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## Research Article

# Whole-Body Lipid Oxidation during Exercise is Correlated to Insulin Sensitivity and Mitochondrial Function in Middle-Aged Obese Men

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## Abstract

Defects in insulin sensitivity are associated with metabolic inflexibility and obesity albeit some obese subjects have preserved insulin sensitivity. A decrease in lipid oxidation during exercise can be found in obesity and type 2 diabetes. This lipid oxidation capacity has been found to be associated in healthy or overweight subjects with insulin sensitivity and mitochondrial function. Thus, we aimed at delineate the interrelationships among the maximal ability to oxidize lipids at exercise (LIPOXmax) insulin sensitivity and mitochondrial function in middle-aged obese men with different levels of insulin sensitivity

After an overnight fast, twenty middle-aged male obese subjects performed: a maximal incremental exercise test allowing the determination of LIPOXmax, two days after a measurement of insulin sensitivity and two days after a muscle biopsy for mitochondrial respiration.

According to the minimal model, we found 9 insulin-resistant and 11 insulin-sensitive middle-aged obese men. LIPOXmax (expressed as power intensity) and whole-body insulin sensitivity are significantly correlated in obese insulin-sensitive but not in insulin-resistant obese men. LIPOXmax and insulin sensitivity are correlated to mitochondrial pyruvate oxidation and mitochondrial density. Multivariate analysis indicates that insulin sensitivity is statistically better explained by the LIPOXmax which is in turn better explained by the mitochondrial function parameter  $V_{max}/V_0$  pyruvate.

Thus, insulin sensitivity, mitochondrial function and the ability of muscle to oxidize lipids during exercise are three closely correlated parameters in middle-aged male obese insulin-sensitive patients.

**Keywords:** Insulin sensitivity; Lipoxmax; Mitochondrial respiration

## Abbreviations

ADP: Adenosine Diphosphate; BMI: Body Mass Index; COP: Crossover Point; CS: Citrate Synthase; HDL: High Density Lipoprotein; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; Hs-CRP: Ultrasensitive-C Reactive Peptide; IR: Insulin Resistance; IVGTT: Intravenous Glucose Tolerance Test; LDL: Light Density Lipoprotein; Lipoxmax: Maximal Lipid Oxidation During Exercise Test; MFO: Maximal Fat Oxidation; MM: Muscle Mass; QUICKI: Quantitative Insulin Sensitivity Check Index; RER: Respiratory Exchange Ratio  $V_{CO2}/V_{O2}$ ; Sg: Glucose Assimilation; SI-MINIMOD Or SI: Insulin Sensitivity determined by Minimal Model; Systolic BP: Systolic Blood Pressure;  $V_0$ : Mitochondrial Respiration Rates without ADP;  $V_{max}$ : Mitochondrial Respiration Rates in the Presence of a Saturating Concentration of ADP;  $V_{max}/V_0$ : Maximal Mitochondrial Respiration with ADP divided by basal respiration;  $VO_{2max}$ : Maximal Oxygen Consumption during Incremental Exercise Test.

## Introduction

Low insulin sensitivity is a common defect in obesity and type 2

diabetes [1] and is associated with a loss of metabolic flexibility [2] and, although this aspect is more controversial [3], with impaired mitochondrial function [4,5]. The report by several teams of a decrease in the ability to oxidize lipids at exercise [6-8], a parameter which is correlated to mitochondrial respiration in type 2 diabetic patients [9], is likely to be related to these pathophysiological mechanisms [10,11]. Moreover, inter-subject variation in maximal fat oxidation during exercise is quite large (49–93%) [12] and not fully explained although gender [13], training [9,14,15] or nutritional status [16] appear to be major determinants of this variability. Moreover, maximal fat oxidation has been shown to be correlated to the distribution of type I fibers [12], lean body mass and maximal oxygen consumption in overweight men [17], 24-hour fat oxidation [18] and surrogates of insulin sensitivity in young, healthy men [18]. Alteration in skeletal muscle lipid oxidation seems to be an early event in the development of Insulin Resistance (IR) [19] and leads to the accumulation of intramyocellular lipid intermediates [10] which in turn inhibits the insulin signaling pathway [20] and insulin sensitivity [21]. Moreover, reduced fat oxidation ability in resting condition has previously been reported to predict future body composition. Thus, high respiratory quotient implying low fat oxidation has been associated with a

high risk of fat mass gain in premenopausal women [11]. Thus, understanding determinant and variation of lipid oxidation is of clinical importance for body weight maintenance [22, 23].

In healthy young men, the ability to oxidize lipids at exercise has been shown to be correlated to a surrogate of insulin sensitivity [18], suggesting that the more an individual is insulin sensitive, the more he is able to oxidize fat during exercise. However, this issue has not yet been addressed in middle-aged obese subjects and remains to be studied with a quantitative and reliable measurement of insulin sensitivity. Therefore, we aimed in this study at delineating the interrelationships among the ability to oxidize lipids at exercise, insulin sensitivity and mitochondrial function in middle-aged obese men with different insulin sensitivity, since there are obese subjects with normal insulin sensitivity [24]. Thus, we performed a measurement of insulin sensitivity with the minimal model, an exercise calorimetry, and a muscle biopsy with ex-vivo assessment of mitochondrial respiration in insulin-sensitive and insulin-resistant middle-aged obese men.

## Materials and Methods

### Ethical approval

Informed consent was obtained from all subjects after explanation about the nature of the study and the risks related to their participation to the experiments. The study was approved by our local Ethics Committee (# 03/10/GESE) and was registered as a clinical trial under the number NCT01644942.

### Experimental design

Twenty middle-aged male obese subjects participated to this study. Subjects were sedentary, measured by the VOORRIPS index [25], and took no medications.

Each subject was asked to come three times to the laboratory at 8 AM after an overnight fast. The first time was for the enrolment visit, which included clinical examination, a physical activity questionnaire, anthropometric measurements and the signature of the informed consent. Weight and height were collected and Body Mass Index (BMI) was calculated as follows: weight in kilograms divided by height in meter squared ( $\text{kg}/\text{m}^2$ ). Two days later, the subjects came back to the laboratory to perform an exercise test (see below). After another two days, measurement of insulin sensitivity and glucose effectiveness with the minimal model and skeletal muscle biopsy of the vastuslateralis were performed.

### Exercise test

All subjects were asked to come and perform the test in the morning after an overnight fast (12 hours). The test consisted of six minute steady-state workloads theoretically set at 20, 30, 40, 50, and 60% of  $P_{\text{max}}$ . However, these intensity levels can be modified during the test according to the evolution of the respiratory exchange ratio ( $\text{RER} = \text{VCO}_2/\text{VO}_2$ ) in order to obtain values of RER below and above 0.9 which is the level of the Crossover Point (COP) defined below. The subjects performed the test on an electromagnetically braked cycle ergometer (Ergoline Bosch 500). Heart rate and electrocardiographic parameters were monitored continuously throughout the test by standard 12-lead procedures. Metabolic and ventilatory responses were assessed using a digital computer based

breath to breath exercise analyzing system (Jaeger MS-CPX). Thus, we could measure  $\text{VO}_2$  and  $\text{VCO}_2$  (in  $\text{ml}/\text{min}$ ). Lipid oxidation (Lipox) and carbohydrate utilization (Glucox) rates were calculated by indirect calorimetry from gas exchange measurements according to the non-protein respiratory quotient technique. This technique provided carbohydrate and lipid oxidation rates at different exercise intensities. Additionally, after smoothing the curves, we calculated the two parameters quantifying the balance between carbohydrates and lipids induced by increasing exercise intensity: the Maximal Lipid Oxidation Point (LIPOX $_{\text{max}}$ ) and the Crossover Point (COP). The LIPOX $_{\text{max}}$  is the exercise intensity at which lipid oxidation reaches its maximal level before decreasing while carbohydrate utilization further increases. It is calculated as previously reported after smoothing of the curve plotting lipid oxidation as a function of power. The maximal lipid oxidation rate at the LIPOX $_{\text{max}}$  was expressed in  $\text{mg}/\text{kg}/\text{min}$ . The Crossover Point (COP) is the exercise intensity at which the proportion of carbohydrate used to provide energy becomes predominant over lipid oxidation [15]. Beyond this point, the subject is referred to as "glucose dependent". It was calculated as the exercise intensity where 70% of the substrates used to provide energy are carbohydrates and 30% are lipids [26].

### Insulin sensitivity

A frequent-sampling intravenous glucose tolerance test was performed according to the protocol usually employed in our unit [27]. After 12-hours fast, at 09h00 a cannula was placed in the cephalic vein at the level of the cubital fossa for blood sampling. A glucose injection (0.5 g/kg, solution at 30%) was administered in the contralateral cephalic vein slowly over precisely 3 min. Blood samples were drawn twice before the glucose bolus and 1, 3, 4, 8, 10, 15, 19, 20, 22, 30, 41, 70, 90, and 180 min after glucose injection. Insulin (0.02 U/kg body weight, i. e. 1 or 2 U) was injected iv immediately after the 19 min sample. The 1 and 3 min samples were used for determination of the insulin early secretory phase. The other samples were necessary for minimal model calculations.

The minimal model analysis of the IVGTT was performed according to Bergman's method [28,29] with the software TISPAG from University of Montpellier (Montpellier, France) [30], which uses a nonlinear least square estimation. SI-MINIMOD was calculated from the following equations:

$$dG(t)/dt = (p_1 \cdot X(t) - G(t)) \cdot p_1 \cdot G_b;$$

$$G(0) = G_0;$$

$$dX(t)/dt = p_2 \cdot X(t) - p_3 \cdot (I(t) - I_b);$$

$$X(0) = 0,$$

where  $G(t)$  and  $I(t)$  are plasma glucose and insulin concentrations,  $X(t)$  is the insulin concentration in a compartment remote from plasma (insulin action), and  $p_1$  to  $p_3$  are model parameters.  $G_0$  is the glucose concentration that would be obtained immediately after injection if there were instantaneous mixing in the extracellular fluid compartment.  $G_b$  and  $I_b$  are the basal values of glucose and insulin. Parameter  $p_1$  represents the fractional disappearance rate of glucose independent of any insulin response.  $p_3$  and  $p_2$  determine the kinetics of insulin transport into and out of, respectively, the remote insulin compartment where insulin action is expressed. SI is an index of the

**Table 1:** Subjects characteristics and comparison of balance of substrates and exercise performance parameters for all subject and insulin-sensitive and insulin-resistant subjects.

	All subjects	Insulin-sensitive	Insulin-resistant
N	20	11	9
Age (years)	53.1 ± 1.6	51.2 ± 2.6	55.5 ± 1.6
VOORRIPS index	3.3 ± 0.5	3.5 ± 0.8	3.0 ± 0.8
Body weight (kg)	97.2 ± 2.3	94.0 ± 2.7	101.1 ± 3.6
BMI (kg/m <sup>2</sup> )	32.9 ± 0.6	31.9 ± 0.7	33.9 ± 1
Body fat mass (kg)	30.0 ± 1.6	29.0 ± 2.1	31.2 ± 2.5
Body fat-free mass (kg)	67.1 ± 1.2	64.9 ± 1.5	69.8 ± 1.5*
Total water (l)	50.9 ± 1.1	48.5 ± 1.3	54.0 ± 1.3*
Waistcircumference (cm)	109.6 ± 1.7	106.3 ± 2.0	113.6 ± 2.3*
Hip circumference (cm)	106.6 ± 1.2	104.1 ± 1.5	109.6 ± 1.4*
Waist/Hip ratio	1.03 ± 0.01	1.02 ± 0.01	1.03 ± 0.01
Fasting plasma insulin (μU/ml)	11.6 ± 1.4	8.9 ± 1.2	15.3 ± 2.3*
Fasting plasma glucose (mM)	5.4 ± 0.1	5.2 ± 0.1	5.7 ± 0.2*
SI (min <sup>-1</sup> /(μU/ml)×10 <sup>4</sup> )	3.6 ± 0.5	5.5 ± 0.6	1.5 ± 0.2*
HOMA-IR	2.9 ± 0.3	2.1 ± 0.3	3.9 ± 0.5*
Sg (%/min)	2.3 ± 0.2	2.6 ± 0.3	1.9 ± 0.1*
Fasting plasma cholesterol (mM)	5.39 ± 0.34	5.52 ± 0.39	5.21 ± 0.63
Fasting plasma HDL-cholesterol (mM)	1.11 ± 0.06	1.16 ± 0.06	1.04 ± 0.11
Fasting plasma LDL-cholesterol (mM)	3.49 ± 0.23	3.40 ± 0.28	3.60 ± 0.39
Systolicblood pressure (mmHg)	130.9 ± 2.3	125.5 ± 2.4	138.8 ± 2.3*
Diastolicblood pressure (mmHg)	80.9 ± 1.4	81 ± 1.8	80.9 ± 2.3
hs-CRP (mg/l)	2.7 ± 0.4	3.4 ± 0.6	1.9 ± 0.4
Vmax/V0 pyruvate	3.17 ± 0.50	4.03 ± 0.86	2.22 ± 0.23
CS (μmol/min/g tissue)	12.83 ± 1.52	15.32 ± 2.39	9.72 ± 0.95
VO <sub>2</sub> max (ml/min/kg)	23.2 ± 1.2	24.6 ± 1.8	21.6 ± 1.2
Max power ( watt)	150.8 ± 7.6	160.9 ± 12.2	138.4 ± 6.6
LIPOX <sub>max</sub> (watt)	53.1 ± 3.7	57.0 ± 5.7	48.8 ± 4.3
COP (watt)	54.4 ± 5.8	62.3 ± 7.8	45.6 ± 8.2

\*significantly different from obese insulin-sensitive subjects, p<0.05.

BMI: Body Mass Index; COP: Crossover Point; CS: Citrate Synthase; HDL: High Density Lipoprotein; HOMA-IR: Homeostatic Model Assessment Of Insulin Resistance; Hs-CRP: Ultrasensitive-C Reactive Peptide; LDL: Light Density Lipoprotein; Lipoxmax: Maximal Lipid Oxidation During Exercise Test; Sg: Glucose Assimilation; SI-MINIMOD Or SI: Insulin Sensitivity Determined by Minimal Model; Vmax/V0: Maximal Mitochondrial Respiration with ADP Divided by Basal Respiration.

influence of plasma insulin on glucose's own effect on the glucose concentration. Thus, SI-MINIMOD is equal to -p3/p2.

### Skeletal muscle biopsies

Vastus lateralis muscle biopsies were taken according to the percutaneous Bergström technique after local anesthesia (xylocaine), as routinely used in our laboratory [10]. The muscle samples (200-300mg) were divided in two portions. One part was immediately frozen in liquid nitrogen and stored at -80°C for enzymatic determinations (i. e., citrate synthase: CS activity). The other portion was used for the in situ mitochondrial respiration studies and was immediately placed

in an ice-cold relaxing solution containing (mM): EGTA-calcium buffer: 10 (free Ca<sup>2+</sup> concentration 100 nM), Imidazole: 20, KH<sub>2</sub>PO<sub>4</sub>: 3, MgCl<sub>2</sub>: 1, Taurine: 20, DTT: 0. 5, MgATP: 5 and, PCr: 15.

### Mitochondrial respiration

Respiratory parameters of the total mitochondrial population were analysed in situ on fresh skeletal muscle fibres as previously described [31]. Bundles of muscle fibres were manually isolated and saponin-skinned (50 μg/ml saponin for 30 min at 4°C). In order to completely remove saponin and free ATP, the fibers were then washed with continuous stirring in relaxing solution for 10 min (4°C) followed by 2 x 5min washes in respiration solution at 4°C (same composition as the relaxing solution, except that MgATP and phosphocreatine were replaced by 3 mM phosphate and 2mM fatty acid-free bovine serum albumin). After washing, the fibers were stored on ice till used. Respiration rates were determined at 27°C with a Clark electrode (Strathkelvin Instruments, Glasgow, Scotland) in an oxygraphic cell with continuous stirring in respiration solution. Respiration rates were recorded in the presence of pyruvate (10 mM)/malate (2 mM) and palmitoyl-L-carnitine (40 μM). For each sample, basal oxygen consumption without ADP (V0) was first recorded, and then the ADP-Stimulated Maximal Respiration (Vmax) was determined in the presence of a saturating concentration of ADP (2 mM). At the end of each measurement, cytochrome c was added to investigate the outer mitochondrial membrane integrity [32]. After the following respiratory measurements, the fibre bundles were dried overnight, and weighed the next day. Respiration rates were expressed in micromoles of O<sub>2</sub> per minute per gram of dry weight.

### Citrate Synthase (CS) activity

Frozen muscle biopsies were homogenized in 40 volumes of a solution (pH 8. 2) containing (mM): Tris HCl: 50, EDTA: 1 and MgSO<sub>4</sub>: 5 in a glass homogenizer, as described by Srere et al. The crude homogenates were then centrifuged for 10 min at 500g and supernatants assayed for and CS activities. CS activity was measured with 0. 38mM oxaloacetate and 1.2 mM acetyl-CoA. Enzyme activity was monitored by recording the changes in absorbance at 412 nm over 2.5 minutes at 30°C.

### Blood analysis

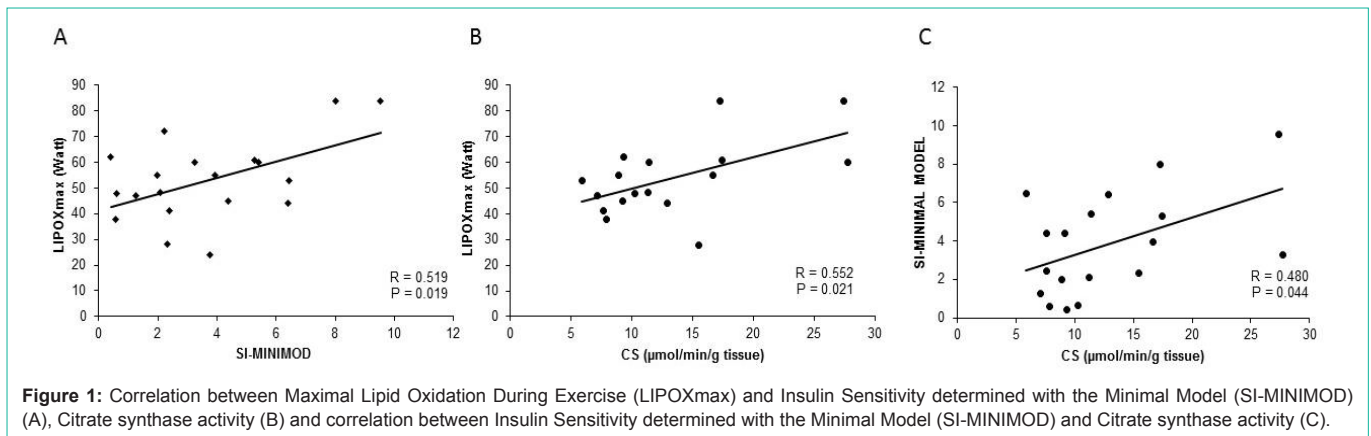
Fasting insulin was assessed by radioimmunoassay (kit Bi Insuline IRMA, Schering CIS bio international, Marcoule, France) and plasma glucose concentrations with an automate (AU2700, Olympus, Rungis, France).

### Data analysis

All statistical analyses were performed using a commercial software package (SigmaStat). Data are presented as means (±SEM). Significance of differences among obese insulin sensitive and obese insulin resistant were determined by using Student t-test. Correlations were determined by Spearman analysis. Multivariate analysis was performed using stepwise regression analysis. Statistical significance was accepted at p<0. 05.

## Results

Comparison of anthropometric, metabolic parameters, whole body oxidative capacity and balance of substrate during exercise in obese insulin-sensitive and obese insulin-resistant subjects.



The characteristics of all subjects are shown in Table 1. Subjects were classified accordingly to their insulin sensitivity measured with the minimal model (SI minimal model). Minimal model-derived insulin sensitivity ranged between 0.41 and 9.53  $\text{min}^{-1}/(\mu\text{U}/\text{ml})$ . 10-4 i.e. all the usual spectrum of insulin sensitivity. There were only 9 values below the cut-off value for the lowest quartile of SI-MINIMOD (i.e. 2.5  $\text{min}^{-1}/(\mu\text{U}/\text{ml})$ . 10-4 in our population). Thus, in the samples studied here, there are 9 obese insulin-resistant and 11 insulin-sensitive obese patients.

Age, sedentarity, metabolic parameters, and mitochondrial function were similar in obese insulin-sensitive and obese insulin-resistant subjects. The main differences were related to insulin sensitivity and related parameters. Obese insulin-resistant had higher levels of insulin, glucose and HOMA-IR index than obese insulin-sensitive subjects. Obese insulin-resistant subjects exhibited also higher fat-free mass, larger hip and waist circumferences and had a highersystolic blood pressure. Whatever the parameters studied, we did not found any difference in exercise capacity (VO<sub>2</sub>max or maximum power elicited) or substrate utilization (LIPOXmax, COP) between both groups as illustrated in Table 1.

LIPOXmax is correlated with parameters related to insulin sensitivity.

As shown of Figure 1A, when subjects were examined as a whole, positive correlations were found between LIPOXmax (expressed in as power intensity) and whole-body insulin sensitivity (SI-MINIMOD;  $R = 0.519$ ,  $p = 0.019$ ). Similarly, LIPOXmax is significantly correlated to muscle oxidative parameters such as citrate synthase activity ( $R = 0.552$ ,  $p = 0.021$ , Figure 1B) and mitochondrial respiration ( $V_{\text{max}}/V_0$  pyruvate;  $R = 0.482$ ,  $p = 0.043$ ). There is also a positive correlation between LIPOXmax and COP ( $R = 0.538$ ,  $p = 0.026$ ) as well as VO<sub>2</sub> max expressed in ml/min/kg ( $R = 0.750$ ,  $p < 0.001$ ).

SI-MINIMOD was also correlated with oxidative parameters such as citrate synthase activity ( $R = 0.480$ ,  $p = 0.044$ , Figure 1C) and mitochondrial respiration ( $V_{\text{max}}/V_0$  pyruvate;  $R = 0.613$ ,  $p = 0.005$ ). We found a negative correlation between SI-MINIMOD and BMI ( $R = -0.498$ ,  $p = 0.026$ ) and systolic blood pressure ( $R = -0.664$ ,  $p = 0.004$ ).

Metabolic and insulin sensitivity parameters are not correlated in obese insulin-resistant men.

**Table 2:** Simple linear correlation analyses between LIPOXmax and oxidative parameters, metabolic variables for insulin-sensitive (n=11) and insulin-resistant (n=9) middle-aged obese men.

LIPOXmax (watt) is related to				
Variables	Insulin-sensitive		Insulin-resistant	
	r	p	r	p
SI-minimal model	0.701	0.024	-0.088	0.821
VO <sub>2</sub> max (ml/min/kg)	0.828	0.003	0.48	0.191
Vmax/V0 pyruvate	0.632	0.068	-0.558	0.118
CS (µmol/min/g tissue)	0.597	0.089	-0.458	0.254

CS: Citrate Synthase; Lipoxmax: Maximal Lipid Oxidation During Exercise Test; SI-MINIMOD Or SI: Insulin Sensitivity Determined by Minimal Model; Vmax/V0: Maximal Mitochondrial Respiration with ADP Divided by Basal Respiration; VO<sub>2</sub>max: Maximal Oxygen Consumption During Incremental Exercise Test.

When subjects were separated according to their insulin sensitivity we found again a strong correlation between LIPOXmax and SI-MINIMOD or VO<sub>2</sub> max and closed to significance for mitochondrial parameters only in obese insulin-sensitive subjects whereas these relations are lost in obese insulin-resistant subjects, Table 2.

### Multivariate analysis

Astepwise regression analysis of the determinants of insulin sensitivity selects among correlated parameters (LIPOXmax, Vmax/V0 pyruvate, CS) only LIPOXmax ( $R = 0.636$ ,  $p = 0.008$ ) and excludes mitochondrial function parameters Vmax/V0 and CS. A stepwise regression analysis of the determinants of LIPOXmax selects among correlated parameters (insulin sensitivity, Vmax/V0 pyruvate, CS) only Vmax/V0 pyruvate ( $R = 0.674$ ,  $p = 0.004$ ) and excludes insulin sensitivity and CS. These two multivariate analyses indicate that insulin sensitivity is statistically better explained by LIPOXmax which is in turn better explained by the mitochondrial function parameter Vmax/V0 pyruvate.

### Discussion

This study evidences for the first time in middle-aged obese nondiabetic male subjects that insulin sensitivity measured with the minimal model is proportional to the ability to oxidize lipids during exercise, which is in turn statistically explained by the mitochondrial function parameter Vmax/V0 pyruvate.

All methods employed in this study are largely validated. The

measurement of insulin sensitivity minimal model analysis of FSIVGTT, first introduced by RN Bergman in 1979 [28], is usually considered as an 'alternative gold standard' beside the most widely used glucose clamp [33,34]. The measurement of the maximal lipid oxidation rate during exercise has been simultaneously introduced by several teams at the very end of the past century [7,35,36]. There have been a few discussions about its reproducibility but if assessed in well standardized conditions this measurement is highly reproducible [37] and stable over years [38]. Most authors employ the procedure with 3 min steps [35] but longer steps have been reported to yield a better steady state of gas exchange and thus to avoid possible bias in the estimation of the lipid oxidation rate particularly in obese subjects [39,40].

Lipid oxidation during exercise reaches a peak at a low to moderate intensity around 40% VO<sub>2</sub> max followed by a decrease when intensity increases. This level is called FATmax in papers using the method of Achten [35] and LIPOXmax in papers using 6 min steps and derivation of the equation of lipid oxidation in order to detect the point where this derivative is equal to zero and indicates the top of the bell-shaped curve [26]. This peak of lipid oxidation is both decreased and shifted to lower intensities in situations of low insulin sensitivity such as obesity and diabetes [7,41]. Low intensity training targeted at this level reverses this defect [9,42] and is an easy way to prescribe efficient intervention to obtain prolonged fat loss and to improve carbohydrate and lipid metabolism [38,42,43]. It has also been suggested that this parameter is a marker of "metabolic fitness" [17,44]. Interestingly, this level of exercise is below that of usual sports, but is spontaneously selected for prolonged activity by most individuals [45] and thus represents the type of exercise that patients will perform when asked to increase their volume of activity over the week.

Therefore our finding of a positive relationship in obese middle-aged male between a reliable measurement of insulin sensitivity and maximal fat oxidation during exercise is not unexpected, although it has not yet been reported in this population. It is consistent with the findings of recent papers [17,18] which evidenced a correlation between maximal fat oxidation during exercise and a surrogate measurement of insulin sensitivity (Quantitative Insulin Sensitivity Check Index, QUICKI) in young, healthy men or with insulin sensitivity measured by hyper insulin micisoglycemic lamp in overweight men, respectively. Our current data lead to think that insulin sensitivity and lipid oxidation during exercise are two biological properties that are closely related to each other. Moreover, our study extends previous finding to obese male subjects in whom a defect in lipid oxidation is frequently associated with skeletal muscle alterations in lipid trafficking [10] and lipid content [10]. Interestingly, here, we found no difference in metabolic parameters other than those related with insulin resistance (blood glucose, insulinemia, systolic blood pressure, anthropometric parameters such as fat-free mass, waist and hip circumferences) between insulin-sensitive and insulin-resistant subjects in our population of middle-aged obese males. The fact that the correlation between insulin sensitivity and LIPOXmax is no longer found in obese insulin-resistant subjects can be due to the narrow range of variation of SI in this sample but underlines the importance of lipid oxidative capacity for the maintenance of insulin sensitivity since insulin-sensitive subjects presented such correlation. This correlation underlines the importance of maintaining lipid

oxidation in middle-aged obese subjects for preserving insulin sensitivity. This is in agreement with data showing that the ability to oxidize lipid at rest is associated with a metabolically healthy phenotype in overweight men [22]. Thus, whatever the condition (rest or exercise) whole-body lipid oxidation capability seems related to insulin sensitivity.

Whole-body fatty acid oxidation has been related to skeletal muscle mitochondrial fatty acid oxidation capacity [12] in young healthy men during low-intensity exercise. Citrate synthase activity and mitochondrial respiration had been shown to be higher in trained type 2 diabetic subjects who had also higher whole-body lipid oxidation rate during exercise [9] compared to untrained state. In our sample of obese subject's representative of all the spectrum of insulin sensitivity, we extend these findings to obese nondiabetic sedentary patients with or without insulin resistance and show that these parameters are correlated to the ability to oxidize lipids during exercise and to insulin sensitivity. The correlation between LIPOXmax and VO<sub>2</sub>max underpins the relation between lipid oxidation and whole-body oxidative capacity. The multivariate analysis suggests that the ability to oxidize lipids during exercise is a determinant of insulin sensitivity, presumably because lipid oxidation may prevent lipid-induced insulin resistance [42] via lipid metabolites [46]. In addition, as previously shown in diabetic subjects, the ability to oxidize lipids during exercise seems to be closely related to skeletal muscle mitochondrial function, which is in line with the fact that most of lipid oxidation occurs in oxidative muscle. Thus, interventions aiming at increasing mitochondrial function are likely to also result into an increase in lipid oxidation and in turn an increase in insulin sensitivity. Furthermore, we showed that only two weeks at this level of maximal lipid oxidation are sufficient for improving insulin sensitivity in class II and III obese man [42].

## Conclusion

In conclusion, we have shown that insulin sensitivity, mitochondrial function, i.e. citrate synthase activity and mitochondrial respiration, and the ability of muscle to oxidize lipids during exercise are three closely correlated parameters in middle-aged male obese insulin-sensitive patients. Whole body insulin sensitivity is related to the ability to oxidize lipids during exercise, which in turn could be explained by skeletal muscle mitochondrial function. This leads to think that a low ability to oxidize lipids at exercise is one of the aspects of the multifaceted syndrome of insulin resistance.

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## Author Contributions

C. A., M. K., and J.M. conception and design of research; C.A., M.K., K.L., JF.B., C.F., A.M., E.Rd M. performed experiments, K.L., A.M., JF.B collected analyzed and interpreted results of experiments, K.L., JF.B drafted manuscript and prepared figures, C.A., M.K., K.L., JF.B., C.F., A.M., E.Rd M. and J.M. edited and revised manuscript.

## References

1. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture. 2009. *Diabetologia*. 2010; 53: 1270-1287.
2. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. *Am J Physiol Endocrinol Metab*. 2008; 295: E1009-1017.
3. Holloszy JO. "Deficiency" of mitochondria in muscle does not cause insulin resistance. *Diabetes*. 2013; 62: 1036-1040.
4. Jelenik T, Roden M. Mitochondrial plasticity in obesity and diabetes mellitus. *Antioxid Redox Signal*. 2013; 19: 258-268.
5. Pagel-Langenickel I, Bao J, Pang L, Sack MN. The role of mitochondria in the pathophysiology of skeletal muscle insulin resistance. *Endocr Rev*. 2010; 31: 25-51.
6. Ghanassia E, Brun JF, Fedou C, Raynaud E, Mercier J. Substrate oxidation during exercise: type 2 diabetes is associated with a decrease in lipid oxidation and an earlier shift towards carbohydrate utilization. *Diabetes Metab*. 2006; 32: 604-610.
7. Perez-Martin A, Dumortier M, Raynaud E, Brun JF, Fedou C, Bringer J, et al. Balance of substrate oxidation during submaximal exercise in lean and obese people. *Diabetes Metab*. 2001; 27: 466-474.
8. Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol*. 2005; 98: 160-167.
9. Bordenave S, Metz L, Flavier S, Lambert K, Ghanassia E, Dupuy AM, et al. Training-induced improvement in lipid oxidation in type 2 diabetes mellitus is related to alterations in muscle mitochondrial activity. Effect of endurance training in type 2 diabetes. *Diabetes Metab*. 2008; 34: 162-168.
10. Aguer C, Mercier J, Man CY, Metz L, Bordenave S, Lambert K, et al. Intramyocellular lipid accumulation is associated with permanent relocation *ex vivo* and *in vitro* of fatty acid translocase (FAT)/CD36 in obese patients. *Diabetologia*. 2010; 53: 1151-1163.
11. Ellis AC, Hyatt TC, Hunter GR, Gower BA. Respiratory quotient predicts fat mass gain in premenopausal women. *Obesity (Silver Spring)*. 2010; 18: 2255-2259.
12. Sahlin K, Mogensen M, Bagger M, Fernstrom M, Pedersen PK. The potential for mitochondrial fat oxidation in human skeletal muscle influences whole body fat oxidation during low-intensity exercise. *Am J Physiol Endocrinol Metab*. 2007; 292: E223-230.
13. Tarnopolsky LJ, MacDougall JD, Atkinson SA, Tarnopolsky MA, Sutton JR. Gender differences in substrate for endurance exercise. *J Appl Physiol*. 1990; 68: 302-308.
14. Stisen AB, Stougaard O, Langfort J, Helge JW, Sahlin K, Madsen K. Maximal fat oxidation rates in endurance trained and untrained women. *Eur J Appl Physiol*. 2006; 98: 497-506.
15. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J Appl Physiol*. 1994; 76: 2253-2256.
16. Bergman BC, Brooks GA. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol*. 1999; 86: 479-487.
17. Nordby P, Rosenkilde M, Ploug T, Westh K, Feigh M, Nielsen NB, et al. Independent effects of endurance training and weight loss on peak fat oxidation in moderately overweight men: a randomized controlled trial. *J Appl Physiol*. 2015; 118: 803-810.
18. Robinson SL, Hattersley J, Frost GS, Chambers ES, Wallis GA. Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *J Appl Physiol*. 2015; 118: 1415-1422.
19. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*. 2004; 350: 664-671.
20. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C  $\theta$  and alterations in the insulin signaling cascade. *Diabetes*. 1999; 48: 1270-1274.
21. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*. 1997; 46: 983-988.
22. Rosenkilde M, Nordby P, Nielsen LB, Stallknecht BM, Helge JW. Fat oxidation at rest predicts peak fat oxidation during exercise and metabolic phenotype in overweight men. *Int J Obes (Lond)*. 2010; 34: 871-877.
23. Astrup A. The relevance of increased fat oxidation for body-weight management: metabolic inflexibility in the predisposition to weight gain. *Obes Rev*. 2011; 12: 859-865.
24. Karelis AD, Faraj M, Bastard JP, St-Pierre DH, Brochu M, Prud'homme D, et al. The metabolically healthy but obese individual presents a favorable inflammation profile. *J Clin Endocrinol Metab*. 2005; 90: 4145-4150.
25. Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA. A physical activity questionnaire for the elderly. *Med Sci Sports Exerc*. 1991; 23: 974-979.
26. Brun JF, Jean E, Ghanassia E, Flavier S, Mercier J. Metabolic training: new paradigms of exercise training for metabolic diseases with exercise calorimetry targeting individuals. *Ann Readapt Med Phys*. 2007; 50: 528-534, 520-7.
27. Brandou F, Brun JF, Mercier J. Limited accuracy of surrogates of insulin resistance during puberty in obese and lean children at risk for altered glucoregulation. *J Clin Endocrinol Metab*. 2005; 90: 761-767.
28. Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. *Am J Physiol*. 1979; 236: E667-77.
29. Bergman RN, Prager R, Volund A, Olefsky JM. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest*. 1987; 79: 790-800.
30. Brun JF, Guinrand-Hugret R, Boegner C, Bouix O, Orsetti A. Influence of short-term submaximal exercise on parameters of glucose assimilation analyzed with the minimal model. *Metabolism*. 1995; 44: 833-840.
31. Thomas C, Sirvent P, Perrey S, Raynaud E, Mercier J. Relationships between maximal muscle oxidative capacity and blood lactate removal after supramaximal exercise and fatigue indexes in humans. *J Appl Physiol*. 2004; 97: 2132-2138.
32. Saks VA, Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, et al. Permeabilized cell and skinned fiber techniques in studies of mitochondrial function *in vivo*. *Mol Cell Biochem*. 1998; 184: 81-100.
33. Henriksen JE, Alford F, Ward G, Thye-Ronn P, Levin K, Hother-Nielsen O, et al. Glucose effectiveness and insulin sensitivity measurements derived from the non-insulin-assisted minimal model and the clamp techniques are concordant. *Diabetes Metab Res Rev*. 2010; 26: 569-578.
34. Rickels MR, Kong SM, Fuller C, Dalton-Bakes C, Ferguson JF, Reilly MP, et al. Insulin sensitivity index in type 1 diabetes and following human islet transplantation: comparison of the minimal model to euglycemic clamp measures. *Am J Physiol Endocrinol Metab*. 2014; 306: E1217-1224.
35. Achten J, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc*. 2002; 34: 92-97.
36. Deriaz O, Dumont M, Bergeron N, Despres JP, Brochu M, Prud'homme D. Skeletal muscle low attenuation area and maximal fat oxidation rate during submaximal exercise in male obese individuals. *Int J Obes Relat Metab Disord*. 2001; 25: 1579-1584.
37. Gmada N, Marzouki H, Haboubi M, Tabka Z, Shephard RJ, Bouhlel E. Crossover and maximal fat-oxidation points in sedentary healthy subjects: methodological issues. *Diabetes Metab*. 2012; 38: 40-45.
38. Romain AJ, Carayol M, Desplan M, Fedou C, Ninot G, Mercier J, et al. Physical activity targeted at maximal lipid oxidation: a meta-analysis. *J Nutr Metab*. 2012; 2012: 285395.

39. Bordenave S, Flavier S, Fedou C, Brun JF, Mercier J. Exercise calorimetry in sedentary patients: procedures based on short 3 min steps underestimate carbohydrate oxidation and overestimate lipid oxidation. *Diabetes Metab.* 2007; 33: 379-384.
40. Lanzi S, Codecasa F, Cornacchia M, Maestrini S, Capodaglio P, Brunani A, et al. Long maximal incremental tests accurately assess aerobic fitness in class II and III obese men. *PLoS One.* 2015; 10: e0124180.
41. Brun JF, Malatesta D, Sartorio A. Maximal lipid oxidation during exercise: a target for individualizing endurance training in obesity and diabetes? *J Endocrinol Invest.* 2012; 35: 686-691.
42. Lanzi S, Codecasa F, Cornacchia M, Maestrini S, Capodaglio P, Brunani A, et al. Short-term HIIT and Fat training increase aerobic and metabolic fitness in men with class II and III obesity. *Obesity (Silver Spring).* 2015 23:1987-1994.
43. Besnier F, Lenclume V, Gerardin P, Fianu A, Martinez J, Naty N, et al. Individualized Exercise Training at Maximal Fat Oxidation Combined with Fruit and Vegetable-Rich Diet in Overweight or Obese Women: The LIPOXmax-Reunion Randomized Controlled Trial. *PLoS One.* 2015; 10: e0139246.
44. Nordby P, Saltin B, Helge JW. Whole-body fat oxidation determined by graded exercise and indirect calorimetry: a role for muscle oxidative capacity? *Scand J Med Sci Sports.* 2006; 16: 209-214.
45. Dasilva SG, Guidetti L, Buzzachera CF, Elsangedy HM, Krinski K, De Campos W, et al. Gender-based differences in substrate use during exercise at a self-selected pace. *J Strength Cond Res.* 2011; 25: 2544-25451.
46. Louche K, Badin PM, Montastier E, Laurens C, Bourlier V, de Glisezinski I, et al. Endurance exercise training up-regulates lipolytic proteins and reduces triglyceride content in skeletal muscle of obese subjects. *J Clin Endocrinol Metab.* 2013; 98:4863-48671.