

Endurance training increases gluconeogenesis during rest and exercise in men

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¹Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley, California, 94720; ²University of Colorado Health Sciences Center, Division of Cardiology, Denver, Colorado 80262; and ³Geriatric Research, Education, and Clinical Center, Palo Alto Veterans Affairs Health Care System, Palo Alto, California 95304

Bergman, Bryan C., Michael A. Horning, Gretchen A. Casazza, Eugene E. Wolfel, Gail E. Butterfield, and George A. Brooks. Endurance training increases gluconeogenesis during rest and exercise in men. *Am. J. Physiol. Endocrinol. Metab.* 278: E244–E251, 2000.—The hypothesis that endurance training increases gluconeogenesis (GNG) during rest and exercise was evaluated. We determined glucose turnover with [6,6-²H]glucose and lactate incorporation into glucose by use of [3-¹³C]lactate during 1 h of cycle ergometry at two intensities [45 and 65% peak O₂ consumption (V_{O₂peak})] before and after training [65% pretraining V_{O₂peak}], same absolute workload (ABT), and 65% posttraining V_{O₂peak}, same relative intensity (RLT). Nine males (178.1 ± 2.5 cm, 81.8 ± 3.3 kg, 27.4 ± 2.0 yr) trained for 9 wk on a cycle ergometer 5 times/wk for 1 h at 75% V_{O₂peak}. The power output that elicited 66.0 ± 1.1% of V_{O₂peak} pretraining elicited 54.0 ± 1.7% posttraining. Rest and exercise arterial glucose concentrations were similar before and after training, regardless of exercise intensity. Arterial lactate concentration during exercise was significantly greater than at rest before and after training. Compared with 65% pretraining, arterial lactate concentration decreased at ABT (4.75 ± 0.4 mM, 65% pretraining; 2.78 ± 0.3 mM, ABT) and RLT (3.76 ± 0.46 mM) (*P* < 0.05). At rest after training, the percentage of glucose rate of appearance (R_a) from GNG more than doubled (1.98 ± 0.5% pretraining; 5.45 ± 1.3% posttraining), as did the rate of GNG (0.11 ± 0.03 mg·kg⁻¹·min⁻¹ pretraining, 0.24 ± 0.06 mg·kg⁻¹·min⁻¹ posttraining). During exercise after training, %glucose R_a from GNG increased significantly at ABT (2.3 ± 0.8% at 65% pre- vs. 7.6 ± 2.1% posttraining) and RLT (6.1 ± 1.5%), whereas GNG increased almost threefold (*P* < 0.05) at ABT (0.24 ± 0.08 mg·kg⁻¹·min⁻¹ 65% pre-, and 0.71 ± 0.18 mg·kg⁻¹·min⁻¹ posttraining) and RLT (0.75 ± 0.26 mg·kg⁻¹·min⁻¹). We conclude that endurance training increases gluconeogenesis twofold at rest and threefold during exercise at given absolute and relative exercise intensities.

exertion; lactate; glucose; stable isotopes

IN LIGHT OF THE IMPORTANCE of maintaining blood glucose homeostasis during prolonged exercise, the effects of endurance training on gluconeogenesis (GNG) in exercising humans have received little attention. Re-

sults of previous investigations suggested that endurance training decreases (7) or does not change (23) GNG in humans during exercise at a given absolute intensity compared with before training. However, increased GNG has been reported in trained compared with untrained rats during hard exercise (10). Additionally, increased glucose carbon recycling in trained compared with untrained rats was reported during the last 30 min of a 60-min exercise bout that followed a 30-h fast to deplete liver glycogen stores (12). Furthermore, data from studies using isolated rat liver perfusion indicated that endurance training increases GNG capacity from lactate (11, 30) and alanine (28). Moreover, norepinephrine-stimulated GNG from lactate was increased in liver slices prepared from endurance-trained compared with untrained rats (25). Thus, in contrast to results of investigations on humans, the more extensive data from studies on rats strongly suggest that endurance training increases GNG capacity at rest as well as during exercise.

The purpose of the present investigation was to evaluate the hypothesis that endurance training increases GNG in men during exercise at given absolute and relative exercise intensities compared with that before training. For this purpose, we utilized a longitudinal design and studied men pre- and posttraining at exercise intensities known to alter circulating lactate concentration and splanchnic circulation.

METHODS

Subjects

Nine healthy sedentary male subjects aged 19–33 yr were recruited from the University of California, Berkeley campus by posted notices. Subjects gave informed consent, were considered untrained if they engaged in no more than 2 h of physical activity per week for the previous year, and had a peak oxygen consumption (V_{O₂peak}) of <45 ml·kg⁻¹·min⁻¹. Subjects were included in the study if they had <25% body fat, were nonsmokers, were diet and weight stable, had a 1-s forced expiratory volume of ≥70% of vital capacity, and were injury/disease free as determined by physical examination. This study was approved by the Committee for the Protection of Human Subjects at Stanford University, and the University of California, Berkeley (CPHS 97–6–34).

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Experimental Design

After interviews and preliminary screening, subjects performed two graded exercise tests to determine $\dot{V}O_{2\text{peak}}$ during leg cycle ergometry. Blood lactate threshold (LT) was determined during the second screening test. Subjects were then tested in a random order at 45 and 65% $\dot{V}O_{2\text{peak}}$, with 1 wk between isotope trials (see *Tracer Protocol*). Two days after the second trial, subjects began training on leg cycle ergometers. Posttraining isotope trials were also performed in a random order at 65% of pretraining $\dot{V}O_{2\text{peak}}$ (same absolute workload, ABT) and at 65% of posttraining $\dot{V}O_{2\text{peak}}$ (same relative intensity, RLT).

Preliminary Testing

All exercise tests were performed on an electronically braked cycle ergometer (Monark Ergometric 829E). For determination of $\dot{V}O_{2\text{peak}}$, exercise started at a power output of 50 W, which was increased by 25 or 50 W every 3 min until exhaustion. Respiratory gases were analyzed via an indirect open-circuit system and recorded by an on-line, real time PC-based system (4). Body composition was determined via both skinfold (21) and underwater weighing techniques. Three-day diet records were kept to obtain baseline dietary habits and to monitor macronutrient composition and energy intake over the course of the study. Dietary analysis was performed using the Nutritionist III software (N-Squared Computing, San Mateo, CA).

Dietary Protocol

On the night preceding each isotope trial, subjects were admitted to the metabolic ward, where they remained until testing was completed the following day. Subjects were fed a standardized dinner [1,174 kcal: 66% carbohydrate (CHO), 21% fat, 13% protein], which was replicated the night before each experimental trial. Later that evening, subjects ate a standardized snack (500 kcal: 53% CHO, 31% fat, and 16% protein) before retiring. Two subjects were tested per day, with morning and afternoon testing randomly assigned to each subject for the first trial and replicated for all subsequent trials. Morning procedures started at 7 AM, and preliminary afternoon procedures began at 1 PM. Morning subjects ate a standardized pretrial meal with a calculated low glycemic index (13) (448 kcal: 72% CHO, 10% fat, 18% protein) at 6 AM, 1 h before procedures started and 4.5–5 h before exercise. Afternoon subjects ate a standardized breakfast in the morning (729 kcal: 57% CHO, 33% fat, 10% protein) and the standardized pretrial meal at noon, again 1 h before procedures began and 4.5–5 h before exercise.

Catheterizations

After local lidocaine anesthesia, the femoral artery was cannulated using standard percutaneous techniques, as previously described (4, 5). One subject experienced blood leaking from catheter placements during the beginning minutes of exercise at 65% pretraining and did not perform further exercise. As a result, a sample size of 8–9 was used for calculations and comparisons.

Tracer Protocol

A venous catheter was placed in an antecubital vein on the morning of each trial for infusion of stable isotope solutions during 90 min of rest and 1 h of exercise. Background blood samples were collected after catheterization of the femoral artery and vein. Subjects then received a primed continuous infusion of [6,6-²H]glucose and [3-¹³C]lactate while resting

semisupine for 90 min. Glucose (4) and lactate (3) kinetics are reported separately. The priming bolus was equal to 23 times the resting lactate infusion rate. Tracer glucose and lactate were infused via a pump (Intelligent 522, Kendall McGaw, Irvine, CA) at 2 and 2.5 mg/min at rest, and at 6 and 7.5 mg/min during exercise, respectively, at 45% pretraining $\dot{V}O_{2\text{peak}}$ and 65% old $\dot{V}O_{2\text{peak}}$ posttraining (ABT), and at 8 and 10 mg/min at 65% pretraining $\dot{V}O_{2\text{peak}}$ and 65% posttraining $\dot{V}O_{2\text{peak}}$ (RLT), respectively. Increases in tracer infusion were intended to elicit similar arterial enrichments between exercise intensities during the last 30 min of exercise. Isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 9% sterile saline, and tested for sterility and pyrogenicity before use (University of California School of Pharmacy, San Francisco, CA).

Blood Sampling

Arterial blood samples were drawn anaerobically over 5 s after 75 and 90 min of rest and at 5, 15, 30, 45, and 60 min of exercise. Blood samples for determination of glucose and lactate concentrations and isotopic enrichments were immediately transferred to tubes containing 8% perchloric acid and were shaken and placed on ice. Blood for determination of arterial plasma lactate concentration was immediately placed on ice. After the final blood sample at the end of exercise, samples were centrifuged at 3,000 g for 10 min, and the supernatant was transferred to storage tubes and frozen at –20°C until analysis.

Metabolite Analyses and Isotope Enrichments

Glucose concentrations were measured in duplicate using a hexokinase kit (Sigma, St. Louis, MO). Lactate concentration was measured in duplicate on plasma by use of the method of Gutmann and Wahlefeld (16) with lactate dehydrogenase. Glucose isotopic enrichment was measured by gas chromatography-mass spectrometry (GC-MS; GC model 5890 series II and MS model 5989A, Hewlett-Packard) of the pentaacetate derivative of mass-to-charge ratio (m/z) 332, indicative of the *M1* glucose isotopomer as described previously (14). Lactate isotopic enrichment was measured by GC-MS of the *N*-propylamide heptafluorobutyrate derivative, as described previously (3).

Training Protocol

Training was performed on stationary cycle ergometers 5 days/wk with workloads adjusted to elicit heart rates corresponding to 75% of $\dot{V}O_{2\text{peak}}$. Subjects were asked to exercise 1 day/wk on their own in addition to cycle ergometry training so that total training was for 6 days/wk. All subjects were exercising at 75% of their $\dot{V}O_{2\text{peak}}$ for 1 h by the end of the 2nd wk of training. After 4 wk of training, subjects performed another maximal exercise test to quantify increases in $\dot{V}O_{2\text{peak}}$, and training workloads were adjusted to maintain relative training intensity at 75% $\dot{V}O_{2\text{peak}}$. Two weeks preceding posttraining testing, subjects began interval training during the last 10 min of each 1-h workout. Interval training was added to develop recruitment patterns conducive to reaching maximal power outputs during posttraining evaluation. Subjects continued training throughout the 1 wk between posttraining testing with 1 day of rest before an experimental trial, and two days of rest after an experimental trial to recover from testing procedures. Thus all posttraining trials were conducted after 8 or 9 wk of endurance training. Subjects were weighed daily and asked to increase energy intake to maintain weight during the training program without changing normal macronutrient composition.

Calculations

%Glucose R_a from GNG. The percentage of glucose R_a from GNG was calculated as previously described by Huie et al. (19) in our laboratory. This approach was derived from that of Zilverstmit et al. (34)

$$\text{glucose } R_a \text{ from GNG (\%)} = \frac{\text{glucose } M1 \text{ IE} \times H}{\text{lactate IE}} \quad (100)$$

where glucose $M1$ IE is the isotopic enrichment of the $M+1$ glucose isotopomer, lactate IE is the isotopic enrichment of lactate, and H is the factor to correct for loss of label in the tricarboxylic acid cycle during GNG (18), which was assumed to be 1.45 at rest (8) and 1.0 during exercise (that is, with no correction) (10).

GNG. The rate of GNG was calculated as shown previously (19)

$$\text{GNG (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = (\% \text{glucose } R_a \text{ from GNG})(\text{glucose } R_a)$$

Glucose kinetics. Glucose R_a values were calculated using equations defined by Steele and modified for use with stable isotopes (33), as previously described (4).

Statistical Analyses

Significance of differences among average arterial glucose and lactate concentrations determined during the last 30 min of exercise were analyzed using a one-factor ANOVA with repeated measures. Differences between training states for body fat, $\dot{V}O_{2\text{peak}}$, and power output at lactate threshold were determined using a paired Student's t -test. Differences between groups for glucose R_a were determined using a repeated-measures factorial ANOVA. Differences between groups for %glucose R_a from GNG and GNG were determined using a repeated-measures ANOVA. Post hoc comparisons were made using Fisher's protected least significant difference test. Statistical significance was set at $\alpha = 0.05$. All data are presented as means \pm SE.

RESULTS

Subject Characteristics

Anthropometric data on subjects pre- and posttraining have been reported previously (4) but are repeated in Table 1. Subjects were weight stable throughout the study period. $\dot{V}O_{2\text{peak}}$ increased significantly by 14.6% as a result of training. Consequently, posttraining trials at $66.0 \pm 1.1\%$ of pretraining $\dot{V}O_{2\text{peak}}$ (the same ABT as pretraining, 150 W) were performed at $54.0 \pm 1.7\%$ of posttraining $\dot{V}O_{2\text{peak}}$; 174 W were required to elicit 65% of $\dot{V}O_{2\text{peak}}$ posttraining. The power output corresponding to LT increased 22% ($P < 0.05$) after training. Specific power outputs and rates of O_2 consumption achieved by subjects before and after training have been reported previously (5).

Glucose and Lactate Concentrations

Glucose concentrations were not different between training states at rest or during exercise at any intensity (Table 2). Resting arterial lactate concentrations were similar before and after training (Table 2). Under all exercise conditions, arterial lactate concentrations

Table 1. Subject characteristics before and after 9 wk of leg cycle endurance training

Variable	Pretraining	Posttraining	%Difference
Age, yr	27.4 \pm 2.0		
Height, in.	70.1 \pm 1.0		
Weight, kg	81.8 \pm 3.3	81.3 \pm 3.2	-0.6
Body fat, %			
Skin folds	19.7 \pm 1.5	17.5 \pm 1.6*	-11.0
Underwater weighing	19.5 \pm 1.5	17.2 \pm 1.4*	-11.6
$\dot{V}O_{2\text{peak}}$			
l/min	3.5 \pm .10	4.02 \pm .15*	14.6
ml \cdot kg ⁻¹ \cdot min ⁻¹	43.5 \pm 1.3	50.1 \pm 1.6*	15.5
Lactate threshold (% $\dot{V}O_{2\text{peak}}$)	60.9 \pm 2.7	65.4 \pm 2.6	7.4
Power output at lactate threshold, W	161.1 \pm 4.4	197.2 \pm 6.5*	22.4
Resting muscle [glycogen], μ mol/g wet weight	94.2 \pm 13.8	152.7* \pm 11.4	62.1

Values are means \pm SE; $n = 9$. $\dot{V}O_{2\text{peak}}$, peak O_2 consumption. *Significantly different from pretraining values at $P < 0.05$.

increased significantly above rest, and the increase was directly related to exercise intensity, both before and after training. Compared with 65% pretraining $\dot{V}O_{2\text{peak}}$, endurance training significantly decreased arterial lactate concentration by 42% at ABT and 21% at RLT.

Glucose R_a

Glucose R_a was similar at rest before and after endurance training (Table 2). Compared with rest, glucose R_a increased during exercise regardless of training state or exercise intensity ($P < 0.05$). Glucose R_a scaled to exercise intensity before and after training. Compared with 65% pretraining $\dot{V}O_{2\text{peak}}$, glucose R_a decreased 23% at ABT ($P < 0.05$) but was unchanged at RLT.

GNG

Arterial glucose enrichments from the $M1$ glucose isotopomer were stable over time throughout rest and exercise but varied due to exercise intensity and tracer infusion rate (Fig. 1). At rest, the percentage of glucose R_a from GNG increased 175% after endurance training ($P < 0.05$; Fig. 2A). However, within a training condition, the percentage of glucose R_a from GNG did not change during exercise compared with rest. Compared with 65% pretraining $\dot{V}O_{2\text{peak}}$, endurance training increased the percentage of glucose R_a from GNG by 230% at ABT and 170% at RLT (Fig. 2A).

Before training, the estimated GNG rate did not increase during exercise compared with rest (Fig. 2B). However, GNG increased 125% during rest after endurance training (Fig. 2B). After training, GNG increased significantly during exercise compared with rest, as well as when compared with exercise before training. Compared with that during the 65% pretraining $\dot{V}O_{2\text{peak}}$ condition, after training GNG increased 300% during exercise under the ABT and RLT conditions (Fig. 2B).

The rate of GNG during exercise relative to arterial lactate concentration followed a saturation-type relationship both before and after training but peaked at arterial lactate concentrations twice as great after training (Fig. 3A). Before training, GNG relative to

Table 2. Arterial glucose and lactate concentrations during rest and exercise before and after training

	Rest Pretraining	Rest Posttraining	45% Pretraining	65% Pretraining	ABT Posttraining	RLT Posttraining
[Glucose] _a , mM	5.16 ± 0.05	4.89 ± 0.06	5.05 ± 0.07	4.9 ± 0.08	4.92 ± 0.08	4.94 ± 0.15
Glucose R _a , mg · kg ⁻¹ · min ⁻¹	2.49 ± 0.18	2.41 ± 0.23	3.95 ± 0.29*	5.71 ± 0.3*†	4.40 ± 0.44*‡	6.10 ± 0.41*§
[Lactate] _a , mM	1.10 ± 0.03	1.13 ± 0.05	1.79 ± 0.16*	4.75 ± 0.40*†	2.78 ± 0.31*‡	3.76 ± 0.46*‡§

Values are means ± SE; *n* = 8–9. ABT, absolute workload; RLT, relative intensity exercise. [Glucose]_a and [lactate]_a, arterial glucose and lactate concentrations, respectively; R_a, appearance rate. *Significantly different from rest at *P* < 0.05; †significantly different from 45% pretraining at *P* < 0.05; ‡significantly different from 65% pretraining at *P* < 0.05; §significantly different from posttraining (ABT) at *P* < 0.05.

whole body oxygen consumption ($\dot{V}O_2$) peaked at 45% pretraining $\dot{V}O_{2\text{peak}}$ and tended to decrease with increasing exercise intensity (Fig. 3B). After training, GNG relative to $\dot{V}O_2$ appeared to plateau at RLT.

DISCUSSION

This investigation is the first to use a longitudinal design to evaluate effects of endurance training on GNG in men exercising at given absolute and relative intensities. Our results indicate that 4.5–5.5 h after a meal, endurance training doubles GNG at rest despite unchanged arterial lactate concentration. Similarly, during exercise after training at given absolute and relative intensities compared with before training, GNG triples despite significantly decreased arterial lactate concentration.

Training Adaptations

Our training program was successful in promoting significant metabolic adaptations (Table 1). During our 9-wk training program, subjects significantly increased $\dot{V}O_{2\text{peak}}$ (15%), decreased respiratory exchange ratio (RER) at a given absolute power output (3.2%), increased the power output eliciting LT by 22%, decreased arterial lactate concentration at the same relative (26%) and absolute (55%) exercise intensities, and increased resting muscle glycogen concentration (62%) (4).

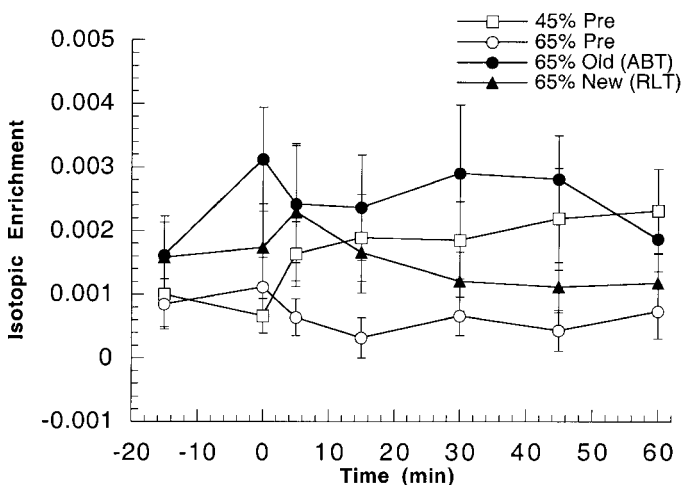


Fig. 1. Arterial glucose enrichment of the M1 glucose isotopomer after [¹³C]lactate infusion during rest and exercise before and after training. See METHODS for description of tracer infusion. Values are means ± SE; *n* = 8–9. ABT, absolute workload. RLT, relative exercise intensity.

Nutritional Controls

We fed subjects to be weight stable and rested them the day before experimentation. Furthermore, we fed standardized meals with low glycemic indexes 4.5–5.5 h

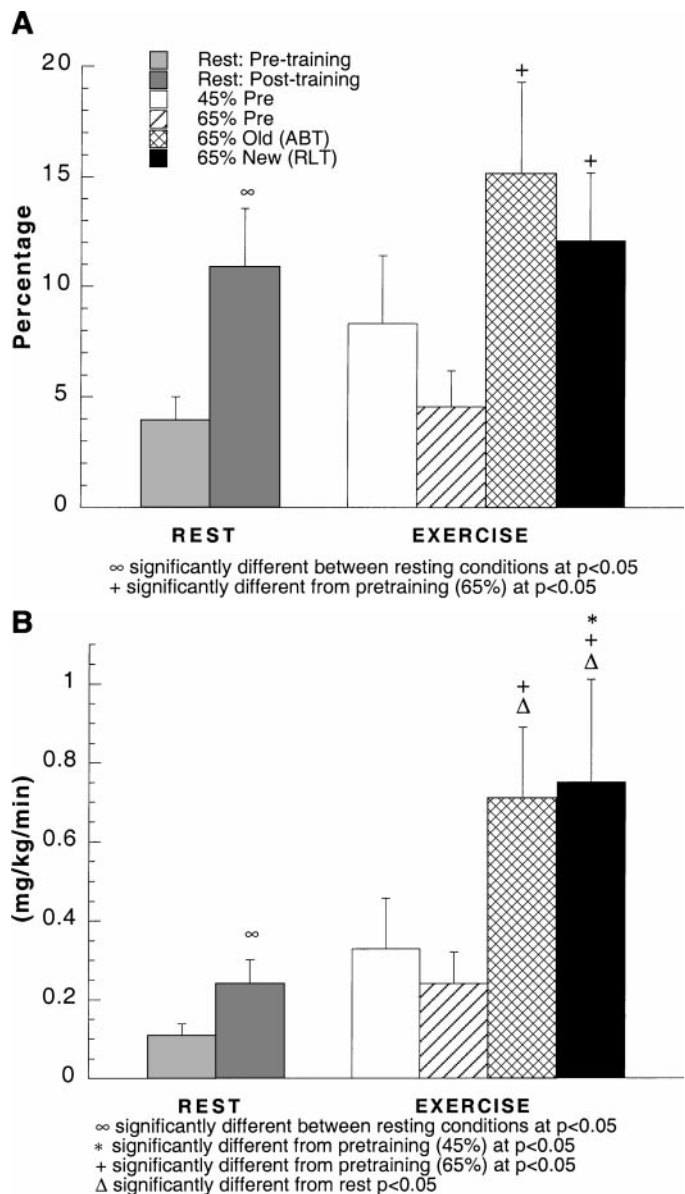


Fig. 2. A: percent glucose appearance rate (R_a) from gluconeogenesis (GNG) during rest and exercise, before and after training. Values are means ± SE; *n* = 8–9. B: estimated GNG during rest and exercise, before and after training. Values are means ± SE; *n* = 8–9.

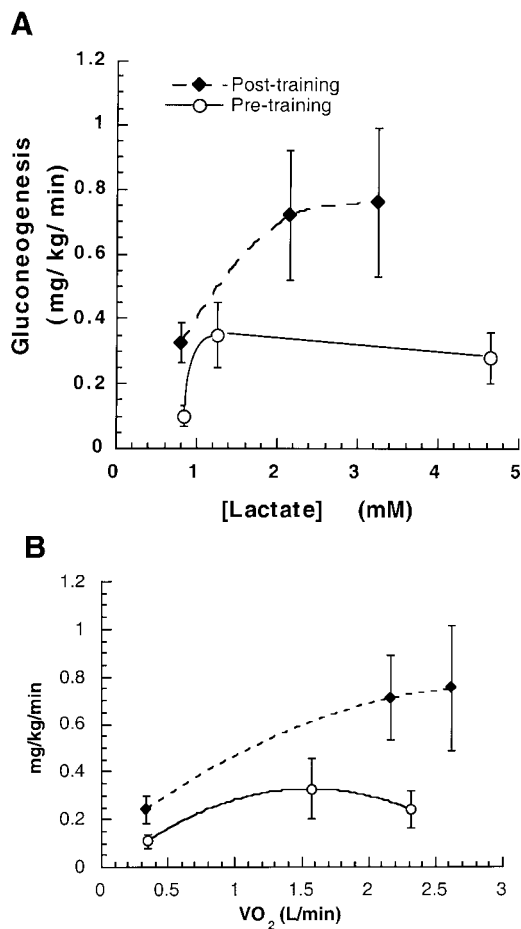


Fig. 3. *A*: estimated GNG relative to lactate concentration during rest and exercise, before and after training. Values are means \pm SE; $n = 8-9$. *B*: estimated GNG relative to oxygen consumption ($\dot{V}O_2$) during rest and exercise, before and after training. Values are means \pm SE; $n = 8-9$.

before exercise studies. Those efforts produced stable blood glucose levels during exercise, suggesting that subjects commenced exercise with normal liver glycogen reserves. Thus observed effects on GNG are attributable to exercise intensity and endurance training and are not confounded by hypoglycemia during exercise.

Table 3 shows values for GNG in the current study compared with literature values in humans estimated by using the lactate-to-glucose precursor-product relationship. Furthermore, Table 3 shows data from investigations using deuterated water and mass isotopomer distribution analysis (MIDA) techniques that reveal the effect of time since last eating on resting GNG in humans. In the current study, preexercise meals dampened rates of GNG compared with values reported after an overnight fast in humans by use of the lactate-to-glucose precursor-product relationship (Table 3). Rates of GNG increase with duration of fast, as shown by studies utilizing deuterated water and MIDA techniques (Table 3). Thus attenuated rates of GNG in the current study were to be expected given the nutritional controls imposed. Our data emphasize the importance of preexercise meals on regulation of GNG and implicate the increasingly important role of GNG for maintenance

of blood glucose homeostasis as duration of fasting progresses.

GNG After Training

Our results indicate the percentages of glucose R_a from GNG (Fig. 2*A*) as well as rates of GNG (Fig. 2*B*) increased at rest after endurance exercise training. However, the relative contribution of GNG to glucose R_a was small, $<10\%$. Only one previous study investigated GNG rates at rest before and after training in humans, and that study reported similar rates of [^{13}C]bicarbonate incorporation into glucose before and after training (7). In contrast, after endurance training, resting whole body GNG increased as estimated from [^{14}C]lactate incorporation into glucose in rats (10, 11). Furthermore, enhanced capacity for GNG from both lactate and alanine was found in liver preparations obtained from endurance-trained rats (24, 25, 28, 30). Thus, although few data on endurance-trained humans are available with which to compare our results, they are consistent with data obtained on rats, indicating increased GNG capacity after endurance training.

In humans exercising at given power outputs, GNG after endurance exercise training has been reported to decrease, as estimated from [^{13}C]bicarbonate incorporation into glucose (7), or remain unchanged, as estimated from the difference between lactate disappearance rate (R_d) and lactate oxidation (R_{ox}) (23). In rats, however, Donovan and Brooks (10) reported increased GNG from lactate at high but not moderate exercise intensities, as estimated from [^{14}C]incorporation into glucose after [^{14}C]lactate infusion. Moreover, Donovan and Sumida (12) reported significantly greater glucose R_a during the last 30 min of a 1-h exercise bout at a given workload in trained compared with untrained rats after a 30-h glycogen-depleting fast, concomitant with an increased rate of glucose recycling. Turcotte and Brooks (31) reported data consistent with increased GNG after training when administration of mercaptopicolinic acid, an inhibitor of phosphoenolpyruvate carboxykinase, abolished the enhanced ability of trained rats to maintain blood glucose homeostasis during treadmill exercise at a given absolute power output. Again, our present data obtained on men are similar to results of studies on rats, as we found a threefold increase in GNG ($P < 0.05$) at ABT compared with 65% pretraining $\dot{V}O_{2peak}$ (Fig. 2*B*). We previously reported that lactate metabolic clearance rate (MCR) tended ($P = 0.06$) to increase at ABT after training compared with 65% pretraining $\dot{V}O_{2peak}$ (3). Our results indicate that the tendency for increased lactate MCR after training may partly be due to enhanced GNG from lactate.

The current study is the first to investigate effects of endurance training on GNG in humans during steady-state relative exercise intensities. GNG tripled at a given relative exercise intensity after endurance training (Fig. 2*B*). In contrast, using a progressive exercise protocol, MacRae et al. (23) found no effect of training on lactate removal by GNG at relative exercise intensities of 65 and 80% $\dot{V}O_{2peak}$. We are unable to

Table 3. Summary of resting and exercise gluconeogenic rates in literature employing lactate to glucose precursor-product, deuterated water, and MIDA techniques

Study	Method	Dietary Condition	Training State	Rate, $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	Exercise Intensity, $\% \text{VO}_{2\text{peak}}$
Bergman et al., 1999	lactate to glucose	4.5–5.5 post-prandial	UT	0.11	Rest
			T	0.24	Rest
Huie et al., 1996	lactate to glucose	12 h fast	UT	0.39	Rest
Jenssen et al., 1990	lactate to glucose	13–15 h fast	UT	0.25	Rest
Consoli et al., 1990	lactate to glucose	12–14 h fast	UT	0.76	Rest
Coggan et al., 1995	Bicarbonate label	12 h fast	UT	0.24	Rest
			T	0.16	Rest
Landau et al., 1996	deuterated water	14 h fast	UT	0.94*	Rest
			UT	1.01*	Rest
			UT	1.40*	Rest
Chandramouli et al., 1997	deuterated water	14 h fast	UT	0.99	Rest
			UT	0.99	Rest
			UT	0.99	Rest
Siler et al., 1998	MIDA with $[2\text{-}^{13}\text{C}]$ glycerol	14 h fast	UT	0.44	Rest
			UT	0.63	Rest
Hellerstein et al., 1997	MIDA with $[2\text{-}^{13}\text{C}]$ glycerol	11 h fast	UT	0.79	Rest
			UT	1.11	Rest
Bergman et al., 1999	lactate to glucose	4.5–5.5 h postprandial	UT	0.33	45
			UT	0.24	65
			T	0.71	54 (ABT)
			T	0.75	65 (RLT)
			T	0.75	50
Huie et al., 1996	lactate to glucose	12 h fast	UT	1.66	50
MacRae et al., 1992	lactate $R_d - R_{ox}$	12–14 h fast	UT	2.8†	65
			UT	1.8†	65
			T	1.3†	53
			T	1.3†	65
Coggan et al., 1995	bicarbonate label	12 h fast	UT	0.99	60
			T	0.39	47
			T	0.39	47

UT, untrained; T, trained; MIDA, mass isotopomer distribution analysis; R_d and R_{ox} , disappearance and oxidation rates. * Glucose R_a was estimated at $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 12 h fast and $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 22 and 42 h fast. † Gluconeogenesis (GNG) was estimated from Fig. 2A in MacRae et al.

explain the differences in results obtained in the two studies, but we note that different exercise protocols (continuous vs. progressive) and methods of estimating GNG were employed (^{13}C incorporation into glucose after lactate infusion vs. difference between lactate R_d and oxidation rate). Possibly, the method of MacRae et al. overestimated GNG, as it is known that in addition to oxidation and GNG, lactate carbons label the bicarbonate, protein, and glycogen pools (6).

The relationship between GNG and arterial lactate concentration ($[\text{lactate}]_a$) (Fig. 3A) also differs from that obtained by MacRae et al. (23). In contrast, the present results are reminiscent of those obtained on laboratory rats (10). In their study, MacRae et al. reported similar GNG for any given $\dot{V}O_2$ before and after training, whereas we found GNG increased after training during exercise at any given $\dot{V}O_2$ (Fig. 3B). Our results are similar to those of others who reported increased GNG in rats exercising at high, but not moderate, RLT values (10). The relationship between GNG and $\dot{V}O_2$, which plateaued with increasing $\dot{V}O_2$ after training (Fig. 3B), was also reported by Donovan and Brooks (10) in rats. Thus our data on effects of endurance training on GNG relative to $\dot{V}O_2$ are similar to previous investigations that employed measurements during isotopic steady state.

We previously reported similar whole body lactate turnover and active muscle lactate production after training at RLT compared with 65% pretraining $\dot{V}O_{2\text{peak}}$ despite dampened arterial lactate concentration (3).

Increased MCR and active muscle lactate uptake promoted dampened $[\text{lactate}]_a$ concentrations at RLT compared with before training. The current findings indicate that part of enhanced lactate MCR after training at RLT (3) can be attributed to increased GNG from lactate.

Mechanisms for Enhanced GNG

Several investigators reported unchanged enzymatic capacity for hepatic GNG after finding similar pyruvate carboxylase (19) and PEPCK activities (30) in livers from trained compared with untrained rats, and so the increased capacity for GNG we observed in men after training may be related to factors such as altered redox and adenine nucleotide energy charge (15). The present data do not allow us to identify the step or steps responsible for enhanced GNG between blood lactate and glucose production, but training effects on lactate transport across cell membranes, one or more steps in the gluconeogenic pathway, or effects on splanchnic blood flow could be involved. As well, the present results do not allow us to discriminate between training effects on liver or the kidneys. Similarly, we need to acknowledge that the glucose flux rates measured were the resultant of hepatic and renal functions that we could not distinguish.

Increased GNG at ABT and RLT after training, compared with 65% pretraining $\dot{V}O_{2\text{peak}}$, occurred de-

spite attenuated $[\text{lactate}]_a$ (Table 2). Because training increased $\dot{V}O_{2\text{peak}}$, the workload at ABT after training was performed at a lower relative exercise intensity (54% posttraining $\dot{V}O_{2\text{peak}}$). Liver blood flow decreases in direct proportion to relative exercise intensity (2). Therefore, greater hepatic blood flow may have enhanced lactate delivery to the liver even with attenuated $[\text{lactate}]_a$ concentration during ABT (Table 2), which may partially explain enhanced GNG at ABT. However, an explanation for enhanced GNG at RLT after training due to alterations in hepatic blood flow is less clear. RLT was unchanged, suggesting similar liver blood flow. However, increased blood volume after endurance training may have promoted enhanced hepatic perfusion after blood shunting to active muscles during exercise. Possibly, greater GNG after endurance training may be due to similar or increased liver lactate delivery as a result of increased hepatic blood flow at both ABT and RLT compared with 65% pretraining $\dot{V}O_{2\text{peak}}$.

Previous investigations reported a decrease in the apparent Michaelis-Menten constant (K_m) for hepatic lactate GNG after endurance training in rats (9, 29). If true for humans, decreased apparent K_m for lactate GNG may also help explain increased GNG observed in the present study despite decreased arterial lactate concentration.

We have previously reported unchanged arterial insulin concentration and significantly decreased arterial glucagon concentration after training at ABT and RLT compared with 65% pretraining $\dot{V}O_{2\text{peak}}$ (4). A lower glucagon-to-insulin ratio after training appears contradictory to increased hepatic GNG. However, Podolin et al. (24) reported that endurance training increased hepatic sensitivity to several glucoregulatory hormones, including glucagon in rats. Thus increased GNG after endurance exercise training observed in our study may be partially attributed to enhanced hepatic sensitivity to glucoregulatory hormones.

Limitations

We employed a dilution factor (Hetenyi, H; see Ref. 18) to correct for loss of label and crossing over in the tricarboxylic acid (TCA) cycle. Loss of label in the TCA cycle was originally reported by Weinmann et al. (32), later quantitated by Strisower et al. (27) by use of incubated liver slices, and further estimated in vivo in dogs and rats (18), followed by humans (8). However, use of a constant correction factor to estimate GNG over a wide range of stresses is likely flawed for several reasons. First, the methodology assumed that acetate is only metabolized in liver, a supposition that is possibly untrue (26). Second, the use of a fixed correction factor accounts only for loss of label from the oxaloacetate (OAA) pool and therefore assumes OAA to be the "true precursor pool" and last site of tracer dilution during GNG. However, we now know that the triose phosphate pool is the true GNG precursor pool and the last site of possible dilution during GNG (17). And finally, H factors are species, nutrition, and (possibly) training state specific; therefore, use of a constant

to correct for isotopic dilution during GNG in a metabolic environment different from that in which the constant was created will likely promote errors in computation.

In resting subjects our data probably underestimated total GNG, as contributions from glycerol are not included in the correction factor we used. Furthermore, our exercise data underestimated GNG because we did not use a correction factor, assuming lactate to be the predominant GNG precursor and no dilution of the precursor pool (1). Thus, although absolute rates of GNG may be inexact, our data are interpretable with respect to the literature because our methodology is similar to that employed by others to study GNG during exercise (10, 19). Moreover, we used identical correction factors before and after training and therefore assumed that endurance training does not affect OAA dilution and loss of label from the TCA cycle. Our data will inaccurately estimate GNG to the extent that endurance training alters hepatic OAA dilution and loss of label. However, from a qualitative standpoint, we are confident of our interpretation that endurance training increases GNG during exercise, because our conclusion is based on uncorrected ($H = 1$) data.

In our report we have made repeated conclusions regarding training effects on hepatic GNG. However, kidneys are the other important site of GNG and have been shown to increase GNG capacity after endurance training (22). Thus, although most enhanced GNG after training likely came from the liver, lactate GNG in kidneys contributed as well.

Conclusions

Data from the secondary labeling of blood glucose from infused carbon-tracer lactate support the conclusion that 9 wk of endurance training increased GNG twofold at rest and threefold during exercise at given absolute and relative exercise intensities. Additionally, increased GNG during exercise at ABT and RLT after endurance training promotes increased lactate MCR and dampens arterial lactate concentrations compared with the untrained state. After training, the capacity for GNG was enhanced in men, even though the circulation concentration of lactate, the main gluconeogenic precursor, was reduced.

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