1 **Comparison of glucose tolerance tests to detect the insulin sensitizing effects of a**
2 **bout of continuous exercise**

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Abstract

The aim of the present study was to determine which of the available glucose tolerance tests (oral, OGTT vs. intravenous, IVGTT) could more readily detect the insulin sensitizing effects of a bout of continuous exercise. Ten healthy moderately-fit young men ($\text{VO}_{2\text{peak}}$ of $45.4 \pm 1.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; age $27.5 \pm 2.7 \text{ yr}$) underwent four OGTT and four IVGTT on different days following a standardized dinner and overnight fast. One test was performed immediately after 55-min of cycle-ergometer exercise at 60% $\text{VO}_{2\text{peak}}$. Insulin sensitivity index was determined during a 50-min IVGTT according to Tura (C_{ISI}) and from a 120-min OGTT using the Matsuda composite index (M_{ISI}). After exercise, M_{ISI} improved $29 \pm 10\%$ without reaching statistical significance ($P=0.182$) due to its low reproducibility (CV $16 \pm 3\%$; intra-class reliability 0.846). However, C_{ISI} significantly improved ($50 \pm 4\%$; $P<0.001$) after exercise showing better reproducibility (CV $13 \pm 4\%$; intra-class reliability 0.955). Power calculation revealed that 6 participants were required for detecting the effects of exercise on insulin sensitivity when using IVGTT while 54 were needed when using OGTT. The superior response of C_{ISI} compared to Matsuda index suggests the preferential use of IVGTT to assess the effects of exercise on insulin sensitivity when using a glucose tolerance test.

Keywords: endurance exercise; glucose tolerance; insulin sensitivity; oral glucose tolerance test; intravenous glucose tolerance test.

1 **Introduction**

2 Exercise is recommended for the treatment and prevention of type 2 diabetes
3 (American-Diabetes-Association. 2011) due to its insulin-sensitizing effects (Roden
4 2012, Mikines et al. 1988, Hawley 2004). Although the euglycemic
5 hyperinsulinemic clamp (EHC) is the gold standard method to assess insulin
6 sensitivity, it requires costly and complex equipment and trained hospital personnel
7 to obtain accurate measures and avoid complications, (Bloomgarden 2006,
8 Muniyappa et al. 2008). Insulin sensitivity indexes derived from oral and
9 intravenous glucose tolerance tests (i.e., OGTT and IVGTT, respectively) are
10 affordable surrogates of the EHC frequently reported in the literature. However, to
11 our knowledge, data comparing the reliability and response to exercise of these
12 indexes are scarce.

13 Although M_{ISI} based on OGTT shows a fairly good agreement with the EHC
14 ($r=0.73$; (Matsuda and DeFronzo 1999)) its reproducibility measured by the
15 coefficient of variation (CV) has been estimated between 14.0 and 20.4%;
16 (Utzschneider et al. 2007)). Glucose ingestion, besides inducing pancreatic insulin
17 release, stimulates gastrointestinal hormones and affects liver metabolism.
18 Furthermore, the ingested glucose would interact with enteric hormones and neural
19 factors capable of altering glucose absorption and insulin secretion (Kahn 1997). We
20 believe that this may be behind the increased within-subject variability when using
21 the OGTT technique. On the other hand, it is worth to mention that the signals
22 initiated from the gastrointestinal system after an oral load of glucose resemble the
23 ones that an individual faces after a meal, justifying the use of OGTT for an
24 ecological, but less reproducible, assessment of insulin sensitivity.

1 To avoid the variable interaction between the ingested glucose and
2 gastrointestinal hormones and neural factors (Kahn 1997) the glucose load is often
3 administered through an intravenous catheter using the intravenous glucose tolerance
4 tests (IVGTT). A mathematical model (i.e. minimal model (Bergman et al. 1987))
5 has been developed to calculate insulin sensitivity from a 180 min IVGTT. When
6 using this model researchers report a high correlation with the EHC ($r=0.89$; $P<0.05$;
7 (Bergman et al. 1987)) and a CV=14%; (Ferrari et al. 1991)). To reduce testing time
8 and costs, Tura and co-workers (Tura et al. 2010) have recently developed a simpler
9 mathematical model to calculate insulin sensitivity from a 50 min IVGTT (i.e., C_{ISI}).
10 They found that C_{ISI} was highly correlated with the EHC ($r=0.91$; $P<0.05$) and the
11 minimal model ($r=0.92$; $P<0.05$; (Tura et al. 2010)) when tested in a mixed group of
12 healthy and insulin resistant participants at rest. However, to our knowledge, C_{ISI}
13 response to exercise or its within-subject reproducibility has not been established.

14 Without knowing the response to exercise of each insulin sensitivity index,
15 researchers often default to use the simpler OGTT technique perhaps limiting the
16 statistical significance of their findings. The purpose of this study was to compare
17 the insulin sensitivity response to exercise from glucose tolerance tests with different
18 routes of glucose administration (i.e., M_{ISI} from OGTT vs. C_{ISI} from IVGTT). We
19 compared the response to exercise of each of these indices as well as their
20 reproducibility. We hypothesized that insulin sensitivity measures derived from
21 IVGTT would be more reproducible and responsive to the effects of exercise than
22 when derived from OGTT.

1 **Materials and methods**

2 **Participants**

3 Ten young, healthy and physically active males with a mean $\pm$ SEM age of
4 27.5 $\pm$ 2.7 years, and a BMI 24.6 $\pm$ 0.9 kg·m⁻² were recruited from the University's
5 student and workers population after medical clearance. After detailed information
6 about the benefits and risks involved with their participation in the study, all gave
7 their written consent which was approved by the local Hospital's Ethics Committee
8 and conformed to the latest revision of the Declaration of Helsinki.

9 **Preliminary testing**

10 A week before participation in the study, under medical supervision,
11 participants underwent a maximal graded exercise test until volitional exhaustion
12 according to the ACSM's guidelines for exercise testing and prescription
13 (American College of Sports Medicine 2006), starting at 100 W and increasing 25
14 W·min⁻¹ in an electrically braked cycle-ergometer (Ergoselect 200, Ergoline,
15 Germany). During the exercise test, O₂ consumption was measured by indirect
16 calorimetry (Quark b², Cosmed, Italy) and peak oxygen consumption and cycling
17 peak power output was assessed. Continuous, standard 12-lead ECG (Quark T12,
18 Cosmed, Italy) was performed to ensure a normal cardiovascular response to
19 exercise.

20 **Experimental design**

21 All participants underwent eight experimental trials, four OGTT and four
22 IVGTT, with a separation of at least 72 hours among them. One of the OGTT (i.e.,
23 OG_{Ex}) and one of the IVGTT (i.e., IV_{Ex}) were performed after a standardized bout of

1 cycling exercise. Trial order was fully randomized, starting the morning (0800-1000
 2 hours) after an overnight 10-h fast that followed a standardized dinner which was
 3 provided by the investigators and was composed by a frozen lasagna, two apples and
 4 an isotonic beverage (703 Kcal, 50% from carbohydrate, 38% from fat, 3% saturated
 5 fatty acids, and 12% from protein. Fiber content: 8.4 g). During the 24-hours prior
 6 to each trial, participants were instructed to ingest at least 7 g of carbohydrate per kg
 7 of body weight to ensure full glycogen storage. This amount of ingested
 8 carbohydrates should avoid the confounding effect of reduced muscle glycogen
 9 levels on insulin sensitivity as previously described (Ivy et al. 1985). In addition, all
 10 participants were asked to refrain from vigorous exercise 48-hours prior to each trial
 11 and to ingest 500 mL of bottled water upon awakening to promote euhydration.

12 **Experimental trials**

13 Upon the participant's arrival to the laboratory, nude body weight (Hawk,
 14 Mettler Toledo, USA) and urine specific gravity (U_{SG} ; Uricon-NE, Atago, Japan)
 15 were measured to assess hydration status. Following this, the participants lay in a
 16 stretcher and a 20G intravenous catheter (BD Insyte, Becton-Dickinson, Spain) was
 17 inserted in an antecubital vein and a Luer-lock three-way stopcock attached
 18 (Vitroway, Vitromed Healthcare, India). After 15 minutes of rest in supine position,
 19 5 ml of blood were withdrawn to obtain a baseline fasting sample. Five minutes
 20 later, the participants underwent the glucose tolerance test (either OGTT or IVGTT)
 21 or started the aerobic exercise bout according to their assigned trial. The glucose
 22 tolerance tests were performed approximately 20 minutes after exercise. We chose
 23 not to delay the glucose tolerance tests beyond 20 min of supine rest because prior
 24 data using hyperinsulinemic euglycemic clamp demonstrated no difference in insulin
 25 sensitivity when assessed either 30 or 180 minutes after an exercise bout similar in

1 intensity and duration to the exercise presently used (Howlett et al. 2008) in subjects
 2 of similar characteristics (i.e., untrained young men).

3 Both exercise trials (i.e., OG_{Ex} and IV_{Ex}) were followed by 20 min of resting supine
 4 after which a new baseline blood sample was withdrawn before starting the glucose
 5 tolerance test. Exercise duration and intensity resembled the ones used by Mikines et
 6 al., (Mikines et al. 1988) in a study in which they showed improved insulin
 7 sensitivity in participants of similar characteristics (male, young, and healthy). In
 8 addition, the chosen exercise bout followed the recommendations of the last
 9 American College of Sports Medicine and American Diabetes Association joint
 10 statement of exercise for type 2 diabetes treatment and prevention (Colberg et al.
 11 2010). Specifically, exercise commenced with a warm-up (5 min, 100 W) followed
 12 by 45-min at 65% VO_{2peak} and a 5 min cool-down (75 W). The average workload for
 13 the 55 min of exercise was 60% VO_{2peak}.

14 **Glucose and insulin analysis**

15 Samples were mixed in tubes with 3K-EDTA (Vacuette®, Greiner Bio-One
 16 GmbH, Austria) and centrifuged at 4000 rpm during 10 min at 4°C (MPW-350R,
 17 Med. Instruments, Poland) to obtain plasma that was stored at -80°C. Glucose was
 18 measured with an enzymatic glucose oxidize assay (Enzymatic Glucose Reagent,
 19 Thermoscientific, USA). Insulin concentration was analyzed by chemiluminescence
 20 (Architect System Insulin, Abbott Diagnostics Division, Germany).

21 **OGTT and IVGTT procedures and insulin sensitivity indexes calculation**

22 After collection of the basal blood sample, OGTT started with the ingestion of
 23 75 g of anhydrous glucose (Guinama Laboratorio, Spain) diluted into 250 mL of
 24 water. Immediately after all fluid was ingested, a timer was started and 5 mL blood

1 samples were obtained after 30, 60, 90 and 120-min of glucose ingestion. For
 2 IVGTT we delivered a glucose load of $0.5 \text{ g} \cdot \text{kg}^{-1}$ body mass with a maximal dose of
 3 35 g. We used a 30% glucose solution (Glucosada 30%, Grifols, Spain) manually
 4 infused at an even pace during 3-min using two 60 mL syringes (BD Plastipak,
 5 Spain). Immediately after delivering the glucose load, the stopcock, catheter and
 6 vein were rapidly flushed with 10 ml of 0.9% saline solution. Following, every ten
 7 minutes (i.e., 10, 20, 30, 40, and 50-min) a 5 mL blood sample was obtained and the
 8 catheter flushed with 5 mL of 0.9% saline. For IVGTT procedure we strictly
 9 complied with the recommendations of the Islet Cell Antibody Register User's
 10 Group (ICARUS; (Bingley et al. 1992)) in all aspects, including the use of a single
 11 catheter for glucose infusion and sample collection.
 12 Basal glucose and insulin concentrations were used to calculate HOMA2-IR index
 13 (Levy et al. 1998) using a specific software (HOMA calculator, Version 2.2.2
 14 Diabetes Trial Unit, University of Oxford, available at
 15 www.dtu.ox.ac.uk/homacalculator).
 16 Data from the OGTT technique was used to calculate M_{ISI} (Matsuda and DeFronzo
 17 1999) as follows:

$$18 \quad M_{\text{ISI}} = 10000 / \sqrt{[(\text{FPI } \mu\text{IU} \cdot \text{mL}^{-1} \cdot \text{FPG mg} \cdot \text{dL}^{-1}) \cdot (\text{PG}_{\text{MEAN}} \cdot \text{PI}_{\text{MEAN}})]}$$

19 where, FPI is the fasting plasma insulin, FPG is the fasting plasma glucose, and
 20 PG_{MEAN} and PI_{MEAN} are respectively the mean of the glucose and insulin
 21 concentration of all the samples collected during the 120 min OGTT, and 10000 is
 22 the constant to obtain numbers ranging between 1 and 12.

23 Data from the IVGTT technique was used to calculate C_{ISI} (Tura et al. 2010) as
 24 follows:

1
$$C_{ISI} = \alpha [K_G / (\Delta AUC_{INS} / T)]$$

2 where α is a scaling factor (0.604), K_G is the rate of glucose disappearance (slope of
3 log glucose), ΔAUC_{INS} is the area under the curve of insulin concentration above
4 basal value and T is the time interval between 10 and 50 min (i.e. 40 min) when
5 glucose and insulin data are computed.

6 **Statistics**

7 We used Shapiro-Wilk to test the normal distribution of data. In the three
8 resting trials, absolute reliability was calculated using coefficient of variation (CV)
9 and relative reliability using intraclass reliability (ICR) (Vincent 1999). Student's T-
10 test identified insulin sensitivity differences between rest (one of the trials randomly
11 chosen) and post-exercise. Nutritional and hydration status stability among trials
12 was evaluated using repeated measures ANOVA comparing pre-trial body weight,
13 U_{SG} , fasting plasma glucose, insulin and HOMA2-IR among trials. Correlations
14 between M_{ISI} and C_{ISI} in all variables were sought using Pearson coefficient. Limits
15 of agreement (LOA) were calculated according to Bland and Altman (Bland and
16 Altman 1986) Sample size estimates for each index were calculated based on the
17 standard deviations of the differences (t test) between rest and post-exercise values
18 using an α -level of 0.05 and a 90% power (Utzschneider et al. 2007). Results are
19 reported as means $\pm$ SEM. Significance value was set at $P < 0.05$. All the tests were
20 performed with SPSS for windows (Version 19, SPSS Inc., USA).

1 **Results**

2 **Participant characteristics and pre-trial hydration and nutritional status**

3 Participants $\text{VO}_{2\text{peak}}$ was $45 \pm 2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and they reached a peak
 4 pedaling power of $300 \pm 18 \text{ W}$ before exhaustion. Body weight and urinary specific
 5 gravity values were not different before any of the eight trials evidencing a similar
 6 pre-test hydration status (Table 1). HOMA2-IR index was not different among any
 7 trial (Table 1), suggesting compliance with the prescribed diet and exercise
 8 refraining.

9 **Reproducibility and response to exercise of insulin sensitivity indices**

10 At rest, the within subject reproducibility of insulin sensitivity measured in
 11 three different days, was superior when using C_{ISI} from IVGTT than when using M_{ISI}
 12 from OGTT, showing lower coefficient of variation and a higher intraclass reliability
 13 (Table 2). HOMA2-IR from fasting glucose and insulin concentrations obtained at
 14 rest before the OGTTs had a coefficient of variation of 15.9% and an intraclass
 15 reliability of 0.827. HOMA2-IR before the IVGTTs had a coefficient of variation of
 16 18.6% and an intraclass reliability of 0.793.

17 After moderate exercise (55 min at 60% $\text{VO}_{2\text{peak}}$), OGTT derived M_{ISI} rose
 18 29% from rest due to a 17% reduction in the product of the mean glucose and insulin
 19 concentration, and a 11% reduction in the product of fasting plasma glucose and
 20 insulin concentration (Table 3). However, that improvement in M_{ISI} did not reach
 21 statistical significance (Figure 1A; $P = 0.141$), likely due to the inconsistent inter-
 22 subject response to exercise since four out of the ten participants did not show
 23 improvement in M_{ISI} (individual responses are dotted lines in Figure 1A). Post-
 24 exercise C_{ISI} increased significantly by 50% (Figure 1B; $P < 0.001$) mainly by a 27%

1 reduction in the area under the curve of plasma insulin above basal value ($P = 0.034$,
2 Table 3). The individual response showed that all ten participants improved their
3 post-exercise C_{ISI} (individual responses are dotted lines in Figure 1B). After exercise
4 HOMA2-IR did not show significant differences from pre-exercise values despite a
5 15% improvement (0.78 ± 0.07 to 0.67 ± 0.12 from pre to post exercise, $P=0.479$).

6 **Correlations between techniques**

7 During resting conditions M_{ISI} and C_{ISI} showed a high and significant
8 correlation ($r = 0.828$; $P = 0.003$; 95% LOA = 4.1 to -3.9). However, the correlation
9 between M_{ISI} and C_{ISI} scores after exercise was not significant ($r = 0.547$; $P = 0.102$;
10 95% LOA = 10.2 to -6.2). In addition, the changes in the insulin sensitivity from rest
11 to exercise obtained from both indices (M_{ISI} and C_{ISI}) were calculated from one
12 randomly chosen resting condition trial (OG_{REST} and IV_{REST}) and the exercise trial
13 (OG_{EX} and IV_{EX}). Correlation of those changes with exercise in each of the
14 techniques (OG and IV) did not show statistical significance ($r = 0.337$; $P = 0.341$;
15 95% LOA = 9.4 to -6.1).

16 **Sample size calculation**

17 Sample size estimates (Utzschneider et al. 2007) revealed that to find the effect
18 of exercise on insulin sensitivity, 54 participants would be required when using M_{ISI}
19 ($SD = 4.1$) while only 6 participants when using C_{ISI} ($SD = 2.1$).

1 Discussion

2 It is well established that exercise stimulates insulin-dependent and insulin
 3 independent glucose uptake in human skeletal muscle (Hayashi et al. 1997). The
 4 OGTT and the IVGTT are two dynamic methods to assess insulin sensitivity when
 5 the euglycemic hyperinsulinemic clamp technique is not affordable. These surrogate
 6 insulin sensitivity indexes from glucose tolerance tests are indistinctly reported in the
 7 scientific literature without much regard to questions of reproducibility or
 8 responsiveness. We tested the reliability of Matsuda index (M_{ISI} , derived from
 9 OGTT) and Tura's insulin sensitivity index (C_{ISI} , derived from IVGTT) at rest
 10 during standardized conditions. In addition, we investigated which index more
 11 readily detects the insulin sensitizing effects of a single bout of prolonged (55 min)
 12 moderately-intense exercise (60% VO_{2peak}). Unlike most of the literature in this
 13 area, we tested reliability and response in the same participants using a cross-over
 14 repeated measures experimental design.

15 A major finding of this study is that C_{ISI} derived from IVGTT showed the
 16 highest reproducibility (Table 2). Due to this higher reproducibility and consistency
 17 (i.e., every subject improved) only with C_{ISI} we detected the improvements in insulin
 18 sensitivity with exercise ($P < 0.001$; Figure 1B). In contrast, M_{ISI} was not responsive
 19 enough to significantly detect the effects of exercise on insulin sensitivity (Figure
 20 1A). Testing another 44 similar participants would be needed to ascertain the effects
 21 of exercise using M_{ISI} . The extra cost and effort of testing that amount of
 22 participants may not be justified in many studies and out of the scope of many
 23 research laboratories with restricted budgets. On the other hand, C_{ISI} is calculated
 24 after a 50 min IVGTT whereas M_{ISI} requires a 120 min OGTT. The reduced time of
 25 study is another advantage when using C_{ISI} instead of M_{ISI} . Thus, our data support

1 the preferential use of the IVGTT technique in comparison to OGTT when
2 evaluating the effects of and exercise bout (or exercise program) on insulin
3 sensitivity.

4 While most studies show a positive effect of exercise on insulin sensitivity
5 when assessed by OGTT (Balducci et al. 2010, Dumortier et al. 2003, Jenkins and
6 Hagberg 2011, Kishimoto et al. 2002, Venables and Jeukendrup 2008), some report
7 no effects. Hansen et al., (Hansen et al. 2009) found in obese type 2 diabetes patients
8 that two exercise training programs of different intensities improved glycated
9 hemoglobin (HbA_{1C}) and whole body oxidative capacity while insulin area under the
10 curve measured during OGTT remained unchanged. Mitchell et al. (Mitchell et al.
11 2011) measured insulin sensitivity using OGTT in obese participants after aerobic
12 exercise finding no improvements. Niakaris and coworkers (Niakaris et al. 2005)
13 recommended caution on the interpretation of OGTT-derived insulin sensitivity
14 results since they seem to be population dependent. Our data suggest that the
15 discrepancies about the effects of exercise on OGTT derived insulin sensitivity
16 calculations originate from the low reproducibility of this technique. In addition, the
17 response was inconsistent since 4 out of the 10 participants did not improve M_{ISI}
18 after exercise.

19 M_{ISI} does not only reflect peripheral insulin sensitivity but also hepatic insulin
20 sensitivity (Matsuda and DeFronzo 1999). Since exercise is mostly known to affect
21 peripheral insulin sensitivity, it is possible that Matsuda index was not that sensitive
22 to the effects of exercise because a portion of the calculation is devoted to hepatic
23 insulin sensitivity. The fasting glucose and insulin concentration portion of the M_{ISI}
24 formulae (see materials and methods) reflects hepatic insulin sensitivity. When we
25 remove that portion of the formula and only the glucose and insulin concentrations

1 during the OGTT are compared, no significant differences are found between the
 2 exercise and rest trials ($P=0.136$, Table 3). We interpret this to indicate that the lack
 3 of improvement in M_{ISI} after exercise is also due to a variable response in peripheral
 4 insulin sensitivity.

5 It is well established that one bout of aerobic exercise improves glycemic
 6 control and insulin sensitivity (Kennedy et al. 1999, Perseghin et al. 1996). In a
 7 recent study healthy non-obese young men were tested twenty minutes after a bout of
 8 30 min of continuous pedaling at 50% of their VO_{2max} . The authors reported a 45%
 9 improvement in insulin sensitivity when using frequently sampled IV glucose
 10 tolerance tests (Hayashi et al. 2005). We used a similar energy demanding bout of
 11 exercise (i.e., 55 min of exercise at 60% VO_{2peak}) and insulin sensitivity improved
 12 50% with C_{ISI} ($P<0.001$; Figure 2(b)). Our results using the 50-min IVGTT
 13 technique described by Tura, agree in the magnitude of insulin sensitivity
 14 improvement with previous studies (Hayashi et al. 2005).

15 Our study design has several limitations that are worth to discuss. We are
 16 favoring the use of IVGTT instead of OGTT. However, it is necessary to note that
 17 OGTT better resembles the physiological challenge that an individual faces after a
 18 meal justifying the use of OGTT especially in large epidemiological studies.
 19 Secondly, we used healthy participants with high baseline insulin sensitivity values
 20 very similar to the ones found by Tura et al. in a similar population (7.5 vs. $5.55 \pm$
 21 $0.25 \times 10^{-4} \text{ min}^{-1} [\mu\text{U}\cdot\text{mL}]^{-1}$) (Tura et al. 2010). However, there is an inverse
 22 relationship between baseline insulin sensitivity and insulin sensitivity improvements
 23 with exercise (Magkos et al. 2008). So it is possible that if a group of overweight or
 24 insulin resistant participants were to perform this study, the increase in insulin

1 sensitivity with exercise would have been more robust and statistically significant
 2 even when using M_{ISI} .

3 Finally, the glucose tolerance tests in resting condition took place 1 hour before
 4 the tests after exercise (i.e., 8 am vs. 9 am). Although circadian rhythm could affect
 5 insulin sensitivity between morning and afternoon trials (Boden et al. 1996), it is
 6 unlikely that circadian rhythm could be a factor when trials are spaced only by one
 7 hour (8 am vs. 9 am). In addition, this difference in the time at the start of the tests
 8 should have not clouded the results of our main aim which was the comparison of
 9 two insulin sensitivity indices with different modes of glucose load delivery (i.e.,
 10 M_{ISI} vs. C_{ISI}), considering that the difference in time between the rest and post
 11 exercise tests was the same for M_{ISI} and C_{ISI} .

12

13 The homeostasis model assessment of insulin resistance (HOMA2-IR) (Levy et
 14 al. 1998) stands out as the more cost and time efficient insulin sensitivity index, since
 15 it requires just fasting glucose and insulin concentrations. However, HOMA2-IR
 16 was unable to detect the insulin sensitivity changes induced by the 55 min bout of
 17 exercise and it was less reproducible than C_{ISI} . Thus, according to our data,
 18 HOMA2-IR is not a recommendable technique to measure the insulin sensitizing
 19 effects of a bout of exercise. In fact, other authors have argued that HOMA-IR is not
 20 indicated to assess insulin sensitivity under non-steady state conditions (Wallace et
 21 al. 2004) like the transitional improvements induced by exercise.

22 In conclusion, exercise prescription to improve carbohydrate metabolism
 23 requires from a reliable and responsive test to gauge the effect of exercise on insulin
 24 sensitivity. We compared two glucose tolerance tests that could be used for insulin

1 sensitivity assessment out of hospital boundaries. We found improved
2 responsiveness and reproducibility when the glucose load is delivered through an
3 intravenous catheter (C_{ISI} , IVGTT-derived) in comparison to oral ingestion (M_{ISI} ,
4 OGTT-derived). Using 10 untrained non-diabetic participants, only the insulin
5 sensitivity index based on IVGTT was fit to detect the acute effects of a bout of
6 prolonged aerobic exercise. In most exercise science research environments, where
7 the use of a euglycemic hyperinsulinemic clamp is not possible and sample size is
8 limited, C_{ISI} based on 50 min IVGTT is a superior index to gauge the acute effects of
9 exercise.

10

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Table 1. Baseline hydration and glucose metabolism before glucose load delivery and before exercise. Values are means $\pm$ SEM for 8 trials in each participant (n=10). *P* values from repeated measures ANOVA among all 8 trials. Urine specific gravity (U_{SG}), fasting plasma glucose (FPG), and fasting plasma insulin (FPI).

	Mean	<i>P</i> value
Body Weight (kg)	78.9 $\pm$ 0.2	0.420
U _{SG}	1.016 $\pm$ 0.002	0.416
FPG (mM·L ⁻¹)	5.0 $\pm$ 0.1	0.088
FPI (μIU·mL ⁻¹)	6.2 $\pm$ 0.3	0.111
HOMA2-IR	0.79 $\pm$ 0.06	0.101

- 1 **Table 2.** Within-subject reproducibility of the three resting trials when using insulin
 2 sensitivity indices derived from oral (OGTT) or intravenous (IVGTT) glucose
 3 tolerance test. Values are means $\pm$ SEM.

	OGTT	IVGTT
	Matsuda Index M_{ISI}	C_{ISI}
Rest 1	7.5 ± 0.7	7.4 ± 1.0
Rest 2	6.8 ± 0.8	7.5 ± 1.0
Rest 3	7.0 ± 0.7	7.6 ± 1.0
Coefficient of variation (%)	16.4 ± 2.5	13.4 ± 3.8
Intraclass reliability (ICR)	0.846	0.955

4

5

Table 3. Components of the M_{ISI} from oral (OGTT) and C_{ISI} from intravenous (IVGTT) during rest and after exercise. Values are means $\pm$ SEM. Fasting plasma insulin (FPI), fasting plasma glucose (FPG), mean plasma glucose (PG_{MEAN}) and insulin (PI_{MEAN}) during OGTT. Rate of glucose disappearance (K_G) and area under the curve of insulin concentration (ΔAUC_{INS}).

	Rest	Exercise	<i>P</i> value
M_{ISI}			
FPI ($\mu IU \cdot L^{-1}$) $\cdot$ FPG($mg \cdot dL^{-1}$)	537 $\pm$ 43	455 $\pm$ 38	0.320
PG_{MEAN} ($mg \cdot dL^{-1}$) $\cdot$ PI_{MEAN} ($\mu UI \cdot mL^{-1}$)	4755 $\pm$ 525	3848 $\pm$ 475	0.136
C_{ISI}			
K_G ($\% \cdot min^{-1}$)	2.9 $\pm$ 0.3	3.1 $\pm$ 0.2	0.138
ΔAUC_{INS} ($\mu IU \cdot mL^{-1}$)	1437 $\pm$ 347	1042 $\pm$ 233	0.034

1 **Figure captions**

2

3 **Figure 1.** (A) Matsuda index (M_{ISI}) from 2 h OGTT during resting (OG_{REST}) and
4 after exercise (OG_{EX}). (B) Tura's insulin sensitivity index (C_{ISI}) from 50 min
5 IVGTT during resting (IV_{REST}) and after exercise (IV_{EX}). Individual responses
6 shown as dotted lines. Values are means $\pm$ SEM. * Different from rest ($P < 0.05$).

7

(A) Matsuda index (M_{ISI}) from 2 h OGTT during resting (OG_{REST}) and after exercise (OG_{EX}). (B) Tura's insulin sensitivity index (C_{ISI}) from 50 min IVGTT during resting (IV_{REST}) and after exercise (IV_{EX}). Individual responses shown as dotted lines. Values are means $\pm$ SEM. * Different from rest ($P < 0.05$).

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