Triacylglycerol infusion does not improve hyperlactemia in resting patients with mitochondrial myopathy due to complex I deficiency1–3

Mark J Roef, Kees de Meer, Dirk-Jan Reijngoud, Helma WHC Straver, Martina de Barse, Satish C Kalhan, and Ruud Berger

ABSTRACT

Background: A high-fat diet has been recommended for correction of biochemical abnormalities and muscle energy state in patients with complex I (NADH dehydrogenase) deficiency (CID).

Objective: This study evaluated the effects of intravenous infusion of isonenergetic amounts of triacylglycerol or glucose on substrate oxidation, glycolytic carbohydrate metabolism, and energy state in patients with CID.

Design: Four CID patients and 15 matched control subjects were infused with triacylglycerol (1.85 mg·kg⁻¹·min⁻¹) or glucose (5 mg·kg⁻¹·min⁻¹) while at rest. Respiratory calorimetry was used to evaluate mitochondrial substrate oxidation. Metabolism of glycolytic carbohydrate was determined on the basis of the rates of appearance and concentrations of plasma lactate from dilution of [1-¹³C]lactate measurements. In addition, high-energy phosphate metabolism was measured in forearm muscle by ³¹P magnetic resonance spectroscopy.

Results: Whole-body oxygen consumption rates were higher in the patients than in the control subjects (P < 0.05). Oxygen consumption and high-energy phosphate metabolism in forearm muscle were not significantly different between the 2 infusion groups. The rates of appearance and concentrations of plasma lactate were higher in each of the 4 patients than in the control subjects (P < 0.05) and were lower during the triacylglycerol infusion than during the glucose infusion (P < 0.05); the differences were comparable in the patients and control subjects.

Conclusions: We conclude that triacylglycerol infusion, relative to glucose infusion, does not improve the oxidation of substrates or the energy state of skeletal muscle and does not lower the rates of appearance and concentrations of plasma lactate to normal values in CID patients at rest.


KEY WORDS Mitochondrial myopathy, hyperlactemia, complex I deficiency, triacylglycerol infusion, glucose infusion, substrate oxidation, stable isotopes, ³¹P magnetic resonance spectroscopy

INTRODUCTION

Complex I (NADH dehydrogenase; EC 1.6.99.3) deficiency (CID), a respiratory chain disorder characterized by impaired mitochondrial oxidation of NADH, is being recognized in an increasing number of patients (1). Clinical manifestations range from pure myopathy restricted to the extraocular muscles (2), the skeletal muscle (2, 3), or both, to multisystemic involvement in which various other tissues are also affected (4, 5). Elevated plasma lactate concentrations are a common laboratory finding in humans with CID (2–5) and are thought to reflect increased oxidation of NADH (coupled to reduction of pyruvate) in the cytosol, resulting from the impaired oxidation of NADH inside the mitochondria of these patients.

A high-fat, low-carbohydrate diet has been recommended for the treatment of CID (6) because high-carbohydrate diets may impose a metabolic challenge in these patients. The recommendation for a high-fat diet is based on the following reasons:

1) Because the mitochondrial oxidation of NADH is thought to be diminished in CID patients, FADH₂ may be an alternative carrier of reducing equivalents and may maintain oxidative phosphorylation because electrons from FADH₂ can enter the respiratory chain distal to complex I.

2) The supply of FADH₂ to the mitochondria can be increased (relative to NADH) by increasing the amount of triacylglycerols and fatty acids in the diet. On the basis of stoichiometry, it follows that oxidation of fatty acids yields a ratio of FADH₂ to NADH of 0.5, whereas glucose yields a much lower ratio of 0.2.

Therefore, we hypothesized that infusion of fatty acids to CID patients would improve mitochondrial substrate oxidation more so than would carbohydrate infusion. The hypothesis is supported by the results of in vitro studies in renal cell cultures loaded with glutamine (an NADH-linked substrate) conducted by Doctor et al (7). Addition of rotenone, a known inhibitor of complex I, in the presence of 2-deoxyglucose, a competitive

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TABLE 1
Physical characteristics of individual patients with complex I deficiency and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Control subjects¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry to study (y)</td>
<td>25</td>
<td>20</td>
<td>22</td>
<td>15</td>
<td>21 (20, 22)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>52</td>
<td>61</td>
<td>57</td>
<td>45</td>
<td>57.4 (54.7, 60.1)</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>41</td>
<td>45</td>
<td>44</td>
<td>35</td>
<td>44.5 (42.5, 46.5)</td>
</tr>
<tr>
<td>Fasting plasma lactate (mmol/L)</td>
<td>2.3</td>
<td>2.7</td>
<td>4.7</td>
<td>2.0</td>
<td>1.0 (0.8, 1.2)</td>
</tr>
<tr>
<td>Maximal exercise performance (W)</td>
<td>60</td>
<td>60</td>
<td>50</td>
<td>55</td>
<td>210 (192, 228)</td>
</tr>
</tbody>
</table>

¹ ± 95% CI in parentheses.

inhibitor of glucose uptake, resulted in near complete depletion of ATP and a significant decrease in oxygen consumption (VO₂). Subsequent addition of heptanoate completely restored ATP concentrations and VO₂. This effect of heptanoate could only be shown in the absence of antymycin A (which inhibits complex II), suggesting that heptanoate oxidation can bypass complex I and that this is mediated by complex II. Thus, CID patients may benefit from fatty acid supplementation because fatty acid oxidation provides more FADH₂ that enters the respiratory chain (distal to complex I). This may result in a lower concentration and rate of appearance (Ra) of plasma lactate.

In the present study, the effects of the triacylglycerol or glucose infusion on substrate oxidation and glycolytic carbohydrate metabolism were studied in 4 CID patients and in 15 healthy control subjects. All patients had documented CID, and exercise intolerance was their main symptom. Glycolytic carbohydrate metabolism was evaluated on the basis of the Ra and concentrations of plasma lactate (from dilution of [1-¹³C] lactate), mitochondrial substrate oxidation was studied indirectly with use of respiratory calorimetry, and high-energy phosphate metabolism in forearm muscle was measured by ³¹P magnetic resonance spectroscopy (³¹P-MRS) (8, 9).

SUBJECTS AND METHODS

Subjects

Four unrelated female CID patients aged 15–25 y were studied (Table 1). The patients had similar clinical histories and their weights ranged from 45 to 61 kg. All the patients had easily fatigable mild muscle weakness dating back to early childhood that had remained stable over time. Patient 1 had experienced a single storkelike episode at age 13 y, which resolved spontaneously after a few hours. Except for patient 4, in whom additional mild cerebellar atrophy and axonal neuropathy were diagnosed, none of the patients showed any signs of central nervous system involvement. Therefore, exercise intolerance was the dominant clinical symptom at the time of the study. Maximal work load performance, assessed from an incremental maximal exercise test on an electrically braked cycle ergometer (Lode Instruments, Groningen, Netherlands), as previously described (10), ranged from 50 to 60 W and was on average only 25% of control values. Baseline fasting plasma lactate concentrations ranged from 2.0 to 4.7 mmol/L (Table 1). Patients 1 and 2 had previously been prescribed riboflavin and carnitine, but discontinued this therapy because they recalled no benefit from this medication. At the time of the study, patients 3 and 4 were taking riboflavin and carnitine, although they did not recall much benefit from this therapy either. All patients were engaged in social activities. Patient 1 was a housewife and had recently given birth to a healthy son, patients 2 and 3 had service jobs, and patient 4 was a student.

CID was diagnosed with microscopic and biochemical investigations in fresh biopsy specimens of the quadriceps (vastus lateralis) muscle, which showed markedly decreased activity of complex I in all patients. Mitochondrial DNA abnormalities (point mutations, eg, 3243A→G MELAS/PEO, 8344A→G MERRF, 8993T→G NARP, and 1178A→G LHON; deletions; duplications; or mitochondrial DNA depletion) were not detected in any of these patients, nor was a maternal pattern of inheritance. A summary of the biochemical investigations is shown in Tables 2 and 3.

Fifteen healthy control subjects matched for age (± 21 y), sex, and body weight were recruited for the tracer infusion and respiratory calorimetry studies. Another 3 control subjects (2 men and 1 woman aged 18–28 y) volunteered for the ³¹P-MRS studies. None of these subjects had a family history of diabetes mellitus or took medications. All subjects were studied after they had fasted overnight; no other dietary restrictions were imposed. Written, informed consent was obtained from all subjects. The experimental protocol was approved by the Medical Ethics Committee of the University Children’s Hospital (Utrecht, Netherlands).

Experimental protocol

The patients and control subjects reported to the Laboratory for Metabolic Diseases (University Children’s Hospital) at 0800 on 2 occasions separated by ≥ 7 d. Two polytetrafluoroethylene catheters were inserted, one into a dorsal hand vein for infusion and the other into a vein draining the dorsum of the contralateral hand for blood sampling. The hand was inserted in a heated box to achieve arterialization of the venous blood (12). After the blood sample was drawn, the catheter was flushed with heparin-containing saline (2.5 kU/L). Subjects were acclimatized to room conditions (temperature: 21–25°C for 30 min before the study began. At time 0, either glucose (10% wt:vol, 5 mg·kg⁻¹·min⁻¹) or a triacylglycerol emulsion (Intralipid, 20% wt:vol, 1.85 mg·kg⁻¹·min⁻¹; Fresenius Kabi, ’s-Hertogenbosch, Netherlands) containing linoleic acid (50%), oleic acid (25%), palmitic acid (10%), linolenic acid (9%), stearic acid (3.5%), and heparin (7.5 U·kg⁻¹·h⁻¹; prime: 14 U/kg) was infused and maintained throughout the 100-min study period, during which time the subjects remained at rest in the supine position. The triacylglycerol and glucose infusions were assigned in random order. All patients and control subjects participated in both the triacylglycerol and glucose infusion studies.

Isotope infusion

Primed, constant infusions of [6,6-²H₂]glucose (98% enriched; Mass Trace, Woburn, MA) were administered in all 4 patients and in 12 control subjects: prime, 20.0 μmol [6,6-²H₂]glucose/kg;
TABLE 2
Microscopic and biochemical investigation of muscle biopsies in 3 individual patients with complex I deficiency (CID) and healthy control subjects

<table>
<thead>
<tr>
<th>Enzyme activity in fresh muscle homogenate</th>
<th>CID patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I (U/g wet wt)</td>
<td>0.18±0.76</td>
<td>3.19±0.26 [10]</td>
</tr>
<tr>
<td>Complex II + III (U/g wet wt)</td>
<td>7.43±1.14</td>
<td>3.99±0.43 [21]</td>
</tr>
<tr>
<td>Complex IV (nU/g wet wt)</td>
<td>305±195</td>
<td>90±7 [16]</td>
</tr>
</tbody>
</table>

Maximal oxygen uptake after substrate stimulation in isolated fresh muscle mitochondria (nanomols O₂·min⁻¹·mg protein⁻¹)

<table>
<thead>
<tr>
<th>NADH-linked substrates</th>
<th>CID patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate + malate + ADP</td>
<td>19±13</td>
<td>92±5 [22]</td>
</tr>
<tr>
<td>Glutamate + malate + ADP</td>
<td>19±11</td>
<td>82±7 [22]</td>
</tr>
<tr>
<td>Palmitoylcarnitine + malate + ADP</td>
<td>21±13</td>
<td>68±5 [22]</td>
</tr>
</tbody>
</table>

Blood sampling and urine collection

Blood samples were drawn at regular intervals, placed on ice, and transferred into sodium fluoride–containing tubes for measurement of plasma glucose, lactate, and triacylglycerol or glucose. Whole blood was deproteinized for measurement of blood pyruvate, β-hydroxybutyrate, and acetoacetate. Blood samples for determination of [6,6-²H₂]glucose and [1,5-¹³C]lactate enrichments were centrifuged at 4°C (1000 × g, 10 min) and stored at -70°C. Urine voided at time 0 and 100 min was collected for measurement of nitrogen excretion.

Respiratory calorimetry

After the subjects had been infused with triacylglycerol or glucose for 245 min, open-circuit indirect calorimetry under a ventilated hood began and continued for 40 min while the subjects were at rest. Stable VO₂ and carbon dioxide production (VCO₂) values were reached within 5 min of recording. Computerized, continuous gas and air volume measurements were performed (Oxycon Champion; Jaeger, Breda, Netherlands) as previously described (13). Atmospheric pressure and temperature calibration of oxygen and carbon dioxide sensor measurements and air volume calibrations (with a standard 3000-mL cylinder) were performed before each measurement. VO₂ relative to VCO₂ was standardized with use of alcohol burning at regular intervals:

theoretical respiratory exchange ratio (0.67) was closely approached in all standardizations (3: 0.66; CV: 1.6%; n = 10).

1 The enzyme assays and polarographic studies were performed according to reference 2. RRF, ragged red fibers; L, lipidosis; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine.

2 SD; n values in brackets.

Three of the CID patients (patients 1, 2, and 3) and 3 control subjects reported to the MRS facility at 0800 for the triacylglycerol or glucose infusion on 2 separate days, as described above. Triacylglycerol or glucose was infused during a 100-min basal period during which the subjects remained at rest. The infusions were maintained at the same rate throughout the following 30-min study period. Studies were conducted on the superficial mass of the flexor digitorum profundus (FDP) muscle of the right forearm. The FDP muscle is affected adversely in CID patients, as
evidenced by observed decreases in maximal voluntary contraction output of the muscle comparable with decreases in maximal workload performance measured on a cycle ergometer (MJ Roef, K de Meer, unpublished observations, 1996). $^{31}$P-MRS spectra of the FDP were obtained at 1.5 T on an S15 HP whole-body MR spectrometer (Philips, Eindhoven, Netherlands), as described in detail elsewhere (14). Briefly, subjects were positioned prone and head first on the patient bed with their right arm extended forward, supported by cushions. Guided by palpation of the ulnar bone directly adjacent to the muscle, the forearm was placed into a support such that the FDP overlaid a 2-turn 25-mm diameter surface coil tuned to a frequency of 25.86 MHz and attached with straps. Correct positioning of the FDP over the $^{31}$P surface coil was checked by $^1$H MR imaging. Resting $^{31}$P-MRS spectra of the superficial region of the FDP were obtained with a frequency-modulated adiabatic 90° excitation pulse. Sixty free induction decays (1024 data points with 333-μs dwell times) were collected with a repetition time of 3 s.

Sample analysis

Plasma glucose, lactate, and triacylglycerol concentrations were measured enzymatically with autoanalyzers (Dimension AR and ACA SX, respectively; Dimension, Dade, FL). Concentrations of [6,6-$^2$H$_2$]glucose and [1-13C]lactate used for the tracer infusions and for the standard curves were measured with the use of calibrated curves from weighed, water-free glucose (Merck, Darmstadt, Germany) and zinc lactate (Sigma, St Louis), respectively. Plasma insulin and cortisol concentrations were measured with the use of a microparticle enzyme immunosay method (IMX analyzer; Abbott, Chicago) and a fluorescence polarization immunoassay (TDX analyzer; Abbott), respectively. Blood pyruvate, β-hydroxybutyrate, acetocetate, plasma fatty acids, and glycerol were measured with use of automated enzymic colorimetric methods (Cobas Paragon II; Hoffmann-La Roche, Montpellier, France). Urinary nitrogen was assayed according to a micro-Kjeldahl method (15).

The isotopic enrichments of glucose and lactate in plasma were measured with the use of gas chromatography–mass spectrometry (model 5890; Hewlett-Packard, Palo Alto, CA). Enrichment of plasma [6,6-$^2$H$_2$]glucose was measured in the penta-acetate derivative with use of positive chemical ammonia ionization, selectively monitoring ions at mass-to-charge ratios of 408 and 410 (16). The peak ratios (408 and 410) were compared with those of a standard curve prepared by diluting 98% tracer [6,6-$^2$H$_2$]glucose (Mass Trace) with weighed amounts of glucose and were reported as molar tracer-trace ratios (TTRs). The enrichment of [1-13C]lactate in plasma was measured in the N-propylamide heptafluorobutyrate derivative with use of electron impact ionization, selectively monitoring ions at mass-to-charge ratios of 86 and 87 (17). The TTRs were likewise derived from the peak ratios and compared with a standard curve prepared by diluting 98% tracer sodium[1-13C]lactate (Mass Trace) with weighed amounts of zinc lactate.

Free induction decays were processed with use of the LAB ONE NMR 1 (New Methods Research, Detroit) spectroscopy processing software as described in detail elsewhere (14). Estimates of the relative peak areas of the various metabolites were obtained by curve fitting the spectrum to Lorentzian line shapes: singlets of both inorganic α-phosphate (P$_i$) and phosphocreatine (PCr), doublets of γ-ATP and α-ATP, and a triplet of β-ATP.

Calculations

Whole-body glucose Ra

In steady state experiments, the whole-body glucose Ra was calculated as follows (18):

$$\text{Whole-body glucose Ra} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = I / \text{TTR} \quad (1)$$

where $I$ is the infusion rate of the [6,6-$^2$H$_2$]glucose tracer (in $\mu$mol·kg$^{-1}$·min$^{-1}$). Isotopic enrichments were considered to be at steady state when the TTRs of the 4 consecutive samples from time 70 to 100 min had a CV < 10% and a slope not significantly different from 0. When the isotopic enrichments were not in steady state, the Ra was calculated with Steele’s equations for non–steady state conditions (19) as follows:

$$\text{Whole-body glucose Ra} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{(I - V_d) [(G_i + G_j) / 2] \times (\Delta \text{TTR} / \Delta T)}{((\text{TTR}_i + \text{TTR}_j) / 2)} \quad (2)$$

where $V_d$ is the volume of distribution of glucose in mL/kg (200 mL/kg), $(G_i + G_j) / 2$ is the mean plasma glucose concentration (in mmol/L) measured at time points $T_i$ and $T_j$, $\Delta \text{TTR}$ is the change in TTR from $T_i$ to $T_j$, $\Delta T$ is the time interval ($T_j - T_i$) in min, and $(\text{TTR}_i + \text{TTR}_j) / 2$ is the mean TTR measured at time points $T_i$ and $T_j$. Steady state [6,6-$^2$H$_2$]glucose TTRs were attained in 3 of the 4 patients and in 8 of the 12 control subjects during the triacylglycerol infusion and in 3 of the 4 patients and in 5 of the 12 control subjects during the glucose infusion.

Whole-body lactate+pyruvate Ra

A single common pool approach for lactate and pyruvate was used to calculate the whole-body lactate+pyruvate Ra (in $\mu$mol·kg$^{-1}$·min$^{-1}$). Wolfe et al (20, 21) reported that the enrichment of plasma pyruvate in anesthetized dogs was 92% of the enrichment of plasma lactate at steady state when [1-13C]lactate was infused. The findings of Large et al (22) suggest that this approach is also applicable to humans. Therefore, we assumed that the Ra values calculated from dilution of labeled lactate in plasma represent the Ra of both lactate and pyruvate:

$$\text{Whole-body lactate+pyruvate Ra} = I / \text{TTR} \quad (3)$$

where $I$ is the infusion rate of [1-13C]lactate (μmol·kg$^{-1}$·min$^{-1}$) and TTR is the molar ratio of tracer to trace in lactate. Steady state conditions for lactate TTRs were defined as described for glucose. When isotopic enrichments were not at steady state, the Ra was calculated with Steele’s equations for non–steady state conditions (23) as described for glucose in Equation 2. The $V_d$ for lactate+pyruvate was assumed to be 500 mL/kg (24, 25). Steady state lactate TTRs were attained in 3 of the 4 patients and in 7 of the 12 control subjects during the triacylglycerol infusion and in all 4 patients and in 10 of the 12 control subjects during the glucose infusion.

Respiratory calorimetry

Carbohydrate oxidation was calculated according to Ferrannini (26):

Carbohydrate oxidation

$(\mu$mol·kg$^{-1}$·min$^{-1}) = (4.55 \times \dot{V}O_2 - 3.21 \times \dot{V}CO_2 - 2.87 \times N/(1000/180)) \times BW \quad (4)$
where \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) are expressed in mL/min, \( N \) is urinary nitrogen excretion (in mg/min), and \( BW \) is body weight (in kg). The calculated whole-body rate of substrate oxidation was assumed to reflect mitochondrial oxidation, neglecting nonmitochondrial peroxisomal and microsomal \( \dot{V}O_2 \). This nonmitochondrial \( \dot{V}O_2 \), which is insensitive to cyanide, was estimated in rats and shown to be 15–20% of the \( \dot{V}O_2 \) of perfused rat skeletal muscle (27). Data on nonmitochondrial \( \dot{V}O_2 \) in humans are not available. The relative contributions of nonmitochondrial \( \dot{V}O_2 \) processes to total \( \dot{V}O_2 \) were assumed to not be different whether triacylglycerol or glucose was infused.

**Outcome parameters**

Triacylglycerol or glucose infusion were expected to affect outcome parameters differently, not only in the patients but also in the control subjects. Thus, the hypothesized effects in the patients should be additional to those in the control subjects. The effects of the triacylglycerol infusion (TG) compared with those of the glucose infusion (GL) on outcome parameters in the individual patients and the control subjects is described as follows:

\[
\Delta V_O_2 = V_{O_2}^{TG} - V_{O_2}^{GL} \quad (5)
\]

\[
\Delta \text{Plasma lactate concentration} = \text{plasma lactate}_{TG} - \text{plasma lactate}_{GL} \quad (6)
\]

\[
\Delta \text{Lactate+pyruvate Ra} = \text{lactate+pyruvate}_{TG} - \text{lactate+pyruvate}_{GL} \quad (7)
\]

When the values for \( \Delta V_{O_2} \), \( \Delta \text{plasma lactate} \), and \( \Delta \text{lactate+pyruvate Ra} \) in the individual patients exceed the upper limit of the 95% CI for the effect of the triacylglycerol infusion (compared with the glucose infusion) in the control subjects, the effect of the triacylglycerol substrate in the patients is considered additional compared with that of glucose.

**Metabolite concentrations**

The average free ADP concentration in fibers within the sampled muscle mass was calculated from the creatine kinase equilibrium as follows:

\[
[\text{ADP}] = [\text{ATP}] ([\text{TCr}] - [\text{PCr}])/(1.66 \times 10^9) (10^{-9} \text{th}) [\text{PCr}] \quad (8)
\]

where 1.66 \( \times \) 10\(^9\) is the value of the equilibrium constant (28). It was assumed that the concentrations of ATP and total creatine ([TCr]) in the skeletal muscle of the patients were unchanged from normal values (8.2 and 42.7 mmol/L for [ATP] and [TCr], respectively; 8, 23). The concentrations of \( P_i \) and \( P_Cr \) were calculated from measured peak ratios of \( P_i \) to \( \beta \)-ATP and of \( P_Cr \) to \( \beta \)-ATP, respectively. Because the peak ratios of \( P_Cr \) to \( \beta \)-ATP in the patients were well outside the normal range, an individual value for the ratio of \( [P_Cr] \) to [TCr] was calculated for each patient:

\[
(\frac{[P_Cr]}{[TCr]} \text{patient}) = \frac{(P_Cr/\beta-ATP) \text{patient}}{(P_Cr/\beta-ATP) \text{controls} \times 0.85} \quad (9)
\]

where (\( P_Cr/\beta-ATP \) \text{controls}) is the mean (\( \pm \)SD) peak ratio of \( P_Cr \) to \( \beta \)-ATP in the control subjects (3.15 \( \pm \) 0.25) and [\( P_Cr \)/[TCr]] is the peak ratio of [\( P_Cr \)] to [TCr] in CID patients (0.85), as described elsewhere (29).

**Statistical analysis**

The results are presented as means \( \pm \) SDs. The data were analyzed by two-factor analysis of variance to identify main effects of group (CID patients compared with control subjects) and infusion condition (triaclyglycerol or glucose) and their interaction. When there was a significant (\( P < 0.05 \)) interaction, post hoc Bonferroni correction was conducted. The outcome parameters \( \dot{V}O_2 \), plasma lactate, and lactate+pyruvate Ra in the individual patients were compared with the 95% CIs of the control subjects. SPSS for WIN-DOWS (version 7.5; SPSS Inc, Chicago) was used for the analyses.

**RESULTS**

**Comparison between the CID patients and the control subjects at the group level: plasma concentrations and substrate utilization**

As expected, plasma glucose, insulin, and lactate and blood pyruvate concentrations in the control subjects were significantly higher during the glucose infusion than during the triacylglycerol infusion (Table 4). Likewise, plasma fatty acid, plasma triacylglycerol, blood \( \beta \)-hydroxybutyrate, blood acetoacetate, and plasma glycerol concentrations were higher during the triacylglycerol infusion.
infusion than during the glucose infusion. Plasma cortisol concentrations were similar after infusion of both substrates. Plasma glucose, insulin, and cortisol and blood pyruvate concentrations were not significantly different between the patients and the control subjects during infusion of either substrate, but plasma lactate concentrations and lactate-pyruvate ratios were significantly higher in the patients than in the control subjects during infusion of both substrates. During the triacylglycerol infusion, plasma fatty acids were significantly lower in the patients than in the control subjects, blood acetoacetate concentrations were lower in the patients than in the control subjects (NS), and the ratio of blood ß-hydroxybutyrate to acetoacetate was significantly higher in the patients than in the control subjects.

Whole-body VO₂ rates were significantly higher during the triacylglycerol infusion than during the glucose infusion in the control subjects, but were not significantly different in the patients (Table 5). However, VO₂ rates were significantly higher in the patients than in the control subjects during both the triacylglycerol (4.41 ± 0.38 compared with 3.70 ± 0.24 mL·kg⁻¹·min⁻¹) and glucose (4.20 ± 0.45 compared with 3.51 ± 0.23 mL·kg⁻¹·min⁻¹) infusions. Respiratory exchange ratios and total carbohydrate oxidation rates were not significantly different from control values during infusion of either substrate. In the control subjects, no significant changes in background enrichment of glucose and lactate were found after infusion of either substrate (data not shown). Plasma glucose Ra was not significantly different between the patients and the control subjects during infusion of either triacylglycerol or glucose.

Comparison of outcome parameters between the individual CID patients and the control subjects

Changes in VO₂, plasma lactate, and lactate+pyruvate Ra in the individual patients and the respective 95% CIs in the control subjects are shown in Table 6 and Figure 1. The triacylglycerol infusion was associated with substantially higher whole-body VO₂ rates than was the glucose infusion in patients 2 and 4 only. However, no additional effect of the triacylglycerol infusion on VO₂ outside the range of the effects of the triacylglycerol infusion in the control subjects (ΔVO₂ = −0.20 to 0.43 mL·kg⁻¹·min⁻¹; data not shown) was observed in these 2 patients. A significant additional effect of the triacylglycerol infusion on plasma lactate concentrations was shown in patients 3 and 4 only. In patient 4, the substantially lower plasma lactate concentration during the triacylglycerol infusion was associated with an increase in whole-body VO₂, in agreement with our hypothesis. In patient 3, the opposite was true; a significant additional effect of the triacylglycerol infusion on lactate+pyruvate Ra was observed. However, in both patients 3 and 4, the triacylglycerol infusion failed

### TABLE 5

Respiratory calorimetry and rate of appearance (Ra) of whole-body glucose and lactate+pyruvate in patients with complex I deficiency (CID) and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>CID patients (n = 4)</th>
<th>Control subjects (n = 15)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triacylglycerol infusion</td>
<td>Glucose infusion</td>
<td>Triacylglycerol infusion</td>
</tr>
<tr>
<td>VO₂ (mL·kg⁻¹·min⁻¹)</td>
<td>4.41 ± 0.38</td>
<td>4.20 ± 0.45</td>
<td>3.70 ± 0.24</td>
</tr>
<tr>
<td>VCO₂ (mL·kg⁻¹·min⁻¹)</td>
<td>3.36 ± 0.28</td>
<td>3.70 ± 0.45</td>
<td>2.96 ± 0.23</td>
</tr>
<tr>
<td>RER</td>
<td>0.76 ± 0.04</td>
<td>0.88 ± 0.06</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>CHox (µmol·kg⁻¹·min⁻¹)</td>
<td>4.8 ± 3.3</td>
<td>16.4 ± 6.2</td>
<td>7.3 ± 1.8</td>
</tr>
<tr>
<td>Glucose Ra (µmol·kg⁻¹·min⁻¹)</td>
<td>14.5 ± 1.9</td>
<td>31.1 ± 2.7</td>
<td>13.9 ± 2.6</td>
</tr>
<tr>
<td>Lactate+pyruvate Ra (µmol·kg⁻¹·min⁻¹)</td>
<td>31.1 ± 4.0</td>
<td>43.8 ± 15.2</td>
<td>22.3 ± 4.9</td>
</tr>
</tbody>
</table>

1x ± SD; VO₂, oxygen consumption; VCO₂, carbon dioxide production; RER, respiratory exchange ratio; CHox, carbohydrate oxidation.

Significantly different from control group, P < 0.05 (ANOVA followed by Bonferroni correction).

Significantly different from glucose infusion, P < 0.05 (ANOVA followed by Bonferroni correction).

n = 12.

### TABLE 6

Whole-body oxygen consumption (VO₂), plasma lactate concentrations, and rate of appearance (Ra) of lactate+pyruvate during triacylglycerol or glucose infusions in 4 patients with complex I deficiency (CID) and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>CID patients</th>
<th>Control subjects²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Whole-body VO₂ (mL·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol infusion</td>
<td>4.03</td>
<td>4.82</td>
</tr>
<tr>
<td>Glucose infusion</td>
<td>3.95</td>
<td>4.40</td>
</tr>
<tr>
<td>ΔVO₂²</td>
<td>0.08</td>
<td>0.42</td>
</tr>
<tr>
<td>Plasma lactate (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol infusion</td>
<td>1.30</td>
<td>1.03</td>
</tr>
<tr>
<td>Glucose infusion</td>
<td>1.73</td>
<td>1.75</td>
</tr>
<tr>
<td>ΔPlasma lactate²</td>
<td>−0.43</td>
<td>−0.72</td>
</tr>
<tr>
<td>Whole-body lactate Ra (µmol·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol infusion</td>
<td>27.6</td>
<td>29.7</td>
</tr>
<tr>
<td>Glucose infusion</td>
<td>35.8</td>
<td>34.7</td>
</tr>
<tr>
<td>ΔLactate+pyruvate Ra²</td>
<td>−8.2</td>
<td>−5.0</td>
</tr>
</tbody>
</table>

¹x; 95% CI in parentheses and n in brackets.

²Difference between triacylglycerol and glucose infusion studies.
to lower plasma lactate concentrations and the lactate+pyruvate Ra to values that were within the range for the control subjects. In neither of these 2 patients did the triacylglycerol infusion lower plasma lactate concentrations or the lactate+pyruvate Ra to values that were within the 95% CIs of the control subjects.

31P-MRS measurements of FDP muscle showed lower PCr-Pi ratios in the individual patients than in the control subjects during infusion of both substrates (Table 7), reflecting a lower muscle energy state due to impaired mitochondrial function. In both the patients and the control subjects, PCr-Pi ratios were not significantly different during the triacylglycerol infusion than during the glucose infusion. In addition, the triacylglycerol infusion failed to increase PCr-Pi ratios to control values.

DISCUSSION

Whole-body oxygen consumption

Our finding of significantly higher whole-body VO2 rates in all 4 patients than in the control subjects during both the triacylglycerol and glucose infusions agrees with data on whole-body VO2 in fasting CID patients previously reported by us (30) and others (4). We believe that the higher VO2 rates in the CID patients is a compensatory mechanism necessary to maintain the resting ATP synthetic rate when mitochondrial oxidative phosphorylation is less efficient as a result of the defective respiratory chain complex (MJ Roef, K de Meer, unpublished observations, 1999).

Plasma lactate and lactate+pyruvate Ra and muscle bioenergetics

Our finding in healthy subjects that the triacylglycerol infusion was associated with lower plasma lactate concentrations than was the glucose infusion was also shown by others (31–34). The failure of the triacylglycerol infusion to increase PCr-Pi ratios to control values is the most direct in vivo evidence that the triacylglycerol infusion does not change the energy state in the affected resting skeletal muscle of CID patients.

Relevance of the use of a high-fat diet in CID patients

The results of the present study suggest that the biochemical abnormalities observed in the 4 CID patients in the present study are not likely to improve after consumption of a high-fat diet. This lack of effect is worthy of remark. First, fatty acid availability for oxidation may have been lower in the patients than in the control subjects. However, our finding of a lower respiratory exchange ratio during the triacylglycerol infusion in the patients than in the control subjects suggests that fatty acid oxidation was unimpaired or even stimulated by the respiratory chain defect.

TABLE 7

<table>
<thead>
<tr>
<th>Substrate infusion</th>
<th>CID patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>5.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.1</td>
<td>5.6</td>
</tr>
</tbody>
</table>
The lower plasma fatty acid concentrations in the CID patients than in the control subjects during the triacylglycerol infusion may have been the consequence of preferential oxidation. Second, the type of fatty acids administered may have been important. In the studies by Doctor et al (7), heptanoate—a short-chain, odd-numbered fatty acid—was used to bypass retenone-inhibited complex I in renal cell cultures. However, we administered a lipid emulsion containing only a small amount of esterified short-chain, odd-numbered fatty acids (see Methods). To address the issue, triacylglycerol infusions containing different fatty acids should be studied in a larger group of CID patients. Limited numbers of available patients make it difficult to increase the sample size, however.

Finally, the findings in the 4 CID patients do not necessarily preclude prescription of a high-fat diet to other CID patients. We selected patients with documented CID for whom exercise intolerance was a main feature of their abnormality. At rest, the CID patients showed abnormalities of a biochemical nature only; no clinical signs, such as muscle wasting, were observed. It may be that our hypothesis, the aim of which was to improve mitochondrial substrate oxidation in CID patients, was not valid in the patients that we selected, at least not in the resting condition. It is tempting to speculate, however, that in resting CID patients with clinical signs and symptoms due to impaired mitochondrial substrate oxidation, the triacylglycerol infusion may be beneficial. In addition, the triacylglycerol infusion may be useful in CID patients when energy demands are increased, such as during exercise.

Conclusion

In resting CID patients in the present study, no additional benefit of the triacylglycerol infusion over glucose infusion was shown. The triacylglycerol infusion failed to improve mitochondrial substrate oxidation, as evidenced by both whole-body $\dot{V}_{O_2}$ and high-energy phosphate values in resting muscle, and failed to lower plasma lactate concentrations and the lactate+pyruvate Ra to values that were within the range for the healthy control subjects.

We thank R Ouwerkerk for his technical assistance with the 31P-MRS measurements and HD Bakker, JBC de Klerk, and GPA Smit for their referral of patients for the studies.

REFERENCES