Insulin Sensitivity Measured by the Minimal Model: No Associations With Fasting Respiratory Exchange Ratio in Trained Athletes

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The aim of this study was to examine the role of fasting insulin concentrations and tissue insulin sensitivity on whole-body substrate oxidation in 61 well-trained subjects. Subjects underwent a frequently sampled intravenous glucose tolerance test (FSIVGT) after a 10- to 12-hour overnight fast. Minimal model analysis was used to determine insulin sensitivity (S_i). A week later, fasting (10- to 12-hour) respiratory exchange ratio (RER) was measured at rest and during exercise at 25%, 50%, and 70% of peak power output (W_{peak}). Prior to these measurements, training volume, dietary intake, and muscle fiber composition, substrate concentrations, and enzyme activities were determined. The average fasting plasma insulin concentration was 7.3 ± 2.4 μU/mL (4.0 to 10.5 μU/mL), and the mean S_i was 14.0 ± 6.1 × (10^{-4} $min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$) (2.6 to 26.3 × $10^{-4}$$min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$). There was no significant correlation between fasting plasma insulin concentration and S_i ($r = -.14$, $P = .336$) or between these measurements and fasting RER, measured at rest and during exercise at 25%, 50%, and 70% W_{peak}. Only VO_{2max} and the proportion of type 1 muscle fibers were significantly correlated with S_i ($r = .30$, $P = .045$ and $r = .34$, $P = .026$, respectively), and waist-to-hip ratio (WHR) was significantly correlated with fasting plasma insulin concentration ($r = .35$, $P = .006$). In conclusion, S_i and fasting plasma insulin concentration were not associated with fasting RER at rest and during exercise of increasing intensity in trained athletes who have high S_i.

RESEARCH HAS SHOWN that low fasting relative rates of fat oxidation and the failure to increase fat oxidation in response to a high-fat diet may predispose an individual to weight gain and obesity.[1,2] However, there is a large variability in substrate oxidation, with 3- to 4-fold differences in the relative rates of fat oxidation being shown in both healthy lean and obese subjects.[3-5]

Body composition (waist circumference, percent body fat, and waist-to-hip ratio [WHR]),[4-6], energy balance,[4] muscle fiber composition,[7] and the activity of enzymes involved in fat oxidation[8,9] are among the major determinants of the variability in substrate oxidation in sedentary subjects. In addition, fasting plasma insulin concentration is a major determinant of 24-hour carbohydrate oxidation rates,[5,6] but the degree of insulin resistance in the subject population studied influences the strength of the relationship between insulin sensitivity (S_i) and the rate of carbohydrate oxidation.[5,6] This suggests that the circulating plasma insulin concentrations, as well as the degree of insulin resistance and/or sensitivity, are important determinants of substrate oxidation in these subjects. In fact, Flatt[10] proposed that increased insulin resistance might ultimately result in increased relative rates of fat oxidation, which may protect against further weight gain.

In contrast to sedentary obese subjects, trained athletes are remarkably insulin sensitive. Despite this, recent studies from our laboratory have demonstrated a large variability in substrate utilization in trained athletes.[11] The major determinants of fasting substrate utilization at rest in these trained subjects included muscle glycogen content, training volume, proportion of type 1 muscle fibers, plasma free fatty acid (FFA) and lactate concentrations, and percent dietary fat intake.[11] However, the role of insulin in nutrient partitioning in these trained athletes was not investigated. In athletes, insulin plays an important role in both oxidative and nonoxidative glucose disposal,[12] as well as attenuating lipid oxidation via its effects on lipolysis and reesterification.[13]

Studies that have examined the determinants of S_i and the relationship between S_i and substrate oxidation have largely been undertaken in sedentary, often overweight individuals. In these studies, S_i was found to be inversely associated with waist circumference,[14] percent body fat,[14] and dietary fat intake[14,15] and positively associated with physical fitness or training.[14,16-18] Skeletal muscle characteristics, including fiber composition,[19-21] muscle fatty acid composition,[22,23] triglyceride content,[23,24] oxidative capacity,[19,22] and capillary density[21] have also been associated with S_i in sedentary subjects.

These determinants of S_i may not be applicable in physically trained athletes who have different skeletal muscle characteristics. Furthermore, the differences in the determinants of S_i may alter the relationship between S_i and substrate utilization in this population.

To address these issues, this study examined the relationships between whole-body substrate utilization and fasting plasma insulin concentrations and S_i, as determined by minimal model analysis, in physically trained athletes with above average performance ability. Factors that had previously been shown to be associated with S_i in overweight, untrained subjects were also examined in this group of physically trained athletes.

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MATERIALS AND METHODS

Subject Selection and Sampling

Forty-five male and 16 female healthy endurance-trained cyclists took part in this investigation, which was approved by the Research and Ethics Committee of the Faculty of Health Science of the University of Cape Town. Participants were required to fulfill the following entry criteria: (1) to have completed a local 104-km cycle race in less than 3.5 hours for men and in less than 4 hours for women; (2) to be currently training; (3) to have no known metabolic conditions, which may have adversely affected intermediary metabolism (eg, diabetes, thyroid hormone disorder, hyperlipidemia); (4) not be taking any medications for chronic conditions such as high blood pressure (eg, β-adrenergic receptor antagonists) or stimulants for conditions such as asthma (eg, β-adrenergic receptor agonists). The subjects were informed of the nature of the study, and all potential risks and benefits were explained to them. Informed written consent was obtained prior to the start of the study.

Preliminary Testing

Anthropometry. Anthropometric measurements, including the sum of 7 skinfolds (biceps, triceps, subscapular, suprailiac, abdomen, thigh, and calf), waist and hip circumference were determined. Percentage body fat was estimated using the equations of Durnin and Womersley.[25]

Peak power output and peak oxygen consumption. Peak oxygen consumption (VO_{2peak}) and sustained peak power output (W_{peak}) were measured on an electronically braked cycle ergometer (Lode, Groningen, Holland) modified with toe clips and racing handle bars, as described previously by Hawley and Noakes.[26] Work rates were started at 3.33 W/kg body mass for men and 150 W for women. After 150 seconds, the workload was increased by 50 W and then by 25 W every 150 seconds until the subjects were exhausted. Exhaustion was defined as a more than 10% reduction in pedaling frequency or a respiratory exchange ratio (RER) of more than 1.10 or both. W_{peak} was defined as the highest exercise intensity the subjects completed for 150 seconds in W, plus the fraction of time spent in the final work rate multiplied by 25 W. The W_{peak} values were used to determine the relative workloads of the subsequent experimental tests.

During the progressive exercise test, ventilation volume (V_E), oxygen uptake (VO_2), and CO_2 production (VCO_2) were measured over 15-second intervals using a breath-by-breath Oxycon Alpha analyzer (Jaeger, Wuerzburg, Netherlands). Before each test, the gas meter was calibrated with a Hans Rudolph 3 L syringe (Vacumed, Ventura, CA), and the analyzers were calibrated with room air and a 4% CO_2, 96% N_2 gas mixture.

Training history. A detailed retrospective training history was obtained from each subject. In addition, the subjects completed a training diary during the 2 weeks preceding the experimental trial. Training was quantified according to intensity and duration in metabolic equivalents (per week).

Dietary analysis. Three days before the experimental trial, the subjects completed a weighed dietary record. The dietary records were analyzed with the Food Finder program (Medtech (Pty), MRC, Tygerberg, South Africa) to determine the subjects' energy intake, macronutrient consumption, and polyunsaturated:saturated fat ratio (PS ratio).

Frequently Sampled Intravenous Glucose Tolerance Test

The frequently sampled intravenous glucose tolerance test (FSIVGT) was used to estimate S_i. The subjects were instructed not to exercise on the day before the FSIVGT. On the subsequent day, the subjects reported to the laboratory after a 10- to 12-hour overnight fast. A flexible 20-gauge cannula, attached to a 3-way stopcock (Industrias Palex, Barcelona, Spain) was inserted into the forearm antecubital vein of each arm. One cannula was used for the intravenous infusion of 0.3 g/kg body weight of 50% dextrose solution. This was infused over a 2-minute period. The contralateral arm was used for blood sampling. This arm was heated by an electric blanket during the entire test to obtain arterialization of venous blood. The cannula was kept patent by flushing with 1 mL sterile saline. Blood samples (6 mL) for the determination of plasma glucose and insulin concentrations were drawn at times –15, -5, -1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 19, 22, 25, 28, 32, 36, 40, 44, 48, 52, 56, 60, 65, 70, 80, 90, 100, 110, and 120 minutes from the start of the dextrose infusion. One aliquot (4 mL) was placed in a tube containing lithium heparin for the subsequent determination of plasma insulin concentrations. The remaining aliquot (2 mL) was placed in a tube containing potassium oxalate and sodium fluoride for the subsequent analysis of plasma glucose concentrations. All samples were kept on ice, until centrifuged at 3,000 rpm at 4°C for 10 minutes, upon completion of the trial. The plasma was then stored at -20°C for later analyses.

Plasma glucose concentrations were determined using the Glucose Oxidase method (Glucose Analyser 2; Beckman Instruments, Fullerton, CA), and plasma insulin concentrations were determined by nonspecific insulin radioimmunoassay (Count-A-Coat Insulin; Diagnostic Products, Los Angeles, CA) with an intra-assay and interassay coefficient of variation (CV) of 4.5% and 6.6%, respectively.

Minimal model analysis of the FSIVGT was performed according to the method reported by Bergman et al[27] using the software MINMOD (Copyright Bergman, 1986). A shortened blood sampling protocol of 120 minutes was used due to the high S_i of the subjects in this study. During the FSIVGT, plasma glucose concentrations returned to baseline values (5.14 $\pm$ 0.51 mmol/L) within 90 minutes (5.05 $\pm$ 0.90 mmol/L) of the glucose administration and decreased further at 120 minutes postglucose infusion (4.75 $\pm$ 0.51 mmol/L), dropping below 4 mmol/L after 90 to 100 minutes in 2 subjects. Plasma insulin concentrations returned to baseline within 120 minutes of the intravenous glucose administration (7.57 $\pm$ 2.50 *v* 7.80 $\pm$ 2.70 μU/mL at time 0 and 120, respectively). Only S_i values with a fractional standard deviation (FSD) of less than 50% were accepted and included in subsequent analyses.[28] Accordingly, the results of 14 of 61 FSIVGTs did not fit the criteria and were excluded. Therefore, the data of 47 subjects are presented.

Experimental Trial

Muscle fiber type, substrate content, and enzyme activities. A week after the FSIVGT, subjects returned to the laboratory to complete the experimental trial. On the day before the experimental trial, the subjects were instructed to train routinely for not longer than 1 hour prior to 6:00 PM. They subsequently fasted overnight for 10 to 12 hours and reported to the laboratory in the morning. Resting muscle biopsies were taken from the vastus lateralis muscle of 56 of the subjects, using the percutaneous needle biopsy technique. A portion of the muscle sample was frozen rapidly in liquid N_2 and stored at -80°C for subsequent analysis of glycogen and triglyceride content and citrate synthase (CS) activity. The remaining sample was orientated and imbedded in Tissuetek (Miles Laboratories, Naperville, IL), frozen in liquid nitrogen-cooled n-pentane (Saarchem, Muldersdrift, South Africa) and stored at -20°C for subsequent fiber type determination using the myofibrillar adenosine triphosphatase (ATPase) method. Briefly, serial sections (10 μm for pH 9.4 and 20 μm for pH 4.3 and 4.6) were cut in a cryostat at -20°C. Adjacent muscle sections were assayed for myofibrillar adenosine triphosphatase (mATPase) at pH 9.4 after acidic (pH 4.3 or 4.6) and alkaline (pH 10.4) preincubation to identify the 3 major fiber types.[29]

Prior to biochemical analysis of muscle glycogen and triglyceride content, a portion of the frozen muscle biopsy sample ($\approx$ 50 mg) was

freeze-dried and dissected free of any visible fat or connective tissue. Muscle glycogen content was determined as glucose residues (glucose oxidase method; Glucose Analyzer 2, Beckman Instruments) after hydrolysis of the muscle sample in 2 mol/L HCl at 95°C for 3 hours.[30] Muscle triglyceride content was measured by hydrolyzing the triglyceride to glycerol and FFAs as described by Kiens and Richter.[31] Glycerol concentrations were measured using a commercial glycerol kit (Boehringer, Mannheim, Germany).

For the determination of skeletal muscle CS activity, 15 to 50 mg (wet weight) of muscle was homogenized on ice in a phosphate buffer (1:19 wt/vol), sonicated on ice 3 times for 10 seconds (Virsonic 60, The Vitrus Co, Gardiner, NY), and measured using the technique described by Srere et al.[32] CS activity is expressed relative to protein content, as assayed in duplicate by the Biorad Protein assay (Bio-Rad Laboratories GmbH, Munich, Germany) using bovine serum albumin as a standard.

Steady-State RER. After the muscle biopsy, the subjects rested for 30 minutes and/or until their heart rate returned to prebiopsy levels. Oxygen consumption (VO_2), carbon dioxide production (CO_2), and RER were then measured for 15 minutes at rest and during a steady-state cycle ride at 25%, 50%, and 70% of W_{peak}, respectively, maintaining a pedaling frequency of 90 revolutions/minute. These workloads corresponded to 41%, 63%, and 80% VO_{2peak}, respectively. Gas exchange measurements were recorded for 5 minutes at rest and at each exercise workload, after a 10-minute stabilization period, as described above.

The reliability of the Oxycon Alpha analyzer was tested on a weekly basis by burning absolute ethanol (99% AR; Associated Chemical Enterprises (Pty), Glenvista, South Africa) as a reference. The reliability of RER, at rest and during exercise at different exercise intensities, has been previously tested in our laboratory.[32a] The CV for RER at rest, tested on 13 healthy male subjects on 3 occasions, was 2.1%. The CV for RER at 25%, 50%, and 70% of W_{peak}, tested on 9 endurance-trained male cyclists on 3 occasions, was 2.0%, 1.5%, and 1.4%, respectively. The intraclass correlation coefficients for RER at rest and at 25%, 50%, and 70% W_{peak} were 0.847, 0.658, 0.814, and 0.843, respectively.

Blood sampling and analysis. On the day of the experimental trial, a fasting, resting blood sample ($\approx$ 3 mL) was drawn from a forearm vein after resting RER had been measured for the subsequent determination of serum FFA concentration. The sample was placed in a tube containing gel and clot activator and kept on ice, until centrifuged at 3,000 rpm at 4°C for 10 minutes upon completion of the trial. The serum was then stored at -20°C for later analysis via enzymatic spectrophotometric measurement using a commercial kit (FFA Half-micro test; Boehringer).

Statistical Analysis

Bivariate correlations were used to explore the relationships between fasting resting plasma insulin concentrations and S_i, as determined by minimal model analysis, RER, body composition, training volume, dietary intake, plasma and muscle substrates, and muscle CS activity. All results are presented as means $\pm$ standard deviations (SD) and an alpha level of $P < .05$ was considered to be statistically significant.

RESULTS

The subject characteristics are presented in Table 1. Although the subjects performed at similar levels (completing a 104-km cycle race in the top 20% of the field), there was a large intersubject variation in body composition and physical work capacity (Table 1). The subjects' reported energy and macronutrient intakes for the 3 days preceding the experimental trial are also presented in Table 1. The mean RER at rest and during exercise at 25%, 50%, and 70% of W_{peak} was 0.82 $\pm$ 0.05, 0.86 $\pm$ 0.04, 0.90 $\pm$ 0.04, and 0.98 $\pm$ 0.04, respectively. As there were no differences between men and women in RER at rest and during exercise, fasting resting plasma insulin concentration or S_i (Table 1), all data were combined for all analyses.

Table 1. Characteristics of the Subjects

	Males (n = 45)	Females (n = 16)	Combined Group (n = 61)
Age (yr)	32 ± 19 (19-46)	29 ± 5 (20-39)	31 ± 7 (19-46)
Weight (kg)	77.3 ± 9.3 (57.2-103.5)	60.4 ± 5.3 (47.7-67.1)	72.9 ± 11.2 (47.7-103.5)
Waist (cm)	83.7 ± 7.4 (68.6-110.0)	74.7 ± 4.5 (67.9-84.8)	81.3 ± 7.9 (67.9-110.0)
% Body fat	15.9 ± 4.1 (9.1-25.9)	22.1 ± 3.6 (15.3-29.6)	17.5 ± 4.8 (9.1-29.6)
Sum 7 (mm)	69.7 ± 23.5 (38.5-130.1)	89.3 ± 19.5 (46.7-126.8)	74.9 ± 24.0 (38.5-130.1)
W_{peak} (W)	359 ± 36 (278-420)	243 ± 32 (179-300)	328 ± 62 (179-420)
VO_{2peak} (mL/kg/min)	57.6 ± 6.7 (44.0-70.0)	50.8 ± 6.3 (41.7-64.1)	55.9 ± 7.2 (41.7-70.0)
Energy intake (kJ)	12,642 ± 3,247 (6,477-19,200)	8,377 ± 2,654 (4,112-13,112)	11,504 ± 3,619 (4,112-19,200)
% CHO	45.6 ± 6.9 (31.8-62.0)	53.4 ± 3.4 (36.3-69.9)	47.7 ± 8.2 (31.8-69.9)
% Fat	32.6 ± 6.5 (19.4-46.2)	27.0 ± 9.0 (9.5-40.2)	31.1 ± 7.6 (9.5-46.2)
% Protein	15.5 ± 3.5 (8.5-24.2)	14.5 ± 2.0 (11.1-18.8)	15.2 ± 3.2 (8.5-24.2)
% Alcohol	3.4 ± 4.3 (0-21.4)	2.4 ± 3.4 (0-11.1)	3.1 ± 4.1 (0-21.4)
PS ratio	0.63 ± 0.33 (0.19-1.96)	0.66 ± 0.29 (0.25-1.46)	0.64 ± 0.32 (0.19-1.96)
Plasma [insulin] (μU/mL)	7.4 ± 2.7 (4.0-10.5)	7.2 ± 1.2 (5.7-9.0)	7.3 ± 2.4 (4.0-10.5)
S_i ($\times 10^{-4}$ min^{-1} · μU^{-1} · mL^{-1})	14.3 ± 6.6 (2.6-26.3)	13.3 ± 5.5 (6.4-24.0)	14.0 ± 6.1 (2.6-26.3)

NOTE. Values are means ± SD. Range is in parentheses.

Abbreviations: CHO, carbohydrate; PS ratio, polyunsaturated to saturated fat ratio; S_i, insulin sensitivity.

The average fasting plasma insulin concentration of the subjects in this study was 7.3 $\pm$ 2.4 μU/ml, ranging from 4.0 to 10.5 μU/mL (Table 1). The mean S_i was 14.0 $\pm$ 6.1 $\times$ 10^{-4} min^{-1} · μU^{-1} · mL^{-1}, ranging from 2.6 to 26.3 $\times$ 10^{-4} min^{-1} · μU^{-1} · mL^{-1} as seen in Table 1. There was no significant correlation between fasting plasma insulin concentration and S_i (r = -.14, P = .34).

There was also no significant correlation between fasting plasma insulin concentration or S_i and fasting RER, measured at rest and during exercise at 25%, 50%, and 70% W_{peak}; sum of 7 skinfolds, percent body fat, waist circumference, training volume, and dietary intake, including energy intake, macronutrient composition, and PS ratio; as well as plasma and muscle substrates, including plasma FFA concentration and muscle triglyceride and glycogen contents (Table 2).

In contrast, maximal oxygen uptake (VO_{2max}) and the proportion of type 1 muscle fibers were weakly, but significantly positively correlated with S_i (r = .30, P = .045 and r = .34, P = .026, respectively, Table 2).

Table 2. Correlation Matrix for Fasting, Resting Plasma Insulin Concentration and S_i as Determined by Minimal Model Analysis

		Resting RER (fasted)	Body Fat (%)	Waist (cm)	VO_{2max} (L/min)	Training Volume (METS/d)	Dietary Fat Intake (%)	PS Ratio	Plasma [FFA] (mmol/L)	Type 1 Muscle Fibers (%)	Muscle [TG] (mmol/kg dw)	Muscle [glycogen] (mmol/kg dw)
Fasting [insulin]	*r*	.022	−.001	−.53	.049	.159	−.133	−.194	−.154	−.141	−.130	.178
(μU/mL)	*P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S_i ($\times 10^{-4}$ min^{-1} ·	*r*	.162	−.133	.232	.297	.165	−.001	−.207	−.083	.343	−.001	.079
$\mu U^{-1} \cdot mL^{-1}$)	*P*	NS	NS	NS	.045	NS	NS	NS	NS	.026	NS	NS

Abbreviations: S_i, insulin sensitivity; VO_{2max}, maximal oxygen uptake; TG, triglyceride; PS ratio, polyunsaturated-to-saturated fat ratio; METS, metabolic equivalents; NS, not significant.

DISCUSSION

The major finding of this study was that fasting plasma insulin concentration and S_i, as determined by minimal model analysis, were not associated with fasting whole-body RER at rest or during low, moderate, or high-intensity exercise in physically trained athletes with above average performance ability. An additional finding was that fasting plasma insulin concentration and S_i were not correlated.

These findings differ from those of Astrup et al[5] and Toubro et al[3] who found a positive correlation between fasting plasma insulin concentration and 24-hour carbohydrate oxidation, measured in a metabolic chamber. However, in the present study, the range of fasting plasma insulin concentrations was narrow, and the mean concentration was half that measured in the study of Astrup et al[5] and Toubro et al[3] (7.1 ± 1.4 *v* 14 ± 7, 15 ± 8 μU/mL, respectively). Conversely, Zurlo et al[6] found a negative correlation between fasting plasma insulin concentrations and 24-hour RER in obese Pima Indians in whom the mean fasting plasma insulin concentration was 45 ± 21 μU/mL, clearly representing an insulin-resistant state.

The narrow range of fasting plasma insulin concentrations was associated with a high, but variable range of S_i in these well-trained cyclists (14.0 ± 6.1 × 10^{-4} min^{-1} · μU^{-1} · mL^{-1}). Kahn et al[33] described the relationship between fasting plasma insulin concentration and S_i (using minimal model analysis) as negatively hyperbolic.[33] This relationship suggests that lower fasting insulin concentrations are associated with higher S_i, whereas increased fasting insulin concentrations are associated with greater insulin resistance (lower S_i).[33] However, because the relationship is hyperbolic, when S_i is high (as in the present study), relatively small changes in fasting plasma insulin concentration (4.0 to 10.5 μU/mL) are associated with large changes in S_i (2.6 to 26.3 × 10^{-4} min^{-1} · μU^{-1} · mL^{-1}).[33]

Previous studies have also attributed the wide variability and particularly high S_i values to increased S_i in response to a recent bout of exercise.[34,35] However, this is unlikely to be a contributing factor in the present study, as the subjects refrained from physical exercise for at least 24 hours before the FSIVGT and for at least 12 hours before the fasting RER measurements. Alternatively, the wide variability and high S_i values may relate to the large variability in VO_{2max} (41.7 to 70.0 mL/kg/min), notwithstanding these values being in different ranges from the studies on sedentary, overweight subjects (Table 3).

Another possible explanation for the wide variability in S_i may be the FSIVGT protocol used, as only glucose and not glucose and tolbutamide or insulin was infused. When comparing the glucose-only protocol with the tolbutamide protocol, Prigeon et al[28] found that the glucose-only protocol was more variable with a CV of 19.3% compared with 6.3% for the tolbutamide protocol. In addition, Steil et al[36] have found that the average interday CV for S_i measured using the minimal model in 11 normal men was 20.2% ± 3.2%.

We also cannot rule out the possibility that the muscle biopsy procedure itself may have partly altered the relationship between S_i and RER. There are limited data to suggest that the concentrations of stress hormones, such as cortisol and norepinephrine, may remain elevated in plasma for at least 20 minutes postbiopsy.[37,38] However, in these studies, the changes in plasma cortisol and norepinephrine concentrations were not directly associated with changes in fasting plasma metabolite concentrations.[37,38] Furthermore, our measurements took place a minimum of 40-minutes postbiopsy and were conducted in the entire group, thereby minimizing possible bias.

Notwithstanding these possible limitations of the study, both high S_i and wide variation in S_i have previously been reported,[14,33] but only in lean, exercise-trained individuals (Table 3). Tokuyama et al[16] documented high S_i in very lean, endurance-trained athletes (Table 3). Slightly lower S_i values were reported in strength-trained athletes, with extremely low body fat, but relatively low VO_{2max} values (Table 3). Furthermore, in a population study of 380 young subjects with varying degrees of obesity, Clausen et al[14] found that the variation in S_i (9.1 ± 5.3 × 10^{-4} min^{-1} · μU^{-1} · mL^{-1}) was highest among the leanest subjects and low in the obese subjects, as their level of S_i was at the lowest level of S_i measurement.[14] They found a significant negative correlation between body fatness and S_i, with body mass index (BMI) explaining 29% and 12% of the variation in men and women, respectively.[14] VO_{2max} was the second strongest determinant of S_i, accounting for 17% of the variance in the regression model.[14] Thus, based on the results from these studies and those listed in Table 3, it appears that aerobic fitness and percent body fat are important determinants of S_i.

Indeed, we found a weak, but significant, positive correlation between VO_{2max} and S_i in the present study ($r = .30$, $P = .045$, Table 2). This finding has been supported by numerous cross-sectional studies that have demonstrated that endurance- or strength-trained individuals have higher S_i than untrained individuals.[16,17,39,40] Moreover, longitudinal training studies have shown that as little as 7 days[18] of exercise training improves S_i,[41] even in the elderly.[18,42] Exercise training increases the responsiveness of muscle glucose uptake to insulin via an increase in GLUT4 expression,[43] but S_i and GLUT4 concen-

Table 3. Summary of Studies That Have Measured S_i Using Minimal Model Analysis

Reference No.	Method	No.	Subject Population	Age (yr)	Gender	BMI (kg/m^2)	Body Fat (%)	VO_{2max} (mL/kg/min)	S_i ($\times 10^{-4}$ $min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$)
Goedecke et al	Glucose-only	47	Endurance trained	30.9 ± 6.6	Male + female	23.5 ± 2.3	17.5 ± 4.8	55.9 ± 7.2	14.0 ± 6.1
Hickey[19]	Insulin	22	Healthy, sedentary	32.8 ± 8.4	Male	25.8 ± 2.8	22.9 ± 4.7	39.6 ± 6.1	3.31 ± 1.9
Brun[57]	Insulin	7	Healthy, untrained	29.6 ± 5.4	Male + female	22.8 ± 2.9	—	—	6.23 ± 2.6
Clausen[14]	Tolbutamide	380	Healthy population	25.5 ± 3.5	Male + female	24.2 ± 3.5	20 ± 6	44 ± 9	9.1 ± 5.3
				25.0 ± 3.5		23.0 ± 3.9	26 ± 7	38 ± 8	9.1 ± 5.8
Houmard[41]	Insulin	11	Sedentary, middle-aged	48.4 ± 3.3	Male + female	29.06	30.1 ± 6.3	28.1 ± 5.0	2.76 ± 2.0
Araujo-Vilar[58]	Glucose-only	12	Healthy, sedentary	31.8 ± 10.8	Male + female	22.6 ± 7.6	—	34.1 ± 7.9	5.1 ± 2.1
Tokuyama[16]	Insulin	14	Sedentary	22.2 ± 2.6	Males	20.9 ± 1.9	16.3 ± 7.4	40.9 ± 5.2	6.2 ± 2.6
		12	Endurance trained	20.6 ± 1.0	Males	20.1 ± 1.4	10.3 ± 2.8	56.2 ± 4.2	14.6 ± 12.4
Fujitani[17]	Insulin	20	Sedentary	23.0 ± 4.5	Males	22.0 ± 2.3	14.2 ± 5.9	43.2 ± 6.7	6.2 ± 3.1
		11	Strength trained	20.0 ± 3.3	Males	25.4 ± 2.3	9.0 ± 3.0	44.4 ± 6.6	10.5 ± 4.0
Cox[18]	Insulin	9	Young, active	22.4 ± 2.4	Female	22.7 ± 1.2	22.0 ± 3	30.8 ± 6.0	5.1 ± 2.7
		9	Young, active	20.9 ± 2.7	Male	24.3 ± 2.4	12.3 ± 3.6	41.4 ± 5.1	3.8 ± 2.1
		10	Old, active	60.9 ± 3.2	Female	25.5 ± 3.2	31.2 ± 4.8	21.2 ± 7.4	4.5 ± 1.9
		8	Old, active	56.5 ± 5.3	Male	30.1 ± 5.3	27.2 ± 5.0	22.5 ± 5.3	2.1 ± 1.4
Kahn[42]	Tolbutamide	14	Old, sedentary	68.1 ± 5.6	Male	25.3 ± 3.0	22.6 ± 3.7	35.2 ± 3.3	3.1 ± 1.5
		11	Young, sedentary	28.0 ± 2.3	Male	25.7 ± 3.3	20.6 ± 6.2	51.8 ± 5.6	3.9 ± 1.3

NOTE. Values are means ± SD.

Abbreviations: S_i, insulin sensitivity; BMI, body mass index; VO_{2max}, maximal oxygen uptake.

trations return to pretraining levels within 14 days of training cessation.[41]

In contrast to the findings of Clausen et al[14] and Kahn et al,[33] we found no correlation between S_i and body fatness, including measures of central obesity, such as WHR and waist circumference (Table 2). Clausen et al[14] found that WHR and waist circumference were negatively associated with S_i and accounted for 19% and 27% of the variance in the men and 8% and 13% of the variance in S_i in the women, respectively.[14] The lack of association between measures of body fatness and S_i in the present study may be the result of the narrow range in waist circumference or the relatively high VO_{2max} values for a given body fat value. Furthermore, the relationship may have been influenced by the skeletal muscle composition of these endurance-trained subjects. The mean muscle fiber composition of the subjects was 50.8% ± 13.5% type 1, 44.5% ± 13.3% type 2a, and 2.6% ± 3.6% type 2b, displaying a smaller proportion of type 2b fibers compared with studies on untrained, sedentary subjects with 15% to 30% type 2b fiber.[19,21,22]

Previous research has demonstrated a positive association between the proportion of type 1 muscle fibers and S_i and a negative association between the proportion of type 2b muscle fiber and S_i.[19,21,22] We only demonstrated a significant association between S_i and the proportion of type 1 muscle fibers (r = .34, P = .026, Table 2) and not the type 2b fibers (r = -.13, P = .411). This lack of association may be related to the low levels and narrow range of type 2b fiber (1% to 14.9%) in our study, with nearly a quarter of the subjects possessing no measurable type 2b fibers.

The precise mechanism for the relationship between muscle fiber type distribution and S_i is not known, but could be related to the oxidative capacity of the muscle. Type 1 fibers are rich in mitochondria with a higher oxidative capacity than the mitochondria-poor type 2b fibers that have a low oxidative capacity.[44,45] Studies on sedentary individuals have shown a positive association between S_i and the oxidative capacity of the muscle as assessed by nicotinamide adenine dinucleotide (reduced form) (NADH) staining and the activity of CS.[19,22] Hickey et al[19] demonstrated a positive correlation between CS activity and S_i in 22 sedentary male subjects (Fig 1B), which was not supported by our study (r = -.13, P = .785, Fig 1A). Skeletal muscle CS activity was higher and more variable in the present study than the study of Hickey et al,[19] probably due to differences in the training status of the 2 populations (VO_{2max} = 55.9 ± 7.2 *v* 39.6 ± 1.3 mL/kg/min). These findings suggest that in trained subjects, the association between muscle fiber distribution and S_i cannot be explained by CS activity as a proxy for the oxidative capacity of the muscle.

An alternate explanation for the relationship between muscle fiber type distribution and S_i could be the membrane phospholipid composition of skeletal muscle. Kriketos et al[22] demonstrated a relationship between muscle fiber type and phospholipid fatty acid composition, with type 1 fiber membranes having a greater proportion of unsaturated fatty acids. This may have an effect by influencing the fluidity of the membrane and/or the glucose transporter intrinsic activity and/or the intrinsic activity of ion transport molecules.[46]

Rat studies have demonstrated that changes in skeletal mus-

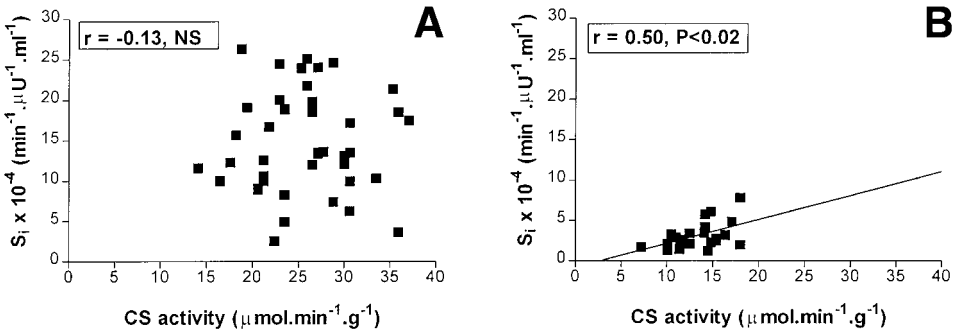

Fig 1. Relationship between S_i and skeletal muscle CS activity in (A) the present study and (B) that of Hickey et al (reprinted with permission[19]).

cle membrane phospholipid composition can be altered by dietary fat intake and can therefore influence S_i.[47,48] However, we found no significant correlation between the PS fat ratio or total fat intake and S_i (Table 2). Human studies examining this association have been conflicting and were most likely influenced by the degree of fatness of the subjects.[49-51] Lovejoy et al[49] found that 3 weeks of an isoenergetic high fat (50% fat by energy) diet reduced S_i by 6% in a group of obese African-American and Caucasian women. In contrast, Yost et al[50] found no effect of 16 days of a high-fat diet on S_i in 25 normal weight subjects. Furthermore, results of the Insulin Resistance Atherosclerosis Study showed that total dietary fat intake was inversely related to S_i, but only in the obese subjects and not the lean subjects.[51] The relationship in the obese subjects was attenuated and no longer significant when S_i was adjusted for BMI.[51]

Increased dietary fat intake has also been associated with increased muscle triglyceride storage,[52-54] which has been shown to reduce S_i. Pan et al[24] found an inverse association between skeletal muscle triglyceride content and S_i, derived by the euglycaemic-hyperinsulinaemic clamp technique, in a group of obese Pima Indians. Together with waist-thigh ratio, intramuscular triglyceride content accounted for 44% of the variance in S_i in these subjects.[24] In contrast, we failed to show a relationship between skeletal muscle triglyceride content and S_i (Table 2), despite the relatively high muscle triglyceride content in these trained subjects (37.1 ± 18.6 mmol/g dry weight). Although the variability in triglyceride content in human skeletal muscle biopsy samples may explain these findings,[55] the discrepancy between our results and others may be associated with the training status of the subjects in our study. Training increases both the subsarcolemmal mitochondria and intracellular lipid deposits, leading to a possible improved usage of these fat stores as a fuel source.[56] Moreover, exercise training has been shown to result in far fewer, but much larger lipid droplets that are closely associated with the mitochondria (Helge and Storlien, unpublished data in Helge et al[46]). Therefore, exercise training, via its ability to alter the location of the lipid droplet in relation to the mitochondria, may eliminate the observed association between intramuscular triglyceride storage and S_i.

In summary, fasting plasma insulin concentrations and S_i, measured using minimal model analysis, were not associated with fasting whole-body RER at rest or during low-, moderate-, or high-intensity exercise in trained subjects with above average performance ability. S_i in these trained subjects was higher and more variable than that demonstrated in studies of obese, sedentary subjects. S_i was only associated with the proportion of type 1 muscle fibers and VO_{2max} and not with body fatness, intramuscular triglyceride content, or dietary fat intake as demonstrated in obese, sedentary subjects. However, it is not known how S_i would alter nutrient partitioning in other situations, for example, during recovery, under conditions of low glycogen, or while ingesting exogenous carbohydrate or fat during exercise.

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