Insulin Sensitivity During Exercise

Postprandial Glucose Fluxes and Insulin Sensitivity during Exercise:

A Study in Healthy Individuals

Michele Schiavon*1
Ling Hinshaw1
Ashwini Mallad
Chiara Dalla Man*
Giovanni Sparacino*
Matthew Johnson
Rickey Carter#
Rita Basu
Yogish Kudva
Claudio Cobelli*
Ananda Basu.

Running Title: Insulin sensitivity during exercise

Key Words: oral minimal model, exercise, insulin sensitivity

1 denotes equal contribution
# Department of Health Sciences Research, Mayo College of Medicine,
*Department of Information Engineering,
University of Padova, Via Gradenigo 6A, Padova, Italy
5-194 Joseph, Endocrine Research Unit,
Division of Endocrinology and Metabolism, Mayo College of Medicine,
Rochester, MN 55905, USA
Corresponding Author: basu.ananda@mayo.edu
Tel: 507-255-6515; Fax 507-255-4828.
Insulin Sensitivity During Exercise

LH, AM, RB, YK and AB assisted in study conduct, data gathering and analyses, manuscript writing and editing. MS, CDM, GS, MJ, RC and CC assisted in data analyses, manuscript writing and editing.
Abstract:

Quantifying the effect size of acute exercise on insulin sensitivity (SI_exercise) and simultaneous measurement of glucose disappearance (Rd), endogenous glucose production (EGP) and meal glucose appearance (MRa) in the postprandial state has not been developed in humans. To do so, we studied 12 healthy subjects (5 men, age 37.1±3.1 yrs, BMI 24.1±1.1 kg/m², Fat Free Mass [FFM] 50.9±3.9 kg) during moderate exercise at 50% VO2 max for 75 minutes, 120-195 min after a triple tracer mixed meal consumed at time 0. Tracer infusion rates were adjusted to achieve constant tracer-tracee ratio and minimize nonsteady state errors. Glucose turnover was estimated by accounting for the non-stationary kinetics introduced by exercise. Insulin sensitivity index was calculated in each subject both in absence (t=0-120 min, SI_rest) and presence of physical activity (t=0-360 min, SI_exercise). EGP at t=0 min (13.4±1.1 μM/kgFFM/min) fell at t=120 min (2.4±0.4 μM/kgFFM/min) then rapidly rose almost eight fold at t=180 min (18.2±2.6 μM/kgFFM/min) before gradually falling at t=360 min (10.6±0.9 μM/kgFFM/min). Rd rapidly peaked at t=120 min at start of exercise (89.5 ±11.6 μM/kgFFM/min) then gradually declined at t=195 min (26.4 ±3.3 μM/kgFFM/min) before returning to baseline at t=360 min. SI_exercise was significantly higher than SI_rest (21.6±3.7 vs. 12.5±2.0 10^-4 dl/kg/min per μU/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.
Insulin Sensitivity During Exercise

Introduction:

It is well established that exercise increases rates of glucose uptake (Rd) and that rates of endogenous glucose production (EGP) must increase to meet the increased metabolic demands of the exercising muscle to prevent hypoglycemia (16, 38) (13, 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 Rd and EGP during physical activity in individuals with and without diabetes (19, 25) in the post absorptive state, very few have examined the effects of exercise in the post prandial state in individuals with and without T2D (9, 23) (8, 26-28) and none in individuals with T1D. Furthermore, very few studies (12) have utilized methods that minimize fluctuations in tracer/tracee specific activity to enable accurate continuous (every 10 minutes) 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_{exercise}), especially in the post prandial 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
Insulin Sensitivity During Exercise

quantification of SI_{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, two hours 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_{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_{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 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.

**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 years, BMI
<40 kg/m², HbA1c ≤ 5.5%, creatinine ≤ 1.5 mg/dl, normal fasting glucose and standard 75 grams
oral glucose tolerance test 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 hours prior to 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 CTSA for a history, physical examination, screening laboratory tests, a 75 grams standard oral glucose tolerance test (OGTT), standard urinalysis and resting ECG. All women of childbearing potential had a negative pregnancy test within 24 hours of study visit. A dietary history was taken to ensure adherence to a weight maintaining diet consisting of at least 200 grams of carbohydrates per day and that diet met ADA guidelines for protein, fat, and carbohydrates. Body composition was also measured using DXA (35). After completion of the OGTT, participants performed a graded exercise test on a treadmill to determine VO2max 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. VO2max was determined when at least two of three criteria were met: 1) Participant too tired to continue exercise 2) respiratory exchange ratio exceeded 1.0; 3) a plateau was reached in oxygen consumption with increasing workload. The purpose of this test was to use individual VO2max data to determine workload during the moderate intensity (~50% of VO2 max) protocol during the study day.

**Screen visit 2:** Using established scintigraphic techniques (5), gastric emptying of solids and liquids were assessed in all subjects who were eligible after the first screening visit; results were
Insulin Sensitivity During Exercise

summarized as the time required for 50% of solids and separately liquids to empty (Ge T\(^1/2\)).

Thereafter, subjects who had normal gastric emptying for solids and liquids proceeded to the
inpatient study visit within 3 weeks of the second screening visit.

**Study Visit:** All subjects spent ~ 40 hours in the CRU.

**Day 1:** Subjects were admitted to the CRU at ~1600 hours. 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 (MSR) accelerometer (MSR Electronics GmbH, 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. Intravenous cannula was
inserted into a forearm vein at approximately 2000 hours for tracer infusion and periodic blood
draws during the study day the following morning.

**Day 2:** At ~ 0400 hours a primed continuous infusion of [6,6-\(^2\)H\(_2\)] glucose was started.
At ~ 0600 hrs., an intravenous cannula was inserted retrogradely into a hand vein for periodic
blood draws. The hand was placed in a heated (55°C) plexiglass box to enable drawing of
arterialized-venous blood for glucose, glucose tracer and hormone analyses. At approximately
0700 hours a triple tracer mixed meal study was performed (4). Briefly, a mixed meal containing
75 grams of glucose labeled with [1-\(^13\)C] glucose was ingested at time 0. Simultaneously, an
intravenous infusion of [6-3H] glucose was started and continued for the next six hours at a
variable rate to mimic the anticipated rate of appearance of the ingested [1-\(^13\)C] glucose.
Concurrently, the [6,6-\(^2\)H\(_2\)] glucose infusion rate was varied to mimic the anticipated rate of
Insulin Sensitivity During Exercise

endogenous glucose. The meal provided ~33% of daily estimated calorie intake. At ~ 120
minutes following the first bite, subjects stepped on a treadmill to exercise at moderate intensity
activity (~50% of VO₂ max): i.e., 4 bouts of walking at 3-4 mph for 15 minutes with rest periods
of 5 minutes between each walking bout: total duration 75 minutes. The workload during
physical activity was continuously monitored by heart rate responses and measurements of VO₂
during exercise to maintain target VO₂ max ~50% exercise intensity. The [6,6-²H₂] glucose
infusion rate was modified from the start of physical activity at 120 min for the next three hours
to mimic the anticipated changes in EGP during physical activity. To determine the optimal [6,6-
²H₂] glucose infusion rate necessary to minimize changes in tracer/tracee concentration for
determination of post prandial EGP we analyzed data from the first 2 subjects and modified the
tracer infusion rates accordingly to minimize changes in tracer/tracee ratios. We did the same to
also optimize the [6-³H] glucose infusion rate to minimize changes in [6-³H] glucose / [1-¹³C]
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 removed. Lunch at 1300
hours and dinner at 1900 hours were provided, each meal contributing 33% of daily estimated
caloric intake and having similar macronutrient composition as the breakfast meal.

From 1500 hrs until 2200 hrs, subjects were asked to walk at 1.2 miles/hour on the
treadmill for 26.5 minutes in an hour, i.e., 26.5 minutes on, 33.5 minutes off with time off during
dinner at 1900. This activity represents low grade activity levels that mimic activities of daily
living (ADL) (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 approximately 0630 hours. After completion of the study at 0800 h, all cannulae were removed and subjects 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 hours) 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 $[6,6 \, ^2\text{H}_2]$ glucose ($11.84 \, \text{mg/kg FFM prime;} \, 0.1184 \, \text{mg/kg FFM/min continuous;} \, \text{Mass-Trace, Woburn, MA}$) was started three hours (~180 min) prior to the first bite of the breakfast mixed meal used to estimate post prandial glucose kinetics (4). Jell-O containing $[1\,-\,^{13}\text{C}]$ glucose was consumed within fifteen minutes along with the rest of the mixed meal of eggs and ham/steak. An infusion of $[6\,-\,^{3}\text{H}]$ glucose was started at time 0, and the rate varied to mimic the anticipated rate of appearance of the $[1\,-\,^{13}\text{C}]$ glucose contained within the meal. Simultaneously, the rate of infusion of $[6,6\,-\,^{2}\text{H}_2]$ glucose was altered to approximate the anticipated pattern of change in endogenous glucose production (4, 35) with modifications to both the tracer infusion rates as discussed earlier. Blood was sampled at periodic intervals for measurement of tracer-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 2-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, Inc., Yellow Springs, OH). Plasma [6-3H] glucose specific activity was measured by liquid scintillation counting. Plasma enrichment of [1-13C] glucose and [6,6-2H2] glucose were measured using GCMS (Thermoquest, San Jose, CA) to simultaneously quantitate C1,2 and C3-6 fragments (35).

Calculations:

Glucose Turnover: Fasting and postprandial rates of glucose turnover were calculated as previously described (4). Briefly, the systemically infused [6-3H] glucose was used to trace the systemic rate of appearance of [1-13C] glucose contained in the meal whereas [6,6-2H2] glucose was used to trace the rate of appearance of endogenously produced glucose. The ratio of plasma concentration of [6-3H] glucose to [1-13C] glucose (specific activity, SA(t)) was used to calculate the rate of appearance of ingested [1-13C] glucose using a single compartment model (33):

\[
Ra_{13C}(t) = \frac{INF_{3H}(t)}{SA(t)} - \frac{p \cdot V \cdot G_{13C}(t)}{SA(t)} \cdot \frac{dSA(t)}{dt}
\]

(1)
where $INF_{3H}$ is the infusion rate of $[6^{-2}H]$ glucose, $G_{13C}$ is the plasma concentration of $[1^{-13}C]$ 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:

$$Ra_{meal}(t) = Ra_{13C}(t) \cdot \left(1 + \frac{1}{TTR_{meal}}\right)$$  \hspace{1cm} (2)

where $TTR_{meal}$ is the ratio of $[1^{-13}C]$ glucose and unlabeled glucose in the meal.

Similarly, the ratio of plasma concentration of $[6,6^{-2}H_2]$ glucose to endogenously produced glucose (tracer-to-tracee ratio, TTR) was used to calculate EGP:

$$EGP(t) = \frac{INF_{2H2}(t)}{TTR(t)} - p \cdot V \cdot G_{end}(t) \cdot \frac{dTTR(t)}{dt}$$  \hspace{1cm} (3)

where $INF_{2H2}$ is the infusion rate of $[6,6^{-2}H_2]$ glucose, $G_{end}$ is the plasma concentration of endogenous glucose (calculated by subtracting the concentration of exogenously derived (ingested) glucose (i.e. plasma $[1^{-13}C]$ glucose concentration multiplied by TTR meal $[1^{-13}C]$ 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:

$$R_d(t) = Ra_{meal}(t) + EGP(t) - p \cdot V \frac{dG(t)}{dt}$$  \hspace{1cm} (4)

As evident from equations (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 non-trivial 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 (1, 3, 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 A1, for details). However, due to physical activity, the TTR and glucose may represent markedly non-stationary 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 presence or absence of physical activity (32) (see Appendix A1, for details).

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

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

where \(D\) is the amount of ingested glucose per kg body weight, \(f\) is the fraction of the absorbed dose which actually reaches plasma, \(G\) is plasma glucose concentration, \(\Delta G\) its deviation from basal level, \(\Delta I\) 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 (10)).
Insulin Sensitivity During Exercise

SI was calculated for each subject, both in absence (i.e. for t=0-120 min, SI_{rest}) and presence of physical activity (t=0-360 min, SI_{exercise}). 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 which reached plasma in the first two hours after the meal ingestion (estimated from the reconstructed Ra_{meal}). In the second case, AUC was calculated from the beginning of the meal to the end of the experiment, and \( f \) was fraction of the dose which reached plasma in the six hours following meal ingestion.

**Statistical Analyses:** Student’s paired t-tests were performed to determine effects of exercise on insulin sensitivity index. A p value of < 0.05 was considered significant.

**Results:**

**Subject Characteristics (Tables 1 and 2):**

A total of 17 participants were screened for the study. There were 3 screen failures (1 subject withdrew after screen visit, 1 had abnormal rates of gastric emptying, 1 could not consume study meals). Two additional participants were withdrawn after being successfully screened due to 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, HbA1c and OGTT were normal. Gastric emptying rates for liquids (\( T^{1/2}: M=28.3, \text{SEM}=6.0 \) min) and solids (\( T^{1/2}: M=112.7, \text{SEM}=12.1 \) min) were normal in all subjects.

Glucose, insulin, C-peptide and glucagon concentrations (Figure 1).
Plasma glucose concentrations rose from 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±0.6mM 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±0.2mM at t=195 min (end of exercise) before returning to baseline 5.0±0.16mM at t=250 min, remaining constant thereafter until t=360 min.

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 was 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 two-fold 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-Tracee ratios (Figure 2):

To reduce non-steady state errors in calculating post prandial glucose turnover, it is important to minimize changes to tracer-tracee ratios. The systemically infused [6-3H] glucose was used to trace the systemic rate of appearance of [1-13C] glucose contained in the meal to enable measurement of rate of appearance of meal glucose (MRa). As shown in Figure 2 (top panel), the
specific activity, i.e., ratio $[6^{-3}\text{H}]$ glucose (tracer)/$[1^{-13}\text{C}]$ glucose (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, $[6,6^{-2}\text{H}_2]$ glucose was used as the tracer while endogenous glucose concentration was the tracee in order to calculate rate of endogenous glucose production. As shown in figure 2 (bottom panel), the ratio $[6,6^{-2}\text{H}_2]$ glucose (tracer)/endogenous glucose (tracee) concentrations was also fairly constant apart from a slight fall at ~ 120 min at the start of exercise. This non-stationary signal characteristic makes it necessary to modify the calculations of the derivative based on deconvolution, as detailed in the Appendix A1.

Meal Appearance, Endogenous Glucose Production and Glucose Disappearance (Figure 3):

Rate of appearance of meal glucose (MRa) into the systemic circulation reached a peak of $76.1\pm9.1 \mu\text{mol/kg/min}$ at $t=60$ min. The MRa gradually dropped to $57.8\pm9.8 \mu\text{mol/kg/min}$ at $t=120$ min (start of exercise), then drifted down to $29.5\pm5.0 \mu\text{mol/kg/min}$ at $t=180$ min before rapidly tapering off by $t=250$ min.

Rates of endogenous glucose production (EGP) dropped rapidly in response to the meal from baseline of $13.4\pm1.1 \mu\text{mol/kg/min}$ at $t=0$ min to a nadir of $0.3\pm0.7 \mu\text{mol/kg/min}$ at $t=60$ min. At $t=120$ min (start of exercise) it was $2.4\pm0.4 \mu\text{mol/kg/min}$, then quickly rose almost eight-fold in response to exercise to $18.2\pm2.6 \mu\text{mol/kg/min}$ at $t=180$ min. Thereafter EGP rate gradually dropped down to baseline finally reaching rates of $10.6\pm0.9 \mu\text{mol/kg/min}$ at $t=360$ min.
Insulin Sensitivity During Exercise

Rates of glucose disappearance (Rd) rose to a peak of $89.1 \pm 11.6 \, \mu\text{mol/kg/min}$ at $t=120 \, \text{min}$ (start of exercise), then declined to $26.4 \pm 3.3 \, \mu\text{mol/kg/min}$ at $t=195 \, \text{min}$ (end of exercise) after reaching a plateau ($44.9 \pm 6.2$ to $45.4 \pm 6.5 \, \mu\text{mol/kg/min}$) during exercise ($t=140$ to $180 \, \text{min}$ respectively). Thereafter, rates of Rd returned to baseline at $t=360 \, \text{min}$.

Insulin Action (Figure 4):

$SI_{\text{exercise}}$ was significantly higher than $SI_{\text{rest}}$ ($21.6 \pm 3.7 \, \text{vs.} \, 12.5 \pm 2.0 \, 10^{-4} \, \text{dl/kg/min per \muU/ml, p<0.0005}$). Of note, all subjects demonstrated the same increasing trend of SI (Figure 4, upper panel) 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 a $\sim 75\%$ increase in model-derived whole body insulin sensitivity ($SI_{\text{exercise}}$) 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-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 Rd 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
Insulin Sensitivity During Exercise

advance clinically for the nationwide NIH 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 (Rd) increases through insulin independent mechanisms including increases in muscle blood flow, capillary recruitment and exercise induced translocation of GLUT4 protein (39). Further, it is well established that a single endurance exercise bout increases insulin action on skeletal muscle (20, 21) in the post exercise period. While the mechanism underlying the acute post exercise 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 which use glucose concentration and its change over time to predict future insulin needs.

Concomitant with changes to peripheral glucose uptake, endogenous glucose production (EGP) also has to increase to meet the increased metabolic demands of the exercising muscle to prevent hypoglycemia (16, 38) (13, 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 permit accurate and continuous quantitation of post prandial glucose fluxes during moderate intensity exercise. By modifying the tracer infusion rates, we helped minimize changes to tracer-tracee ratios to the best of our ability hence minimizing nonsteady state errors in calculation of glucose turnover. The ratio of [6-3H] glucose to [1-13C] glucose applied to measure MRa was minimally affected by exercise. On the
other hand although the ratio of [6,6-²H₂] glucose to endogenous glucose applied to calculate EGP, fell at the start of exercise, the fall was gentle and the ratio leveled off quickly.

Glucose concentrations rapidly fell by almost 2.3mM (~41 mg/dl) from the start of exercise to a mean nadir of ~4 mM (~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 (~60 mg/dl) during exercise with the lowest glucose of 2.5 mM (~44 mg/dl) in a subject. However, none of the participants experienced any symptoms of hypoglycemia. These changes were accompanied by rapid fall in insulin concentrations together with greater than a two-fold rise in glucagon concentrations. Predictably the consequent change in insulin/glucagon ratio resulted in an almost eight fold 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 intra-muscular 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 Rd 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 eight-fold increase in rates of EGP.

Rate of appearance of meal glucose (MRa) fell rapidly during the first ten minutes 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, MRa rapidly declined to near zero by $t=240$ min (i.e., within 35 minutes) after completion of exercise. In contrast, MRa continued to decline steadily after $t=120$ min in the prior report when subjects were resting (35). The transient plateau in MRa during exercise could be due to 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, MRa 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 MRa. Therefore, an earlier start to the exercise after the meal could have had a greater effect on MRa. Furthermore, humoral factor/s that could have contributed to
the plateau in MRa 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. Since exercise is known to increase the rate of glucose leaving the circulation (i.e., Rd), rates of glucose entering the circulation in the postprandial state (i.e., EGP and/or MRa) must also increase to compensate for the physiological increase in Rd in order 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. Since glucose concentrations fell during exercise, the rate of glucose leaving the circulation (i.e., Rd) must have exceeded the rate of glucose entering the circulation (i.e., combined rates of EGP and MRa). It is noteworthy however that these dynamic changes were occurring in the presence of rapidly falling insulin concentrations during exercise. While the rise in EGP could be explained by both falling insulin and rising glucagon concentrations, the plateau in Rd can be explained by increasing muscle glucose uptake by both insulin independent and dependent mechanisms. As discussed above, the sustained rate of Rd during exercise was only in part compensated by the eight-fold increase in EGP and a transient plateau in the rate of MRa 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 utilizing 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 minutes of 50% VO$_2$ max exercise with 5 minute rest periods. This study design was chosen for several reasons. Firstly, the Juvenile Diabetes Research Foundation (JDRF) 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 three hours after a solid (23) or liquid meal (9) resulted in a three-fold increase in glucose Rd, while recovery from exercise in the post-exercise period is distinguished by a continuous decline in glucose Rd for up to 180 minutes (23). Our results show an exercise induced increase in glucose Rd in comparison to our initial measurement (time point 10), but 120 minutes from the start of the meal was not enough time to allow the participants to clear the meal (Figure 3a). Our absolute values for glucose Rd are higher than those with similar protocols (9, 23) likely attributed to both the absorption of the meal, as exogenous meal glucose during exercise elevates glucose Rd (8), and mathematical modeling differences between the studies. Furthermore, the blood sampling time to measure glucose fluxes was every ten minutes during the first hour of exercise in this study. Hence, an earlier change in Rd induced by exercise, as described by Coggan et al (11) during 80% VO$_2$ max exercise where sampling time
Insulin Sensitivity During Exercise

was every five minutes, would have been missed by us. Our post exercise time period does show
a steady decline in glucose Rd 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_{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 uses insulin and
glucose as their inputs and as such measure the net effect of insulin on glucose disposal and
production before, during and post exercise. However, the inhibitory effect of insulin on EGP is
relatively minor compared to 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 Rd, one must realize that apart from the skeletal muscle, Rd
also includes glucose uptake into the liver, splanchnic tissues, central nervous system, red blood
cells etc. Therefore, in order 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
Insulin Sensitivity During Exercise

tracer study without exercise; 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_{rest} (estimated from 0-120 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_{rest} vs. SI_{exercise} in each subject 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 eight fold increase in rates of EGP, with a plateau in the rates of MRa and Rd during exercise in these individuals. We have also introduced the index SI_{exercise} that reflects a parameter of whole body insulin sensitivity (the ability of insulin to promote glucose uptake and inhibit endogenous glucose production) 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 two hours into the postprandial state. Therefore, by virtue of the index SI_{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.
According to regularized deconvolution (14), the derivative of the noisy TTR (and glucose concentration) can be calculated by solving the following optimization problem:

\[
\hat{u} = \arg \min_u (y - Hu)^T \Sigma_v^{-1} (y - Hu) + u^T \Sigma_u^{-1} u
\]

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

\[
\Sigma_u = F^{-1} \Lambda F^{-T} \quad \text{with} \quad \Lambda = \lambda^2 \cdot I
\]

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

However, due to physical activity, both the TTR and glucose represents marked non-stationary 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 non-
stationary white noise process, with different variance in absence ($\lambda_1^2$) or presence ($\lambda_2^2$) of physical activity (32), leading to:

$$\Lambda = \begin{bmatrix} \lambda_1^2 & 0 & \ldots & 0 & 0 \\ 0 & \lambda_1^2 & \ldots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \ldots & \lambda_2^2 & 0 \\ 0 & 0 & \ldots & 0 & \lambda_2^2 \end{bmatrix}$$

Figure 5 shows an example of the performance of the new method vs. that not accounting for non-stationary factor.
Acknowledgements:

We are deeply indebted to the research participants. Our sincere thanks to the staff of the Mayo Clinic Center for Translational Science Activities (CTSA) Clinical Research Unit (CRU); to the GI Motility Core; the CRU Mass Spectroscopy Laboratory; CRU Immunochemical Core Laboratory; Pamela Reich (research assistant), Chad Clark (Laboratory Technician), Brent McConahey (research assistant), and Shelly McCrady Spitzer (research assistant). All persons mentioned above are at Endocrine Research Unit, Mayo Clinic, Rochester.

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.

The work was supported by NIH DK R01 085561, DK DP3 094331 and Grant Number UL1 TR000135 from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH). M.S., C.D.M., G.V. and C.C. are partially funded by Italian Ministero dell’Istruzione, dell’Università e della Ricerca (Progetto FIRB 2009).

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.
References:


Figure Legends:

Figure 1a: Glucose (upper panel) and Insulin (lower panel) concentrations obtained from time 0-360 minutes. Shaded box between 120-195 minutes represents exercise period at 50% VO₂ max.

Figure 1b: C-peptide (upper panel) and Glucagon (lower panel) concentrations obtained from time 0-360 minutes. Shaded box between 120-195 minutes represents exercise period at 50% VO₂ max.

Figure 2: [6-³H] glucose / [1-¹³C] glucose ratio (upper panel) and [6,6-²H₂] glucose / endogenous glucose ratio (lower panel) obtained from time 0-360 minutes. Shaded box between 120-195 minutes represents exercise period at 50% VO₂ max.

Figure 3: Rates of Meal Appearance (upper left panel), Endogenous Glucose Production (upper right panel) and Glucose Disappearance (lower panel) obtained from time 0-360 minutes. Shaded box between 120-195 minutes represents exercise period at 50% VO₂ max.

Figure 4: Individual values of SI_rest and SI_exercise (upper panel) and mean SI_rest and SI_exercise (lower panel) obtained during the study.

Figure 5: An example of comparison between the performance of the deconvolution algorithm accounting (new method) vs., not accounting for non-stationary tracer-tracee ratio (TTR) signal (standard method). TTR (upper panel) weighted residuals (middle panel) and estimated EGP (lower panel). Grey area represents the period of exercise.
Fig 1a

**Glucose**

- **X-axis**: Time (min)
- **Y-axis**: mM

**Insulin**

- **X-axis**: Time (min)
- **Y-axis**: pmol/L
Fig 1b

C-peptide

Time (min)

Glucagon

Time (min)
Fig 2


[6,6-2H2] Glucose/Endogenous Glucose
Fig 3

Rate of Meal Appearance

Endogenous Glucose Production

Glucose Disappearance

μmol/kg/min

0 60 120 180 240 300 360

Time(min)
* $p < 0.0005$

![Graph showing SI (10^-4 dL·kg⁻¹·min⁻¹ per μU/mL) values at rest and during exercise.](image)

- **SI** (10^-4 dL·kg⁻¹·min⁻¹ per μU/mL)
- **SI<sub>rest</sub>**
- **SI<sub>exercise</sub>**
Fig 5

Tracer-to-tracee ratio

Weighted residuals

Endogenous Glucose Production
Table 1. Subject Characteristics:

Anthropometric and screening test characteristics of enrolled subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F, %)</td>
<td>7 (58%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>37.1</td>
<td>3.8</td>
<td>[20.0, 55.0]</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.9</td>
<td>4.8</td>
<td>[54.9, 115.8]</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.7</td>
<td>0.03</td>
<td>[1.6, 1.9]</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.1</td>
<td>1.1</td>
<td>[19.1, 34.2]</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td>50.9</td>
<td>3.9</td>
<td>[37.2, 81.0]</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>29.1</td>
<td>2.0</td>
<td>[20.5, 41.3]</td>
</tr>
<tr>
<td>Lab results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4.7</td>
<td>0.2</td>
<td>[3.4, 5.4]</td>
</tr>
<tr>
<td>HBA1c, %</td>
<td>5.1</td>
<td>0.1</td>
<td>[4.7, 5.5]</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.9</td>
<td>0.4</td>
<td>[12.0, 16.6]</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8</td>
<td>0.04</td>
<td>[0.7, 1.1]</td>
</tr>
<tr>
<td>BUN</td>
<td>14.4</td>
<td>1.4</td>
<td>[9.0, 27.0]</td>
</tr>
<tr>
<td>TSH (IU/L)</td>
<td>2.4</td>
<td>0.4</td>
<td>[0.5, 4.4]</td>
</tr>
<tr>
<td>Gastric emptying test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid (T50, min)</td>
<td>112.7</td>
<td>12.1</td>
<td>[54.4, 195.0]</td>
</tr>
<tr>
<td>Liquid (T50, min)</td>
<td>28.3</td>
<td>6.0</td>
<td>[13.0, 86.0]</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>32.3</td>
<td>2.1</td>
<td>[19.1, 43.4]</td>
</tr>
</tbody>
</table>
Table 2. Breakfast Content on Study Day

Meal composition of the breakfast meal that preceded exercise.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>637.5</td>
<td>55.7</td>
<td>[505.6, 879.5]</td>
</tr>
<tr>
<td>Carbs (g)</td>
<td>76.1</td>
<td>0.2</td>
<td>[75.6, 78.6]</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>36.6</td>
<td>4.2</td>
<td>[20.5, 52.8]</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>19.6</td>
<td>1.6</td>
<td>[13.5, 38.5]</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Meal Macronutrient Composition as a proportion of total meal calories

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbs (%)</td>
<td>53.3</td>
<td>1.5</td>
<td>[50.3, 56.3]</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16.3</td>
<td>0.4</td>
<td>[15.5, 17.1]</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>30.4</td>
<td>1.7</td>
<td>[30.6, 33.8]</td>
</tr>
</tbody>
</table>