Skeletal muscle mitochondrial function and exercise capacity in HIV-infected patients with lipodystrophy and elevated p-lactate levels

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Objective To investigate the skeletal muscle mitochondrial function in HIV-infected patients with lipdystrophy or elevated p-lactate levels.

Design Eight HIV patients treated with highly active antiretroviral therapy, with lipodystrophy or elevated p-lactate, and eight healthy controls were exposed to incremental exercise until exhaustion.

Methods Blood samples and gas analysis were performed at rest, during exercise and in recovery. Oxygen consumption, workload and blood lactate were assessed. Before and immediately after exercise muscle biopsies were obtained, in which citrate synthase (CS), hydroxyacyl-coenzyme A dehydrogenase (HD), glycogen and nucleotides were measured.

Results Maximal workload was significantly lower in patients compared with controls [171 Watt (88–206) versus 235 Watt (118–294) $P = 0.05$]. A trend towards lower maximal oxygen consumption (VO_{2max}) was detected in patients [2136 ml/min (1221–2598) versus 2985 ml/min (1506–3959) $P = 0.11$]. Patients had significantly elevated levels of blood lactate at rest [1.55 mmol/l (1–2.5) versus 0.8 mmo/l (0.37–1.1) $P < 0.01$), but no significant difference in maximal blood-lactate values was found. The decline in blood lactate in the recovery period was similar between groups. There was no significant difference in CS, HD, glycogen or nucleotides.

Conclusion The significantly lower working capacity and the trend towards reduced VO_{2max} in patients could be caused by mitochondrial dysfunction, but may also be caused by impaired physical fitness. The similar levels of nucleotides, CS, HD, and glycogen and the normal increase in blood lactate during exercise indicates a normal oxidative phosphorylation. No evidence of serious damage to skeletal muscle mitochondrial function was found.

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Introduction

Serious side-effects of the antiretroviral treatment of HIV are lipodystrophy and lactic acidosis. Lactic acidosis is a relatively rare and life-threatening condition [1–4], whereas chronic and asymptomatic hyperlactatemia occur very frequently [5–7]). To what extent chronic hyperlactatemia evolves into acute, uncompensated lactic acidosis is not yet clear. The pathogenesis of elevated lactate levels is supposed to be nucleoside reverse transcriptase inhibitor (NRTI)-induced mitochondrial toxicity, which is also assumed

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to be the pathogenic mechanism behind other NRTI-related side-effects such as polyneuropathy, myopathy, hepatic steatosis and pancreatitis [8,9].

NRTI's were suggested to contribute to the lipodystrophy syndrome, because it was diagnosed in patients who had never received treatment with protease inhibitors [10,11]. In particular the form of lipodystrophy predominantly with fat loss (fat atrophy) has been attributed to the effect of nucleoside analogues, and to treatment with stavudine in particular [12–14].

Therefore, a link between mitochondrial toxicity and lipodystrophy was inferred, supported by the interesting observation of morphological and metabolic similarities between highly active antiretroviral therapy (HAART)-related lipodystrophy and benign or multiple symmetrical lipomatosis type I (MSL-I) [8]. One of the main causes of MSL-I is believed to be mitochondrial dysfunction, as point mutations and deletions in the mitochondrial DNA (mtDNA) of MSL-I patients have been detected [15–17]. Mitochondrial toxicity could therefore be a plausible common cause of the different side-effects seen with NRTI treatment.

The most important function of the mitochondria is oxidative phosphorylation and the production of adenosine triphosphate (ATP). NRTI are thought to exert their mitochondrial toxicity by the inhibition of DNA polymerase γ, which is the only DNA polymerase involved in mtDNA replication [9]. The mtDNA encodes subunits of the enzyme complexes in the respiratory chain [18]. By depleting mtDNA and mtDNA-encoded enzymes, NRTI could cause deterioration of oxidative phosphorylation and increase lactate production. The degree to which the mitochondria are affected can differ between tissues, and the consequences of inhibition might depend on the extent of the ATP requirements of the cells.

The different disease presentations in relation to NRTI-induced toxicity could be explained by this tissue specificity. The precise pathogenesis and tissue specificity of NRTI in lipodystrophy as well as in hyperlactatemia are at present unknown.

Skeletal muscle cells have abundant mitochondria and high oxidative requirements when activated. In theory, a skeletal muscle mitochondrial dysfunction could be uncovered by increasing the demands of oxidative phosphorylation during exercise, and hepatic mitochondrial function could be mirrored by the clearance of lactate, especially in the post-exercise period.

The aim of the present study was to investigate skeletal muscle mitochondrial function in HIV-infected patients with lipodystrophy or elevated p-lactate levels, at rest and in response to exercise.

Materials and methods

The study took place at the Copenhagen Muscle Research Center, Rigshospitalet, from November 1999 to May 2000. Patients were recruited from the outpatient clinic of the Department of Infectious Diseases, Rigshospitalet in Copenhagen. The study was approved by the local ethical committee, and written informed consent was obtained from all the participants.

The inclusion criteria were: (i) age 18 years or over; (ii) treatment with HAART for one year or more; (iii) previous or ongoing treatment with stavudine for at least 10 months; (iv) clinical lipodystrophy; or (v) hyperlactatemia. Inclusion was based on the treating physician's clinical judgement of lipodystrophy, and patients with lipodystrophy presenting as predominantly fat accumulation were excluded. Also excluded were patients with: (i) a history of arteriosclerosis or ischaemic heart disease; (ii) a CD4 cell count of less than 200 cells/mm^3 ; (iii) anaemia; (iv) secondary infections; (v) pregnancy; (vi) antihypertensive medication; and (vii) treatment with growth hormone.

At the time of the study, no distinction was made between asymptomatic and symptomatic hyperlactatemia. The study protocol reflected this situation, and patients with hyperlactatemia were included indiscriminately. Retrospectively, patient number 6 (Table 1) had complaints that were consistent with symptomatic hyperlactatemia (weight loss, abdominal discomfort, fatigue and anorexia). Eight patients were included and matched according to age, sex and height, with eight healthy, non-HIV-infected, control individuals. The controls were chosen from among individuals who did not perform intensive exercise on a regular basis.

The participants were examined at rest, during standardized incremental exercise on a cycle ergometer until exhaustion, and during a recovery period of 60 min. In the resting state, blood samples and muscle biopsies were obtained and gas analysis performed. During exercise, blood samples were drawn at times of load change and at maximal exercise. Gas exchange was continuously measured until the end of exercise. Immediately after the termination of exercise, a second biopsy with muscle tissue representative of the exhausted state was obtained. During a recovery period of 60 min, blood samples were drawn at regular intervals.

Oxygen consumption (VO_2), carbon dioxide production, pulmonary ventilation (Ve), and the respiratory quotient (RER) were measured using an on-line system (Medical Graphics CPX, Saint Paul, Minneapolis, MN, USA) while the subjects breathed through a

low-resistance breathing valve. Gases with known VO_2 and carbon dioxide consumption concentrations were used for gas analyser calibration. The heart rate was obtained from a continuously recorded electrocardiogram signal. Blood samples were drawn without stasis from a venous catheter in the forearm. In the blood samples the following parameters were assessed: blood lactate, p-pyruvate, free fatty acids (FFA), p-glycerol, p-norepinephrine and p-epinephrine. The blood-lactate levels were immediately measured using an electrolyte metabolite analyser (EML 105; Radiometer, Copenhagen). The samples were immediately frozen for later analyses of FFA, glycerol, p-pyruvate and plasma lactate using Cobas Fara 2 (Roche Diagnostics, Basel, Switzerland), whereas values of p-epinephrine and p-norepinephrine were assessed by high performance liquid chromatography with electrochemical detection (Hewlett Packard, Waldbronn, Germany).

Muscle biopsies were obtained from the quadriceps muscle of the leg and immediately freeze-dried. In the muscle biopsies, the following parameters were measured: (i) the activity of citrate synthase (CS), a citric acid cycle enzyme; (ii) the activity of hydroxyacyl-coenzyme A dehydrogenase (HD), a mitochondrial enzyme catalysing the oxidative degradation of fatty acids to acetyl-CoA; (iii) the concentration of inosine monophosphate (IMP), adenosine monophosphate (AMP), adenosine 5'-diphosphate (ADP) and ATP; and (iv) the glycogen content.

CS, HD and glycogen were analysed using Cobas Fara 2 (Roche Diagnostics), and assessed at dry weight and standard temperature (25°C for CS and HD, 37°C for glycogen). The nucleotides were analysed using high performance liquid chromatography. The patients and controls were dual-energy X-ray absorptiometry (DEXA)-scanned to support the clinical diagnosis of lipodystrophy and to assess the amount of lean leg mass (LLM). The study participants were advised to have fasted or to have just eaten a light meal before performing the test. No other food restrictions were prescribed.

Statistics

The non-parametric Mann–Whitney test and the Pearson correlation analysis were used. Data are presented as median and range, unless otherwise specified, and results are considered significant if $P < 0.05$.

Results

Patient characteristics

One female and seven male HIV-infected patients were included in the study. Seven patients had clinically diagnosed moderate or severe lipodystrophy (patient nos. 1–7, Table 1.). Three suffered from mild to severe peripheral neuropathy (patient nos. 1, 3, 6, Table 1). Three patients had elevated p-lactate levels at the time of inclusion (patient nos. 5, 6, 8, Table 1), and one patient (no. 8) had compensated lactic acidosis as the sole NRTI-related side-effect at the time of inclusion (pH 7.34, plasma-bicarbonate 20.4 mmol/l, base excess −5.0 mmol/l, blood lactate 8.1 mmol/l) (Table 1).

In the latter patient, lactate levels had been measured both in venous and arterial blood and had accumulated over months. This patient had no subjective symptoms of hyperlactatemia, and he was kept on his current medication for another 5 days until the exercise test could be performed. When he presented on the study day his lactate level had spontaneously normalized (blood lactate 1.8 mmol/l).

Table 1. Patient characteristics.

Patient number	1	2	3	4	5	6	7	8	Median
Lipodystrophy	Severe	Severe	Severe	Severe	Moderate	Moderate	Severe	None	
Resting p-lactate at time of inclusion	2.9	ND	ND	ND	3.3	4.5	1.2	8.1	
Resting p-lactate (mmol/l) on study day	2.1	1	1.2	1.3	1.5	2.5	1.6	1.8	1.6
Neuropathy	Mild	None	Mild	None	None	Severe	None	None	
Age (years)	69	50	55	41	64	52	57	43	53.5
Duration of HIV (months)	108	96	108	168	84	192	180	168	138
Months on HAART	28	36	27	41	24	12	34	42	31
Months on stavudine	28	36	27	36	32	11	41	36	34
CD4 cell count (mio/l)	625	460	960	492	520	390	530	540	525
HIV RNA (copies/ml)	19	693	19	229	21	19	19	19	19
Weight (kg)	63	49	67	73	71	53	61	62	62.5
BMI	21.8	16.6	21.5	20.9	22	18.6	20.9	20.3	20.9
p-Alanin-aminotransferase (U/l)	30	13	50	22	45	17	31	374	30.5
P-triglyceride (mmol/l)	8.58	1.99	1.23	8.22	3.15	7.1	3	3.49	3.32

BMI, body mass index; HAART, highly active antiretroviral therapy.

The patients had been HIV positive for a median of 138 months, and had been treated with HAART for a median of 31 months (Table 1).

All the patients except number 4 were on treatment with stavudine and lamivudine. Patient numbers 1, 5 and 7 received ritonavir and saquinavir, number 2 ritonavir and indinavir, number 8 nelfinavir, whereas numbers 3 and 6 were on treatment with efavirenz as a supplement to the nucleoside analogues. The duration of stavudine therapy was 11–41 months. Patient number 4 had been on stavudine for 36 months, but because of treatment failure his medication was changed 11 months before the study day, to a five-component regimen without stavudine, consisting of abacavir, didanosine, efavirenz, indinavir and ritonavir.

Patient number 6 received tolbutamide 1000 mg a day, because of type II diabetes. The diabetes had been diagnosed approximately 2 years before he started antiretroviral therapy. Neither patients nor controls were on any other medication.

The patients and controls were well matched according to sex, age (median 54 years in patients versus 51 years in controls) and height (median 175 versus 176 cm in patients and controls, respectively). However, they differed in weight, with a median of 62.1 kg in patients versus 73.2 kg in controls, and a body mass index (BMI) of 20.9 (16.6–22) versus 24.2 (18.5–26.3) in patients and controls, respectively.

Fat and muscle distribution

The DEXA scan showed a significant lower total fat mass [median 8.20 kg (5.0–12.5) versus 15.9 kg (7.3–21.9) $P = 0.028$] and a significant lower total fat percentage [12.5% (10–18) versus 21.5% (10–32) $P = 0.038$] in patients compared with controls.

The regional DEXA scans revealed a significantly higher abdominal fat content in patients [abdominal fat fraction 26.6% (1.9–35.0) versus 18.6% (8.1–23.4) $P = 0.015$], and a significantly lower leg fat content in patients compared with controls [27.0% (18.7–33.3) versus 33.8% (29.1–42.0%) $P < 0.01$]. There was no significant difference in LLM [18 kg (13.5–20) versus 20.5 kg (13–23.7) $P = 0.083$, in patients and controls, respectively].

Exercise performance

The patients had significantly lower working capacity, with a maximal workload of 171 Watt (88–206) compared with 235 Watt (118–294) in controls ($P = 0.05$) (Fig. 1a). Workload per kg LLM was 9.1 Watt/kg LLM in patients versus 11.2 Watt/kg LLM in controls ($P = 0.03$).

There was a trend towards reduced maximal oxygen consumption (VO_{2max}) in patients compared with controls, although the difference was not significant [2136 ml/min (1221–2598) versus 2985 ml/min (1506–3959) $P = 0.11$] (Fig. 1a). Likewise, there was a non-significant trend towards reduced VO_{2max} per kg LLM [114.6 ml/kg LLM/min (90.8–140.5) versus 144.6 ml/kg LLM/min (96.0–178.7) $P = 0.11$, in patients and controls, respectively]. The fold increase in VO_2 above resting levels (VO_{2max}/VO_{2rest}) was significantly different between groups [6.2-fold (3.8–8.6) in patients versus 9.9-fold (5.4–13.7) in controls, $P = 0.03$]. There was no significant difference in VO_2 at a given workload (Fig. 1a).

The Ve at rest, at 50% VO_{2max} and at VO_{2max} was similar between the groups [Ve at VO_{2max} : 98.5 l/min (54.6–129) versus 127.6 l/min (66.2–155.3) $P = 0.20$ in patients and controls, respectively]. Also no significant difference in the ventilatory coefficient (Ve/VO_2) at rest or at VO_{2max} was found [Ve/VO_2at rest: 4.1 (3.5–4.8) versus 4.1 (3.2–5.9) $P = 0.88$, in patients and controls, respectively, Ve/VO_2at VO_{2max}: 4.5 (3.2–5.6) versus 4.0 (3.6–4.6) $P = 0.20$, in patients and controls, respectively].

Metabolic parameters

Patients had significantly elevated levels of blood lactate and p-pyruvate at rest and at 50% of VO_{2max}, but there was no significant difference in maximal blood lactate and p-pyruvate levels, or at VO_{2max} (Fig. 2b and c).

The median blood-lactate levels at rest were: 1.55 mmol/l (1–2.5) versus 0.8 mmol/l (0.37–1.1) $P < 0.01$, and the median p-pyruvate levels at rest were: 141 μmol/l (66–309) versus 49.5 μmol/l (9-62) $P < 0.01$ in patients and controls, respectively. The maximal p-pyruvate levels were: 327 μmol/l (213–439) versus 296 μmol/l (168–505) $P = 0.34$, in patients compared with controls. The maximal blood lactate levels were: 8.8 mmol/l (2.9–14.8) versus 8.5 mmol/l (3.3–11.2) $P = 0.72$, in patients and controls, respectively.

To compare the rate of decline in blood-lactate levels in the recovery period between patients and controls, the change in blood lactate from baseline values (Δ blood lactate) from 5 to 60 min post-exercise was plotted against time (Fig. 1d). As the relationship between Δ blood lactate and time was clearly non-linear, Δ blood lactate was log-transformed to permit calculation of the individual $T\frac{1}{2}$. Pearson's correlation coefficient R, as calculated for each individual, was −0.99 (mean; range 0.97–1.00). $T\frac{1}{2}$ did not differ between groups ($P = 0.16$).

Resting levels of p-norepinephrine and p-epinephrine did not differ significantly between the groups, but a trend towards lower p-epinephrine levels in patients

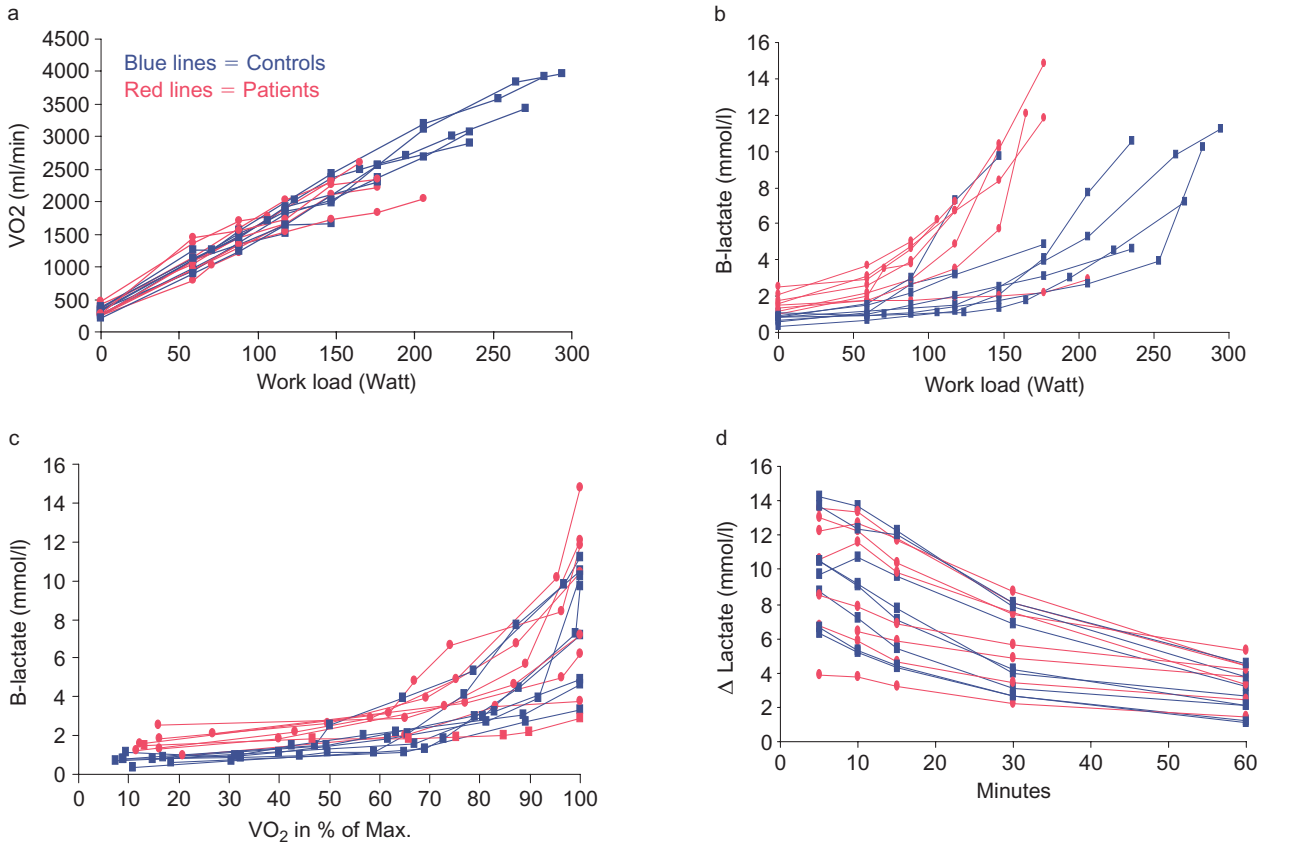

Fig. 1. (a) Relationship between oxygen consumption and exercise intensity. (b) Relationship between blood lactate and exercise intensity. (c) Blood lactate in relation to percentage of maximal oxygen consumption. (d) Decline in blood lactate minus baseline values (Δ lactate) in recovery period. Blue lines, Controls; red lines, patients.

as compared with controls was observed [resting p-norepinephrine 4.04 nmol/l (2.08–6.97) in patients versus 2.88 nmol/l (1.84–5.67) in controls, $P = 0.20$, resting p-epinephrine 0.33 nmol/l (0.14–0.63) in patients versus 0.50 nmol/l (0.25–0.76) in controls, $P = 0.08$].

Likewise, there was no significant difference in the chatecholamine response to exercise, and peak values of p-epinephrine and p-norepinephrine were similar [maximal p-norepinephrine 27.2 nmol/l (8.61–42.52) in patients versus 27.09 nmol/l (12.02–43.52) in controls, $P = 0.96$; maximal p-epinephrine 1.56 nmol/l (0.71–7.77) in patients versus 4.94 nmol/l (0.89–22.13) in controls, $P = 0.38$].

There was a tendency, although it was not significant, towards a reduced heart rate at maximal workload in patients compared with controls [median heart rate 160 (131–194) versus 179 (156–187) $P = 0.083$].

There was no difference in the concentrations of FFA and p-glycerol at rest or during exercise [FFA at rest 497 μmol/l (274–891) versus 473 μmol/l (244–624) $P = 0.5$; FFA at VO_{2max}: 284 μmol/l (211–792) versus 302 μmol/l (64–416) $P = 0.8$, in patients and controls, respectively]. The values for p-glycerol at rest: 82 μmol/l (53–161) versus 77 μmol/l (53–92) $P = 0.38$; p-glycerol at VO_{2max}: 123 μmol/l (74–268) versus 123 μmol/l (91–384) $P = 0.88$, in patients compared with controls. The respiratory quotient displayed a similar distribution in patients and controls [RER at rest: 0.83 (0.77–1.06) in patients versus 0.81 (0.74–1.06) controls, $P = 0.72$; RER at VO_{2max}: 1.09 (1.04–1.21) in patients versus 1.15 (1.06–1.31) controls, $P = 0.16$].

Muscle biopsies

In the muscle tissue there was no significant difference in the activity of CS and HD at rest or during exercise (Fig. 2a and b). The muscle glycogen content and the concentration of IMP, AMP, ADP, and ATP in the muscle biopsies at rest and exercise were similar among the groups (Fig. 2c and Fig. 3a–d).

Discussion

Our study investigated the hypothesis that clinical lipodystrophy and elevated p-lactate levels are asso-

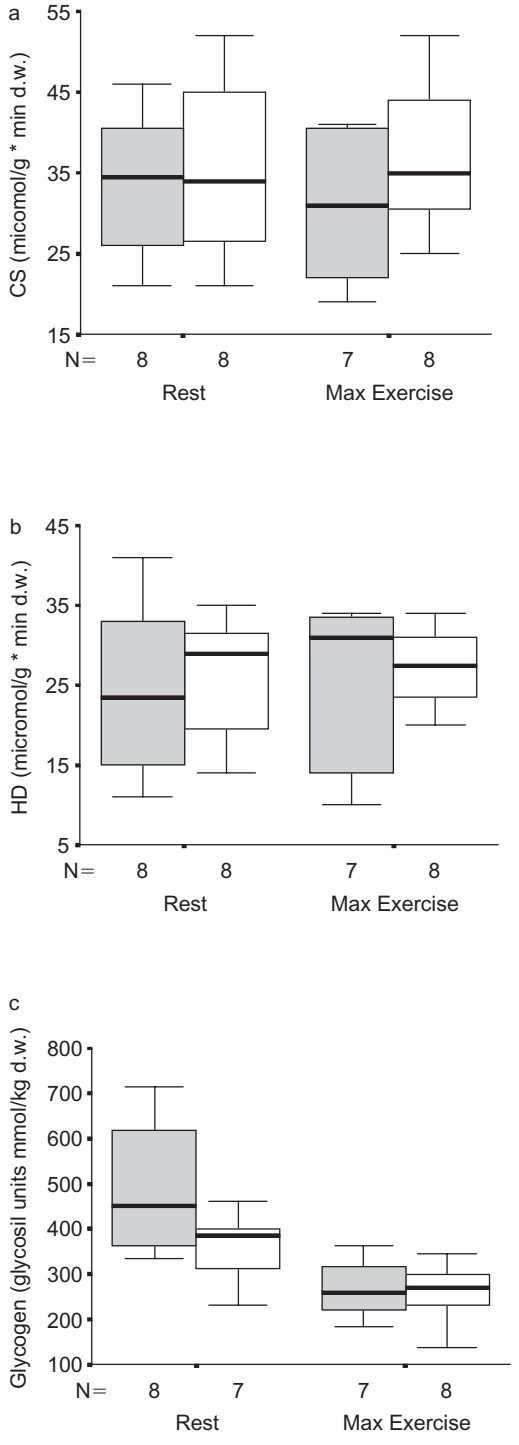

Fig. 2. Results of muscle biopsies. (a) Activity of citrate synthase (CS) at rest and at maximal oxygen consumption for patients and controls. (b) Activity of hydroxyacyl-coenzyme A dehydrogenase (HD) at rest and at maximal oxygen consumption for patients and controls. (c) Glycogen content at rest and at maximal oxygen consumption for patients and controls. Grey boxes, Patients; white boxes, controls. The boxes represent the interquartile range, which contains the 50% of values. The whiskers extend from the box to the highest and lowest values, excluding outliers. The horizontal line indicates the median.

ciated with mitochondrial dysfunction in skeletal muscle tissue or in the liver. Although a mitochondrial defect is likely to be restricted to certain tissues, no data on tissue specificity regarding lipoatrophy or hyperlactatemia were available at the time the present study was conducted.

The skeletal muscle tissue has a great influence on lactate homeostasis because of high oxidative requirements, high mitochondrial density and its capability to utilize lactate. Furthermore, a possible latent mitochondrial dysfunction, for which the tissue is able to compensate in the resting state, could be disclosed during exertion. Also, the rate of lactate clearance in the post-exercise period is a possible marker of hepatic mitochondrial function. However, it is important to emphasize that the results of the present study only apply to the function of mitochondria in skeletal muscle tissue and, to a lesser extent, in the liver.

We included seven patients with lipodystrophy and one with compensated lactic acidosis. The lack of a consensus case definition of lipodystrophy is a main weak point of the investigation. To address the emerging awareness of different subtypes of the syndrome, with possible different pathogenic backgrounds, and the impression that fat atrophy is the predominant expression of NRTI-related lipodystrophy [12–14,22], we standardized the patient group by excluding patients with predominantly fat accumulation. Because studies have shown evidence of stavudine as a risk factor for elevated lactate levels and fat atrophy [3,6,10,11], we included patients with a history of prolonged stavudine treatment. Three of the patients suffered from neuropathy, which is considered an additional expression of NRTI toxicity. Given the conditions of poor definitions and speculative hypotheses, we selected a group of patients who we had reason to suspect would show signs of defects of mitochondrial function if the hypothesis were valid.

In order to match a population with chronic disease, the control group might have been chosen from among individuals in an obvious bad training condition. However, that would have left us with the problem of possible confounding by silent morbidity in the control subjects. This would also be the case if we had chosen controls who were matched by BMI, because patients with fat atrophy generally have very low body weight. Therefore, matching by BMI would be difficult and would potentially select controls with undiagnosed disease. In an attempt to compensate for the difference in weight, we calculated the working capacity and VO_2 per kg LLM.

The patients showed significantly reduced working capacity, a trend towards reduced VO_{2max} and a significantly lower fold increase in VO_2. This pattern could

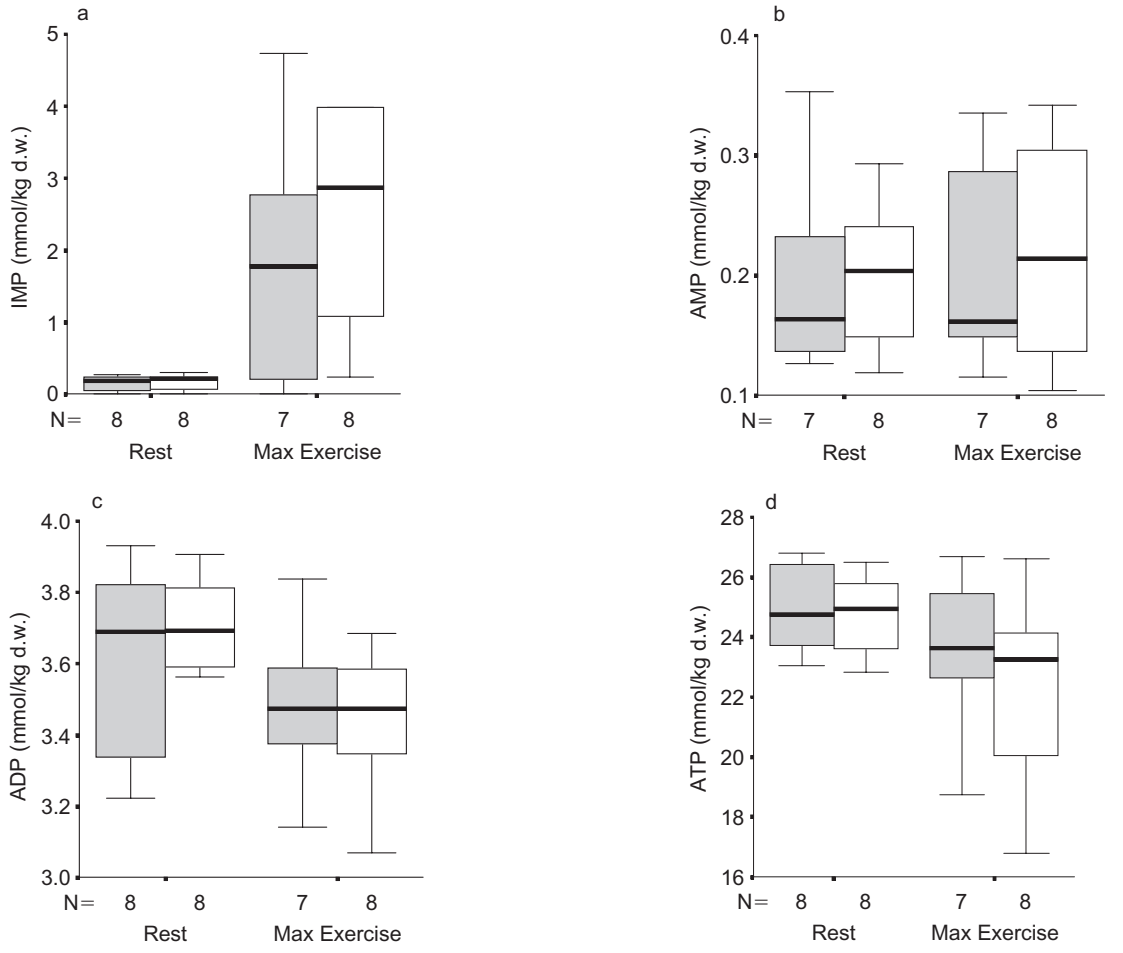

Fig. 3. (a) Inosine monophosphate (IMP) levels at rest and at maximal exercise for patients and controls. (b) Adenosine monophosphate (AMP) levels at rest and at maximal exercise for patients and controls. (c) Adenosine 5′-diphosphate (ADP) levels at rest and at maximal exercise for patients and controls. (d) Adenosine triphosphate (ATP) levels at rest and at maximal exercise for patients and controls. Grey boxes, Patients; white boxes, controls. The boxes represent the interquartile range, which contains 50% of values. The whiskers extend from the box to the highest and lowest values, excluding outliers. The horizontal line indicates the median.

be caused by mitochondrial dysfunction, but might also be a consequence of impaired physical fitness, caused by underlying chronic disease [23–27]. In a study by Hooper *et al.* [28] of exercise performance, normal-appearing adults with mitochondrial enzyme deficiencies demonstrated a reduced maximum work capacity with a rapid heart rate and excessive ventilation for the work performed. The patients in the present study showed a tendency towards reduced heart rate at maximal workload, as well as a normal ventilatory response, which favours the assumption that the observed differences are caused by impaired training status.

Glycogen and fatty acids are important substrates for ATP production in skeletal muscle tissue; therefore, a reduction in glycogen stores, limited oxidation of fatty acids, or impaired function of the citric acid cycle may reduce the ATP-generating capacity of the cell.

HD is a mitochondrial enzyme that catalyses the first step of the β-oxidation of fatty acids, whereas CS catalyses the conversion of acetylcoenzyme A and oxaloacetate to citrate in the citric acid cycle. No difference in glycogen content in skeletal muscle tissue was found at rest or at maximal exercise, and the activity of HD and CS were similar between groups, suggesting that the substrate supply for glycolysis and the respiratory chain was normal (see Fig. 2).

At maximal exercise, VO_2 in patients was elevated more than sixfold above the resting level. Despite this severely increased demand for ATP, patients maintained the same concentrations of nucleotides as controls; in particular, ATP levels in skeletal muscle tissue did not differ between groups (see Fig. 3). Also of interest is the fact that the concentration of IMP, which will accumulate in cases of accelerated ATP

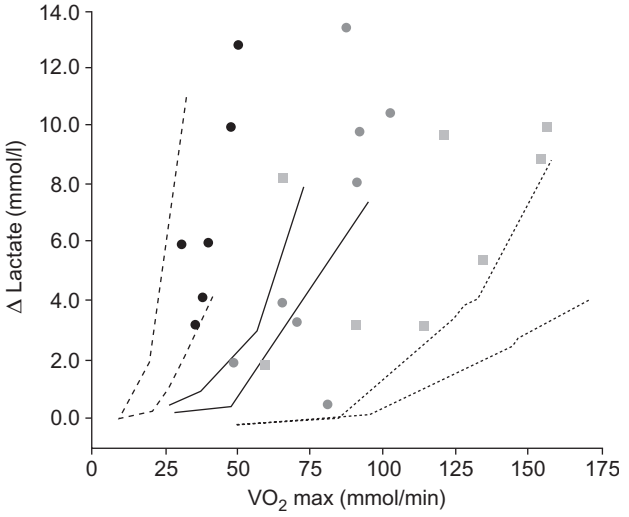

Fig. 4. Change in blood lactate levels from resting conditions to maximal oxygen consumption. VO_2, Oxygen consumption. ● HIV patients; ■ control group from present study; ● patients with diagnosed mitochondrial enzyme deficiency (adapted with kind permission from Bogaard *et al.* [19]). – – – – Patients with heart disease; —— sedentary/normal; ···· well trained (adapted with kind permission from Wasserman [20,21]).

degradation, was similar. This indicates that the oxidative phosphorylation and metabolism of nucleotides, even during strenuous conditions, were normal.

During intensive exercise, the oxidative capacity of the respiratory chain is exceeded, and lactate accumulates as a result of anaerobic ATP production. Lactate levels may therefore reflect the function and capacity of the respiratory chain in the mitochondrial inner membrane. Two studies [19,28] have shown that the maximal blood lactate levels during exercise in patients with mitochondrial dysfunction display wide inter-patient differences. Therefore, the normal distribution of blood lactate levels in response to exercise in our patients compared with controls does not definitely rule out the possibility of mitochondrial damage. However, patients with diagnosed mitochondrial disorders achieved maximal blood lactate levels at much lower workloads and VO_{2max} than the patients in the present study, supporting the notion that the mitochondrial function of skeletal muscle tissue in our patients is not seriously impaired.

To elucidate further the relative contribution of a possible underlying mitochondrial defect from the effect of physical unfitness, we compared the performance of our subjects with results obtained by Bogaard *et al.* [19] and Wasserman [20,21] (see Fig. 4). The authors measured lactate levels during incremental exercise until exhaustion in patients with diagnosed mitochondrial disease, in patients with heart disease as well as in sedentary and well-trained individuals. As shown in Fig. 4, the results of our patients resemble the performance of untrained individuals, whereas the results of our controls fall in between the performance of well-trained and sedentary subjects.

Contracting skeletal muscle tissue is able to use different substrates to generate ATP. The pattern of substrate utilization during exercise can be described well from the respiratory exchange ratio, which differs when lipids (RER 0.7) and carbohydrate (RER 1.0) are metabolized [29]. We compared the changes in RER and found no difference between the groups. This finding, together with the normal profile of FFA and glycerol, suggests that the metabolic response to exercise is normal in this group of patients.

As an indication of the hepatic mitochondrial function, we measured the clearance of lactate in the recovery period. Lactate is transported to the liver and converted into glucose in series of reactions, depending on the mitochondrial function of the liver cells [30]. To a lesser extent, this reaction also takes place in heart, kidney and muscle tissue [31–35]. The capacity of these tissues, however, is too small to compensate fully in the case of severely decreased hepatic clearance [31,36,37]. The similar clearance of blood lactate in the two groups during the recovery period indicates that hepatic mitochondrial respiratory function is sufficient to maintain gluconeogenesis and thus blood lactate metabolism.

One of the inclusion criteria was elevated lactate levels, and not surprisingly, the patients had significantly elevated p-lactate at rest and at 50% VO_{2max}. This finding, however, is inconsistent with the demonstrated normal accumulation and clearance of lactate, unless the raised lactate levels are generated in other tissues, or the degree to which the liver is affected is too modest to be detected by differences in lactate clearance.

In contrast to the apparently normal hepatic function in our patients is the liver involvement in patients suffering from severe lactic acidosis [2,4]. This raises the question as to whether aymptomatic hyperlactatemia and lactic acidosis do not necessarily represent a continuum, but might be two independent clinical and pathological entities.

Several studies have shown that NRTI are capable of causing damage to the mitochondria [38–41], and from studies of genetic mitochondrial disorders [42] it is well known that different tissues are affected to a varying extent. This has been proposed to be caused by mtDNA heterosplasmy (the random segregation of wild-type and mutant mtDNA), an explanation that does not hold for the acquired mitochondrial disorders.

In the case of NRTI toxicity, factors such as varying degrees of NRTI uptake into the cells, triphosphorylation, incorporation in mtDNA, and removal of the NRTI, could be possible explanations of inter-tissue and inter-drug differences, and of variations in patient susceptibility.

In theory, a possible explanation of the elevated resting lactate levels in patients could be elevated levels of catecholamines in the resting state. Epinephrine is known to increase lactate concentrations, as shown by Laurent *et al.* [43]. Moreover, Renard and colleagues [44] demonstrated a significantly higher 24 h urinary output of chatecholamines in HIV-infected patients with fat redistribution, compared with HIV-infected patients without alterations in body fat. In the present study, however, we did not detect any differences in catecholamine levels. Plasma concentrations of catecholamines are very labile and may exhibit wide fluctuations during the day in the same individual. We did not measure 24 h urinary output, but whereas the values of the resting state may have been influenced by individual fluctuations, the normal response to exercise argues against elevated sympathetic activity as the cause of hyperlactatemia in this group of patients. Upregulated glycolysis, at least on the basis of elevated sympathetic activity, is therefore also unlikely.

Conclusion

The results of the present study refute the theory that a skeletal muscle mitochondrial defect is the cause of NRTI-related lipodystrophy and hyperlactatemia. The observed differences in response to exercise may be well explained by differences in general fitness. Several aspects of the skeletal muscle mitochondrial function were investigated, and all variables were consistent with normal oxidative phosphorylation and glycolysis. Furthermore, the normal decline in post-exercise lactate levels suggests that the mitochondrial function of the liver cells is not seriously disturbed in this group of patients.

The significant elevated lactate levels in the resting state could be caused by mitochondrial defects in other tissues, or by minor impairments of the hepatic mitochondrial function.

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References

1. Lenzo NP, Garas BA, French MA. **Hepatic steatosis and lactic acidosis associated with stavudine treatment in an HIV patient: a case report.** *AIDS* 1997, **11**:1294–1296.
2. Olano JP, Borucki MJ, Wen JW, *et al.* **Massive hepatic steatosis and lactic acidosis in a patient with AIDS who was receiving zidovudine.** *Clin Infect Dis* 1995, **21**:973–976.
3. Mokrzycki MH, Harris C, May H, *et al.* **Lactic acidosis associated with stavudine administration: a report of five cases.** *Clin Infect Dis* 2000, **30**:198–200.
4. Bissuel F, Bruneel F, Habersetzer F, *et al.* **Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy.** *J Intern Med* 1994, **235**:367–371.
5. Harrris M, Tesiorowski A, Montaner JSG. **Screening for nucleoside-associated lactic acidosis.** In: *XIIIth International AIDS Conference*. Durban, 9–12 July 2000 [Abstract TuPpB1233].
6. John M, Moore CB, James IR, *et al.* **Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy.** *AIDS* 2001, **15**:717–723.
7. Vrouenraets S, Treskes M, Regez RM, *et al.* **The occurence of hyperlactatemia in HIV-infected patients on NRTI treatment.** In: *XIIIth International AIDS Conference*. Durban, 9–12 July 2000 [Abstract TuPpB1234].
8. Brinkman K, Smeitink JA, Romijn JA, *et al.* **Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy.** *Lancet* 1999, **354**:1112–1115.
9. Brinkman K, ter Hofstede HJM, Burger DM, *et al.* **Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway.** *AIDS* 1998, **12**:1735–1744.
10. Carr A, Miller J, Law M, *et al.* **A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome.** *AIDS* 2000, **14**:F25–F32.
11. Mallal SA, John M, Moore CB, *et al.* **Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection.** *AIDS* 2000, **14**: 1309–1316.
12. Rozenbaum WGS, Salhi Y, Adda N, *et al.* **Clinical and laboratory characteristics of lipodystrophy in a French cohort of HIV-infected patients treated with protease inhibitors.** In: *1st International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV.* San Diego, 26–28 June, 1999 [Abstract 012].
13. Saint-Marc T, Partisani M, Poizot-Martin I, *et al.* **A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy.** *AIDS* 1999, **13**:1659–1667.
14. Saint-Marc T, Partisani M, Poizot-Martin I, *et al.* **Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: preliminary results of the LIPOCO study.** *AIDS* 2000, **14**:37–49.
15. Klopstock T, Naumann M, Schalke B, *et al.* **Multiple symmetric lipomatosis: abnormalities in complex IV and multiple deletions in mitochondrial DNA.** *Neurology* 1994, **44**:862–866.
16. Campos Y, Martin MA, Navarro C, *et al.* **Single large-scale mitochondrial DNA deletion in a patient with mitochondrial myopathy associated with multiple symmetric lipomatosis.** *Neurology* 1996, **47**:1012–1014.
17. Becker-Wegerich P, Steuber M, Olbrisch R, *et al.* **Defects of mitochondrial respiratory chain in multiple symmetric lipomatosis.** *Arch Dermatol Res* 1998, **290**:652–655.
18. Wallace DC. **Diseases of the mitochondrial DNA.** *Annu Rev Biochem* 1992, **61**:1175–1212.
19. Bogaard JM, Scholte HR, Busch HF, *et al.* **Anaerobic threshold as detected from ventilatory and metabolic exercise responses in patients with mitochondrial respiratory chain defect.** *Adv Cardiol* 1986, **35**:135–145.
20. Wasserman K. **The anaerobic threshold: definition, physiological significance and identification.** *Adv Cardiol* 1986, **35**:1–23.

21. Wasserman K, Hansen J, Sue D, *et al.* **Principles of exercise testing and interpretation, 3rd Edition**. 1999. Lippincott Williams & Wilkins, Philadelphia.
22. Safrin S, Grunfeld C. **Fat distribution and metabolic changes in patients with HIV infection [Editorial].** *AIDS* 1999, **13**: 2493–2505.
23. Gaesser GA, Poole DC. **Blood lactate during exercise: time course of training adaptation in humans.** *Int J Sports Med* 1988, **9**:284–288.
24. Hurley BF, Hagberg JM, Allen WK, *et al.* **Effect of training on blood lactate levels during submaximal exercise.** *J Appl Physiol* 1984, **56**:1260–1264.
25. Bassett DR Jr., Howley ET. **Limiting factors for maximum oxygen uptake and determinants of endurance performance.** *Med Sci Sports Exerc* 2000, **32**:70–84.
26. Hickson RC, Hagberg JM, Ehsani AA, *et al.* **Time course of the adaptive responses of aerobic power and heart rate to training.** *Med Sci Sports Exerc* 1981, **13**:17–20.
27. Gaesser GA, Poole DC, Gardner BP. **Dissociation between VO_{2max} and ventilatory threshold responses to endurance training.** *Eur J Appl Physiol* 1984, **53**:242–247.
28. Hooper RG, Thomas AR, Kearl RA. **Mitochondrial enzyme deficiency causing exercise limitation in normal-appearing adults.** *Chest* 1995, **107**:317–322.
29. van Hall G, Gonzalez-Alonso J, Sacchetti M, *et al.* **Skeletal muscle substrate metabolism during exercise: methodological considerations.** *Proc Nutr Soc* 1999, **58**:899–912.
30. Ahlborg G, Felig P, Hagenfeldt L, *et al.* **Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids.** *J Clin Invest* 1974, **53**:1080–1090.
31. Woll PJ, Record CO. **Lactate elimination in man: effects of lactate concentration and hepatic dysfunction.** *Eur J Clin Invest* 1979, **9**:397–404.
32. Consoli A, Nurjhan N, Reilly JJ Jr, *et al.* **Contribution of liver and skeletal muscle to alanine and lactate metabolism in humans.** *Am J Physiol* 1990, **259**:E677–E684.
33. Young LH, Zaret BL, Barrett EJ. **Physiologic hyperinsulinemia stimulates lactate extraction by heart muscle in the conscious dog.** *Metabolism* 1989, **38**:1115–1119.
34. Cersosimo E, Molina PE, Abumrad NN. **Renal glucose production during insulin-induced hypoglycemia [published erratum appears in *Diabetes* 1997 Sept; 46:1532].** *Diabetes* 1997, **46**:643–646.
35. Cersosimo E, Garlick P, Ferretti J. **Renal substrate metabolism and gluconeogenesis during hypoglycemia in humans.** *Diabetes* 2000, **49**:1186–1193.
36. Record CO, Chase RA, Williams R, *et al.* **Disturbances of lactate metabolism in patients with liver damage due to paracetamol overdose.** *Metabolism* 1981, **30**:638–643.
37. Almenoff PL, Leavy J, Weil MH, *et al.* **Prolongation of the half-life of lactate after maximal exercise in patients with hepatic dysfunction.** *Crit Care Med* 1989, **17**:870–873.
38. Chariot P, Drogou I, Lacroix-Szmania I, *et al.* **Zidovudine-induced mitochondrial disorder with massive liver steatosis, myopathy, lactic acidosis, and mitochondrial DNA depletion [see Comments].** *J Hepatol* 1999, **30**:156–160.
39. Pan-Zhou XR, Cui L, Zhou XJ, *et al.* **Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells.** *Antimicrob Agents Chemother* 2000, **44**:496–503.
40. McCurdy DT III, Kennedy JM. **AZT decreases rat myocardial cytochrome oxidase activity and increases beta-myosin heavy chain content.** *J Mol Cell Cardiol* 1998, **30**:1979–1989.
41. Benbrik E, Chariot P, Bonavaud S, *et al.* **Cellular and mitochondrial toxicity of zidovudine (AZT), didanosine (ddI) and zalcitabine (ddC) on cultured human muscle cells.** *J Neurol Sci* 1997, **149:**19–25.
42. Moraes CT, Shanske S, Tritschler HJ, *et al.* **mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases.** *Am J Hum Genet* 1991, **48**: 492–501.
43. Laurent D, Petersen KF, Russell RR, *et al.* **Effect of epinephrine on muscle glycogenolysis and insulin-stimulated muscle glycogen synthesis in humans.** *Am J Physiol* 1998, **274**:E130–E138.
44. Renard E, Fabre J, Paris F, *et al.* **Syndrome of body fat redistribution in HIV-1-infected patients: relationships to cortisol and catecholamines.** *Clin Endocrinol (Oxford)* 1999, **51**:223–230.