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Relationship between blood lactate concentration and substrate utilization during exercise in type 2 diabetic postmenopausal women

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Abstract

Increased blood lactate concentration and alterations of substrate utilization have been shown to be partly involved in development of insulin resistance in obese and type 2 diabetic patients. As blood represents the first great distribution space and participates to lactate exchange in whole body, we investigated lactate transport in red blood cells at rest and the potential relationships between elevated blood lactate and substrate utilization in 7 obese controls and 7 obese type 2 diabetic postmenopausal women during an incremental exercise test. Blood samples were collected at rest, 30%, 50%, and 60% of maximal power and at 8 and 20 minutes of recovery time. Baseline lactatemia and its increase during exercise were higher in the diabetic group ($P < .05$). We found a negative correlation between basal and 30% maximal power lactatemia and 2 indexes of substrate utilization (crossover point: $r = -0.79$, $r = -0.82$ and maximal lipid oxidation point: $r = -0.83$, $r = -0.80$; $P < .05$) in diabetic group only. Furthermore, there were positive correlations between the affinity constant, maximal velocity transport, and basal lactate level in diabetic subjects ($r = 0.91$ and $r = 0.73$, respectively; $P < .05$). These results show that the elevation of blood lactate is associated with a greater carbohydrate oxidation in type 2 diabetes, but the mechanisms underlying the alteration of substrate utilization need to be clarified. Furthermore, increased lactate levels cannot be explained by alterations of lactate transport in red blood cells, but it could affect monocarboxylate transporter 1 properties.

1. Introduction

The pathophysiological mechanism of insulin resistance in obese and type 2 diabetic patients is multifactorial. Substrate availability and substrate competition for oxidation represent 2 factors that contribute to insulin resistance and that have gained importance recently [1-3]. In fact, it has been shown that obese and type 2 diabetic patients present alterations in substrate utilization [3,4]. In obese people, these alterations are characterized by both a decrease of the crossover point of carbohydrate and lipid oxidation and the maximal lipid oxidation (lipox max) point during exercise [3]. According to the concept proposed by Brooks and Mercier [5], the crossover point of substrate utilization is defined as the power at which energy from carbohydrate-derived fuels predominates over energy from lipids.

Substrate competition between lactate, free fatty acids, and glucose has been suggested as a mechanism to exacerbate muscle insulin resistance and to lead to glucose intolerance [6]. As a consequence, lactate metabolism in obese and type 2 diabetic patients has received considerable interest recently. Indeed, a significant inverse correlation between overnight fasting lactate level and insulin sensitivity has been found in obese and type 2 diabetic subjects [7]. Moreover, it has been observed that patients with type 2 diabetes have a greater elevation in basal lactate levels than obese subjects [8]. An association between elevated basal lactate level, insulin resistance, and diabetes is supported by epidemiological studies in healthy subjects in whom an elevated fasting lactate level was found to be a significant and independent risk factor for the development of type 2 diabetes [9]. Lactate is now considered as an intermediary metabolite that can be oxidized by muscle [10]. Because it has also been shown in animals that an increase in plasma lactate can alter free fatty acid mobilization [11], a primary goal in this study was to investigate the relationship between substrate utilization and

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lactatemia in type 2 diabetes. As diabetic patients present a greater increase in basal blood lactate than obese people, we hypothesized that substrate utilization in diabetic patients would be more affected by lactatemia.

In rats, chronic elevation of lactate level is accompanied by alterations of lactate transport and lactate transporter (ie, monocarboxylate transporter [MCT]) in skeletal muscle [12,13]. The volume occupied by red blood cells (RBCs) represents a large place for acceptance of anion lactate (La^-) during increased lactatemia [14]. The RBC membrane contains the MCT1 isoform that is responsible of the major part of lactate transport across RBC membrane. The mechanism underlying the chronic elevation of lactate level in pathological states such as diabetes is not yet well understood, and it could be hypothesized that alterations of lactate transport into RBC in type 2 diabetic patients could contribute to the increased lactate level found in diabetes.

Therefore, we investigate if blood lactate concentration could interfere with substrate utilization during exercise (a situation of increased lactate production) in type 2 diabetic patients. Furthermore, we have investigated lactate transport capacity and quantified MCT1 in RBCs.

2. Materials and methods

2.1. Population

Seven obese (controls) and 7 obese type 2 diabetic (diabetic subjects) women were selected from patients undergoing routine hospital tests. All were postmenopausal, without any hormonal substitution. Diabetes was defined on the basis of fasting glucose concentration ($\geq$7 mmol/L) and glycated hemoglobin (HbA1c) ($\geq$7 %) levels. Type 2 diabetic patients were on diet and/or were treated with oral drugs (2 diet, 3 sulfonylurea alone, 3 metformin + sulfonylurea). All subjects were examined to allow them to undergo an exercise protocol. All were sedentary, reporting that they did not exercise on a regular basis.

2.2. Bioelectrical impedance

Measurements were performed at rest with a multifrequency (1, 5, 10, 50, 100 kHz) device (Human IM-Scan, Dietosystem, Milan, Italy). Data were analyzed with the software manufacturer provided.

2.3. Exercise testing

After an overnight fast, subjects underwent an exercise test on a cycle ergometer to calculate carbohydrate and lipid oxidation. The workload of each step was calculated from the theoretical maximal power (Wmax), that is, power corresponding to the theoretical maximum oxygen consumption [15]. The protocol consisted of a 3-minute warm-up at 20% of theoretical Wmax, followed by four 6-minute, steady-state workloads at 30%, 40%, 50%, and 60% of Wmax [4,16,17]. Maximal power per kilogram of fat-free mass and oxygen consumption ($\dot{V}O_2$) were compared between the 2 groups to make sure that both control and diabetic subjects were tested at similar relative intensities. The crossover point and the lipox max point were expressed both in absolute values (watt) and in percentage of theoretical Wmax.

2.4. Calculation of substrate oxidation

Oxygen consumption, CO_2 production ($\dot{V}CO_2$), and ventilation during the test were measured by a breath-by-breath device (Cardio-O_2 CPX, Medicalgraphics, Minn, USA) that analyzes respiratory gases with a zirconium cell for O_2 and an infrared analyzer for CO_2. Lipid and carbohydrate oxidation were measured by indirect calorimetry according to the nonprotein respiratory quotient (R) technique [18]. Two variables were measured to characterize the balance of carbohydrate and lipid oxidation: the crossover point as previously described by our group and the lipox max point [4,16,17]. According to the concept proposed by Brooks and Mercier [5], the crossover point is defined as the power at which energy from carbohydrate-derived fuels predominates over energy from lipids. The maximal fat oxidation rate point is the power at which the increase in lipid oxidation induced by the workload reaches a maximum. It is calculated from gas exchange measurements according to the nonprotein respiratory quotient technique [18], considering the empirical formula: fat = 1.6946 $\dot{V}O_2$−1.7012 $\dot{V}CO_2$, which can be simplified as fat = 1.7 (1-R) $\dot{V}O_2$, in which R is the respiratory quotient.

2.5. Influx assay into RBCs

Preparation of RBC and the lactate influx measurements were performed according to Skelton et al [19]. Briefly, blood samples were taken at rest and then the cell pellet was washed with chloride buffer and the hematocrit adjusted to 30% with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (90 mmol/L NaCl, 50 mmol/L HEPES, pH 7.4). Two stocks of the cell suspension were made, one with 1 mL of suspension, the other containing 1 mL of cell suspension with 18 μL of p-chloromercuribenzene acid. These stocks were incubated in a 37°C water bath for 30 minutes.

A 25-μL sample of stock cell suspension was added to 75 μL of HEPES influx buffer. The HEPES influx buffer contained (^{14}C) lactate at 5 unlabeled (La) values of 2, 10, 50, and 100 mmol/L. Because of dilution with the stock cell suspension, the actual final (La) values were 1.6, 8.1, 41, and 81.1 mmol/L. After 20 seconds, influx was stopped and washed 2 times with a cold stop solution (150 mmol/L NaCl, 10 mmol/L Na-2-(N-Morpholino) ethanesulfonic acid, pH 6.5). The RBC pellet was lysed and deproteinized with 0.5 mL of 4.2% perchloric acid, followed by a centrifugation of the sample (15 minutes, 2000g, 4°C). A 0.4-mL sample was pipetted into scintillation vials containing 6 mL of aqueous counting fluid and counted in a liquid scintillation counter (Packard 2200, Rungis, France). In all

Table 1
Anthropometrics and glycemic characteristics of subjects

	Controls (n = 7)	Diabetic subjects (n = 7)	P
Age (y)	56.2 ± 2.1	51.1 ± 3.4	NS
BMI (kg/m^2)	31.3 ± 2.7	32.1 ±1.7	NS
Waist-hip ratio	0.82 ± 0.03	0.88 ± 0.03	NS
$\dot{V}O_2$max (mL min^{-1} kg^{-1})	18.2 ± 0.9	16.9 ± 1.5	NS
Maximal power (W)	113.6 ± 5.5	105.4 ± 6.5	NS
Fat mass (%)	42.1 ± 2.8	43.2 ± 2.4	NS
Fat-free mass (%)	57.8 ± 2.8	56.7 ± 2.4	NS
Basal lactate (mmol/L)	0.90 ± 0.26	1.64 ± 0.49	<.05
Glycemia (mmol/L)	5.1 ± 0.15	8.0 ± 2.0	<.05
HbA1c (%)	–	8.01 ± 0.01	–

Data are expressed as mean ± SEM. $\dot{V}O_2$max indicates maximum oxygen consumption.

conditions, blank controls and the radioactivity of this sample were subtracted from the radioactivity in the normal influx assays to determine the amount of radioactivity that had actually entered RBCs.

Calculation of influx was done with Lineweaver-Burk kinetics curve; we estimated a maximal velocity (Vmax) value and an affinity constant (Km) value corresponding to the protein affinity for its substrate.

2.6. Membrane isolation and detection of MCT1

Red blood cell membranes were isolated as previously described [20]. Protein samples of membrane preparations were separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels (150 V for 1 hour) and transferred onto polyvinylidene difluoride membranes (100 V, 1 1/2 hours). Membranes were incubated on a shaker overnight in 20 mmol/L Tris-base, 137 mmol/L NaCl, and 0.1 mol/L HCl, pH 7.5, 0.1% (vol/vol) Tween 20, and 10% (wt/vol) nonfat dried milk at room temperature (buffer D). Membranes were then incubated with diluted NH_2-terminus MCT1 antibody (1:2500) in buffer D for 3 hours followed by 3 washes in buffer E (ie, buffer D without dried milk: 15-minute wash and two 5-minute washes) and by incubation for 1 hour with rabbit immunoglobulin G horseradish peroxidase–conjugated secondary antibody (1:2500, Sigma, L'isle d'Abeau Chesnes, France) in buffer E. Membranes were washed as before with buffer E and then MCT1 was detected by using an enhanced chemiluminescence detection method. Western blots and quantification of results were performed in duplicate for each sample.

2.7. Biochemical analyses

With subjects in the supine position, blood samples were taken via an indwelling catheter in a vein of the forearm at rest, 30%, 50%, and 60% of Wmax and at 2 times of recovery (8 and 20 minutes) [11]. No blood sample was taken at 40% to limit constraints for patients. Blood lactate concentration was determined using the method of Gutmann and Wahlefeld [21]. To guarantee a rapid sampling and immediate sample processing, every working operation was always performed by the same person. Blood samples for lactate analysis were immediately deproteinized in cold perchloric acid. The perchloric acid extracts were frozen (−80°C) for subsequent analysis. Plasma glucose was determined with a Vitros Product Chemistry analyzer (Johnson and Johnson, Clinical Diagnostics, Rochester, NY).

2.8. Statistical analysis

Results are presented as mean ± SE. After checking the normality of distribution with the Komolgorov-Smirnov test, values between and within groups were tested using unpaired Student *t* tests and analysis of variance analysis. Scheffé post hoc tests were used to determine where significant differences occurred. Correlations were assessed with Pearson coefficient. All calculations were performed with Statview software (France). Statistical significance was set at $P < .05$.

3. Results

3.1. Subjects characteristics

The anthropometrics and metabolic data at rest are shown in Table 1. Basal lactate concentration was greater in diabetic subjects than in controls (1.64 vs 0.90 mmol/L; $P < .05$). A positive correlation between basal lactatemia and HbA1c in the diabetic group ($r = 0.83$; $P < .05$) was found. Lactatemia during exercise was higher in the diabetic group than in controls ($P < .05$) as shown in Fig. 1.

There was no significant difference in crossover and lipox max respectively expressed in percentage of Wmax (42.1 ± 6.3 for controls vs 36 ± 2.4 for diabetic subjects and 38.4 ± 5 for controls vs 33.4 ± 2.4 for diabetic subjects) or in absolute values of power (42.4 ± 4.5 for controls vs 37.4 ± 4.4 W for diabetic subjects and 38.5 ± 4.4 for controls vs 34 ± 3.6 W for diabetic subjects). In the diabetic group, a negative correlation was observed between both basal lactatemia and lactatemia measured at 30% of Wmax and with the 2 indexes of substrate utilization: the

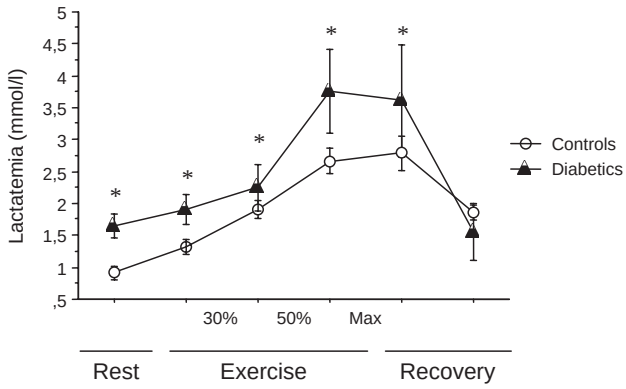

Fig. 1. Basal, exercise, and recovery levels of circulating lactate. Asterisk indicates significant difference between groups ($P < .05$).

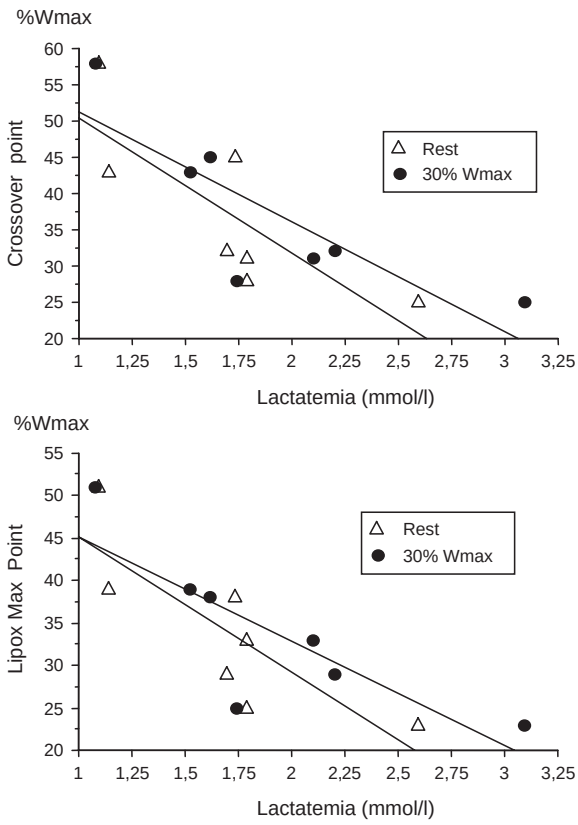

Fig. 2. Correlations between basal lactatemia and crossover point and lipox max point in diabetic patients. For crossover point, $r = -0.79$ (rest) and $r = -0.82$ (30% Wmax) ($P < .05$). For lipox max point, $r = -0.83$ (rest) and $r = -0.80$ (30% Wmax) ($P < .05$).

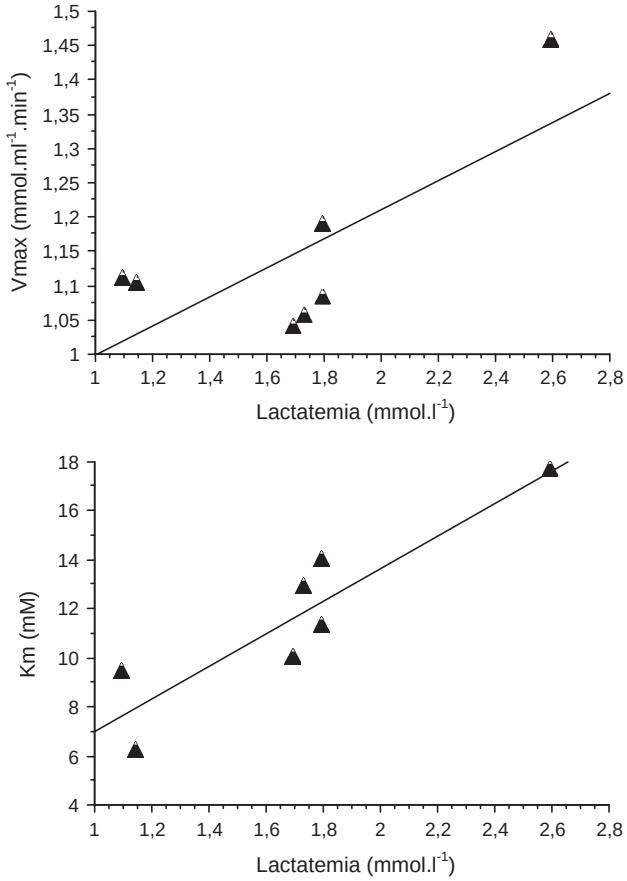

Fig. 4. Correlation between basal lactatemia and kinetics parameters of lactate influx in diabetic patients. For Vmax, $r = 0.73$; for Km, $r = 0.91$ ($P < .05$).

crossover point ($r = -0.79$, $r = -0.82$; $P < .05$) and lipox max ($r = -0.83$, $r = -0.80$; $P < .05$) as shown in Fig. 2. There was no such relation in control subjects between these 2 lactate and crossover points ($r = 0.42$, $r = 0.17$; $P > .05$) and lipox max ($r = 0.28$, $r = 0.36$; $P > .05$).

3.2. Lactate transport and MCT1 content

There was no difference between controls and diabetic subjects in Vmax and Km, respectively. Influx for different concentrations was not different between the 2 groups despite a slight increase in diabetic group at 41 (0.896 ± 0.09 vs 0.998 ± 0.04 μmol mL^{-1} min^{-1}) and 81.1 mmol/L (1.069 ± 0.12 vs 1.219 ± 0.06 μmol mL^{-1} min^{-1}). Fig 3 shows a Western blot of MCT1 on RBCs. Quantification of the Western blot did not show significant differences of MCT1 quantity between control and diabetic subjects. A positive correlation was found between Km, Vmax transport, and lactate level in diabetic subjects ($r = 0.91$, $r = 0.73$; $P < .05$) (Fig. 4), but not in controls ($r = 0.05$, $r = 0.09$; $P > .05$).

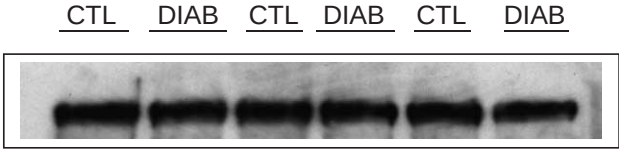

Fig. 3. Monocarboxylate transporter 1 expression in RBCs of representative control and diabetic subjects.

4. Discussion

The major finding of this study is that basal and exercise-induced elevations of lactate level in type 2 diabetic patients are negatively correlated with 2 indexes of substrate utilization (crossover and lipox max points). Moreover, despite the lack of difference in MCT1 activity and expression between the 2 groups, there was a positive relation between resting blood lactate level and kinetics parameters of MCT1 lactate transport in RBC of type 2 diabetic patients.

Different factors have been shown to influence lactate levels in diabetic patients, including body mass index (BMI), adipose tissue, and blood glucose level [6]. It has been suggested that metformin medication could induce lactic acidosis [22]. Three of our subjects were taking metformin for medication. However, it has been clearly

shown that this treatment has a limited effect on lactate level in diabetic type 2 people without cardiac failure or renal insufficiency [22]. Metformin could explain an increase lactate levels of 0.16 mmol/L [22]. Our subjects did not present cardiac or renal alterations, and although we cannot rule out the possibility of an effect of metformin on our lactate results, it can not explain per se the major increase of lactate levels. Because we have compared 2 groups with no significant differences for BMI, fat mass, and waist-hip ratio, we investigated the specific effect of diabetes on lactate metabolism and substrate utilization. The correlation between basal lactate levels and HbA1c found in our study is not surprising and confirms previous findings [23]. Differences in lactate levels cannot be explained by different workloads because there was no difference in absolute value. We also compared Wmax per kilogram of fat-free mass and $\dot{V}O_2$ during exercise and none of these parameters differed between the 2 groups. We thus demonstrate that in type 2 diabetic patients, altered substrate utilization is associated with hyperlactatemia in comparison with controls matched for BMI.

An interesting result is the relationship between lactate levels (both basal and at 30% Wmax) and the 2 indexes of substrate utilization (crossover point and lipox max). These relations are even more interesting because crossover and lipox max points were approximately determined around 30% Wmax. Although there was no relationship in the control group between these variables, we could hypothesize that an elevated lactate level in pathological conditions such as diabetes could potentially act on substrate utilization by 2 mechanisms. First, the increased lactate level could act by promoting lactate utilization and, by the same way, carbohydrate oxidation. Recent evidence has shown that lactate infusion in healthy humans increases the rate of lactate oxidation while decreasing glucose oxidation [24]. The consequence could be a dependence on carbohydrate utilization more pronounced in type 2 diabetic patients. This could explain the decrease of the crossover point in diabetic women in our study. A second explanation is that lactate could inhibit lipid oxidation as already suggested in animals [11]. This could explain the decrease in the lipox max point found in our study in the diabetic group. Most of the studies on the interaction between lactate and other substrates [11,25] have been studied in healthy animals or humans using a lactate infusion protocol. Although there are no data about the effect of chronic elevated blood lactate on substrate utilization in human pathological conditions such as type 2 diabetes, our study is in accordance with previous results found in animals and healthy humans. We show for the first time that in a pathological condition with increased blood lactate levels, lactate could be implicated in the altered substrate utilization seen in human type 2 diabetic patients.

In addition to the effect of hyperlactatemia on substrate utilization during exercise, we have investigated lactate transport in RBC and quantified MCT1. Indeed, blood represents the first great distribution space and, furthermore, serves as a rapid transport medium from the lactate-producing organs to the places where lactate is eliminated. Red blood cells contribute to lactate metabolism in terms of production, but also uptake during exercise when lactatemia increases. Furthermore, it has been suggested that lactate transport and MCT expression could partly explain abnormal lactate levels in diabetic animals [12]. Here we report the first data on lactate transport and MCT1 expression in RBCs from type 2 diabetic postmenopausal women. Although lactatemia is increased at rest and during exercise in diabetic patients, we found no alteration in lactate transport into RBCs. It has been shown that animal species with different glycolytic or oxidative capacities present different rates of lactate influx; however, these differences seem to be less pronounced in humans. Skelton et al [19] found no differences when comparing lactate influx into RBCs between sedentary subjects, sprinters, and cross-country runners. Our results agree with those of Skelton et al [19] because despite a known decrease in oxidative metabolism in type 2 diabetes [26], there was no change in lactate influx into RBCs when compared with controls. However, the positive correlation found between Vmax, Km, and basal lactate level suggests that we can not exclude that the RBC MCT1 biochemical characteristics could be influenced by elevated lactate levels and, consequently, potentially increased by itself lactate level. This relationship suggests that chronically increased lactate level could decrease the affinity of lactate for the MCT1 transporter. This result agrees with data reported by Py et al [12] on a model of sarcolemmal membranes. Indeed, these authors have shown that insulin-resistant rats presenting an increased lactate level also had increased Km during lactate transport measurements on sarcolemmal vesicles compared with control. The lower proportion of MCT1 in RBC compared with skeletal muscle could explain why we found such a relation without differences in lactate transport capacity between our 2 groups. However, it seems that mechanisms other than lactate transport could contribute to increased lactate levels in type 2 diabetes. Alterations of oxidative metabolism in type 2 diabetes could contribute to alterations of lactate metabolism, as lactate can be oxidized by mitochondria.

It is now well known that the activity of mitochondria is altered in type 2 diabetes [27], and recent research has highlighted the involvement of numerous genes [28] and, in particular, the importance of transcription factor and their coactivators in metabolic disturbances characteristic of insulin resistance and diabetes [29-31]. In this context, peroxisome proliferator–activated receptor-γ coactivator 1α (PGC-1α) has been implicated in the down-regulation of oxidative phosphorylation in type 2 diabetes [29], which could have consequences on lactate metabolism. It could be hypothesized that altered expression and activity of PGC-1α are present in our diabetic subjects and that this alteration could be related to disturbances in substrate utilization. Moreover, a possible down-regulation of PGC-1α could influence pyruvate dehydrogenase complex expression [31],

which could contribute to increase lactate levels in diabetic subjects. Alterations of both lactate metabolism and the coactivation of transcription factor such as PGC-1α could be linked and would be interesting to study in the skeletal muscle of type 2 diabetic patients.

In conclusion, this study shows an increased lactatemia at rest and during moderate exercise in type 2 diabetic postmenopausal women, which is associated with an early dependence on carbohydrate oxidation. This alteration could not be explained by alterations of lactate transport in RBC. We also show that increased lactate level cannot be explained by alterations of lactate transport in RBC, but is linked with RBC MCT1 properties.

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