IN HUMANS, AS HEPATIC GLYCOGEN stores are reduced during fasting, glucose production (GP) is maintained by an increased contribution of gluconeogenesis (GNG) (28, 29, 38, 39). During exercise, GNG is important also because GP is significantly increased and greater demands are placed on liver glycogen stores. Liver glycogen stores (~72 g in the overnight-fasted condition; Refs. 38, 39, 41) would be completely depleted in <3 h of exercise at ~65% maximal O2 consumption if hepatic glycogen was the only source of GP (4, 11, 12, 47). Elevated rates of GNG and the fraction of GP from GNG under postabsorptive (PA) conditions are accomplished by alterations in the hormonal milieu (33) and increased delivery and extraction of gluconeogenic precursors across the splanchnic bed (38). During exercise, mobilizations of lactate (6, 35, 44) and glycerol (13, 47) increase in an intensity-dependent manner, thus increasing GNG precursor supply. Results from splanchnic catheterization (1–3, 49, 50) and stable-isotope infusion studies (5, 24–26) suggest that extraction and conversion of gluconeogenic precursors to glucose increases in a concentration-dependent manner during rest and exercise. Consequently, GNG has been estimated to provide as much as 60% of GP after 4 h of moderate exercise (1, 2), and GNG would also be expected to increase with increasing exercise intensity. However, because of the difficulties associated with the measurement of GNG in vivo (27, 46, 51), the effects of exercise intensity on absolute (GNGab) and fractional (fGNG) gluconeogenic rates in humans remains unclear.

Previous investigations employing carbon tracers and the measurements of precursor-to-product ratios have reported variable effects of exercise intensity on GNG (5, 7, 11, 45, 47). The discrepancies are likely the result of methodological limitations because the estimation of GNG by use of carbon tracers and the precursor-to-product ratio is made uncertain by dilution of precursor carbon label in the TCA cycle (27, 46, 48, 51). In an attempt to account for isotopic dilution, our laboratory (48) and others (20–22, 51) have derived correction factors calculated from the recovery of infused carbon-labeled acetate. However, correction fac-
tors are species and treatment specific and are affected by extrahepatic metabolism of acetate (22).

A recent technique developed by Hellerstein and colleagues (15, 17, 37), termed mass isotopomer distribution analysis (MIDA), estimates true precursor pool enrichment (p) and subsequently fGNG without the necessity of correction factors. Previously, we showed that the infusion of unlabeled glycerol at rates required to perform MIDA did not affect GP during rest and exercise (47). In the present investigation, we used MIDA to evaluate the effects of exercise intensity on the apparent rates of GNG and hepatic glycogenolysis (Gly) during 90 min of leg cycling exercise. We hypothesized that coordinated adjustments in GNG and Gly would permit GP to match glucose utilization during moderate (45% \( V_{O2peak} \)) and hard (65% \( V_{O2peak} \)) exercises. Previously, we showed that these exercise intensities raise GP two- to threefold (4, 11, 12, 47).

**METHODS**

**Subjects.** Eight endurance-trained male subjects were recruited from the University of California, Berkeley campus by posted notice and electronic mailing. Trained cyclists were used to extend the period of time that constant metabolic flux rates could be sustained during exercise. Subjects were considered endurance trained if they had been competing in United States Cycling Federation or collegiate mountain or road cycling competitions for more than 3 yr and had a peak \( O_2 \) consumption (\( V_{O2peak} \)) >60 ml·kg\(^{-1}\)·min\(^{-1}\) during leg cycling exercise. Subjects were nonsmokers, were diet and weight stable, had a percent body fat <10%, had a 1-s forced expiratory volume of 70% or more of vital capacity, and were injury and disease free as determined by medical questionnaire and physical examination. The protocol was approved by the University of California Committee for the Protection of Human Subjects (CPHS 97-5-79), and subjects gave informed, written consent to participate.

**Screening tests.** \( V_{O2peak} \) was determined on three occasions by means of a progressive leg cycle ergometer protocol (Monark Ergometric 839E) beginning at 100 W and increasing 25 or 50 W every 3 min until voluntary cessation. Two \( V_{O2peak} \) tests were performed before the isotope trials to ensure a true maximum effort, and blood was collected from a forearm vein during the second test for determination of lactate threshold. A third evaluation of \( V_{O2peak} \) was conducted 1 wk after the last tracer trial to confirm that \( V_{O2peak} \) was unchanged over the 6-wk experimental period. For indirect calorimetry, respiratory gases were continuously collected and analyzed via an open-circuit indirect calorimetry system (Ametek S-3A1 \( O_2 \) and Ametek CD-3A \( CO_2 \) analyzers) and respiratory parameters, including respiratory exchange ratio (\( CO_2 \) production/\( O_2 \) consumption), were determined every minute by a real-time, on-line personal computer-based system (11). Heart rate was monitored throughout the experimental protocols by using a modified 12-lead electrocardiogram. Three-day dietary records were collected before and after completion of the testing period to assess dietary habits and monitor individual caloric intake and macronutrient composition. Analysis of dietary records was performed using the Nutritionist III program (N-Squared Computing, Salem, OR). Body composition was determined by skinfold measurements, as previously reported (11).

**Experimental design.** After screening, two stable-isotope infusion trials were performed on each subject under each exercise condition. Previously, our laboratory (47, 48) reported on trials using \([6,6-^2H_2]glucose (D_2-glucose)\) and \([1,13C]glucose\) tracers, with and without exogenous glycerol infusion. Now we report results of separate trials on the same subjects, but infused with \([3,13C]glycerol\) and D_2-glucose. During the 24 h preceding each isotope trial, subjects refrained from exercise and consumed a standardized diet (3,240 kcal; 66% carbohydrate, 19% fat, and 14% protein) prepared by the laboratory staff. The dietary protocol included a final snack (609 kcal; 54% carbohydrate, 29% fat, and 17% protein) consumed exactly 12 h before the onset of exercise. Subjects reported to the laboratory at 7:00 AM on the morning of the isotope trial, 7.5 h after their last meal. After collection of background samples, tracer infusion begun and subjects rested for 3.75 h followed by 90 min of leg ergometer cycling at either 45% (moderate) or 65% \( V_{O2peak} \) (hard) intensity exercise. Trials were performed in a randomized order with no fewer than 5 days between experiments. Subjects were instructed to maintain their habitual dietary and training regimes throughout the testing period.

**Tracer protocol.** All trials were conducted at the same time of day. On the morning of isotope trials, a catheter was inserted into a dorsal hand vein that was subsequently warmed by a heating pad for collection of “arterialized” blood. A second catheter was placed into the antecubital or forearm vein of the contralateral arm for continuous infusion of the isotope solutions. After collection of background blood and breath samples, \([6,6-^2H_2]glucose\) and \([2-13C]glycerol\) were continuously infused (Baxter Travenol 6200 infusion pump). The \([6,6-^2H_2]glucose\) was infused at 4.0 and 8.0 mg/min during rest and exercise, respectively; these rates were previously demonstrated by our laboratory to maintain stable plasma isotopic enrichments under the conditions studied (47). The glycerol isotope infusion rates were selected after pilot studies indicated that stable and adequate precursor pool enrichment (p) values were achieved during rest and exercise at the specified infusion rates. Previous studies (18, 30, 37) have shown that measurement of GNG by MIDA requires \( p \) in excess of 12% for accurate measurement of fractional GNG. In the present investigation, \([2,13C]glycerol\) was infused at 20.0 and 40.0 mg/min during rest and exercise, respectively. Glucose and glycerol isotope tracers (Cambridge Isotope Laboratories, Andover, MA) were diluted in 0.9% sterile saline and were pyrogenicity and sterility tested (University of California, San Francisco, School of Pharmacy). Furthermore, on the day of the experiment, the solutions were passed through a 0.2-μm Millipore filter (Nalgene, Rochester, NY) before infusion.

**Blood sampling and analysis.** Blood was sampled at minutes 0, 180, 195, 210, and 225 of the 230-min rest period and at 30, 45, 60, 75 and 90 min of exercise. Samples were immediately chilled on ice and centrifuged at 3,000 g for 18 min, and the supernatant was collected and frozen until analysis. Blood samples for determination of glucose and glycerol isotope enrichments and glucose and lactate concentrations were collected in 8% perchloric acid. Samples for free fatty acid and glycerol concentrations were transferred to vacutainers containing EDTA, thoroughly mixed, and chilled on ice before centrifugation. Glucose and lactate concentrations were measured enzymatically in duplicate or triplicate using hexokinase (Sigma Chemical, St. Louis, MO) and lactate dehydrogenase (11), respectively. Plasma free fatty acid and glycerol concentrations were determined as previously reported (11) (NEFA-C, Wako, Richmond, VA, and GP O-Trinder, Sigma Chemical). Hematocrit was measured at each sampling point, and subjects were instructed to drink tap
water to prevent changes in plasma volume that would affect metabolite and hormone concentrations.

Isolation of metabolites and preparation for mass spectrometry. Glucose and glycerol samples for isotopic analyses were prepared by using ion-exchange chromatography as previously described (47). After lyophilization and resuspension in methanol, three aliquots were removed from each sample for glucose-pentaacetate, glucose-saccharic acid tetraacetate, and glycerol-triacetate derivatizations. The glucose-pentaacetate and glycerol-triacetate derivatizations have been previously described (47). Glucose samples to be converted to saccharic acid derivatives were lyophilized in a 2-ml microreaction vial, resuspended in 35 μl of concentrated nitric acid and 25 μl of sodium nitrate (0.5 g/ml), and heated at 60°C for 1 h. Samples were then lyophilized, and the two carbonyl groups of saccharic acid were methylated by adding 500 μl of MeOH/HCl, heated to 80°C for 1 h, and then dried under a stream of nitrogen. Acetylation of the hydroxyl groups was performed by adding 300 μl of a 2:1 acetic-anhydride-pyridine solution to each vial, which were then heated for 20 min at 60°C. Samples were dried under nitrogen, resuspended in 200 μl of ethylacetate, and filtered through glass wool (18).

When the glucose was converted to saccharic acid, the two deuterium atoms on the sixth carbon of the infused D2-glucose were removed. The resulting saccharic acid tetraacetate derivative contained only excess isotope mass from [13C]glycerol incorporated into glucose by GNG. The fGNG was measured by MIDA as described previously (14, 37).

Isotopic enrichments were measured by using gas chromatography-mass spectrometry (GC/MS; GC model 5890 or 6890 series II and MS model 5989A or 5973, Hewlett-Packard, Palo Alto, CA) analyses of the glucose-pentaacetate, saccharic acid tetraacetate, and glycerol-triacetate derivatives. The GC/MS analyses of the glucose-pentaacetate and glycerol-triacetate derivatizations have been previously described (47). For analyses of the saccharic acid tetraacetate derivative, injector temperature was set at 260°C and initial oven temperatures at 160°C. Oven temperature was increased 60°C/min until a final temperature of 270°C. Helium was used as the carrier gas for all analyses; transfer line temperature was set at 280°C, source temperature at 250°C, and the quadrupole temperature at 106°C. Positive chemical ionization was performed by use of methane gas (flow 18 ml/min), and selective ion monitoring was set at the ion mass-to-charge ratios of 347, 348, and 349 for the saccharic acid tetraacetate isotopomers.

Plasma glucose Ra. To calculate glucose rate of appearance (Ra) by dilution of D2-glucose, the contribution of 13C from the gluconeogenic conversion of infused [13C]glycerol was “subtracted” from the excess M2 glucose enrichment before use in the Steele equation. For this purpose, a calculation algorithm was used as described elsewhere (8).

Triose phosphate pool enrichment and fractional GNG. Triose phosphate pool enrichments were estimated by using MIDA and combinatorial probability calculations described briefly herein and in detail previously (15, 37). Combinatorial probabilities were used to predict the expected mass excess (EM)1- and EM2-glucose (saccharic) isomer enrichments for a theoretical precursor pool enrichment (p) with the following equation

\[
\text{Frequency } M_x = \frac{(n)!}{(n-x)!x!} \frac{[p]^x(1-p)^{n-x}}{(n-x)!(x)!} \tag{1}
\]

where M represents 13C glucose enrichment above background; n is the number of monomers in the glucose polymer; x is the number of labeled monomers in the glucose polymer, and p is the theoretical precursor pool enrichment. Because the EM2/EM1 ratio is uniquely determined by p, endogenous p was calculated by substitution of observed mass excess (M2/M1) ratios into a regression equation derived from theoretical M2/M1 values calculated using Eq. (1) (15).

The fGNG was calculated for a given p by comparison of the GC/MS-measured EM1 isotopomer to theoretical values by using the following equation

\[
f_{\text{GNG}} = \frac{\text{ObsM}_1 - [\text{EM}_0 + \text{EM}_1 + \text{EM}_2]}{\text{ExpM}_1} \times 100 \tag{2}
\]

where ObsM1 represents GC/MS-measured molar fractions and ExpM1 represents the theoretical EM1 for a given p when 100% of GP is derived from GNG (16). GNGab and Gly were then calculated by using the following equations

\[
\text{Absolute GNG}_{\text{ab}} \text{(mg kg}^{-1}\text{min}^{-1}) = \text{glucose } R_a \times f_{\text{GNG}} \tag{3}
\]

\[
\text{Absolute GLY} \text{(mg kg}^{-1}\text{min}^{-1}) = \text{glucose } R_a - \text{absolute GNG} \tag{4}
\]

where glucose Ra represents corrected [6,6-D2]glucose-measured appearance (see above) and fGNG is fractional GNG (Eq. 2).

Statistical analyses. Data are presented as means ± SE. Representative values for metabolite concentration and substrate kinetics were obtained by averaging values from the final 30 min of rest and exercise. Significance of mean differences between exercise intensities was determined with one-factorial ANOVA measures. Significance of changes over time was determined by using repeated-measures factorial ANOVA and post hoc analysis. Post hoc comparisons were made with Fisher’s protected least significant difference test. Statistical significance was set at α = 0.05.

RESULTS

Subject characteristics, dietary records, and physiological responses to exercise. During the course of the 8-wk experimental period, body weight and composition and VO2 peak were unchanged, as previously reported (47, 48). As well, 3-day dietary records indicated that macronutrient and energy contents of individual diets were unchanged during the experimental period. Heart rate, respiratory exchange ratio, and O2 consumption increased as a function of exercise intensity in a similar manner as reported previously (47, 48).

Metabolite and hormone concentrations. Plasma glucose concentration was not different between rest and 90 min of exercise at 45% VO2 peak but decreased over time during exercise at 65% VO2 peak and was reduced ~10% after 75 min of continuous exercise compared with rest and 45% VO2 peak (Fig. 1). Plasma glycerol concentration increased in the transition from rest to exercise in a time- and intensity-dependent manner (Ref. 47; Table 1). The increase in glycerol was not the result of the increase in glycerol infusion alone. Our laboratory has previously reported that glycerol concentration increases in the transition from rest to exercise of the same intensity (13, 47). Lactate concentrations remained constant during rest and 45% VO2 peak: During exercise at 65% VO2 peak, lactate concentrations were increased approximately twofold compared with rest and 45% VO2 peak (Table 1). Compared
with rest, plasma insulin concentration decreased in an intensity-dependent manner during exercise (Table 1). Conversely, plasma glucagon concentration increased in the transition from rest to exercise at 65% \( V_{\text{O}2_{\text{peak}}} \) (Table 1). As a result of the exercise-induced changes in the glucoregulatory hormones, compared with rest, the mean insulin-to-glucagon ratio was reduced 23 and 55% at 45 and 65% \( V_{\text{O2_{peak}}} \) respectively (Table 1, \( P < 0.05 \)).

**Glucose enrichments and kinetics.** Stable D2-glucose isotopic enrichments, corrected for incorporation of \([^{13}C]\)glycerol (see above), were obtained during the final 30 min of rest and exercise (Fig. 2A). During exercise, plasma glucose enrichments were significantly different (Fig. 2A). Glucose \( R_d \) increased approximately two- and threefold (\( P < 0.05 \)) during the transition from rest to exercise at 45 and 65% \( V_{\text{O2_{peak}}} \), respectively (Fig. 2B). Mean glucose rate of disappearance (\( R_d \)) also scaled to exercise intensity (Fig. 2C). However, during exercise at 65% \( V_{\text{O2_{peak}}} \), the increase in glucose \( R_d \) was 12% greater than the gain in \( R_a \), resulting in lower blood glucose concentrations (Fig. 1A). Because of the intensity effect on glucose disposal, glucose metabolic clearance rate increased in an intensity-dependent manner during the transition from rest to exercise (rest, 2.28 ± 0.21; 45%, 4.81 ± 0.53; 65%, 7.78 ± 1.13, ml·kg\(^{-1}·min^{-1}, P < 0.05 \)).

**Triose phosphate pool.** Estimated \( p \) was stable throughout rest and both exercise intensities (Fig. 3) and in excess of the minimum required enrichment of 12% for the accurate measurement of fractional GNG at all time points (29). There were no observed differences in \( p \) between rest (16.98 ± 0.33) and either exercise intensity (45% \( V_{\text{O2_{peak}}} \), 17.85 ± 0.62; 65% \( V_{\text{O2_{peak}}} \), 16.49 ± 0.57). However, \( p \) was higher during the final 30 min of exercise at 45% \( V_{\text{O2_{peak}}} \) compared with 65% \( V_{\text{O2_{peak}}} \) (45%, 17.92 ± 0.62 vs. 65%, 16.29 ± 0.60, \( P < 0.05 \)).

**GNG and glycogenolysis.** The fraction of GP from GNG (fGNG) increased (\( P < 0.05 \)) over time during rest, reaching 25.6 ± 0.9% during the final minutes of rest; 12 h after the last meal (Fig. 4A). Despite the effect of fasting duration, compared with the last rest sample, fGNG remained constant and was reduced throughout exercise at 45 and 65% \( V_{\text{O2_{peak}}} \), respectively. In addition, an intensity effect was observed during the first 60 min of exercise (Fig. 4A).

Despite the relatively minor effects of exercise on fGNG, GNG\(_{ab}\) increased ~76 and 128% (\( P < 0.05 \)) compared with rest during exercise at 45 and 65% \( V_{\text{O2_{peak}}} \), respectively (Fig. 4B). The exercise-induced increase in GNG\(_{ab}\) resembled the intensity effect on GP (Fig. 2B). In addition, GNG\(_{ab}\) was increased at 65 compared with 45% \( V_{\text{O2_{peak}}} \) during the first 45 min of exercise (Fig. 4B). However, the exercise intensity effect on GNG\(_{ab}\) was not apparent during the final 30 min of exercise. The plateau in GNG\(_{ab}\) at about 1.2 mg·kg\(^{-1}·min^{-1}\)

### Table 1. Metabolite and hormone concentrations during the final 30 minutes of rest and exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th>45% ( V_{\text{O2_{peak}}} )</th>
<th>65% ( V_{\text{O2_{peak}}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol, mM</td>
<td>0.22 ± 0.02</td>
<td>0.52 ± 0.04*</td>
<td>0.69 ± 0.03†</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>3.5 ± 0.6†</td>
</tr>
<tr>
<td>Insulin, pg/ml</td>
<td>366 ± 30.4</td>
<td>292 ± 53.5*</td>
<td>225 ± 28.4†</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>102 ± 6.7</td>
<td>107 ± 6.7</td>
<td>132 ± 16.2†</td>
</tr>
<tr>
<td>Insulin/glucagon</td>
<td>3.56 ± 0.62</td>
<td>2.71 ± 0.52†</td>
<td>1.62 ± 0.48†</td>
</tr>
<tr>
<td>Cortisol, µg/d</td>
<td>13.4 ± 1.3</td>
<td>16.4 ± 1.8</td>
<td>17.3 ± 1.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE recorded during the final 30 min of rest and exercise; \( n = 8 \). \( V_{\text{O2_{peak}}} \), peak \( O_2 \) consumption. *Significantly different from rest; †significantly different from 45% (\( P < 0.05 \)).
during exercise at 65% VO₂_peak coincided with the decline in blood glucose concentration (Fig. 1A).

Similar to GNG_ab, the absolute rate of Gly increased during the transition from rest to exercise (rest, 1.4 ± 0.2; 45%, 2.9 ± 0.3; 65%, 4.6 ± 0.7 mg·kg⁻¹·min⁻¹, P < 0.05). The absolute and relative increase in Gly during exercise was greater than the exercise effect on GNG, contributing to >70% of GP. Our results suggest that Gly is quantitatively more important for maintaining glycemia during exercise compared with GNG. However, the combined increase in GNG_ab and Gly was not sufficient to prevent the fall in plasma glucose observed during the final 60 min of exercise at 65% VO₂_peak (Fig. 1A).

**DISCUSSION**

We report the first attempt using MIDA to differentiate between the contributions of GNG and Gly to GP in men exercising after an overnight fast. Our results are consistent with those of previous studies indicating...
that the \( f_{\text{GNG}} \) contribution to GP gradually increases during fasting. However, we did not observe a time-dependent increase in \( f_{\text{GNG}} \) during 90 min of exercise. Rather, the \( f_{\text{GNG}} \) to GP remained constant during moderate-intensity (45% \( \dot{V}_O_2\text{peak} \)) exercise and decreased compared with rest during hard exercise at 65% \( \dot{V}_O_2\text{peak} \). Because of augmentations in absolute GNG and Gly rates, GP increased during both exercise intensities compared with rest. Despite these compensations, peripheral glucose utilization exceeded GP, and blood glucose concentration decreased in the 12-h fasted men during the final 60 min of hard exercise (65% \( \dot{V}_O_2\text{peak} \)). Thus our data extend the scope of knowledge to include the effects of exercise intensity on absolute and fractional GNG and Gly rates measured by use of the MIDA technique. In this regard we note that, in our laboratory’s previous investigations (4, 5, 14, 52), blood glucose concentration was well maintained over 60 min of hard (65% of \( \dot{V}_O_2\text{peak} \)) exercise when subjects were given a standardized breakfast several hours before exercise. Again, Gly appears to be more important than GNG for maintaining euglycemia in men during high-intensity exercise.

**GNG during rest.** Our results indicate that euglycemia after an overnight fast is maintained by a time-dependent increase in \( f_{\text{GNG}} \) such that GNG contributed 25% (0.56 mg kg\(^{-1}\) min\(^{-1}\)) of GP after a 12-h fast. Previous studies employing a variety of measurement techniques reported that GNG provided 25–40% of GP after a 10–14 h fast (21, 31, 32, 41, 44, 47), and as the fast progressed GNG increased to account for 60–70% of GP by 22 h of fasting (13, 34, 41, 44, 45) (Fig. 4A). Linear extrapolation of the time-dependent increase in \( f_{\text{GNG}} \) in the present study [\( \% \text{GNG} = (0.157 + 0.0239\ h) \times 100 \)] predicted that GNG would account for 67% of GP after a 22-h fast.

Recently Magnusson et al. (34), employing NMR techniques, concluded that hepatic glycogen content regulated hepatic glycogen turnover. Similar findings were reported by Nilsson and Hultman (39) from liver biopsies. In the resting PA men we studied, plasma cortisol, insulin, and glucagon concentrations (Table 1), as well as concentrations of the gluconeogenic precursors glycerol and lactate (Table 1), were unchanged over the course of measurement. Thus our results are consistent with the conclusion that during the first 12 h of fasting, hepatic glycogen turnover rate is regulated by mechanisms intrinsic to the liver.

Because there is no “gold standard” method to measure GNG in vivo (even hepatic vein catheterization requires assumptions about contributions of the gut and kidneys to GP), in a sense all current methods to estimate GNG are “indirect.” Hence, a comparison of values obtained on subjects studied under similar conditions can be informative. The rates we report are elevated compared with those in which GNG was estimated from the incorporation of \(^{13}\)C-label into glucose after infusion of \(^{13}\)C-lactate (5, 23, 26, 28, 29, 45). Such differences may be attributable to the inability to know, a priori, how to correct for isotope carbon dilution in the TCA cycle. Retrospectively, comparison of GNG estimated by using MIDA with rates obtained using the precursor-product method on trained subjects (5, 23) studied under comparable conditions suggests that a dilution factor of 1.86 should be applied to gluconeogenic rates estimated during rest from the incorporation of labeled alanine, lactate, and glycerol.

Gluconeogenic rates that we determined by using MIDA were 25–50% less than those reported on fasting subjects employing NMR, dideuterated water, and liver biopsy methods. Differences in GNG rates between studies could be due to variations in subject populations (in the present study trained cyclists were examined, compared with untrained subjects in the NMR, dideuterated, and liver biopsy studies) or to intrahepatic cycling between gluconeogenic glucose and glycogen. Previously, using MIDA techniques, Hellerstein et al. (18) reported simultaneous deposition and degradation of glycogen in fasting humans. Consequently, the absolute rate of hepatic glycogenolysis is greater than the time-dependent net reduction in hepatic glycogen. This realization permits a plausible explanation of the differences observed when GNG is estimated by using MIDA compared with NMR or biopsy. Because GNG is derived from the difference between GP and net glycogen change when NMR or biopsy procedures are used, an underestimation of Gly rate will result in an overestimation of GNG. Possible limitations of this method have been recently described (36).

**GNG during exercise.** Glucose production (\( R_g \)) scales exponentially to relative exercise intensity (4, 11, 12, 47). Our results on exercising men with MIDA indicate that \( f_{\text{GNG}} \) was constant during exercise and inversely related to relative exercise intensity. Despite the effect of exercise intensity on \( f_{\text{GNG}} \), because of the positive effect of exercise intensity on glucose \( R_g \), \( GNG_{\text{imb}} \) increased 54 and 103% (\( P < 0.05 \)) over that shown at rest during exercises at 45 and 65% \( \dot{V}_O_2\text{peak} \), respectively.

The rate of GNG during PA conditions has been reported to be dependent on mobilization and delivery of gluconeogenic substrate as well as the conversion efficiency within the liver (24–26, 44, 45). The relationship between GNG and precursor supply during exercise has been demonstrated by incorporation of carbon-labeled gluconeogenic precursors (5, 45) and splanchnic catheterization (1–3, 50). Increased precursor extraction across the splanchnic bed was attributed to increased delivery and fractional extraction of gluconeogenic precursors during exercise compared with rest (1, 3, 50). Previously, our laboratory (6, 13) reported on similarly treated subjects that glycerol and lactate appearance rates increased approximately three- and sixfold, respectively (6, 13). In the present investigation, circulating lactate and glycerol levels increased significantly (Table 1). Given these observations, it is unlikely that GNG was limited by mobilization of GNG precursor availability in the exercising men we studied. Also, because cell membrane lactate transport follows Michaelis-Menten kinetics (9, 39), if anything hepatic fractional extraction of lactate probably rose as a function of arterial lactate concentration.
More likely, reduction of splanchnic blood flow due to redistribution of cardiac output to working muscles limits GNG during hard exercise.

In the present study, a transient, intensity-dependent increase in GNG was observed during the first 45 min of exercise (Fig. 4B). The intensity effect reported in the present study corresponded to the exercise period when the largest differences in lactate concentrations were observed between moderate and hard exercise (Table 1). Because lactate is the predominant gluconeogenic precursor during exercise and lactate incorporation into glucose increases in a dose-dependent manner (5, 20), it seems reasonable to conclude that increased hepatic lactate delivery was responsible for the intensity effect observed at the onset of exercise. In like manner, the time-dependent reduction in lactate concentration during 65% \( \dot{V}O_2 \) peak (Fig. 1B) may have been responsible for the attenuation in GNG after 60 min of exercise at \( \dot{V}O_2 \) peak.

A reduction in the insulin-to-glucagon ratio (I/G), in addition to changes in gluconeogenic precursor availability (5, 47) during hard exercise, may further explain the increase in GNG during the transition from rest to exercise. Previously, Lavoie et al. (33) reported that, during exercise, a reduction in I/G is essential for increased GNG. In the present study, I/G decreased 23 and 54% (\( P < 0.05 \)) during exercise at 45 and 65% \( \dot{V}O_2 \) peak, respectively (Table 1). The reduction in I/G during exercise at 45% \( \dot{V}O_2 \) peak resulted from a 20% decrease in insulin, rather than a change in the glucagon concentration. During exercise at 65% \( \dot{V}O_2 \) peak, insulin fell 39% and glucagon rose 30%, a response that accompanied the fall in blood glucose concentration.

We interpret our data to mean that reductions in insulin stimulated GNG during exercise and that, when glucose \( \text{Ra} \) failed to match \( \text{Re} \) after prolonged hard exercise, rising glucagon concentrations amplified the change in I/G, thus increasing the signal to increase GNG. Despite the change in I/G during the last 30 min of exercise at 65% \( \dot{V}O_2 \) peak, the capacity to raise GNG was inadequate to prevent the time-dependent decline in blood glucose concentration during prolonged exercise. Our results suggest that the capacity to support GP from GNG during exercise is somewhat less than 1.5 mg·kg\(^{-1}\)·min\(^{-1}\).

**Comparison of gluconeogenic rates previously reported during exercise.** The GNG rates reported for exercising men in the present study are increased with values previously published by our laboratory (5, 11, 23, 45) and others (1-3, 7, 33, 49) at similar exercise intensities. However, MIDA was not used to measure GNG in the previous studies. Rather, GNG was estimated from the incorporation of tracer carbon from “surrogate” precursors, including alanine (33) and lactate (5, 23, 45). However, dilution of surrogate \( ^{13} \text{C} \) label in the mitochondrial oxaloacetate pool results in the underestimation of GNG (27, 51). Comparing the present results obtained with the use of MIDA with results obtained on similar subjects in which GNG was estimated from the appearance of \( ^{13} \text{C} \) in glucose after infusion of [\( ^{3-13} \text{C} \)]lactate (5, 23, 45) suggests that dilution factors of 1.30 and 1.45 should be applied during exercise eliciting 45 and 65% of \( \dot{V}O_2 \) peak, respectively. The dilution factors for exercise compare with that for resting men (1.86, see above).

The present experimental protocol differed from our previous studies (5, 23, 45) in which GNG was estimated from secondary labeling in that the most recently studied subjects were 12-h PA as opposed to 3-4 h PA. As already discussed, time since last eating affects GNG in resting subjects. The question then arises: does time since last eating also affect GNG during exercise? Although at present we cannot answer this question definitively, we are of the opinion that methodological considerations are more important than immediate dietary history for assessing GNG during exercise. In our companion report (47), subjects were 12-h PA and GNG was estimated from glucose carbon recycling. GNG estimated by MIDA was 30 and 45% greater during exercises eliciting 45 and 65% of \( \dot{V}O_2 \) peak, respectively. Because MIDA estimates the contributions to GNG from all, not just \( ^{13} \text{C} \), precursors, GNG\(_{ab} \) estimated from MIDA should be systematically greater than that estimated by other methods. In this regard, it is interesting to note that secondary labeling and carbon-recycling techniques yield similar but consistently lower values for GNG\(_{ab} \) in exercising men.

**Mass isotopomer distribution analysis.** Although we and others (18, 19, 37, 42, 43) have employed MIDA to estimate enrichment of the “true” gluconeogenic precursor pool, several groups have challenged the accuracy of MIDA. Concerns are that heterogeneity among gluconeogenic triose phosphate pools results in the inaccurate measures of \( p \) and nonphysiological calculations of GNG (10, 30). Those challenges have been addressed by Hellerstein and colleagues (18, 36, 37), who maintain that MIDA accurately estimates GNG when \( [2-^{13} \text{C}] \)glycerol is infused at rates sufficient to raise \( p \) above 12%. As noted previously, \( p \) was stable and in excess of 15% throughout rest and exercise in the present study (Fig. 3).

An additional concern regarding the use of MIDA for the measurement of GNG is the potential effects of the \( [^{13} \text{C}] \)glycerol load on GP. Although increased concentrations of a gluconeogenic precursor, such as glycerol, can influence its fractional contribution to GNG, our laboratory (47) and others (24-26) have shown that a gluconeogenic precursor load of the magnitude used in the present study does not increase the GNG\(_{ab} \) rate or absolute GP. Rather, increases in the contribution of a specific gluconeogenic precursor are likely compensated for by decreased contributions from other precursors. Previously, we have observed a decrease in glucose carbon recycling rate (an index of gluconeogenic flux through PEPCK) during glycerol infusion. Thus it is likely that the elevated glycerol concentrations resulting from exogenous infusion increased the contribution of glycerol and decreased the contributions of other precursors to GNG; exogenous glycerol infusion did not change GP (4, 11, 12, 47).

**Summary and conclusions.** The rates of GNG we observed in resting, PA men by using MIDA are in...
agreement with values previously obtained by others using similar methodology. Furthermore, results obtained by MIDA and other methods support the conclusion that the $f_{\text{GNG}}$ increases in resting individuals over time after eating. Results obtained by using MIDA to estimate GNG in exercising men yielded results 30–45% higher than obtained on similarly treated subjects in whom GNG was estimated by using carbon-labeled precursor product methods. Although the GNG$_{\text{ab}}$ increases during exercise compared with those at rest, as a fraction of GP, the relative role of GNG to GP remains the same during moderate-intensity exercise and decreases during hard exercise. In the exercise conditions we studied, GNG provided only a minor portion (20–25%) of glucose production; hence, Gly was more important than GNG in maintaining blood glucose homeostasis. The combined increases in GNG and Gly in 12-h PA men during 90 min of hard (65% $V_o_2$ peak) exercise were insufficient to prevent a decline in blood glucose concentration despite elevations in precursor (lactate and glycerol) supply. Our results indicate that combined Gly and GNG cannot continuously compensate for high rates of peripheral glucose uptake ($R_d > 6.0 \text{ mg-kg}^{-1}\text{-min}^{-1}$) in exercising PA men whose liver glycogen content has been compromised by fasting and exercise.

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