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## Postprandial glucose fluxes and insulin sensitivity during exercise: A study in healthy individuals

Michele Schiavon,<sup>1\*</sup> Ling Hinshaw,<sup>3\*</sup> Ashwini Mallad,<sup>3</sup> Chiara Dalla Man,<sup>1</sup> Giovanni Sparacino,<sup>1</sup> Matthew Johnson,<sup>3</sup> Rickey Carter,<sup>2</sup> Rita Basu,<sup>3</sup> Yogish Kudva,<sup>3</sup> Claudio Cobelli,<sup>1</sup> and Ananda Basu<sup>3</sup>

<sup>2</sup>Department of Health Sciences Research, Mayo College of Medicine, Rochester, Minnesota; <sup>1</sup>Department of Information Engineering, University of Padova, Padova, Italy; and <sup>3</sup>Endocrine Research Unit, Division of Endocrinology and Metabolism, Mayo College of Medicine, Rochester, Minnesota

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**Schiavon M, Hinshaw L, Mallad A, Man CD, Sparacino G, Johnson M, Carter R, Basu R, Kudva Y, Cobelli C, Basu A.** Postprandial glucose fluxes and insulin sensitivity during exercise: A study in healthy individuals. *Am J Physiol Endocrinol Metab* 305: E557–E566, 2013. First published July 2, 2013; doi:10.1152/ajpendo.00182.2013.—Quantifying the effect size of acute exercise on insulin sensitivity ( $SI_{\text{exercise}}$ ) and simultaneous measurement of glucose disappearance ( $R_d$ ), endogenous glucose production (EGP), and meal glucose appearance in the postprandial state has not been developed in humans. To do so, we studied 12 healthy subjects [5 men, age  $37.1 \pm 3.1$  yr, body mass index  $24.1 \pm 1.1$  kg/m<sup>2</sup>, fat-free mass (FFM)  $50.9 \pm 3.9$  kg] during moderate exercise at 50%  $\dot{V}O_{2\text{max}}$  for 75 min, 120–195 min after a triple-tracer mixed meal consumed at *time 0*. Tracer infusion rates were adjusted to achieve constant tracer-to-tracee ratio and minimize non-steady-state errors. Glucose turnover was estimated by accounting for the nonstationary kinetics introduced by exercise. Insulin sensitivity index was calculated in each subject both in the absence [*time* (*t*) = 0–120 min,  $SI_{\text{rest}}$ ] and presence (*t* = 0–360 min,  $SI_{\text{exercise}}$ ) of physical activity. EGP at *t* = 0 min ( $13.4 \pm 1.1$   $\mu\text{M}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$ ) fell at *t* = 120 min ( $2.4 \pm 0.4$   $\mu\text{M}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$ ) and then rapidly rose almost eightfold at *t* = 180 min ( $18.2 \pm 2.6$   $\mu\text{M}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$ ) before gradually falling at *t* = 360 min ( $10.6 \pm 0.9$   $\mu\text{M}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$ ).  $R_d$  rapidly peaked at *t* = 120 min at the start of exercise ( $89.5 \pm 11.6$   $\mu\text{M}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$ ) and then gradually declined at *t* = 195 min ( $26.4 \pm 3.3$   $\mu\text{M}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$ ) before returning to baseline at *t* = 360 min.  $SI_{\text{exercise}}$  was significantly higher than  $SI_{\text{rest}}$  ( $21.6 \pm 3.7$  vs.  $12.5 \pm 2.0$   $10^{-4}$  dl·kg<sup>-1</sup>·min<sup>-1</sup> per  $\mu\text{U/ml}$ ,  $P < 0.0005$ ). Glucose turnover was estimated for the first time during exercise with the triple-tracer technique. Our results, applying state-of-the-art techniques, show that moderate exercise almost doubles postprandial insulin sensitivity index in healthy subjects.

oral minimal model; exercise; insulin sensitivity

IT IS WELL ESTABLISHED THAT exercise increases rates of glucose uptake ( $R_d$ ) and that rates of endogenous glucose production (EGP) must increase to meet the increased metabolic demands of the exercising muscle to prevent hypoglycemia (13, 16, 38, 40). These changes in glucose fluxes are facilitated by falling insulin and rising glucagon and catecholamine levels during exercise in healthy individuals (43). Although numerous studies have demonstrated increased  $R_d$  and EGP during physical activity in individuals with and without diabetes (19, 25) in the postabsorptive state, very few have examined the effects of exercise in the postprandial state in individuals with and

without type 2 diabetes (8, 9, 23, 26–28) and none in individuals with type 1 diabetes. Furthermore, very few studies (12) have used methods that minimize fluctuations in tracer-to-tracee specific activity to enable accurate continuous (every 10 min) measurement of glucose turnover and during the transition from rest to exercise in nondiabetic subjects. This latter point is important when developing a mathematical model for the next-generation artificial pancreas for type 1 diabetes.

Models of insulin action and secretion in response to physiological perturbations (e.g., oral glucose or mixed-meal challenges) have been developed and validated in humans with and without type 2 diabetes (1, 4). However, none of these methods have been tested or validated when these perturbations are further challenged by exercise. This is an important practical issue related to the model that needs to be tested since many people, with and without diabetes, exercise a few hours after a meal. Furthermore, lack of development and validation of such physiological models of whole body insulin sensitivity during exercise ( $SI_{\text{exercise}}$ ), especially in the postprandial state, precludes quantification of the effect and effect size of exercise on insulin sensitivity. This represents a significant knowledge gap and is important because accurate quantification of  $SI_{\text{exercise}}$ , especially in people with type 1 diabetes, could then perhaps be incorporated into currently available artificial pancreas control algorithms, thus extending their applicability and wider use to treat people with type 1 diabetes. However, such models will first need to be developed and tested in healthy subjects before validating in those with diabetes. Therefore, we conducted a triple-tracer mixed-meal study in healthy individuals where the subjects underwent moderate-intensity exercise, 2 h after meal ingestion. We used the tracer-tracee clamp method to estimate postprandial glucose fluxes during exercise and an integral formula (10) to estimate  $SI_{\text{exercise}}$ . The primary goal of this study therefore was to establish a novel method to quantify glucose turnover and derive a robust index of whole body insulin sensitivity when exercise is part of the postprandial period (viz.,  $SI_{\text{exercise}}$ ) using state-of-the-art methods. This would help consequently to quantify the effect size of exercise first in healthy individuals before applying such methods to quantify the effect size of exercise in those with type 1 diabetes. In addition, the availability of postprandial glucose fluxes in the presence of physical activity would enable development of a physiological model quantitatively describing the effect of exercise on glucose turnover. Once successful, the ultimate goal is to incorporate such a model into the next-generation closed-loop control algorithms to better manage individuals with type 1 diabetes.

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## RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board and following signed informed consent, 12 nondiabetic subjects were recruited. Inclusion criteria were age 18–60 yr, body mass index <40 kg/m<sup>2</sup>, HbA<sub>1c</sub> ≤5.5%, creatinine ≤1.5 mg/dl, normal fasting glucose and standard 75 grams oral glucose tolerance test (OGTT), and normal gastric emptying to solids and liquids. Exclusion criteria were significant gastrointestinal symptoms by questionnaire, documented recent upper gastrointestinal disorder, medications affecting gastric motility (e.g., erythromycin), pregnancy or breast feeding, or other comorbidities precluding participation. Medications (except stable thyroid hormone or hormone replacement therapy) that could influence glucose tolerance, history of diabetes in first-degree family members, or prior history of diabetes were also exclusionary. Subjects did not engage in vigorous physical activities for 72 h before screen and study visits. Each subject underwent two screen visits.

### Screen Visit 1

Subjects reported in the morning after an overnight fast to the Clinical Research Unit (CRU) of the Mayo Center for Translational Science Activities for a history, physical examination, screening laboratory tests, a 75-gram standard OGTT, standard urinalysis, and resting ECG. All women of childbearing potential had a negative pregnancy test within 24 h of the study visit. A dietary history was taken to ensure adherence to a weight-maintaining diet consisting of at least 200 g of carbohydrates/day and that diet met American Diabetes Association guidelines for protein, fat, and carbohydrates. Body composition was also measured using dual-energy X-ray absorptiometry (35). After completion of the OGTT, participants performed a graded exercise test on a treadmill to determine  $\dot{V}O_{2max}$  according to guidelines (American College of Sports Medicine Guidelines for Exercise Testing and Prescription, 7th Edition) and ensure stable cardiac status. Expired gases were collected and analyzed using indirect calorimetry.  $\dot{V}O_{2max}$  was determined when at least two of the following three criteria were met: 1) participant too tired to continue exercise, 2) respiratory exchange ratio exceeded 1.0; or 3) a plateau was reached in oxygen consumption with increasing workload. The purpose of this test was to use individual  $\dot{V}O_{2max}$  data to determine workload during the moderate-intensity (~50% of  $\dot{V}O_{2max}$ ) protocol during the study day.

### Screen Visit 2

With the use of established scintigraphic techniques (5), gastric emptying of solids and liquids was assessed in all subjects who were eligible after the first screening visit; results were summarized as the time required for 50% of solids and separately liquids to empty ( $T_{1/2}$ ). Thereafter, subjects who had normal gastric emptying for solids and liquids proceeded to the inpatient study visit within 3 wk of the screening visit.

### Study Visit

All subjects spent ~40 h in the CRU.

**Day 1.** Subjects were admitted to the CRU at ~1600. A point-of-care urine pregnancy test was performed where appropriate to ensure that the test was negative before proceeding any further. Thereafter, two Dexcom 7 plus continuous glucose monitors (CGM) and a Modular Signal Recorder accelerometer (MSR Electronics, Seuzach, Switzerland) were placed and maintained for the rest of the study period. They were then provided a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat) consumed between 1700 and 1730. No additional food was provided until the next morning. A heart rate monitor was also attached to capture heart rate during the study. An intravenous cannula was inserted in a forearm vein at ~2000 for tracer infusion and periodic blood draws during the study day the following morning.

**Day 2.** At ~0400 a primed continuous infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose was started. At ~0600, an intravenous cannula was inserted retrogradely into a hand vein for periodic blood draws. The hand was placed in a heated (55°C) Plexiglas box to enable drawing of arterialized-venous blood for glucose, glucose tracer, and hormone analyses. At ~0700 a triple-tracer mixed-meal study was performed (4). Briefly, a mixed meal containing 75 grams of glucose labeled with [1-<sup>13</sup>C]glucose was ingested at *time 0*. Simultaneously, an intravenous infusion of [6-<sup>3</sup>H]glucose was started and continued for the next 6 h at a variable rate to mimic the anticipated rate of appearance of the ingested [1-<sup>13</sup>C]glucose. Concurrently, the [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion rate was varied to mimic the anticipated rate of endogenous glucose. The meal provided ~33% of daily estimated calorie intake. At ~120 min following the first bite, subjects stepped on a treadmill to exercise at moderate-intensity activity (~50% of  $\dot{V}O_{2max}$ ): i.e., four bouts of walking at 3–4 miles/h for 15 min with rest periods of 5 min between each walking bout: total duration 75 min. The workload during physical activity was continuously monitored by heart rate responses and measurements of  $\dot{V}O_2$  during exercise to maintain target  $\dot{V}O_{2max}$  ~50% exercise intensity. The [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion rate was modified from the start of physical activity at 120 min for the next 3 h to mimic the anticipated changes in EGP during physical activity. To determine the optimal [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion rate necessary to minimize changes in tracer/tracee concentration for determination of postprandial EGP, we analyzed data from the first two subjects and modified the tracer infusion rates accordingly to minimize changes in tracer-to-tracee ratios. We did the same to also optimize the [6-<sup>3</sup>H]glucose infusion rate to minimize changes in [6-<sup>3</sup>H]glucose/[1-<sup>13</sup>C]glucose concentrations to enable accurate estimation of meal glucose appearance. CGM and accelerometer recordings were collected throughout the study period to monitor interstitial fluid glucose and quantitate activity levels, respectively, as part of another study. Following the last blood draw, all tracer infusions were stopped, and the hand vein cannula was removed. Lunch at 1300 and dinner at 1900 were provided, each meal contributing 33% of daily estimated caloric intake and having similar macronutrient composition as the breakfast meal.

From 1500 until 2200, subjects were asked to walk at 1.2 miles/h on the treadmill for 26.5 min in 1 h, i.e., 26.5 min on, 33.5 min off with time off during dinner at 1900. This activity represents low-grade activity levels that mimic activities of daily living (31). The continuation of the study for the rest of the day and overnight, including use of the CGM and accelerometer data, was part of additional investigation that is being currently analyzed for future reporting.

**Day 3.** Basal resting metabolic rate was measured at ~0630. After completion of the study at 0800, all cannulas were removed, and subjects were dismissed from the CRU.

### Study Meals

All meals were provided by the CRU metabolic kitchen. Study participants received three weighed meals (0700, 1300, and 1900), with each meal comprising 33% of total estimated calorie intake based on Harris Benedict calorie requirements (~75 grams of carbohydrate in each meal) adjusted for the level of physical activity. The macronutrient contents for the labeled and unlabeled meals that each participant consumed were identical. No snacks or calorie-containing drinks were permitted between meals.

**Triple-tracer mixed meal.** A primed-continuous infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose [11.84 mg/kg fat-free mass (FFM) prime; 0.1184 mg/kg FFM<sup>-1</sup>·min<sup>-1</sup> continuous; Mass-Trace, Woburn, MA] was started 3 h (~180 min) before the first bite of the breakfast mixed meal used to estimate postprandial glucose kinetics (4). Jell-O containing [1-<sup>13</sup>C]glucose was consumed within 15 min along with the rest of the mixed meal of eggs and ham/steak. An infusion of [6-<sup>3</sup>H]glucose was started at *time 0*, and the rate was varied to mimic the anticipated rate of appearance of the [1-<sup>13</sup>C]glucose contained within the meal. Simul-

taneously, the rate of infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose was altered to approximate the anticipated pattern of change in EGP (4, 35) with modifications to both tracer infusion rates as discussed earlier. Blood was sampled at periodic intervals for measurement of tracer-to-tracee ratios, glucose, insulin, glucagon, and C-peptide concentrations.

#### Analytical Techniques

**Hormone analyses.** C-peptide was measured on the Cobas e411 (Roche Diagnostics, Indianapolis, IN) using a two-site electrochemiluminescence immunometric assay. Insulin was measured by a two-site immunoenzymatic assay performed on the DxI automated system (Beckman Instruments, Chaska, MN) and glucagon by a direct, double-antibody radioimmunoassay (Linco Research, St. Charles, MO) (35).

**Glucose tracers.** Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at -80°C until assay. Plasma glucose concentration was measured using a glucose oxidase method (YSI, Yellow Springs, OH). Plasma [6-<sup>3</sup>H]glucose specific activity was measured by liquid scintillation counting. Plasma enrichment of [1-<sup>13</sup>C]glucose and [6,6-<sup>2</sup>H<sub>2</sub>]glucose was measured using GCMS (Thermoquest, San Jose, CA) to simultaneously quantitate C-1 to C-2 and C-3 to C-6 fragments (35).

#### Calculations

**Glucose turnover.** Fasting and postprandial rates of glucose turnover were calculated as previously described (4). Briefly, the systemically infused [6-<sup>3</sup>H]glucose was used to trace the systemic rate of appearance of [1-<sup>13</sup>C]glucose contained in the meal, whereas [6,6-<sup>2</sup>H<sub>2</sub>]glucose was used to trace the rate of appearance of endogenously produced glucose. The ratio of plasma concentration of [6-<sup>3</sup>H]glucose to [1-<sup>13</sup>C]glucose [specific activity, SA(*t*)] was used to calculate the rate of appearance of ingested [1-<sup>13</sup>C]glucose using a single-compartment model (33):

$$R_{a13C}(t) = \frac{INF_{3H}(t)}{SA(t)} - \frac{p \cdot V \cdot G_{13C}(t)}{SA(t)} \cdot \frac{dSA(t)}{dt} \quad (1)$$

where INF<sub>3H</sub> is the infusion rate of [6-<sup>3</sup>H]glucose, G<sub>13C</sub> is the plasma concentration of [1-<sup>13</sup>C]glucose, V is the volume of distribution, and p is the pool fraction, fixed to 200 ml/kg and 0.65, respectively, according to previous studies in normal subjects (33).

The total rate of appearance can be thus calculated as:

$$R_{a\text{ meal}}(t) = R_{a13C}(t) \cdot \left(1 + \frac{1}{TTR_{\text{meal}}}\right) \quad (2)$$

where TTR<sub>meal</sub> is the ratio of [1-<sup>13</sup>C]glucose and unlabeled glucose in the meal.

Similarly, the ratio of plasma concentration of [6,6-<sup>2</sup>H<sub>2</sub>]glucose to endogenously produced glucose (tracer-to-tracee ratio, TTR) was used to calculate EGP:

$$EGP(t) = \frac{INF_{2H2}(t)}{TTR(t)} - \frac{p \cdot V \cdot G_{\text{end}}(t)}{TTR(t)} \cdot \frac{dTTR(t)}{dt} \quad (3)$$

where INF<sub>2H2</sub> is the infusion rate of [6,6-<sup>2</sup>H<sub>2</sub>]glucose, G<sub>end</sub> is the plasma concentration of endogenous glucose [calculated by subtracting the concentration of exogenously derived (ingested) glucose (i.e., plasma [1-<sup>13</sup>C]glucose concentration multiplied by TTR<sub>meal</sub> [1-<sup>13</sup>C]glucose enrichment) from total plasma glucose concentration (4)], V is the volume of distribution, and p is the pool fraction, fixed to 200 ml/kg and 0.65, respectively.

Glucose rate of disappearance can then be calculated as:

$$R_d(t) = R_{a\text{ meal}}(t) + EGP(t) - p \cdot V \cdot \frac{dG(t)}{dt} \quad (4)$$

As evident from Eqs. 1, 3, and 4, calculation of glucose fluxes requires estimation of derivatives of SA, TTR, and glucose concentration. The presence of measurement error in the data makes the calculations nontrivial because of ill-conditioning. The derivative of a noisy signal can be obtained by solving, via regularized deconvolution, an input estimation problem (14), already employed in Refs. 1, 3, and 37, where the system is an integrator, the unknown input is the derivative, and the available output is the noisy signal itself. A key implicit assumption of standard regularized deconvolution is that the unknown input is a stationary signal (see APPENDIX for details). However, because of physical activity, the TTR and glucose may represent markedly nonstationary signals. Thus, in the present calculations, the problem has been reformulated relaxing this critical assumption. In particular, in a Bayesian framework, a priori expectations on the smoothness of the unknown derivative are formalized by modeling it as the single (or double) integration of a white noise process, with different variance in the presence or absence of physical activity (32) (see APPENDIX for details).

**Estimation of insulin sensitivity.** Insulin sensitivity, i.e., the ability of insulin to stimulate glucose disposal and suppress EGP, was estimated from plasma glucose and insulin concentrations with the integral formula (10), derived from the minimal model:

$$SI = \frac{f \cdot D \cdot \frac{AUC[\Delta G(t)/G(t)]}{AUC[\Delta G(t)]} - GE \cdot AUC[\Delta G(t)/G(t)]}{AUC[\Delta I(t)]} \quad (5)$$

where D is the amount of ingested glucose per kilogram body weight, f is the fraction of the absorbed dose that actually reaches plasma, G is plasma glucose concentration, ΔG its deviation from the basal level, ΔI is the above basal insulin concentration, AUC denotes the area under the curve, calculated with the trapezoidal rule, and GE is glucose effectiveness (fixed to population value according to Ref. 10).

Insulin sensitivity index (SI) was calculated for each subject, both in the absence (i.e., for *t* = 0–120 min, SI<sub>rest</sub>) and presence (*t* = 0–360 min, SI<sub>exercise</sub>) of physical activity. In the first case, the AUC was calculated from the beginning of the meal to the beginning of the exercise session, and f was the fraction of the dose that reached plasma in the first 2 h after the meal ingestion (estimated from the reconstructed R<sub>a meal</sub>). In the second case, AUC was calculated from the beginning of the meal to the end of the experiment, and f was the fraction of the dose that reached plasma in the 6 h following meal ingestion.

#### Statistical Analyses

Student's paired *t*-tests were performed to determine the effects of exercise on insulin sensitivity index. A *P* value of <0.05 was considered significant.

## RESULTS

#### Subject Characteristics

A total of 17 participants were screened for the study. There were three screen failures (1 subject withdrew after screen visit, 1 had abnormal rates of gastric emptying, and 1 could not consume study meals). Two additional participants were withdrawn after being successfully screened because of the inability to obtain adequate intravenous access. The remaining 12 subjects completed the study and comprised the study group. Subject characteristics are provided in Table 1 and breakfast composition in Table 2. Fasting glucose concentrations, HbA<sub>1c</sub>, and OGTT were normal. Gastric emptying rates for liquids (*T*<sub>1/2</sub>: M = 28.3, SE = 6.0 min) and solids (*T*<sub>1/2</sub>: M = 112.7, SE = 12.1 min) were normal in all subjects.

Table 1. *Subject characteristics*

Variable	Mean	SE	Range
Gender (F, %)		7 (58%)	
Age, yr	37.1	3.8	20.0, 55.0
Weight, kg	70.9	4.8	54.9, 115.8
Height, m	1.7	0.03	1.6, 1.9
Body mass index, kg/m <sup>2</sup>	24.1	1.1	19.1, 34.2
Fat-free mass, kg	50.9	3.9	37.2, 81.0
Body fat, %	29.1	2.0	20.5, 41.3
Lab results			
Fasting blood glucose, mmol/l	4.7	0.2	3.4, 5.4
HBA <sub>1c</sub> , %	5.1	0.1	4.7, 5.5
Hemoglobin, g/dl	13.9	0.4	12.0, 16.6
Creatinine, mg/dl	0.8	0.04	0.7, 1.1
BUN	14.4	1.4	9.0, 27.0
TSH, IU/l	2.4	0.4	0.5, 4.4
Gastric emptying test			
Solid ( <i>T</i> <sub>50</sub> ), min	112.7	12.1	54.4, 195.0
Liquid ( <i>T</i> <sub>50</sub> ), min	28.3	6.0	13.0, 86.0
$\dot{V}O_{2max}$ , ml · kg <sup>-1</sup> · min <sup>-1</sup>	32.3	2.1	19.1, 43.4

Anthropometric and screening test characteristics of enrolled subjects. F, female; BUN, blood urea nitrogen; TSH, thyroid-stimulating hormone; *T*<sub>50</sub>, half-time.

### Glucose, Insulin, C-Peptide, and Glucagon Concentrations

Plasma glucose concentrations rose from a baseline of 5.0 ± 0.18 mM to a peak of 9.1 ± 0.6 mM at *t* = 60 min, then dropped to 6.3 ± 0.6 mM at *t* = 120 min (start of exercise), and reached a nadir of 4.0 ± 0.3 mM at *t* = 140 min. Thereafter, plasma glucose concentrations gradually rose to 4.3 ± 0.2 mM at *t* = 195 min (end of exercise) before returning to baseline 5.0 ± 0.16 mM at *t* = 250 min, remaining constant thereafter until *t* = 360 min (Fig. 1).

Plasma insulin concentrations rose from a baseline of 25.9 ± 4.2 pM to a peak of 375.6 ± 47.4 pM at *t* = 60 min, then dropped to 228.0 ± 47.1 pM at *t* = 120 min (start of exercise), and reached a nadir of 33.8 ± 7.9 pM at *t* = 195 min (end of exercise) before gradually drifting to 31.4 ± 9.8 pM at *t* = 360 min.

Plasma C-peptide concentrations rose from a baseline of 0.55 ± 0.05 nM to a peak of 3.46 ± 0.3 nM at *t* = 90 min, then dropped to 2.9 ± 0.4 nM at *t* = 120 min (start of exercise), and reached 0.9 ± 0.1 nM at *t* = 195 min (end of exercise) before gradually drifting down to 0.6 ± 0.08 nM at *t* = 360 min.

In contrast, plasma glucagon concentrations at baseline were 59.3 ± 4.5 pg/ml, then briefly rose on meal ingestion before falling to a nadir of 54.3 ± 4.2 pg/ml at *t* = 60 min, then slowly rose to 64.0 ± 7.4 pg/ml at *t* = 120 min (start of exercise) before rapidly rising more than twofold to a peak of 135.0 ± 18.9 pg/ml at *t* = 180 min. Subsequently, glucagon concentrations gradually fell to 70.9 ± 6.9 pg/ml at *t* = 360 min.

### Tracer-to-Tracee Ratios

To reduce non-steady-state errors in calculating postprandial glucose turnover, it is important to minimize changes to tracer-to-tracee ratios. The systemically infused [6-<sup>3</sup>H]glucose was used to trace the systemic rate of appearance of [1-<sup>13</sup>C]glucose contained in the meal to enable measurement of the rate of appearance of meal glucose (MR<sub>a</sub>). As shown in Fig. 2, *top*, the specific activity, i.e., ratio of [6-<sup>3</sup>H]glucose (tracer)/[1-<sup>13</sup>C]g-

lucose (tracee) concentration, was fairly constant for almost the entire duration (10–360 min) of the study apart from the initial perturbations (0–10 min) that are unavoidable when both the intravenously infused tracer and orally ingested tracee are entering the systemic circulation. In contrast, physical activity starting at 120 min did not alter specific activity, demonstrating that the plasma concentrations of tracer and tracee changed in parallel throughout the study.

Simultaneously, [6,6-<sup>2</sup>H<sub>2</sub>]glucose was used as the tracer while endogenous glucose concentration was the tracee to calculate the rate of EGP. As shown in Fig. 2, *bottom*, the ratio [6,6-<sup>2</sup>H<sub>2</sub>]glucose (tracer)/endogenous glucose (tracee) concentrations was also fairly constant apart from a slight fall at ~120 min at the start of exercise. This nonstationary signal characteristic makes it necessary to modify the calculations of the derivative based on deconvolution, as detailed in the APPENDIX.

### Meal Appearance, EGP, and Glucose Disappearance

MR<sub>a</sub> in the systemic circulation reached a peak of 76.1 ± 9.1 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 60 min. The MR<sub>a</sub> gradually dropped to 57.8 ± 9.8 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 120 min (start of exercise) and then drifted down to 29.5 ± 5.0 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 180 min before rapidly tapering off by *t* = 250 min (Fig. 3).

Rates of EGP dropped rapidly in response to the meal from a baseline of 13.4 ± 1.1 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 0 min to a nadir of 0.3 ± 0.7 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 60 min. At *t* = 120 min (start of exercise) it was 2.4 ± 0.4 μmol·kg<sup>-1</sup>·min<sup>-1</sup> and then quickly rose almost eightfold in response to exercise to 18.2 ± 2.6 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 180 min. Thereafter, EGP rate gradually dropped down to baseline, finally reaching rates of 10.6 ± 0.9 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 360 min.

R<sub>d</sub> rose to a peak of 89.1 ± 11.6 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 120 min (start of exercise) and then declined to 26.4 ± 3.3 μmol·kg<sup>-1</sup>·min<sup>-1</sup> at *t* = 195 min (end of exercise) after reaching a plateau (44.9 ± 6.2 to 45.4 ± 6.5 μmol·kg<sup>-1</sup>·min<sup>-1</sup>) during exercise (*t* = 140–180 min, respectively). Thereafter, rates of R<sub>d</sub> returned to baseline at *t* = 360 min.

### Insulin Action

SI<sub>exercise</sub> was significantly higher than SI<sub>rest</sub> (21.6 ± 3.7 vs. 12.5 ± 2.0 10<sup>-4</sup> dl·kg<sup>-1</sup>·min<sup>-1</sup> per μU/ml, *P* < 0.0005). Of

Table 2. *Breakfast content on study day*

	Mean	SE	Range
<i>Meal composition of the breakfast meal that preceded exercise</i>			
Energy, kcal	637.5	55.7	505.6, 879.5
Carbs, g	76.1	0.2	75.6, 78.6
Protein, g	36.6	4.2	20.5, 52.8
Fat, g	19.6	1.6	13.5, 38.5
Fiber, g	0	0	0
<i>Meal macronutrient composition as a proportion of total meal calories</i>			
Carbs, %	53.3	1.5	50.3, 56.3
Protein, %	16.3	0.4	15.5, 17.1
Fat, %	30.4	1.7	30.6, 33.8

Carbs, carbohydrates.

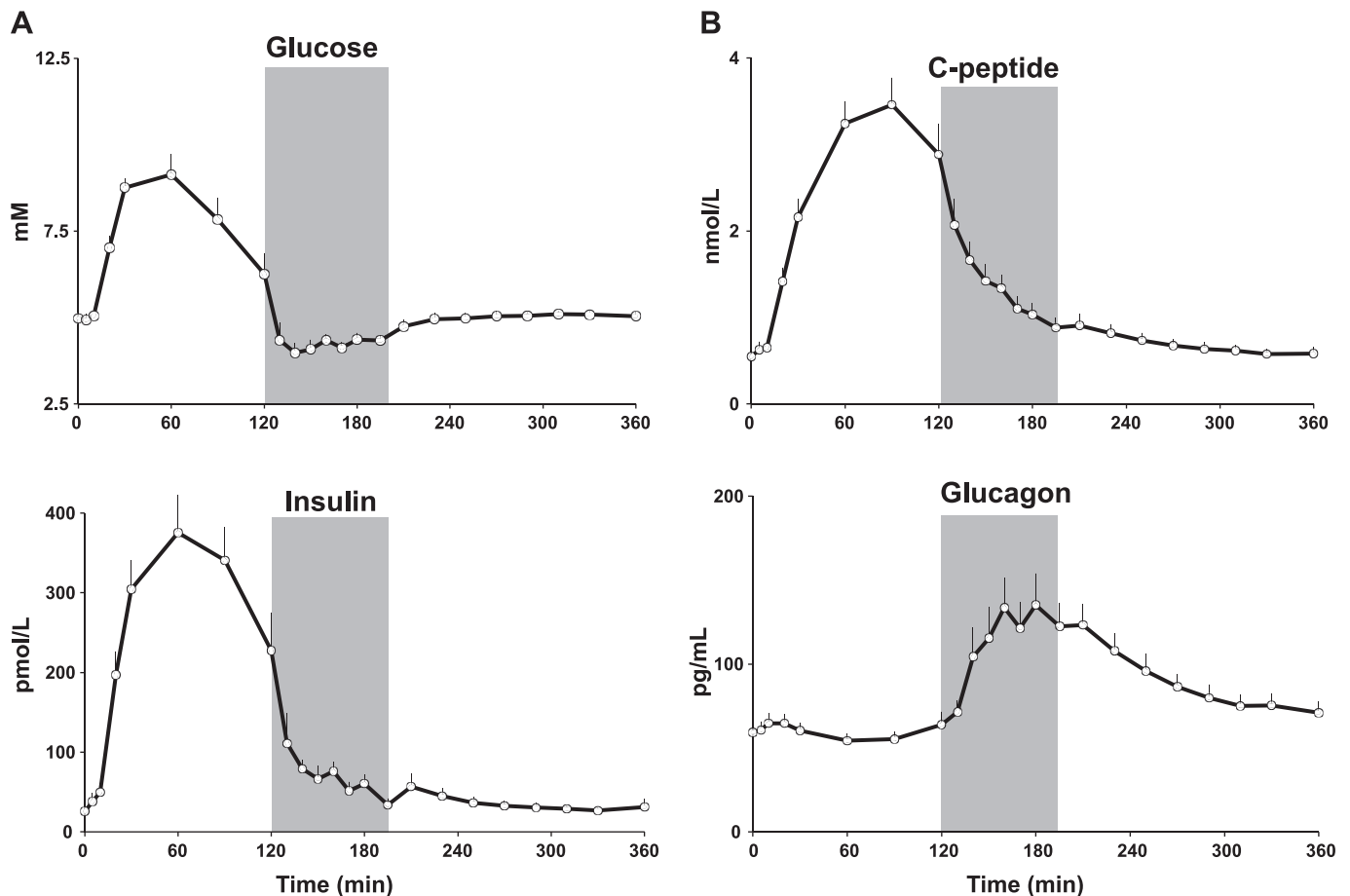


Fig. 1. A: glucose (top) and insulin (bottom) concentrations obtained from time 0 to 360 min. Shaded box between 120 and 195 min represents the exercise period at 50%  $\dot{V}O_{2max}$ . B: C-peptide (top) and glucagon (bottom) concentrations obtained from time 0 to 360 min. Shaded box between 120 and 195 min represents the exercise period at 50%  $\dot{V}O_{2max}$ .

note, all subjects demonstrated the same increasing trend of SI (Fig. 4, top) with exercise.

## DISCUSSION

While exercise is known to improve insulin action, quantification of the effect size on index of whole body insulin sensitivity has been difficult to estimate, especially in the postprandial state. Using a formula derived from the state-of-the-art oral minimal model (10), we have demonstrated an ~75% increase in model-derived whole body insulin sensitivity ( $SI_{exercise}$ ) index with moderate physical activity in healthy subjects. Using the triple-tracer technique, we have accurately estimated postprandial glucose turnover continuously after a meal, during and after exercise by successfully clamping tracer-to-tracee ratios. The unique value of this technique builds on the work of previous investigators who have made kinetic measurements during single discontinuous time points at baseline and during exercise. Our method confirms the findings of others and extends those by showing the time course of change in glucose  $R_d$  and EGP as well as insulin sensitivity. This technique furthers our understanding of glucose kinetics in healthy individuals after a meal, during exercise, and after exercise. These data are also a necessary advance clinically for the nationwide National Institutes of Health-sponsored effort to develop an artificial endocrine pancreas.

Effectively quantifying changes in insulin sensitivity during exercise is essential to the establishment of a mathematical model for the development of an artificial pancreas. It is well established that, during exercise, skeletal muscle glucose uptake ( $R_d$ ) increases through insulin-independent mechanisms, including increases in muscle blood flow, capillary recruitment, and exercise-induced translocation of GLUT4 protein (39). Furthermore, it is well established that a single endurance exercise bout increases insulin action on skeletal muscle (20, 21) in the postexercise period. Although the mechanism underlying the acute postexercise increase in insulin action on skeletal muscle is still under investigation, it is likely downstream of Akt and glycogen synthase kinase 3 or directly involved with glycogen synthase activation (42). Thus, quantification of  $SI_{exercise}$  will be very helpful to improve modern closed-loop algorithms that use glucose concentration and its change over time to predict future insulin needs.

Concomitant with changes to peripheral glucose uptake, EGP also has to increase to meet the increased metabolic demands of the exercising muscle to prevent hypoglycemia (13, 16, 38, 40). These changes in glucose fluxes are facilitated by falling insulin and rising glucagon and catecholamine levels during exercise (43). Our data concur with these observations while applying a triple-tracer method that permits accurate and

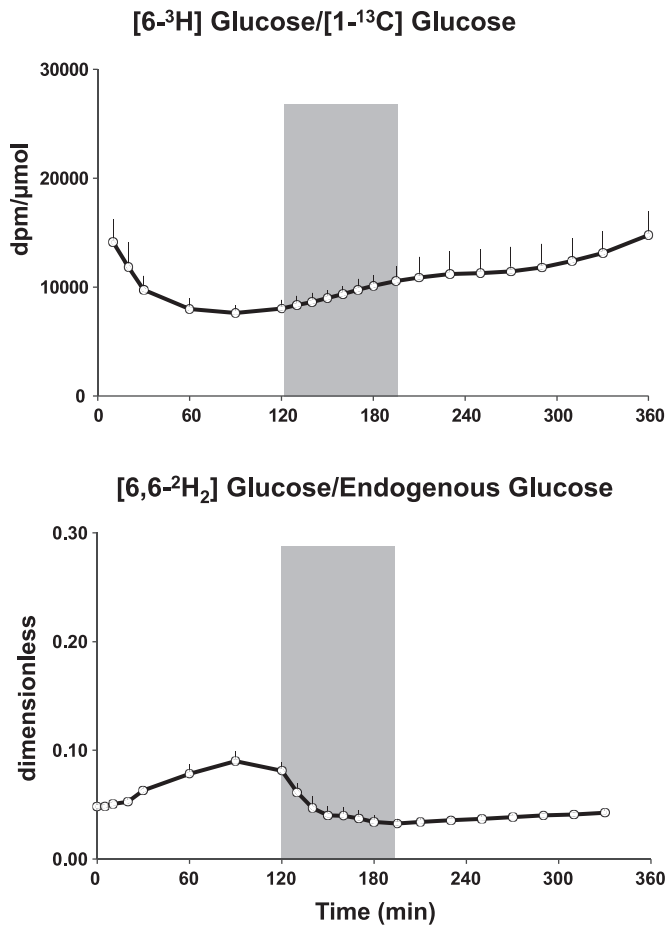


Fig. 2.  $[6\text{-}^3\text{H}]\text{glucose-to-}[1\text{-}^{13}\text{C}]\text{glucose}$  ratio (top) and  $[6,6\text{-}^2\text{H}_2]\text{glucose-to-}$ endogenous glucose ratio (bottom) obtained from time 0 to 360 min. Shaded box between 120 and 195 min represents exercise period at 50%  $\dot{V}O_{2\text{max}}$ .

continuous quantitation of postprandial glucose fluxes during moderate-intensity exercise. By modifying the tracer infusion rates, we helped minimize changes to tracer-to-tracee ratios to the best of our ability, hence minimizing non-steady-state

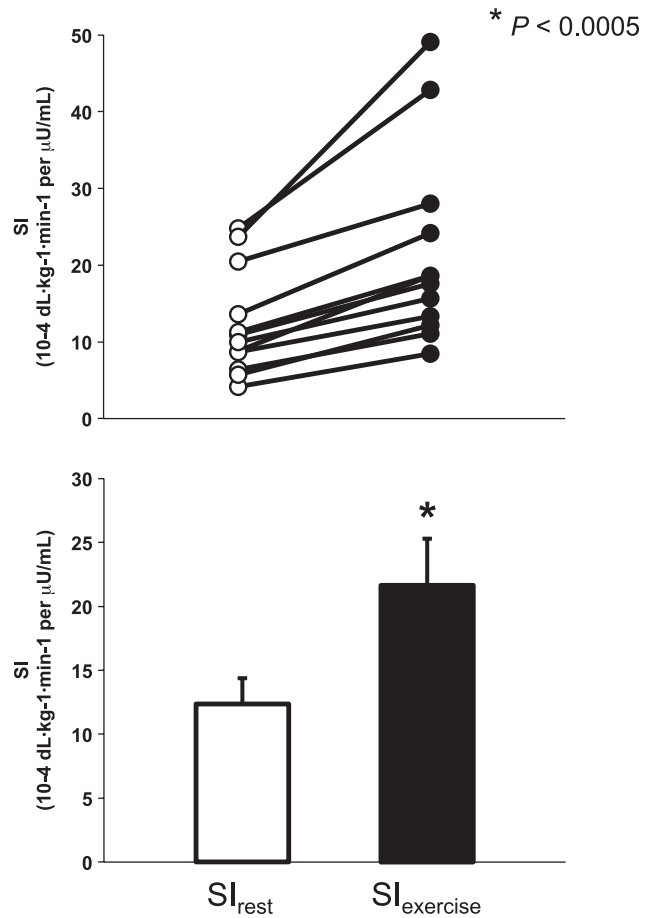


Fig. 4. Individual values of resting sensitivity index ( $SI_{\text{rest}}$ ) and the effect size of acute exercise on insulin sensitivity ( $SI_{\text{exercise}}$ ) (top) and mean  $SI_{\text{rest}}$  and  $SI_{\text{exercise}}$  (bottom) obtained during the study. SI, insulin sensitivity index.

errors in calculation of glucose turnover. The ratio of  $[6\text{-}^3\text{H}]\text{glucose}$  to  $[1\text{-}^{13}\text{C}]\text{glucose}$  applied to measure  $MR_a$  was minimally affected by exercise. On the other hand, although the ratio of  $[6,6\text{-}^2\text{H}_2]\text{glucose}$  to endogenous glucose applied to

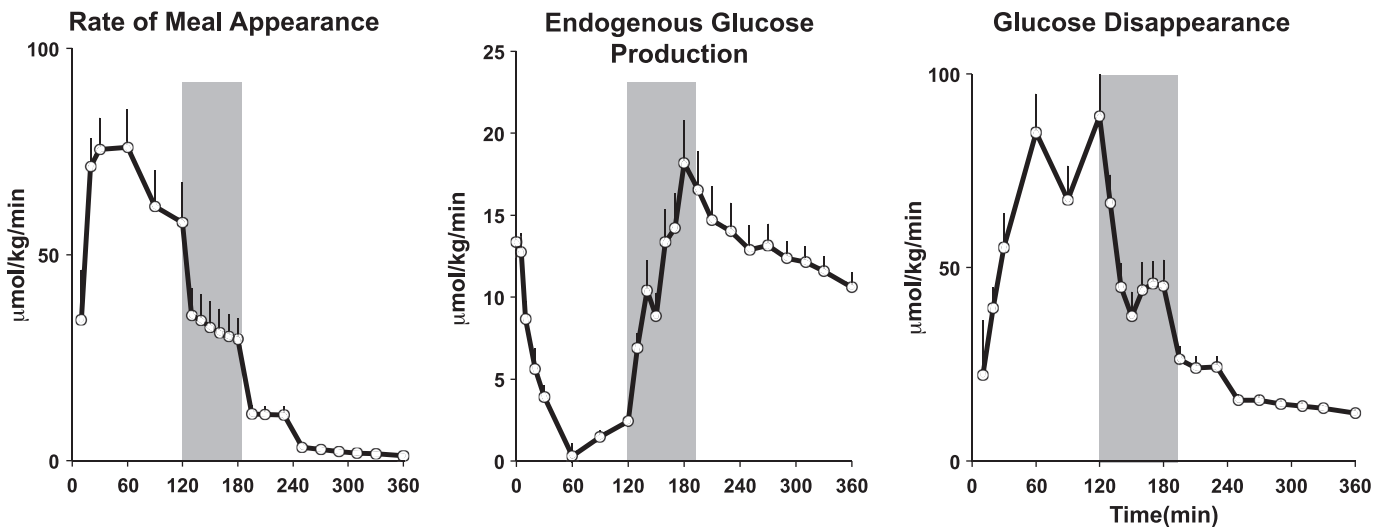


Fig. 3. Rates of meal appearance (top left), endogenous glucose production (top right), and glucose disappearance (bottom) obtained from time 0–360 min. Shaded box between 120 and 195 min represents the exercise period at 50%  $\dot{V}O_{2\text{max}}$ .

calculate EGP fell at the start of exercise, the fall was gentle and the ratio leveled off quickly.

Glucose concentrations rapidly fell by almost 2.3 mM ( $\sim 41$  mg/dl) from the start of exercise to a mean nadir of  $\sim 4$  mM ( $\sim 72$  mg/dl) during exercise, falling below baseline fasting levels in all subjects. In eight subjects, plasma glucose concentrations reached a nadir of  $<3.3$  mM ( $\sim 60$  mg/dl) during exercise with the lowest glucose of 2.5 mM ( $\sim 44$  mg/dl) in a subject. However, none of the participants experienced any symptoms of hypoglycemia. These changes were accompanied by a rapid fall in insulin concentrations together with greater than a twofold rise in glucagon concentrations. Predictably, the consequent change in the insulin-to-glucagon ratio resulted in an almost eightfold rise in rates of EGP from the start to end of exercise to limit hypoglycemia. However, we were unable to determine the relative contributions of glycogenolysis and gluconeogenesis to EGP in the postprandial state during this study. Furthermore, this study was not designed to determine rates of glycolysis or glucose oxidation to better parse out the intracellular fate of glucose. Presumably, acute exercise induced changes to intramuscular organelles, and mediators alluded to above maintained glucose uptake despite rapidly falling insulin concentrations during exercise.

The temporal patterns to postprandial glucose, insulin, and glucagon concentrations are in sharp contrast to our recent report (35) in an albeit different group of anthropometrically similar healthy subjects who underwent a triple-tracer mixed-meal study without postprandial exercise. In the prior report, postprandial glucose and insulin concentrations demonstrated a gradual decline after reaching peak values, and glucose concentrations gradually fell to baseline values within a comparable time frame without exercise. In contrast, in this study, glucose concentrations fell below baseline within a comparable time frame with exercise. Furthermore, plasma glucagon concentrations gently rose after the anticipated postprandial fall (34). These changes led to a gradual change in rates of EGP with a more rapid decline in rates of  $R_d$  after reaching their nadir and peak values, respectively. Although we realize that the subjects and experimental protocols differed between the current and prior (35) reports, the contrasting patterns of postprandial glucose and hormone concentrations and glucose kinetics with and without exercise are noteworthy, especially since the subjects were anthropometrically very similar. In the current study, there were rapid changes to glucose, insulin, and glucagon concentrations with exercise accompanied by an eightfold increase in rates of EGP.

$MR_a$  fell rapidly during the first 10 min of exercise ( $t = 120$ – $130$  min) but thereafter reached a plateau at rates similar to that observed at  $t = 10$  min for the remainder of the exercise period. Subsequently,  $MR_a$  rapidly declined to near zero by  $t = 240$  min (i.e., within 35 min) after completion of exercise. In contrast,  $MR_a$  continued to decline steadily after  $t = 120$  min in the prior report when subjects were resting (35). The transient plateau in  $MR_a$  during exercise could be because of an exercise-induced increase in visceral sympathetic nerve activity delaying gastric emptying rates. Although we did not directly measure rates of gastric emptying during exercise in the current study,  $MR_a$  could be an effective surrogate measure of gastric emptying rates in otherwise healthy adults with

normal gastric emptying rates. Prior reports on the direct effects of exercise on rates of gastric emptying have been conflicting, with reports demonstrating either delayed (29, 30, 34, 36) or no effects (7, 15) on gastric emptying rates in healthy adults. It is possible that the timing of exercise in relation to the meal is an important determinant of  $MR_a$ . Therefore, an earlier start to the exercise after the meal could have had a greater effect on  $MR_a$ . Furthermore, humoral factor(s) that could have contributed to the plateau in  $MR_a$  includes the rise in glucagon concentrations during exercise. Glucagon is known to possess decelerating effects on gastric motility (41).

Glucose concentration at any given time point is a function of the rate of glucose entering and leaving the circulation. This concept is true in both the postabsorptive and postprandial situations. Because exercise is known to increase the rate of glucose leaving the circulation (i.e.,  $R_d$ ), rates of glucose entering the circulation in the postprandial state (i.e., EGP and/or  $MR_a$ ) must also increase to compensate for the physiological increase in  $R_d$  to prevent hypoglycemia. The current approach enabled us to tease out the effects of all of these interactive factors simultaneously when exercise was conducted in the postprandial state. Because glucose concentrations fell during exercise, the rate of glucose leaving the circulation (i.e.,  $R_d$ ) must have exceeded the rate of glucose entering the circulation (i.e., combined rates of EGP and  $MR_a$ ). It is noteworthy, however, that these dynamic changes were occurring in the presence of rapidly falling insulin concentrations during exercise. Although the rise in EGP could be explained by both falling insulin and rising glucagon concentrations, the plateau in  $R_d$  can be explained by increasing muscle glucose uptake by both insulin-independent and -dependent mechanisms. As discussed above, the sustained rate of  $R_d$  during exercise was only in part compensated by the eightfold increase in EGP and a transient plateau in the rate of  $MR_a$  from the gastrointestinal tract. Despite these physiological compensatory mechanisms, biochemical hypoglycemia was not prevented in 8 of 12 subjects. Furthermore, exercise is also known to increase rates of blood flow to exercising muscles, thereby contributing, at least in part, to enhanced muscle glucose uptake (22). However, the extent to which these physiological effects of exercise on the circulation are affected by the postprandial state cannot be determined from this study.

There have been prior publications that have reported on the effect(s) of exercise of varying intensity on aspects of glucose physiology. These have included pioneering work by exercise physiologists using isotope dilution techniques and glucose clamps in healthy adults. Friedlander et al. (17, 18) demonstrated that whole body glucose uptake was proportional to exercise intensity. Interesting gender differences in glucose kinetics and hormonal responses were also observed (18, 24), but not when a meal was given before exercise (9, 23). Our studies were conducted in recreationally active but untrained men and women.

Our exercise trial measured SI during a single fixed level of 15 min of 50%  $\dot{V}O_{2max}$  exercise with 5-min rest periods. This study design was chosen for several reasons. First, the Juvenile Diabetes Research Foundation inpatient control to range automated closed loop trial used this exercise trial protocol (6). Previous reports by other investigators at constant workloads in

the postprandial state have found both similar and divergent results to ours. An acute bout of moderate-intensity exercise 3 h after a solid (23) or liquid (9) meal resulted in a threefold increase in glucose  $R_d$ , whereas recovery from exercise in the postexercise period is distinguished by a continuous decline in glucose  $R_d$  for up to 180 min (23). Our results show an exercise-induced increase in glucose  $R_d$  compared with our initial measurement (*time point 10*), but 120 min from the start of the meal was not enough time to allow the participants to clear the meal (Fig. 3A). Our absolute values for glucose  $R_d$  are higher than those with similar protocols (9, 23), likely attributed to both the absorption of the meal, since exogenous meal glucose during exercise elevates glucose  $R_d$  (8), and mathematical modeling differences between the studies. Furthermore, the blood sampling time to measure glucose fluxes was every 10 min during the 1st h of exercise in this study. Hence, an earlier change in  $R_d$  induced by exercise, as described by Coggan et al. (11) during 80%  $\dot{V}O_{2\max}$  exercise where sampling time was every 5 min, would have been missed by us. Our postexercise time period does show a steady decline in glucose  $R_d$  as has been seen by others (23) under similar conditions. Future studies will need to be conducted to determine if the effects of exercise at various intensities on SI are monotonic and whether training affects this relationship on postprandial insulin sensitivity.

A limitation of the applied method is that it relies on both glucose and insulin inputs to estimate global whole body  $SI_{\text{exercise}}$ . It cannot therefore specifically tease out the relatively insulin-independent effects on muscle glucose uptake induced by exercise. We fully agree that glucose disposal during and after exercise is also determined by insulin-independent effects of exercise, but the model applied cannot determine the effect size and contribution of such insulin-independent effects of exercise. Future more sophisticated protocols will need to be developed to determine the extent of insulin-independent effects of exercise on glucose excursions. In addition, the oral minimal model and the integral formula applied in this paper use insulin and glucose as their inputs and as such measure the net effect of insulin on glucose disposal and production before, during, and postexercise. However, the inhibitory effect of insulin on EGP is relatively minor compared with the stimulatory effect of glucagon on EGP. Thus, the measure of insulin sensitivity most likely reflects the effect of exercise on glucose disposal. Furthermore, although the triple-tracer approach is able to determine, with a fair degree of accuracy, time-dependent changes to whole body  $R_d$ , one must realize that, apart from the skeletal muscle,  $R_d$  also includes glucose uptake in the liver, splanchnic tissues, central nervous system, red blood cells, etc. Therefore, to specifically determine the effects of exercise on postprandial muscle glucose uptake, invasive limb catheterization studies would be necessary. Furthermore, ideally one would have preferred the subjects return on another occasion for a mixed-meal triple-tracer study without exercise, with the two visits conducted in random order. That said, our primary goal was to determine the feasibility of the triple-tracer approach to reliably measure postprandial glucose fluxes and the assessment of the insulin sensitivity index with a formula derived from the oral minimal model during exercise. We reasoned that, for a given individual, his/her  $SI_{\text{rest}}$  (estimated from 0 to 120 min after the meal when subjects were resting) would be a reliable estimate of their resting postprandial SI,

therefore allowing a reasonable comparison of  $SI_{\text{rest}}$  vs.  $SI_{\text{exercise}}$  in each subject and thus enabling assessment of the effect size of exercise on SI. Furthermore, having the subjects return on another day would have added the complexities of day-to-day variability of SI (an uninvestigated factor at this stage) into the mix. However, lack of a control study day without exercise precludes precise quantification of the effect of exercise in the same individual.

In conclusion, we have described, to the best of our knowledge for the first time, glucose-insulin physiology, using the triple-tracer approach, to measure postprandial glucose turnover during moderate-intensity exercise. We observe a rapid eightfold increase in rates of EGP, with a plateau in the rates of  $MR_a$  and  $R_d$  during exercise in these individuals. We have also introduced the index  $SI_{\text{exercise}}$ , which reflects a parameter of whole body insulin sensitivity (the ability of insulin to promote glucose uptake and inhibit EGP) during exercise in the postprandial state. We report that SI increases by  $\sim 75\%$  during moderate-intensity exercise in recreationally active healthy adults when exercise is conducted in starting 2 h into the postprandial state. Therefore, by virtue of the index  $SI_{\text{exercise}}$ , we have successfully quantified the effect size of exercise on model-derived whole body insulin sensitivity index using this approach. Future studies will need to be conducted to determine effect sizes of exercise of various intensities and characteristics on SI, especially in those with diabetes, to better inform closed-loop control algorithms currently being developed to treat type 1 diabetes.

## APPENDIX

According to regularized deconvolution (14), the derivative of the noisy TTR (and glucose concentration) can be calculated by solving the following optimization problem:

$$\hat{\mathbf{u}} = \underset{\mathbf{u}}{\operatorname{argmin}} (\mathbf{y} - \mathbf{H}\mathbf{u})^T \sum_{\mathbf{v}}^{-1} (\mathbf{y} - \mathbf{H}\mathbf{u}) + \mathbf{u}^T \sum_{\mathbf{u}}^{-1} \mathbf{u} \quad (A1)$$

where  $\mathbf{y}$  is the vector containing the available TTR (or glucose) samples,  $\sum_{\mathbf{v}}$  is the covariance matrix of the measurement error vector  $\mathbf{v}$ ,  $\mathbf{H}$  is a Toeplitz matrix whose first column represents the impulse response of the discrete single integrator,  $\mathbf{u}$  is the vector of the unknown derivatives, and  $\sum_{\mathbf{u}}$  is its a priori covariance matrix. As far as the latter matrix is concerned, the deconvolution method presented in Ref. 14, and employed in Refs. 1, 2, and 37, assumes that, in a Bayesian embedding, expectations on the smoothness of the unknown input  $\mathbf{u}$  can be formalized by describing it a priori as the single (or double) integration of a stationary white noise process, leading to:

$$\sum_{\mathbf{u}} = \mathbf{F}^{-1} \Lambda \mathbf{F}^{-T} \quad \text{with} \quad \Lambda = \lambda^2 \cdot \mathbf{I} \quad (A2)$$

where  $\lambda^2$  is the constant variance of the zero-mean white noise process,  $\mathbf{I}$  the identity matrix, and  $\mathbf{F}$  is a lower triangular Toeplitz matrix whose first column contains the impulse response of the first (or second-)order discrete differentiator.

However, because of physical activity, both the TTR and glucose represent marked nonstationary signals, making the above-described method unusable to calculate the derivative. Thus, in the present paper, the problem has been reformulated, relaxing the stationary assumption. In particular, the unknown derivative is modeled as the discrete integration of a nonstationary white noise process, with different variance in the absence ( $\lambda_1^2$ ) or presence ( $\lambda_2^2$ ) of physical activity (32), leading to:

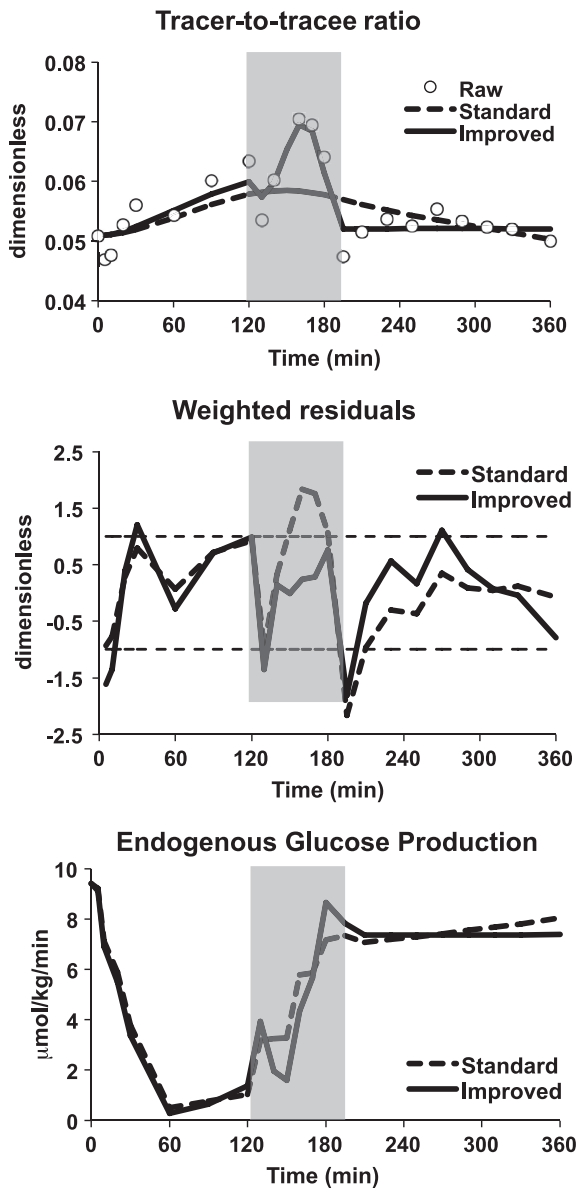


Fig. 5. An example of comparison between the performance of the deconvolution algorithm accounting (new method) vs. not accounting for nonstationary tracer-to-tracee ratio (TTR) signal (standard method). TTR (*top*), weighted residuals (*middle*), and estimated endogenous glucose production (*bottom*). Gray area represents the period of exercise.

$$\Lambda = \begin{bmatrix} \lambda_1^2 & 0 \dots 0 & 0 \dots 0 & 0 & 0 \\ 0 & \lambda_1^2 \dots 0 & 0 \dots 0 & 0 & 0 \\ \dots & \dots & \dots & \dots & \dots \\ 0 & 0 \dots \lambda_2^2 & 0 \dots 0 & 0 & 0 \\ 0 & 0 \dots 0 & \lambda_2^2 \dots 0 & 0 & 0 \\ \dots & \dots & \dots & \dots & \dots \\ 0 & 0 \dots 0 & 0 \dots 0 & \lambda_1^2 & 0 \end{bmatrix} \quad (A3)$$

Figure 5 shows an example of the performance of the new method vs. that not accounting for a nonstationary factor.

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The data has been presented in part at the Diabetes Technology Meeting at Bethesda, MD, in November 2012, at Advanced Technologies and Treatments for Diabetes Meeting at Paris, France, in February 2013, and will be presented in part at the Annual Scientific Meeting of the American Diabetes Association at Chicago, IL, in June 2013.

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DISCLOSURES

Dr. Ananda Basu is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis. There are no conflicts of interest to declare for any of the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.S., L.H., A.M., C.D.M., G.S., Y.C.K., C.C., and A.B. analyzed data; M.S., C.D.M., G.S., M.L.J., R.E.C., R.B., Y.C.K., C.C., and A.B. interpreted results of experiments; M.S., L.H., A.M., C.D.M., G.S., M.L.J., R.E.C., R.B., Y.C.K., C.C., and A.B. edited and revised manuscript; M.S., L.H., A.M., C.D.M., G.S., M.L.J., R.E.C., R.B., Y.C.K., C.C., and A.B. approved final version of manuscript; L.H., Y.C.K., and A.B. performed experiments; L.H., A.M., C.D.M., Y.C.K., and A.B. prepared figures; C.D.M., R.B., Y.C.K., C.C., and A.B. conception and design of research; C.D.M., Y.C.K., and A.B. drafted manuscript.

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