulin-deprived healthy controls and of the insulin-resistant offspring of parents with adult-onset diabetes mellitus (34, 36). Therefore, in the current study, we think of the human gastrocnemius as an isolated system that is buffered by the rest of the body, where metabolism occurs in an abundance of substrates.

Another issue that is of potential importance is fiber-type recruitment by the exercise protocol. The human gastrocnemius muscle is made up of ~60% type I and ~40% type II fibers. Because the MR measurement does not distinguish between fiber types, an exercise

protocol that recruits all fibers equally provides the most comprehensive information. Although skeletal muscle fiber types are differentially recruited at light workloads, exercise at moderate to heavy workloads is known to deplete glycogen in all fibers (21). Hence, the assessment of workload is of primary importance in understanding the results of this type of study. The local exercise employed in many of our studies has been shown to work the gastrocnemius at ~50% of the muscle's maximum voluntary contraction mass, which can be considered a moderate to heavy workload (33, 34, 36). Therefore, we think of the changes in glycogen content that are measured by MR spectroscopy as representing changes that are occurring equally in fast-twitch and in slow-twitch fibers. Based upon these rationales, we postulate that the results of this study represent basic muscle metabolism at the cellular level and therefore suggest that smoking interferes with basic postexercise muscle metabolism in young healthy smokers.

In conclusion, we have used ^{13}C and ^{31}P MR spectroscopy to show that cigarette smoking leads to an impairment of insulin-dependent muscle glycogen recovery from exercise in a young healthy population. We further hypothesize that this impairment is the result of altered insulin-dependent glucose transport/phosphorylation. The question remains as to whether these otherwise healthy young subjects represent a new high-risk group for adult-onset diabetes or whether a group that is already at risk, such as young predabetics, increases its risk by smoking.

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