

ORIGINAL RESEARCH

Kinetics of lactate metabolism after submaximal ergometric exercise in HIV-infected patients

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Objectives

It is unknown whether high levels of lactate result from enhanced production or decreased degradation. We therefore investigated differences in the kinetics of plasma lactic acid in HIV-infected patients receiving or not receiving highly active antiretroviral therapy (HAART) and in uninfected controls after submaximal ergometric exercise.

Methods

Ten healthy controls, 11 HIV-infected therapy-naïve patients, 15 HIV-infected patients on HAART with normal baseline lactate levels, and nine HIV-infected patients on HAART with elevated baseline lactate levels >2 mmol/L performed 10 min of ergometric exercise, with a heart rate of 200 beats/min minus age. Lactate levels were measured at baseline, at the end of exercise and 15, 30, 45, 60 and 120 min thereafter.

Results

Mean baseline lactate levels were 1.4, 1.5, 1.5 and 2.8 mmol/L in the controls, the therapy-naïve patients, the patients on HAART with normal lactate levels and the patients on HAART with elevated lactate levels, respectively. Maximum lactate levels after exercise were similar in all groups (9.7, 9.4, 9.0 and 10.1 mmol/L, respectively). Significant differences were found in the slope of lactate decline between controls and untreated individuals ($P = 0.038$) and between patients on HAART with normal baseline lactate and patients on HAART with elevated baseline lactate ($P = 0.028$).

Conclusions

Differences in lactate metabolism do exist between healthy controls and HIV-infected therapy-naïve individuals. Thus, HIV infection in itself may influence lactate levels. Elevated baseline lactate levels are associated with a delayed decline of lactate after exercise. These results could be explained by impaired lactate clearance. Lactate production upon exercise does not seem to be affected by baseline lactate levels.

Keywords: hyperlactataemia, lactate metabolism under exercise, lactic acidosis, mitochondrial toxicity

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Introduction

Mitochondrial toxicity causing hyperlactataemia, lactic acidosis and hepatic steatosis is a well-recognized adverse effect of nucleoside reverse transcriptase inhibitors (NRTIs). Some evidence suggests that the mitochondrial DNA polymerase gamma function is impaired, resulting in altered mitochondrial DNA and protein function [1]. These toxic effects can range from asymptomatic elevated lactate levels to symptomatic hyperlactataemia leading to mild and nonspecific clinical symptoms such as nausea, vomiting, fatigue or weight loss [2]. In contrast, lactic acidosis represents a relatively uncommon (approximately 2 per 1000 person-years) but life-threatening clinical syndrome in which lactate homeostasis is completely decompensated [3], characterized by a pH <7.25 and a plasma lactate level >5 mmol/L [4]. The syndrome of hyperlactataemia is well known in HIV-infected patients receiving antiretroviral therapy, particularly in those on stavudine [5]. Host risk factors have been identified for NRTI-associated lactic acidosis, including concurrent liver disease, female gender

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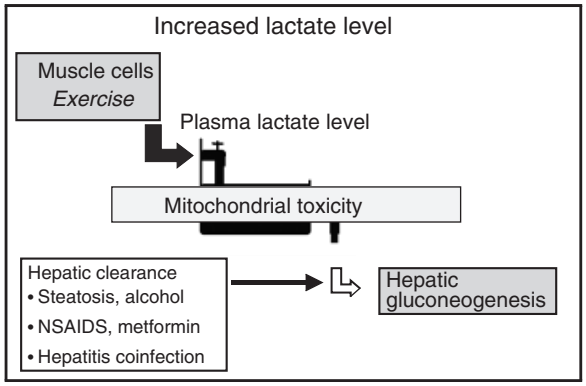

Fig. 1. Hypothesis of lactate metabolism and development of hyperlactataemia.

and obesity [6]. Whether elevated lactate levels are predictive for the development of serious lactic acidosis is still uncertain. However, several data suggested serum lactate concentrations to be poorly predictive for future events [7] (see hypothesis in Fig. 1).

Mitochondria have a pivotal role in cellular energy homeostasis, producing adenosphine biphosphate (APT) by oxidative phosphorylation. Under normal aerobic conditions, glucose is metabolized to pyruvate, which is further degraded in the mitochondrion to CO_2, H_2O and ATP. Lactate production can be induced by anaerobic glycolysis, by a defect in the oxidative phosphorylation of peripheral tissue or as a result of defective mitochondrial DNA (mtDNA)-encoded proteins. The primary site of lactate clearance is the liver and, to a lesser extent, the kidney and the skeletal muscle itself. The sole pathway for lactate utilization leading to stable lactate concentrations of less than 2 mmol/L is conversion back to pyruvate and then to glucose in the Cori Cycle, which depends on ATP and sufficient oxidative phosphorylation. Thus, impaired lactate clearance may be the result of a mitochondrial dysfunction in the liver [8] or in other tissues, for example skeletal muscle.

Skeletal muscle cells, which have high amounts of mitochondria and high oxidative requirements, are the most important producers of lactate, especially during exercise. Elevated lactate levels could also be caused by increased production (see Fig. 1). Whether hyperlactataemia associated with highly active antiretroviral therapy (HAART) and mitochondrial toxicity is caused by increased production or decreased clearance, or both, is not clear.

Mitochondrial dysfunction could be unmasked by increasing demands of oxidative phosphorylation (e.g. during exercise) or by increased stimulus for lactate clearance.

Our purpose was to investigate differences in the kinetics of lactic acid in HIV-infected patients compared with uninfected controls and to determine the influence of HAART on plasma lactate levels at rest and in a post-exercise period. We intended to unmask lactate metabolism disturbances by increasing demands of both oxidative phosphorylation and lactate clearance.

Materials and methods

The study took place at the outpatient department of the Medical Poliklinik Department of Infectious Diseases, Munich, Germany. Patients were recruited from our HIV-infected patient cohort.

Patient screening was performed by routine lactate measurement at rest. Patients were selected to participate if they gave consent to perform an exercise test and did not show any contra-indication for an exercise test [e.g. complete bundle branch block in electrocardiogram (ECG), known coronary heart disease, or congestive heart failure]. Screening included 58 patients on HAART and 23 patients not on HAART. Reasons for non-inclusion were patient's decision (no time for extra test or not willing to participate; $n = 21$), complete left bundle block ($n = 1$) and known coronary heart disease ($n = 1$). For group 2 (no HAART), 23 patients had to be screened. Twelve patients declined to participate. No medical reasons for non-inclusion were present.

Healthy controls were chosen from among volunteer individuals. Every participant gave informed consent. The study was approved by the local institutional board.

Four different groups were formed: (1) HIV-negative controls ($n = 10$), (2) HAART-naïve patients ($n = 11$), (3) patients on HAART with normal baseline lactate ($n = 15$) and (4) patients on HAART with lactate higher than 2 mmol/L ($n = 9$).

Physical fitness levels were assessed using a questionnaire validated by Fonds Soziales Wien, Vienna, Austria [9]. The survey includes questions on lifestyle (sedentary vs. physically active), number of training units (exercise of 1 hour) per week, and type of regular exercise (endurance training vs. power-associated training). A score was computed that had a maximum of 130. The average score was 58.5 and did not vary significantly amongst the four groups.

At rest, the following parameters were assessed: concentrations of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transferase (GGT), alkaline phosphatase (AP), cholesterol, triglycerides and lactate. Body mass index was calculated using the formula weight (kg)/body surface area (m^2).

Under ECG monitoring, ergometric exercise on a cycle ergometer was performed to reach a heart rate of 200 minus

Table 1. Treatment regimens

	n (%)		
	Group 3	**Group 4**	**Total**
Stavudine	8 (53)	4 (44)	12 (50)
Lamivudine	8 (53)	3 (33)	11 (46)
Didanosine	3 (20)	4 (44)	7 (29)
Zidovudine	3 (20)	2 (22)	5 (21)
Abacavir	3 (20)	1 (11)	4 (17)
Nevirapine	5 (33)	3 (33)	8 (33)
Efavirenz	3 (20)	1 (11)	4 (20)
Lopinavir	3 (20)	3 (33)	6 (25)
Nelfinavir	3 (20)	2 (22)	5 (21)
Saquinavir	2 (13)	1 (11)	3 (13)
Didanosine + stavudine	2 (13)	4 (44)	6 (25)

Group 3 ($n = 15$): patients on HAART without baseline hyperlactataemia; Group 4 ($n = 9$): patients on HAART with hyperlactataemia of baseline.

age. This submaximal exercise was performed for 10 min. Venous lactate levels were taken without stasis via an indwelling intravenous catheter at rest, at the end of exercise and at time points 15, 30, 45, 60 and 120 min thereafter. The blood samples were taken in sodium fluoride tubes (Vacutainer®; Becton Dickinson, Heidelber, Germany).

The study was not designed to compare differences in lactate levels for different nucleoside compounds. Nevertheless, we listed all HAART components of the patients' regimens used at the time the study was carried out for a rough descriptive analysis (Table 1). All patients were NRTI-experienced. Intake of concomitant medication affecting lactate metabolism (particularly non-steroidal anti-inflammatory drugs (NSAIDs) and Metformin) and chronic alcohol abuse were exclusion criteria.

Statistical methods

The nonparametric Kruskal–Wallis test and the *t*-test were used. For comparison of mean decline of lactate, we calculated the area under each curve showing the individual kinetics of each participant. We therefore transformed measured data using percentage rate of decline (100% for lactate maximum). Data are presented as average and standard deviation or as median and range. Results are considered significant if $P < 0.05$.

Results

Patient characteristics

The patients had been HIV-positive for a median of 2 (range 1–18) years in group 2, for 9 (1–16) years in group 3 and for 7 (2–14) years in group 4, and both groups 3 and 4 had been treated with HAART for a median of 5 years. Mean CD4 counts were not significantly different amongst HIV-positive groups 2–4.

Concerning AIDS status, 10 patients in group 2 were at Centers for Disease Control (CDC) stage A and 1 patient stage B, in group 3 there were two patients at stage A, six at stage B and seven at stage C, and in group 4 there were six patients at stage A and three patients at stage B.

The patients and controls were matched according to training level, sex and body mass index (BMI), but they differed in age (median 37, 38, 48 and 50 years). Significant differences in mean age were found between groups 2 and 3 ($P = 0.013$).

Four patients in group 3 and two in group 4 showed clinical signs of lipodystrophy. Lipodystrophy was assessed using either the patient's history or physicians' impressions.

Concerning liver parameters, we found no significant differences in liver enzymes, but there was a slight tendency for higher mean gamma glutamyl transferase (GGT) in group 4, the patients with elevated baseline lactate: 45 ($\pm$ 31.7) U/L in group 4 vs. 41 ($\pm$ 25.2) U/L in group 3, 20 ($\pm$ 18) U/L in group 2 and 11 ($\pm$ 2.9) U/L in group 1. All patients in group 4 showed hypertriglyceridaemia (498 $\pm$ 556 mg/dL) and five of them show elevated cholesterol levels (220 $\pm$ 57.1 mg/dL).

Two patients in group 2 suffered from chronic hepatitis B and one from hepatitis C coinfection. In group 4, one patient had chronic hepatitis B infection.

For a summary of patient characteristics see Table 2.

Exercise performance

Average baseline lactate levels were 1.4 ($\pm$ 0.4), 1.5 ($\pm$ 0.6), 1.5 ($\pm$ 0.3) and 2.8 ($\pm$ 0.5) mmol/L in the four groups.

Mean exercise performance in 10 min of submaximal ergometry was 154 ($\pm$ 56.6) W in group 1, 131 ($\pm$ 37.1) W in group 2, 121 ($\pm$ 33.8) W in group 3 and 99 ($\pm$ 23.7) W in group 4. The differences were not significant, although the average in group 4 showed a tendency towards lower performance ($P = 0.09$).

Maximum lactate levels measured immediately after exercise were not significantly different amongst the groups: 9.7 ($\pm$ 3.2) mmol/L in group 1, 9.4 ($\pm$ 3.7) mmol/L in group 2, 9.0 ($\pm$ 2.8) mmol/L in group 3 and 10.1 ($\pm$ 2.2) mmol/L in group 4. Patients in group 4 had slightly higher lactate despite a workload that was only two-thirds of that in controls. 120 min after exercise, lactate levels were 1.4 ($\pm$ 0.3), 1.6 ($\pm$ 0.6), 1.7 ($\pm$ 0.7) and 3.1 ($\pm$ 1.11) mmol/L in groups 1, 2, 3 and 4, respectively. Significant differences were found in the slope of lactate decline both between controls and untreated individuals and between patients on HAART with normal lactate and patients on HAART with elevated lactate (Fig. 2). The

Table 2. Patient characteristics

	Group			
	1	2	3	4
Age (years)	37 (± 10.5)	38 (± 9.8)	48 (± 8.8)	50 (± 16)
BMI	23 (± 1.9)	24 (± 2.9)	25 (± 4.3)	23 (± 2.6)
CD4 count (cells/μL)	_	418 (± 130)	377 (± 132)	472 (± 247)
Duration of HIV (years)	_	5 (± 6.2)	10 (± 5.4)	8 (± 4.4)
Years on HAART	_	_	5 (± 3.2)	5 (± 4.3)
Lipodystrophy	_	_	4	2
Fitness score	67 (± 14)	59 (± 22)	55 (± 19)	53 (± 19)
Hepatitis B	_	2	_	1
Hepatitis C	_	1	_	_
Lactate baseline (mmol/L)	1.4 (± 0.4)	1.5 (± 0.6)	1.5 (± 0.3)	2.8 (± 0.5)
AST (U/L)	10 (± 3.7)	13 (± 4.5)	17 (± 18)	11 (± 2.7)
ALT (U/L)	15 (± 9.8)	20 (± 11.6)	22 (± 10.9)	19 (± 8.4)
GGT (U/L)	11 (± 2.9)	20 (± 18)	41 (± 25.2)	45 (± 31.7)
AP (U/L)	83 (± 24.4)	86 (± 23.8)	110 (± 40.8)	99 (± 26.6)
Cholesterol (mg/dL)	186 (± 38.7)	167 (± 29.4)	217 (± 58.2)	220 (± 51.4)
Triglycerides (mg/dL)	104 (± 34.6)	109 (± 54.4)	227 (± 197.7)	498 (± 556.5)

Group 1 ($n = 10$), healthy controls; group 2 ($n = 11$), HIV-infected treatment-naïve patients; group 3 ($n = 15$), HIV-infected patients on HAART; group 4 ($n = 9$), HIV-infected patients on HAART with hyperlactataemia.

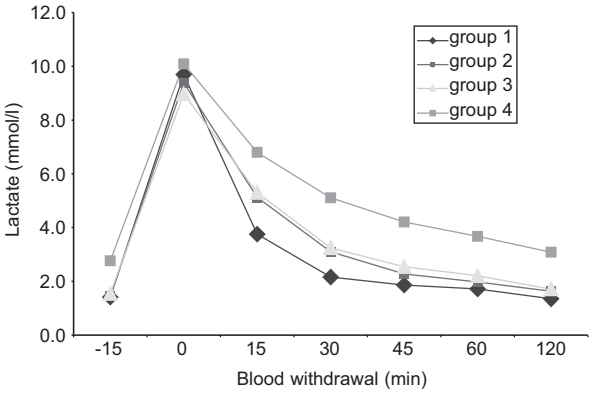

Fig. 2. Kinetics of lactate metabolism in group 1 (10 healthy controls), group 2 (11 HIV-infected therapy-naïve patients), group 3 (15 HIV-infected patients on HAART) and group 4 (nine HIV-infected patients on HAART with hyperlactataemia). Average values are shown for lactate concentrations at time points 15 min before exercise, directly after exercise and 15, 30, 45, 60 and 120 min thereafter.

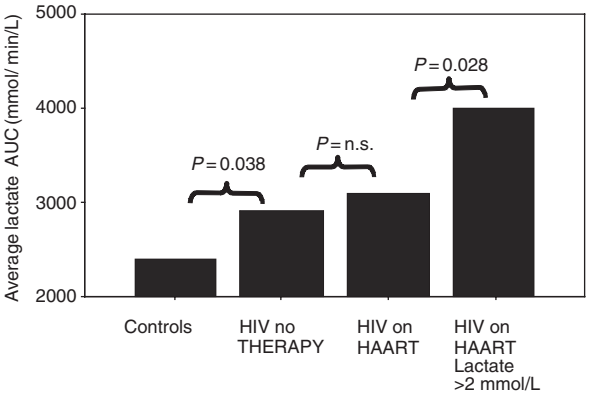

Fig. 3. Decline of plasma lactate after exercise: area under the curve.

decline in lactate values was more rapid in group 1 compared to all other groups and slowest in group 4. Measuring the decline in plasma lactate level by calculating the area under the curve (AUC) for each group, we found significant differences: patients in group 1 seemed to clear lactate more quickly than patients in group 2 ($P = 0.038$), and those in group 3 had a steeper decline compared to those in group 4 ($P = 0.028$). We did not find any difference between group 2 (untreated individuals) and group 3 (patients on HAART with normal lactate at baseline) (Fig. 3).

Discussion

The finding of differences in lactate decline between healthy controls and HIV-infected therapy-naïve patients suggests that HIV infection itself may influence lactate metabolism. One possible explanation is that the virus itself affects mitochondrial DNA, leading to depletion and disturbance of enzyme functions. Direct toxicity of HIV proteins on mitochondrial DNA *in vitro* was shown by Macready *et al.* [10]. The HIV protein Virion-associated protien of HIV-1 (Vpr-1) led to mitochondrial dysfunction in *Sacharomyces cerevisae* [10]. McComsey *et al.* found multiple variations and mutations in mtDNA in both HIV-infected patients with or without therapy and healthy individuals. These pre-existing differences could become

clinically relevant during HIV infection or therapy with NRTIs [11,12].

There is ongoing discussion about the relevance of mtDNA measurements in peripheral blood mononuclear cells (PBMC) to assess mitochondrial toxicities. Some researchers could not find any correlation between hyperlactataemia and mtDNA contents of PBMC [13].

Elevated baseline lactate levels, as found in group 4, resulted in a decreased rate of decline of lactate after exercise. These patients did not reach their mean baseline lactate level after 120 min of rest, which demonstrates a defect in the normal, rapid clearance mechanism of serum lactate. This may be caused by impaired lactate clearance. We did not find any pathological elevation of transaminases AST and ALT. Liver affection could be moderate and not yet reflected in blood liver enzymes. Thus a liver biopsy could reveal any potential alteration in mitochondrial phosphorylation capacity. Brinkman *et al.* found microvesicular or mixed hepatic steatosis in patients with persistent hyperlactataemia who underwent liver biopsies [8]. Therefore, impaired lactate clearance might be the result of mitochondrial dysfunction in the hepatocytes. Hyperlactataemia in patients receiving NRTI-containing therapy would then reflect impaired hepatic clearance and not increased production. Patients with HIV infection may have several reasons for diminished hepatic clearance of lactate: NRTI effects on mitochondria, dyslipidaemia and insulin resistance contributing to steatosis or hepatitis B or C coinfection.

The question of lactate production and clearance was also investigated by Leclercq *et al.* using a pharmacological model to distinguish exogenous and endogenous lactate based on a test using lactate infusion [14]. They found a statistically significant increase in lactataemia and lactate production in symptomatic HIV-infected patients compared with asymptomatic or control patients, but no difference in lactate clearance [14]. Roge *et al.* tested eight HIV-infected patients on HAART with lipodystrophy and elevated plasma lactate levels and eight healthy controls exposed to incremental exercise until exhaustion [15]. The decline in blood lactate in the recovery period was similar in the two groups. This finding may have been attributable to the impaired physical fitness of the HIV-infected patients and to the fact that, rather than exercising submaximally, individuals exercised to exhaustion, which is highly dependent on individual motivation.

If elevated plasma lactate levels reflect mitochondrial dysfunction, a physiological derangement is to be expected. A recent study conducted by Tesiorowski *et al.* demonstrated that physiological abnormalities do exist in HIV-infected patients with nucleoside-associated hyperlactataemia [16]. These patients were found to show a decrease in the aerobic threshold and an increased peak respiratory quotient on exercise testing. As Tesiorowski *et al.* suggested, increased venous lactate levels represent a marker of physiologically meaningful mitochondrial derangement [16]. In our study, individuals in group 4 had a significantly lower workload than those in other groups, but lactate levels after exercise were higher (10.1 mmol/L) compared with groups 1–3 (9.7, 9.4 and 9.0 mmol/L, respectively). Thus, one could hypothesize that in group 4 oxidative phosphorylation in skeletal muscle is impaired and cellular energy deficit is compensated by anaerobic glycolysis earlier than in the other groups. How these values might be affected by the age differences amongst the groups is not known. No literature exists on normal ranges of exercise performance and lactate clearance in relation to age.

An elevated lactate level is a relatively frequent finding in HAART (8–18.3% above 2–2.5 mmol/L), whereas lactic acidosis is rare (0.3–0.4% per patient-year) [6]. Most case reports describe patients as being well and stable while receiving NRTI therapy before lactic acidosis suddenly develops. This suggests that an additional trigger may be needed for the development of this life-threatening event. Lactic acidosis most commonly occurs in persons receiving prolonged therapy (for more than 6 months). Among drug combinations, didanosine + stavudine-containing therapy appears to be over-represented in case reports on hyperlactataemia [7]. In our cohort, 44% of patients in group 4 took didanosine + stavudine combination therapy.

Recent data quantifying mtDNA/nuclear DNA (nDNA) ratios in the PBMCs of patients receiving NRTI therapy showed that this ratio was often diminished for many months before the development of 'symptomatic hyperlactataemia'. Drug withdrawal led to recovery of mtDNA and a fall in lactate levels [17]. Future investigations might test lactate metabolism in patients with diminished mtDNA/nDNA ratios under exercise in order to examine lactate kinetics in situations of increased demands of oxidative phosphorylation.

We conclude that differences in lactate metabolism do exist between healthy controls and HIV-infected therapy-naïve individuals. Thus, HIV infection in itself may influence lactate levels. Elevated baseline lactate levels are associated with a delayed decline of lactate after exercise. These results could be explained by impaired lactate clearance.

Acknowledgements

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