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ORIGINAL RESEARCH ARTICLE

Assessment of Metabolic Flexibility by Means of Measuring Blood Lactate, Fat, and Carbohydrate Oxidation Responses to Exercise in Professional Endurance Athletes and Less-Fit Individuals

Iñigo San-Millán[1,2] · George A. Brooks[3]

Abstract

Background Increased muscle mitochondrial mass is characteristic of elite professional endurance athletes (PAs), whereas increased blood lactate levels (lactatemia) at the same absolute submaximal exercise intensities and decreased mitochondrial oxidative capacity are characteristics of individuals with low aerobic power. In contrast to PAs, patients with metabolic syndrome (MtS) are characterized by a decreased capacity to oxidize lipids and by early transition from fat to carbohydrate oxidation (FATox/CHOox), as well as elevated blood lactate concentration [La^-] as exercise power output (PO) increases, a condition termed 'metabolic inflexibility'.

Objective The aim of this study was to assess metabolic flexibility across populations with different metabolic characteristics.

Methods We used indirect calorimetry and [La^-] measurements to study the metabolic responses to exercise in PAs, moderately active individuals (MAs), and MtS individuals.

Results FATox was significantly higher in PAs than MAs and patients with MtS ($p < 0.01$), while [La^-] was significantly lower in PAs compared with MAs and patients with MtS. FATox and [La^-] were inversely correlated in all three groups (PA: $r = -0.97$, $p < 0.01$; MA: $r = -0.98$, $p < 0.01$; MtS: $r = -0.92$, $p < 0.01$). The correlation between FATox and [La^-] for all data points corresponding to all populations studied was $r = -0.76$ ($p < 0.01$).

Conclusions Blood lactate accumulation is negatively correlated with FATox and positively correlated with CHOox during exercise across populations with widely ranging metabolic capabilities. Because both lactate and fatty acids are mitochondrial substrates, we believe that measurements of [La^-] and FATox rate during exercise provide an indirect method to assess metabolic flexibility and oxidative capacity across individuals of widely different metabolic capabilities.

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Key Points

Measurements of blood lactate concentration and fat oxidation (FATox) provide an indirect method to assess metabolic flexibility, mitochondrial function, and oxidative capacity during exercise in different populations.

The inverse correlations between blood lactate and FATox are quite robust, therefore assessing blood lactate alone could be an effective way to indirectly assess mitochondrial function and metabolic flexibility during exercise in different populations.

Since lactate exerts profound effects on fat and carbohydrate (CHO) metabolism, a poor mitochondrial lactate clearance capacity due to decreased mitochondrial function could greatly affect FATox and CHOox, which could result in metabolic dysregulation, which in turn could lead to different metabolic diseases such as type 2 diabetes.

1 Introduction

Recent studies on very good, but not elite, male cyclists showed that during leg cycle ergometer testing, athletes produced the same, or more, lactate than healthy individuals exercising at given exercise intensities [1]. Lower blood lactate concentrations [La^-] in athletes are attributable to increased lactate clearance via oxidation [2] and gluconeogenesis [3]. Cross-sectional [1] and longitudinal training studies [4] on healthy, young individuals show that training lowers circulating [La^-] by increasing lactate clearance [4], increasing lipid oxidation [5], and decreasing glucose and total carbohydrate (CHO) utilization [6] during exercise at given absolute exercise power outputs (POs). However, there are a paucity of data on energy substrate partitioning in elite professional endurance athletes (PAs). Studies on elite athletes are of interest because they express a robust muscle mitochondrial reticulum, perhaps due to genetic and epigenetic variations [7–13], which allows them to be metabolically flexible [5, 6]. Therefore, PAs readily switch back and forth between lipid and CHO oxidation (CHOox), depending on energy demand and substrate availability at higher absolute workloads.

Insulin resistance (IR) states, such as type 2 diabetes mellitus (T2DM) and metabolic syndrome (MtS), are characterized by mitochondrial dysfunction (MitoD) [14–21] and poor capacity for the cellular uptake and oxidation of glucose [22, 23]. In this paper, MitoD refers to either limited or aberrant mitochondrial protein expression. Similarly, insulin-resistant states are also characterized by poor capacity to oxidize fat [24–29]. Furthermore, those patients with T2DM and MtS are overly reliant on CHO-derived energy sources and possess limited ability to transition between CHOox and FATox. Metabolic inflexibility is ultimately attributable wholly, or in part, to poor mitochondrial respiratory capacity and related dysfunctions [30–32]. For example, individuals with T2DM and obesity show significant reductions in subsarcolemmal and interfibrillar regions of the mitochondrial reticulum, with similar reductions in muscle mitochondrial electron transport chain capacity compared with lean individuals [33]. Although the exact mechanisms of the role of MitoD in the pathogenesis of T2DM are not fully understood, it seems clear that MitoD is associated with metabolic inflexibility, T2DM, MtS, and cardiometabolic diseases (CMDs).

Carbohydrate and lipid metabolism are largely dependent on mitochondrial abundance and function [34]. Under resting and aerobic exercise conditions, glucose is catabolized to pyruvate and lactate in the cytosol, then transported across mitochondria to be oxidized to acetyl-coenzyme A (CoA) and, finally, via the tricarboxylic acid cycle (TCA), to carbon dioxide (CO_2). Therefore, MitoD limits cellular glucose metabolism. Fatty acid derivatives are oxidized in mitochondria, therefore mitochondrial abundance and function are also keys to the capacity for fatty acid metabolism. Beyond difficulties in fatty acid oxidation as a result of MitoD, impaired ability to clear pyruvate and lactate by oxidation and gluconeogenesis leads to the formulation of malonyl-CoA, an inhibitor of CPT1 and mitochondrial fatty acid uptake and oxidation [35]. Hence, MitoD, in the presence of lactatemia, leads to impaired fatty acid clearance and elevated plasma free fatty acid (FFA) concentration [36], another symptom of IR [37–40].

Not only can lactatemia limit FATox by limiting mitochondrial FFA uptake via inhibition of CPT1, but also because FATox can be limited by substrate availability. In this regard, it is noteworthy that lactate may inhibit lipolysis in fat cells through activation of an orphan G-protein-coupled receptor (GPR81) [41–43]. Furthermore, triglyceride metabolism can also be altered by lactate as it has been shown that exercise-induced lactate accumulation regulates intramuscular triglyceride metabolism via transforming growth factor (TGF)-β1-mediated pathways [44].

PAs are on the opposite pole of metabolic characteristics compared with individuals with T2DM and MtS. PAs possess the highest mitochondria density observed in human skeletal muscle [10–13], again probably a result of endurance training on the background of genetic

predisposition [45–50]. Compared with those with T2DM and MtS, or even untrained healthy individuals, in trained endurance athletes FATox is far greater during exercise at given absolute and relative exercise intensities [5, 51–55], giving rise to superb metabolic flexibility in PAs [31]. Moreover, lactate is a key element for performance, and well-trained athletes have a higher lactate clearance capacity and decreased [La^-] levels at the same relative and absolute submaximal exercise intensities [4, 56–62] owing to mitochondrial abundance and function. This enhanced metabolic function and flexibility makes PAs a very good (if not 'gold standard') model to understand the effect of mitochondrial function on energy substrate partitioning in vivo.

For many years, the fact that lactate is a major regulator of intermediary metabolism was not appreciated. Far from being a 'dead end' metabolite produced as the result of oxygen insufficiency, we now know that lactate is a major energy source [4, 63–65], the major gluconeogenic precursor [6], and a signaling molecule, a 'lactormone' [66] responsible for diverse actions such as gene expression [67] and control of lactate-responsive genes such as GPR81 and TREK1 (*vide infra*) [41–43, 68]. In the process of shuttling between sites of production and removal, lactate exerts profound effects on fat and CHO metabolism. Because lactate is a preferred fuel over glucose in the heart [69], working muscle [4, 70, 71] and brain [72], hyperlactatemia affects glucose uptake and oxidation by substituting for pyruvate and lactate produced from glycolysis in widely dispersed tissues. Lactate is oxidized via the mitochondrial lactate oxidation complex (mLOC) comprising the monocarboxylate transporter-1 (MCT1), its chaperone (CD147), mitochondrial lactate dehydrogenase (mLDH), and cytochrome oxidase (COx) [73, 74].

In view of new knowledge of lactate kinetics in resting and exercising individuals, the aim of our study is to examine the relationships among [La^-] and FATox and CHOox rates during exercise in different populations known to express widely different levels of oxidative capacity. Hence, measuring [La^-] along with CHOox and FATox is proposed as a way to indirectly assess mitochondrial function and metabolic flexibility. Furthermore, and because of the metabolic regulation of lactate on fat metabolism is determined at the muscle mitochondrial level, we evaluated the possibility that measuring [La^-] alone could be a simple, efficient, and rapid indirect manner to estimate substrate utilization as well as mitochondrial function in different populations. Finally, we evaluated the possibility of utilizing the measurement of [La^-] for individualized exercise prescription in the same manner that it is done with competitive athletes worldwide, which could be an efficient way to prescribe individual exercise programs for patients with IR, T2DM or MtS.

2 Materials and Methods

We studied 22 international-level male professional cyclists (PAs), 20 moderately active male individuals (MAs) and 10 male individuals with MtS, who performed graded exercise tests to maximal oxygen consumption (VO_2max) on a leg cycle ergometer (Lode Excalibur; Lode B.V., Groningen, The Netherlands) [62]. All subjects were given nutritional recommendations (>50% of kcal in the form of CHO) for the night before the test, as well as for the day of the test. They were instructed to fast and to restrain from caffeine 1:30 h prior to performing the test, and were also advised not to perform any intense or longer form of exercise over 2 h the day before the test, as well as no exercise on the day of the test.

Requirements for entry into the PA cohort was to compete at the highest level of professional road cycling, the Pro Tour Circuit; for the MA group, the requirements were to exercise at least three times/week and a minimum of 150 min/week; and, for the MtS group, the requirements were to have been diagnosed with MtS, including IR or T2DM and exercise less than 1 day/week.

Subjects were told to freely warm up at an intensity below 100 W for 15 min. After the warm-up period, subjects started graded leg cycling exercise to volitional exhaustion. Exercise intensity was increased approximately 35 W every 10 min. The goal was to observe the metabolic responses to exercise during longer (10 min) stages and at a wide range of exercise intensities.

Study procedures were conducted in accordance with the Declaration of Helsinki and in accordance with a predefined protocol that was approved by the Colorado Multiple Institutional Review Board (COMIRB), and written informed consent was obtained from all subjects.

2.1 Gas Exchange Measurements

Oxygen consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio ($RER = VCO_2/VO_2$) were determined using a ParvoMedics TrueOne 2400 Metabolic Measurement System (ParvoMedics, Inc.; Sandy, UT, USA). Subjects were required to wear the mouthpiece to collect respiratory gases, and respiratory gas data were averaged over 15 s throughout the entire test.

2.1.1 Fat and Carbohydrate Oxidation Rate Measurements

For the measurement of total FATox and CHOox, stoichiometric equations were applied according to the methodology described by Frayn [75], where:

$$\text{FATox}\ (\text{g}\cdot\text{min}^{-1}) = 1.67\text{VO}_2(\text{L}\cdot\text{min}^{-1}) - 1.67\ \text{VCO}_2\ (\text{L}\cdot\text{min}^{-1})$$

$$\text{CHOox}(\text{g}\cdot\text{min}^{-1}) = 4.55\text{VO}_2(\text{L}\cdot\text{min}^{-1}) - 3.21\text{VCO}_2\ (\text{L}\cdot\text{min}^{-1})$$

2.2 Lactate Concentration Measurement

At the end of every stage throughout the test, a sample of capillary blood was collected from the earlobe to analyze both intra- and extracellular levels of L-lactate (YSI 1500 Sport; YSI, Yellow Springs, OH, USA). Heart rate was monitored during the whole test with a heart monitor (Polar S725x; Polar Electro, Kempele, Finland).

2.3 Statistical Analysis

Student's *t* tests with Bonferroni corrections for multiple comparisons were used to assess statistical significance of mean values observed on the three cohorts, and Pearson correlation coefficients were used to assess statistical significance of relationships among the variables studied. Statistical significance was set at $p < 0.05$, but are reported as $p < 0.01$ if a lesser p value was calculated.

3 Results

VO_2max was significantly higher in PAs than MAs (74.1 ± 4.7 vs. 49.6 ± 5.8 ml·kg^{-1}·min^{-1}, $p < 0.01$) and patients with MtS (26.9 ± 10.5 ml·kg^{-1}·min^{-1}, $p < 0.01$), and was significantly higher in MAs than patients with MtS ($p < 0.01$). Maximal PO was significantly higher in PAs than MAs ($p < 0.01$) and MtS patients ($p < 0.01$) (Table 1).

[La$^-$] was remarkably lower in the PA group compared with both the MA and MtS groups throughout exercise (Fig. 1).

Throughout the range of exercise POs studied, FATox was remarkably higher in the PA group compared with both the MA and MtS groups (Fig. 2). Maximal FATox (FATmax) was significantly higher in the PA group compared with both the MA and MtS groups (0.66 vs. 0.38 g·min^{-1}, $p < 0.01$; and 0.66 vs. 0.12 g·min^{-1}, $p < 0.01$, respectively).

Strong inverse relationships were observed among the average rates of FATox and the average [La$^-$] across the three groups studied. In the PA group, the correlation between FATox and [La$^-$] was $r = -0.97$ ($p < 0.01$) (Table 2; Fig. 3), while the relationship between FATox and [La$^-$] in the MA group was $r = -0.98$ ($p < 0.01$).

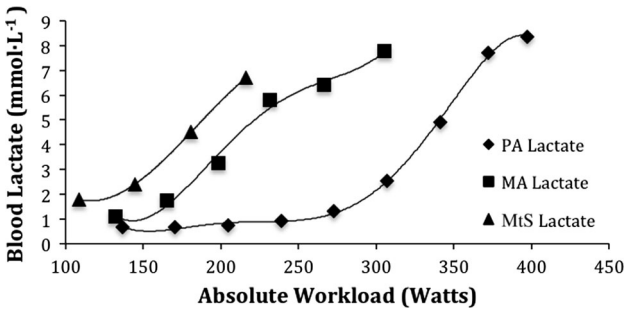

Fig. 1 Relationships between average blood lactate levels and exercise power outputs in international-level PAs, MAs, and individuals with MtS. *PAs* professional endurance athletes, *MAs* moderately active healthy individuals, *MtS* metabolic syndrome

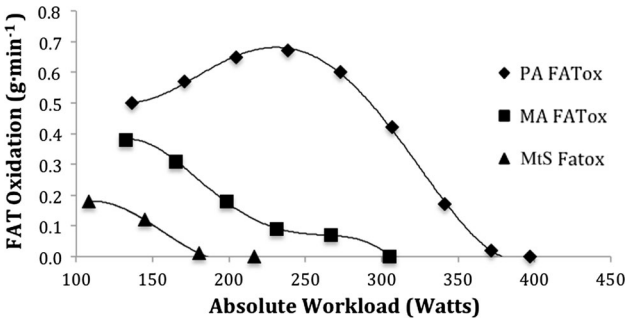

Fig. 2 Relationships between average rates of FATox and exercise power outputs in international-level PAs, MAs, and individuals with MtS. *PAs* professional endurance athletes, *MAs* moderately active healthy individuals, *MtS* metabolic syndrome, *FATox* fat oxidation

Table 1 Subject characteristics

Group	Age (years)	Height (cm)	Weight (kg)	BMI	Body fat (%)	VO_2max (ml·kg^{-1}·min^{-1})	Maximal power output (W)
PAs ($n = 22$)	26.8 ± 2.8	179.5 ± 5	68.8 ± 6	21.25 ± 2.1	9.7 ± 1*	74.1 ± 4.7*	378.5 ± 30*
MAs ($n = 20$)	40.0 ± 4.7	178.3 ± 3	74.2 ± 2	23.34 ± 2.8	14.2 ± 2*	49.6 ± 5.8*	246.3 ± 26*
MtS subjects ($n = 10$)	55.2 ± 5.2	179.1 ± 4	102.8 ± 8	35.05 ± 7.1	43.5 ± 1*	26.9 ± 10.5*	216.0 ± 13*

BMI body mass index, *VO_2max* maximum oxygen consumption, *PAs* professional endurance athletes, *MAs* moderately active individuals, *MtS* metabolic syndrome

* $p > 0.01$

Table 2 Average rates of fat and carbohydrate oxidation and blood lactate levels in an incremental exercise test until volitional exhaustion in international-level professional endurance athletes ($n = 22$)

Power (W)	FATox ($g \cdot min^{-1}$)	[La^-] ($mmol \cdot L^{-1}$)	CHOox ($g \cdot min^{-1}$)
136.5 ± 10.4	0.50 ± 0.12	0.67 ± 0.12	1.46 ± 0.30
170.6 ± 13.2	0.57 ± 0.13	0.67 ± 0.14	1.86 ± 0.34
204/7 ± 15.6	0.65 ± 0.15	0.74 ± 0.18	2.27 ± 0.37
238.8 ± 18.2	0.67 ± 0.12	0.92 ± 0.27	2.78 ± 0.46
272.9 ± 20.8	0.62 ± 0.14	1.32 ± 0.47	3.31 ± 0.51
307.1 ± 23.4	0.42 ± 0.18	2.55 ± 1.11	4.24 ± 0.68
341.2 ± 26.1	0.17 ± 0.17	4.91 ± 1.82	5.35 ± 0.68
371.9 ± 30.2	0.02 ± 0.07	7.77 ± 1.72	6.02 ± 0.75
396.9 ± 35.4	0.00 ± 0.00	8.37 ± 1.90	6.37 ± 1.06

Relationships:

[La^-] and FATox: $r = -0.97$, $p < 0.01$

[La^-] and CHOox: $r = 0.94$, $p < 0.01$

FATox and CHOox: $r = -0.90$, $p < 0.01$

FATox fat oxidation, *CHOox* carbohydrate oxidation, *[La^-]* blood lactate concentration

Finally, the relationship between FATox and [La^-] in the MtS group was $r = 0.92$ ($p < 0.01$).

The correlations between FATox and [La^-] for all groups were strong (PAs: $r = -0.80$, $p < 0.01$; MAs: $r = -0.76$, $p < 0.01$; MtS: $r = -0.78$, $p < 0.01$) (Fig. 4). The relationships were all nonlinear, yielding similar quadratic coefficients (≈ 0.007) and linear coefficients (≈ 0.1); however, the constants ranged from ≈ 0.7 in PAs to ≈ 0.2 in patients with MtS. The correlation between FATox and [La^-] for all data points for all groups together was also significant ($r = -0.76$, $p < 0.01$).

Relationships between the average CHOox and average [La^-] were robust for all groups studied. The correlation in the PA group was $r = 0.94$ ($p < 0.01$) (Table 2; Fig. 5), while the correlation in the MA group was $r = 0.99$ ($p < 0.01$) (Table 3; Fig. 5). Finally, the correlation for the MtS group was $r = 0.99$ ($p < 0.01$) (Table 4; Fig. 5).

Relationships between the average FATox and average CHOox for all groups studied were significant and strong (PAs: $r = -0.90$, $p < 0.01$ (Table 2; Fig. 6); MAs: $r = -0.99$, $p < 0.01$ (Table 3); Mts: $r = -0.96$, $p < 0.01$ (Table 4; Fig. 6)). The correlations between metabolic PO, expressed as VO_2 ($L \cdot min^{-1}$), and PO, expressed as W, were significant and strong: $r = 0.97$ ($p < 0.01$) and $r = 0.94$ ($p < 0.01$) (Fig. 7a, b) in the PA and MA groups, respectively. However, the correlation was moderate for MtS subjects ($r = 0.67$, $p < 0.01$) (Fig. 7c). Nonetheless, there were no significant differences in slopes of the power–power relationships (Fig. 7), indicating similar delta efficiencies in all three groups.

4 Discussion

Our study shows the presence of an overarching major effect of lactatemia on limiting FATox in individuals of widely ranging exercise capacities. Our study also shows the presence of significant differences in the relationships among blood lactate accumulation and CHOox and FATox rates in the three groups of individuals (PAs, MAs, and patients with MtS) studied during graded exercise. As shown in Fig. 3, lactatemia significantly affects and downregulates fat metabolism. In contrast, [La^-] and CHOox rate are highly related because they are part of the same process, i.e. glycolytic flux and oxidative disposal of the products of glycolysis (Fig. 5).

4.1 Blood Lactate Response

Slope of the blood lactate rise in response to exercise PO in PAs was low compared with MAs who, in turn, showed lower [La^-] accumulation curve than MtS subjects (Fig. 1). Based on previous work, we know that while [La^-] is lower in trained, compared with untrained, individuals at a given exercise PO [4], we also know that during high-intensity exercise, lactate production, and hence blood lactate appearance rate, is greater in trained than in untrained individuals, probably as a simple mass effect and higher energy expenditure. However, [La^-] is lower due to the training adaptations that increase [La^-] clearance capacity.

An example of remarkable lactate clearance capacity can be observed in Fig. 1, wherein [La^-] response in the

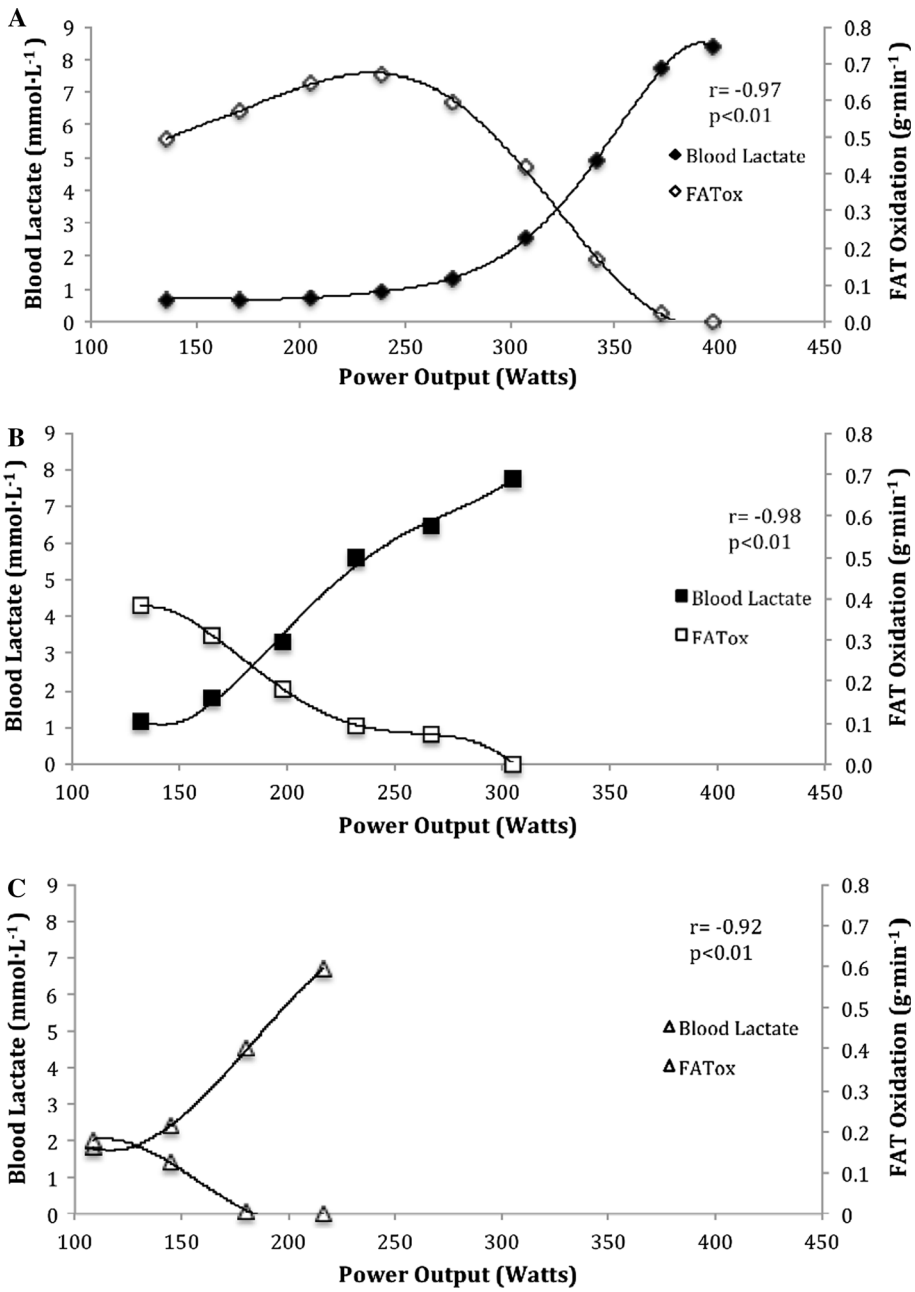

Fig. 3 Relationships between the average blood lactate concentrations and FATox rates as a function of exercise power output in **a** international-level professional endurance athletes, **b** moderately active healthy individuals, and **c** individuals with metabolic syndrome. *FATox* fat oxidation

three cohorts can be observed at common exercise POs. Also remarkable is that the [La^-] levels accumulated in PAs at approximately 300 W are close to the [La^-] observed at rest in individuals with T2DM [76, 77].

4.2 Fat Oxidation and Lactate

Our data show strong inverse relationships between FATox and [La^-] across the range of exercise intensities studied in all three groups (Fig. 3). Our data also show remarkable and significantly higher capacity to oxidize fat in the PAs, followed by the MAs and, finally, by the MtS group with a poor FATox capacity (Fig. 2). Accordingly, our data are in concordance with the scientific literature showing that endurance training increases the capacity to oxidize fatty acids [5, 51–55]. Not surprisingly, our data also show consistent and strong inverse correlations between [La^-] and FATox in all three groups, as expected, as both are mitochondrial substrates and lactate inhibits lipolysis, as previously discussed [42]. Therefore, our correlations between fat and lactate oxidation implicate differences in mitochondrial function across the three categories of fitness level, PA > MA > MtS. As previously shown, increases in FATox and lactate clearance capacity have been observed

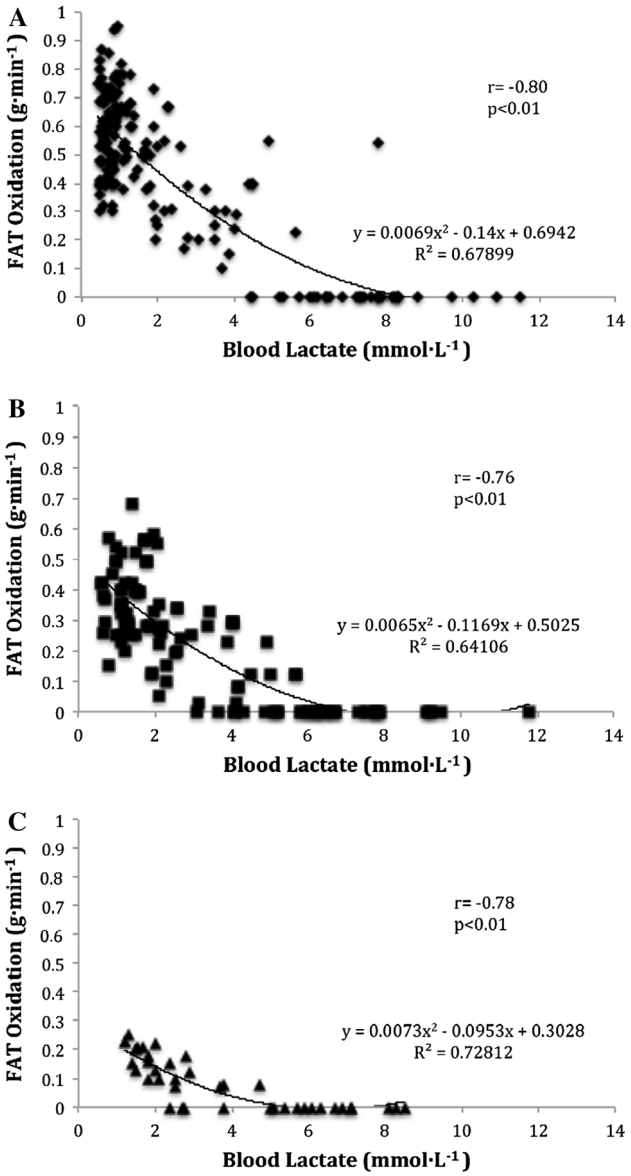

Fig. 4 Relationships between FATox and blood lactate for all data points from all groups together, i.e. international-level professional endurance athletes, moderately active healthy individuals, and individuals with metabolic syndrome. *FATox* fat oxidation

after endurance training [1, 4–6, 51, 53–55, 78]. The relationship among [La^-] and energy substrate partitioning have recently been reviewed [36], but inverse relationships between [La^-] and plasma FFA concentration and oxidation has long been recognized. In 1962 and 1964, Issekutz et al. noted the effect of lactacidemia on diminishing circulating FFA in individuals during hard exercise [79, 80]. Furthermore, several groups of investigators [41–43] have shown that, independent of pH, lactate inhibits lipolysis in fat cells through activation of a G-protein-coupled receptor (GPR81). In mouse, rat, and human adipocytes GPR81 appears to act as a lactate sensor, the response of which was to inhibit lipolysis. In addition, a second, but cytoplasmic, lactate receptor, TREK1, has been discovered in brain, but its role in regulating muscle metabolism is unstudied [68]. Interestingly, we can observe this phenomenon in Fig. 3. For both the PA and MA groups, the first increase in [La^-] over baseline levels coincides with the decrease in FATox (Figs. 3a, b), which is possibly due to the effects of lactate on lipolysis, as described above. Since the metabolic task for the MtS group is already high from the beginning of exercise with higher [La^-] concentrations and low FATox levels, we do not observe this phenomenon in this group (Fig. 3c). In Fig. 4, we can also observe that regardless of the level of aerobic capacity, all groups show that FATox is suppressed at the [La^-] of approximately 4–6 mmol·L^{-1}. This is also possible due to the suppression of lipolysis at high [La^-], and also indicating a 'threshold-like' phenomenon in all subject groups.

In our study, higher FATox levels and lower [La^-] at a given different intensity suggest a highly developed mitochondrial function and mLOC in PAs compared with individuals with MtS. On the other hand, significantly lower FATox, and significantly higher levels of [La^-], at low exercise POs in the MtS group suggest poor mLOC protein expression and MitoD. Therefore, correlating both FATox rates and [La^-] seems to represent a valid indirect method to 'test' mitochondrial power and function during a long bout of exercise in individuals ranging from athletes to MtS patients. The matter of integrity of mitochondrial coupling efficiency is discussed below.

4.3 Carbohydrate Oxidation

Our data show strong relationships between CHOox and [La^-] lactate throughout all three groups (Fig. 6), which was expected as lactate is the obligatory end product of glycolysis and is proportional to glycolytic flux independently of aerobic or glycolytic conditions [66, 81, 82]. The higher the exercise intensity and absolute energy expenditure, the higher the glycolytic flux and lactate production. Consequently, there is a steep rise in [La^-] accumulation if skeletal muscle lactate clearance capacity is inadequate.

4.4 Mechanical and Phosphorylation Coupling Efficiencies

Muscle efficiency is commonly measured by the relationship between energy equivalents of metabolic PO, as determined from VO_2 and RER and PO, where VO_2 increases linearly with PO. Both the PA and MA groups show normal muscle efficiency with very strong correlations between energy equivalents of metabolic PO (calculated from VO_2) and mechanical PO, as determined on the leg cycle ergometer (Fig. 7). Delta efficiencies for PAs, MAs and patients with MtS were 26, 24, and 28%,

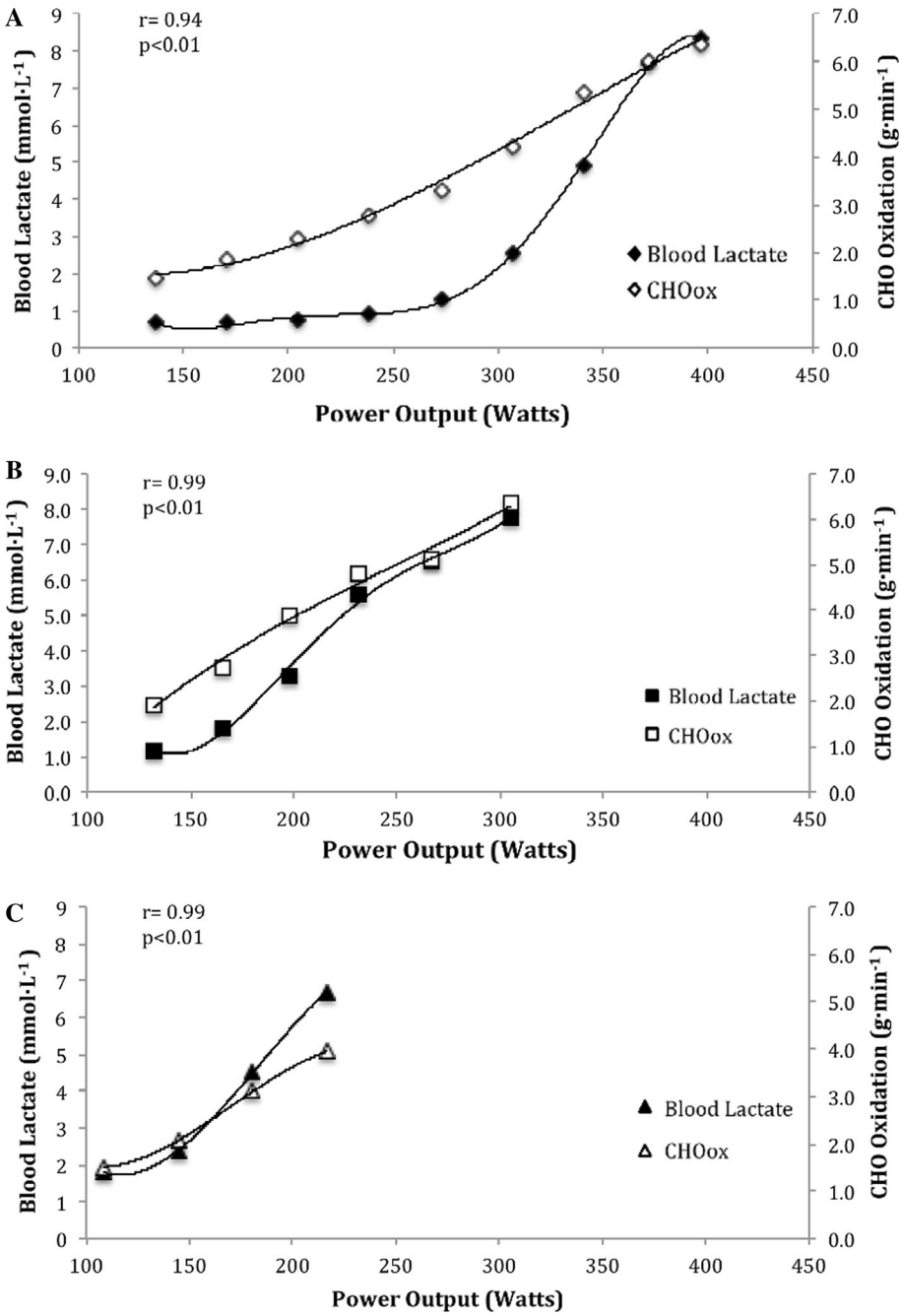

Fig. 5 Relationships between the average blood lactate concentrations and the average CHOox rates as a function of exercise power output in **a** international-level professional endurance athletes, **b** moderately active healthy individuals, and **c** individuals with metabolic syndrome. *CHO* carbohydrate oxidation

respectively. Hence, assuming similar mechanical coupling efficiencies in MtS subjects, our results suggest that MtS subjects suffered from poor mitochondrial mass, and not dysfunctional phosphorylation coupling efficiency.

4.5 Indirect Assessment of Metabolic Flexibility

Metabolic flexibility and inflexibility are terms proposed well over a decade ago by Kelley and colleagues [30–32] that are gaining popularity among researchers and clinicians working with individuals with T2DM and obesity. Metabolic flexibility reflects the ability to oxidize fats and CHOs and is associated with IR and MitoD [18, 30, 31]. In 1994, Brooks and Mercier proposed the 'crossover concept' (CO) [34] as a novel approach to study CHO and fat metabolism during exercise (see Fig. 6). We observed important differences in the CO between all three groups. In the PA group, the CO occurs at high absolute exercise intensities, with approximately 75% of maximal PO denoting an exceptional capacity for FATox. In the MA group, the CO occurs at approximately 55% of maximal PO, denoting decreased capacity to oxidize fat and relying

Table 3 Average rates of fat and carbohydrate oxidation and blood lactate levels in an incremental exercise test until volitional exhaustion in moderately active individuals ($n = 20$)

Power (W)	FATox ($g \cdot min^{-1}$)	[La^-] ($mmol \cdot L^{-1}$)	CHOox ($g \cdot min^{-1}$)
132 ± 4.9	0.38 ± 0.13	1.17 ± 0.37	1.99 ± 0.44
165.3 ± 6.1	0.31 ± 0.15	1.83 ± 0.90	2.75 ± 0.55
198.3 ± 7.3	0.18 ± 0.18	3.35 ± 1.75	3.86 ± 0.83
231.4 ± 8.7	0.09 ± 0.12	5.97 ± 2.49	4.97 ± 0.95
264.5 ± 9.7	0.07 ± 0.12	6.42 ± 1.65	5.13 ± 0.88
300.6 ± 11.8	0.00 ± 0.00	7.79 ± 1.63	6.43 ± 0.27

Relationships:

[La^-] and FATox: $r = -0.98$, $p < 0.01$

[La^-] and CHOox: $r = 0.99$, $p < 0.01$

FATox and CHOox: $r = -0.99$, $p < 0.01$

FATox fat oxidation, *CHOox* carbohydrate oxidation, *[La^-]* blood lactate concentration

Table 4 Average rates of fat and carbohydrate oxidation and blood lactate levels in an incremental exercise test until volitional exhaustion in individuals with metabolic syndrome ($n = 10$)

Power (W)	FATox ($g \cdot min^{-1}$)	[La^-] ($mmol \cdot L^{-1}$)	CHOox ($g \cdot min^{-1}$)
108.4 ± 6.3	0.18 ± 0.04	1.8 ± 0.50	1.51 ± 0.41
144.6 ± 8.5	0.12 ± 0.05	2.61 ± 0.83	2.22 ± 0.74
180.7 ± 10.6	0.01 ± 0.03	5.21 ± 1.80	3.32 ± 0.99
216 ± 13.1	0.00 ± 0.00	6.83 ± 1.27	4.24 ± 1.02

Relationships:

[La^-] and FATox: $r = -0.92$, $p < 0.01$

[La^-] and CHOox: $r = 0.99$, $p = 0.01$

FATox and CHOox: $r = -0.96$, $p < 0.01$

FATox fat oxidation, *CHOox* carbohydrate oxidation, *[La^-]* blood lactate concentration

on CHO earlier in the exercise test. For the MtS subjects, the CO was difficult to determine as FATox was already quite low in the first step of the test, denoting poor FATox and metabolic flexibility. We believe that our method of contrasting FATox rate and [La^-] during exercise (Fig. 3) is an alternative approach to assessing the effect of glycolysis on FATox that may find applicability in assessing metabolic flexibility in ambulatory and clinical settings as similarities between Figs. 3 and 6 are remarkable.

4.6 Indirect Assessment of Mitochondrial Function

On a population-wide basis it could be very helpful to diagnose defects in mitochondrial function years ahead of the development of chronic, mitochondrial-derived metabolic diseases. However, to our knowledge, there are no current tests to measure mitochondrial function in a minimally invasive, cost-effective, ambulatory manner. Moreover, in the same manner that cardiology exercise stress tests are useful to study cardiac electrophysiology to diagnose cardiomyopathies, graded exercise tests as used here could be a surrogate method to stress and assess mitochondrial function and effects on metabolic flexibility in vivo. The results obtained here clearly show that PAs demonstrate superior capabilities to oxidize lactate, as well as CHO- and lipid-derived fuel energy sources, and also retain capacity for lipid oxidation at different exercise intensities where MAs and MtS patients are completely CHO-dependent, which is also expected as, especially for the MtS group, even the initial lower absolute intensities were metabolically tasking.

Finally, because exercise is the only known physiological stimulation of mitochondrial biogenesis, an important application of our method is the possibility of prescribing individualized exercise to patients with IR, T2DM or MtS in the same manner that is done with competitive athletes worldwide. For the application of our method, the incremental stages were longer in comparison with standard short incremental protocols. We believe that longer stages are helpful to elicit a proper 'steady state' status, and are therefore quite useful to gather accurate metabolic data. This is especially useful for populations with higher aerobic capacity levels such as world-class athletes. However, our MtS group responded properly to this protocol and we

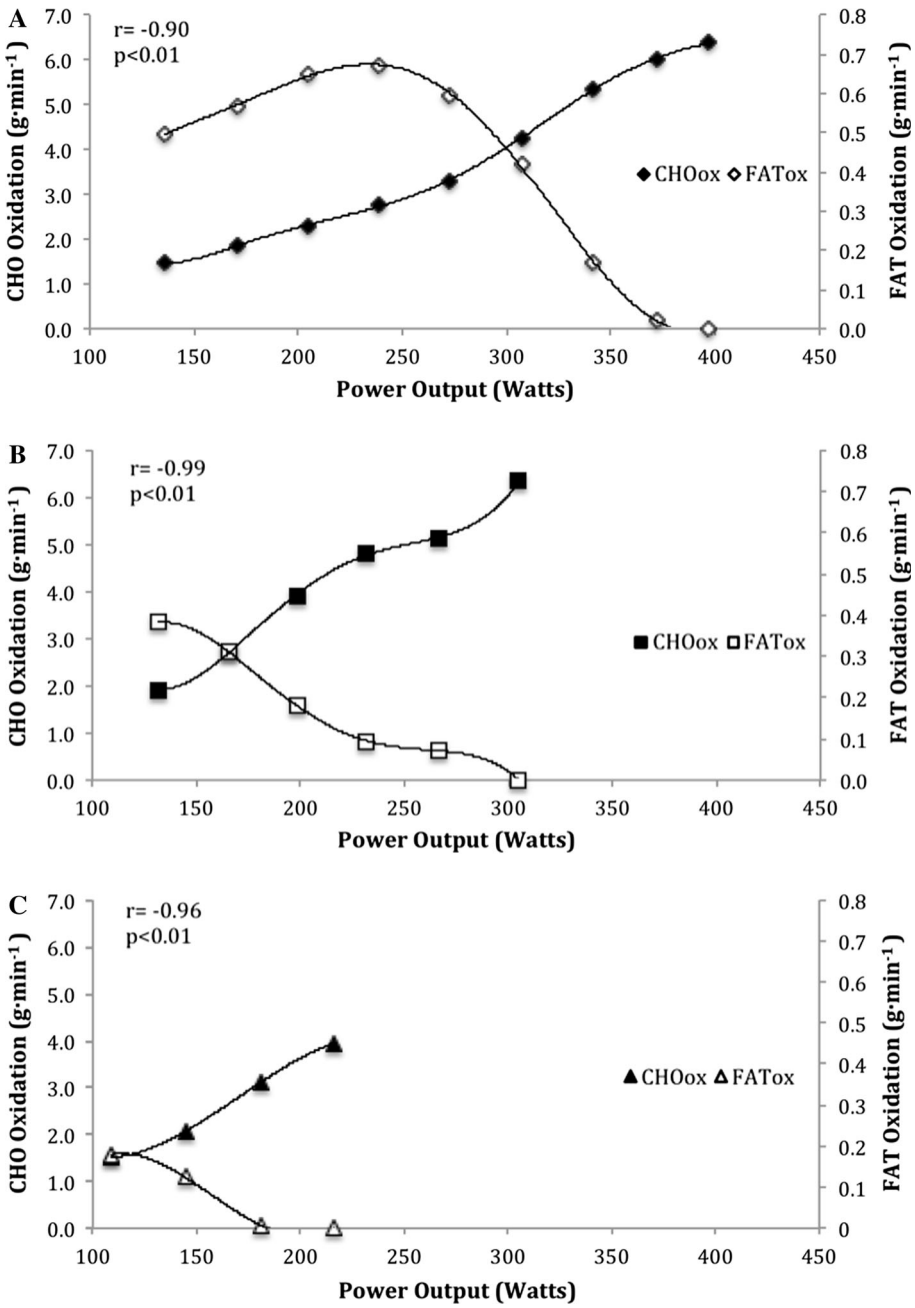

Fig. 6 Relationship between the average FATox and CHOox rates as a function of exercise power output in **a** international-level professional endurance athletes, **b** moderately active healthy individuals, and **c** individuals with metabolic syndrome. *FATox* fat oxidation, *CHOox* carbohydrate oxidation

were able to acquire the data we were anticipating. Furthermore, the inclusion of different 'metabolic points', such as different ventilator thresholds or lactate turn points, into our method and protocol could be very useful to further evaluate mitochondrial function, as well as to discriminate between different levels of performance, especially for sports performance purposes.

4.7 Limitations of the Study

The limitations of our study mainly reside in the indirect nature of the assessment of metabolic flexibility and mitochondrial function. Although we obtained robust data of indirect parameters of metabolic flexibility and mitochondrial function, the ideal study should be to directly study, through muscle biopsies and tracer methodologies, mitochondrial function and metabolic flexibility based on the function to oxidize both fatty acids and lactate. Furthermore, our study does not discriminate between subjects of each of the cohorts, especially PAs and patients with MtS. With a larger population for each of these two cohorts, differences between subjects could possibly be identified. Finally, it could be possible that the duration of the test, according to our protocol, may not fit the time

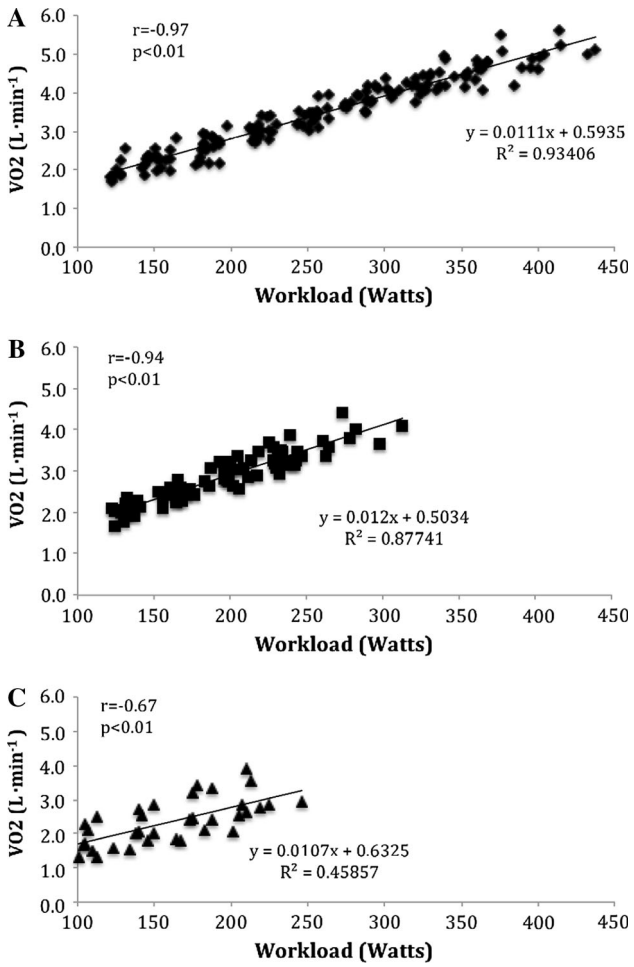

Fig. 7 Correlations between VO_2 and power output (Watts) in **a** international-level professional endurance athletes, **b** moderately active healthy individuals, and **c** individuals with metabolic syndrome. *VO_2* oxygen consumption

limitations of some clinical settings, therefore a shorter version of the protocol with shorter stages could be adequate.

5 Conclusions

Our data show consistent and strong inverse correlations between blood lactate and FATox, as well as between FATox and CHOox, in all three groups studied. Because both fat and lactate oxidation occur in skeletal muscle mitochondria, we advocate for our method as plausible for indirectly measuring mitochondrial function and metabolic flexibility in different populations. Furthermore, as the inverse correlations between blood lactate and FATox are robust, we also show that assessing blood lactate alone is an effective way to indirectly assess mitochondrial function and metabolic flexibility during exercise in different types of populations with completely different metabolic responses to exercise. Finally, because lactate exerts profound effects on fat and CHO metabolism, a poor lactate clearance capacity due to mitochondrial (mLOC) limitations greatly affect FATox and CHOox, which could result in metabolic dysregulation, and which, in turn, may give rise to IR, T2DM, and possibly other chronic diseases, including CMDs.

Compliance with Ethical Standards

Conflict of interest Iñigo San-Millán and George A. Brooks declare that they have no conflicts of interest that are directly relevant to the content of this article.

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