Triacylglycerol infusion improves exercise endurance in patients with mitochondrial myopathy due to complex I deficiency[1–3]

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ABSTRACT

Background: A high-fat diet has been recommended for the treatment of patients with mitochondrial myopathy due to complex I (NADH dehydrogenase) deficiency (CID).

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

Design: Four CID patients and 15 control subjects were infused with triacylglycerol (3.7 $mg \cdot kg^{-1} \cdot min^{-1}$) or glucose (10 $mg \cdot kg^{-1} \cdot min^{-1}$) during low-intensity leg exercise. Respiratory calorimetry was used to evaluate mitochondrial substrate oxidation. The concentration and rate of appearance of plasma lactate (from dilution of [1-^{13}C]lactate) were used to evaluate glycolytic carbohydrate metabolism. ^{31}P magnetic resonance spectroscopy was used to determine ratios of phosphocreatine to inorganic *o*-phosphate in forearm muscle during exercise.

Results: In 3 patients, leg exercise endurance time was better during the triacylglycerol infusion than during the glucose infusion. In all 4 patients, whole-body oxygen consumption rates during exercise were higher during triacylglycerol infusion than during the glucose infusion. In 3 patients, the concentration and rate of appearance of plasma lactate were lower during triacylglycerol infusion than during the glucose infusion. Ratios of phosphocreatine to inorganic *o*-phosphate during exercise were not significantly different between the 2 infusion studies or between the patients and control subjects.

Conclusions: Triacylglycerol infusion is associated with a greater oxidation of substrates, lower rates of appearance and concentrations of plasma lactate, and greater leg exercise endurance time in myopathic CID patients than is glucose infusion. The energy state of muscle during exercise, however, was not significantly different after infusion of triacylglycerol or glucose. *Am J Clin Nutr* 2002;75:237–44.

KEY WORDS Complex I deficiency, exercise, triacylglycerol infusion, mitochondrial myopathy, stable isotopes, ^{31}P magnetic resonance spectroscopy

INTRODUCTION

In our preceding article in this issue, we proposed a potential role for high-fat, low-carbohydrate diets in the treatment of patients with mitochondrial myopathy due to complex I (NADH dehydrogenase; EC 1.6.99.3) deficiency (CID; 1). We hypothesized that supplementation with fatty acids (triacylglycerol) might improve the oxidation of substrates in CID patients by supplying $FADH_2$-linked reducing equivalents (electrons) to the mitochondrial respiratory chain distal to complex I. This action would make these patients' muscle cells less dependent on glycolytic carbohydrate metabolism, ie, the rate of appearance (Ra) and concentration of plasma lactate might be lower during triacylglycerol infusion than during glucose infusion. We documented that substrate oxidation rates were stimulated rather than impaired in resting, myopathic CID patients during infusion of glucose or triacylglycerol. In addition, triacylglycerol infusion did not lower the Ra or concentration of plasma lactate to resting control values. The finding that these patients showed no clinical signs or symptoms of CID at rest indicates that in vivo cellular energy balance was maintained during the triacylglycerol or glucose infusion, despite in vitro mitochondrial impairment. During exercise, when energy requirements in muscle are elevated, in vivo mitochondrial oxidation of NADH may become limiting in CID. Triacylglycerol administration then may be of benefit to these patients because fatty acid oxidation increases the relative supply of $FADH_2$, thereby improving oxygen consumption ($\dot{V}O_2$), and as a result may lower the Ra and concentration of plasma lactate and improve exercise tolerance.

In the present study, the effects of the triacylglycerol or glucose infusion on substrate oxidation and glycolytic metabolism were studied during low-intensity cycling in 4 CID patients and

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TABLE 1
Physical characteristics of individual patients with complex I deficiency (CID) and healthy control subjects at baseline[1]

	CID patients				Control subjects[2]
	1	2	3	4	(n = 15)
Age (y)	25	20	22	15	21 (20, 22)
Body weight (kg)	52	61	57	45	57.4 (54.7, 60.1)
Fat-free mass (kg)	41	45	44	35	44.5 (42.5, 46.5)
Maximal exercise testing					
*W*max (W)	60	60	50	55	210 (192, 228)
$\dot{V}O_2$max					
(mL/min)	721	750	596	797	2461 (2321, 2601)
($mL \cdot min^{-1} \cdot kg^{-1}$)	13.6	12.5	10.5	17.6	42.9 (41.6, 44.2)
Fasting plasma lactate (mmol/L)	2.3	2.7	4.7	2.0	1.0 (0.8, 1.2)

[1] *W*max, maximal workload; $\dot{V}O_2$max, maximal oxygen consumption.
[2] $\bar{x}$; 95% CI in parentheses.

15 healthy control subjects. Respiratory calorimetry was used as a measure of mitochondrial substrate oxidation. Glycolytic carbohydrate metabolism was evaluated on the basis of the Ra and concentration of plasma lactate (from dilution of [1-^{13}C]lactate). In addition, the effects of submaximal exercise on the energy state of forearm muscle was measured by ^{31}P magnetic resonance spectroscopy (^{31}P-MRS).

SUBJECTS AND METHODS

Subjects

Details about the CID patients and healthy control subjects are provided in the preceding article (1). Briefly, the 4 CID patients had similar clinical histories, ie, easily fatiguable mild muscle weakness dating back to early childhood that remained stable over time. Therefore, exercise intolerance was the dominant clinical symptom at the time of the study. 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 (range: 5.6–23.8% of normal). Eighteen healthy control subjects matched for age ($\bar{x}$ age: 21 y), sex, and body weight were recruited: 15 for the tracer infusion and respiratory calorimetry studies and 3 (2 men and 1 woman aged 18–28 y) for the ^{31}P-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).

Preexperiment testing

All participants reported to the Laboratory for Metabolic Diseases (University Children's Hospital, Utrecht, Netherlands) ≥3 d before the onset of the experiments to perform an incremental maximal exercise test on an electrically braked cycle ergometer (Lode Instruments, Groningen, Netherlands). Maximal workload (*W*max) and maximal $\dot{V}O_2$ ($\dot{V}O_2$max) were assessed as previously described (2). The *W*max ranged from 50 to 60 W in the CID patients, 25% of control values on average ($\bar{x}$: 210 W; **Table 1**). The results of this test were used to calculate a workload equal to 15% of each subject's *W*max. Thus, the calculated workloads ranged from 7 to 9 W in the CID patients. From our experience, these workloads can be sustained for a relatively prolonged period of time (>0.5 h) to test exercise endurance. Seven of the control subjects exercised at 15% of their respective *W*max (range: 24–37 W, corresponding to ≈30% $\dot{V}O_2$max). The remaining 8 control subjects were studied during stationary cycling at an absolute workload similar to that for the patients (7 W), to take into account possible confounding effects of the extreme low exercise intensity on exercise mechanics. Both workloads can be easily sustained by healthy subjects for hours.

Experimental protocol

The patients and control subjects reported to the Laboratory for Metabolic Diseases at 0800 on 2 occasions separated by ≥7 d. Before exercise was initiated, all subjects were infused intravenously with either glucose (10% wt:vol, 5 $mg \cdot kg^{-1} \cdot min^{-1}$) or a triacylglycerol emulsion (Intralipid, 20% wt:vol, 1.85 $mg \cdot kg^{-1} \cdot min^{-1}$; Fresenius Kabi, 's-Hertogenbosch, Netherlands) and heparin (7.5 $U \cdot kg^{-1} \cdot h^{-1}$; prime 14 U/kg) for 120 min, during which time the subjects remained at rest. Next, the stationary cycling exercise began at 15% *W*max (all patients and 7 control subjects) or 7 W (8 control subjects). The control subjects exercised for a nominal period of 90 min, whereas the patients exercised until exhaustion ensued. During exercise, the triacylglycerol and glucose infusion rates were doubled to 3.7 and 10 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively.

Isotope infusion

Primed, constant infusions of [6,6-$^2H_2$]glucose (98% enriched; Mass Trace, Woburn, MA) were administered in all patients and in 9 control subjects as previously described (1). An unprimed, constant infusion of [1-^{13}C]lactate (98% enriched; Mass Trace) was administered throughout the nominal 90-min exercise period. The [1-^{13}C]lactate tracer infusion rates for the CID patients (n = 4) ranged from 2.50 to 5.34 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; control subjects ($n$ = 9) received 0.56 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. The remaining 6 control subjects received no lactate tracer, to examine the effects of triacylglycerol or glucose infusion on the background enrichment of lactate.

Blood sampling and urine collection

Blood samples were drawn at regular intervals during exercise and handled as described previously (1). All subjects voided before and after exercise, and the urine was collected for measurement of nitrogen excretion.

Respiratory calorimetry

Open-circuit indirect calorimetry with use of a face mask was performed continuously during the 90-min exercise period. Stable $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) 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 (1, 3).

^{31}P-MRS studies ratios of phosphocreatine to inorganic o-phosphate at rest and during exercise

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 and received an intravenous infusion of either triacylglycerol plus heparin or glucose during a 100-min basal resting period as described above. The infusions were maintained at the same rate throughout the following 30-min study period. Peak ratios of phosphocreatine (PCr) to β-ATP and of inorganic *o*-phosphate (Pi) to β-ATP were measured at rest and during exercise at an average of 6 normalized power output levels; concentrations of P_i and PCr were calculated from each ratio, respectively, as described elsewhere (4).

The results obtained at rest were reported previously (1). Exercise consisted of bulb-squeezing at an audio signal frequency of 0.33 Hz (duration, 300 ms) with use of only the fourth and fifth digits at progressively increasing submaximal workloads in a ramp protocol with feedback of power output (details described previously; 5). Power output was measured and recorded as developed pressure × the displaced volume of air and was normalized to power output during maximal voluntary contraction (MVC). The highest implemented workload in the patients ranged from 30% MVC to 45% MVC and was 50% MVC in the control subjects. All subjects were right-handed.

Studies were conducted on the superficial mass of the flexor digitorum profundus (FDP) muscle of the right forearm, as described in detail elsewhere (5). The FDP muscle is affected in CID patients, as evidenced by observed decreases in MVC output of the muscle comparable with decreases in *W*max measured on a cycle ergometer (MJ Roef, K de Meer, unpublished observations, 1996). Radio frequency pulsing during exercise was gated to the audio signal that synchronized bulb-squeezing with a home-built audiotriggering device. To minimize motion artifacts and improve standardization of the measurements, the radio frequency pulse was set to occur 1250 ms after contraction.

Sample analysis

Blood and urine samples and MR spectra were analyzed as described previously (1).

Calculations

Whole-body glucose rate of appearance

In steady state experiments, the whole-body glucose Ra was calculated as follows (6):

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

where I is the infusion rate of the [6,6-$^2H_2$]glucose tracer (in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and TTR is the molar tracer-tracee ratio in plasma. Isotopic enrichments were considered to be at steady state when the TTRs of the final 4 consecutive samples (from time 175 to 205 min) had a CV < 10% and a slope not significantly different from zero. When the isotopic enrichments were not at steady state, the Ra was calculated with Steele's equations for non–steady state conditions (7), as previously described (1). Steady state [6,6-$^2H_2$]glucose TTRs were attained in 3 of the 4 patients and in 7 of the 9 control subjects during triacylglycerol infusion and in 3 of the 4 patients and in 6 of the 9 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 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Wolfe et al (8, 9) reported that plasma pyruvate enrichment was 92% of plasma lactate enrichment at steady state in anesthesized dogs when [1-^{13}C]lactate tracer was infused. Results of Large et al (10) suggest that this approach could also be applicable to humans. Therefore, we assumed that the turnover rates calculated from dilution of labeled lactate in plasma represent not only lactate but also pyruvate turnover:

$$\text{Whole-body lactate+pyruvate Ra} = I/\text{TTR} \quad (2)$$

where I is the infusion rate of [1-^{13}C]lactate (in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). 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 (7), as previously described (1). The volume of distribution for lactate+pyruvate was assumed to be 500 mL/kg (11, 12). During exercise, steady state lactate TTRs were attained in only 1 of the 4 patients during both the triacylglycerol and glucose infusions and in 7 of the 9 control subjects during the triacylglycerol infusion and in 6 of the 9 control subjects during the glucose infusion.

Respiratory calorimetry

Substrate oxidation rates were calculated as described previously (1). Calculated whole-body substrate oxidation rates during exercise were assumed to reflect mitochondrial oxidation, neglecting nonmitochondrial peroxisomal and microsomal $\dot{V}O_2$.

Outcome parameters

We compared whole-body $\dot{V}O_2$ rates, plasma lactate concentrations, lactate+pyruvate turnover rates, and leg exercise endurance time between the patients and the control subjects. The triacylglycerol or glucose infusion can be 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:

$$\Delta\dot{V}O_2 = \Delta\dot{V}O_{2TG} - \Delta\dot{V}O_{2GL} \quad (3)$$

$$\Delta\text{Plasma lactate concentration} = \text{plasma lactate}_{TG} - \text{plasma lactate}_{GL} \quad (4)$$

$$\Delta\text{Lactate+ pyruvate Ra} = \text{lactate+pyruvate}_{TG}\ \text{Ra} - \text{lactate+pyruvate}_{GL}\ \text{Ra} \quad (5)$$

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

TABLE 2
Plasma substrate concentrations during cycling exercise and infusion of triacylglycerol or glucose in patients with complex I deficiency (CID) and healthy control subjects[1]

	CID patients exercising at 15% *W*max (7–9 W) (*n* = 4)		Control subjects exercising at 7 W (*n* = 8)		*P* (ANOVA)		
	Triacylglycerol infusion	Glucose infusion	Triacylglycerol infusion	Glucose infusion	Infusion condition	Group	Interaction
Plasma glucose (mmol/L)	5.4 ± 0.6[2]	9.4 ± 1.8	5.0 ± 0.3[2]	9.4 ± 1.1	<0.001	NS	0.001
Plasma lactate (mmol/L)	7.34 ± 3.88[3]	7.30 ± 0.84[3]	0.90 ± 0.37	1.18 ± 0.34	NS	<0.001	<0.001
Blood pyruvate (μmol/L)	207 ± 96[3]	177 ± 66[3]	64 ± 20[2]	85 ± 11	NS	<0.001	<0.001
Lactate:pyruvate	34.6 ± 8.4[3]	38.4 ± 14.2[3]	15.6 ± 7.4	13.7 ± 4.2	NS	<0.001	<0.001
Plasma fatty acids (mmol/L)	0.86 ± 0.18[2,3]	0.10 ± 0.06	1.44 ± 0.42[2]	0.08 ± 0.06	<0.001	<0.05	<0.001
Plasma triacylglycerol (mmol/L)	2.16 ± 0.78[2]	0.71 ± 0.22	2.06 ± 0.79[2]	0.52 ± 0.17	<0.001	NS	<0.05
Blood β-hydroxybutyrate (mmol/L)	0.48 ± 0.38	0.09 ± 0.10	0.40 ± 0.17	ND	NS	NS	NS
Blood acetoacetate (mmol/L)	0.11 ± 0.06	ND	0.19 ± 0.08	ND	NS	NS	NS
β-Hydroxybutyrate:acetoacetate	4.5 ± 2.0	—	2.1 ± 0.3	—	NS	NS	NS
Plasma glycerol (mmol/L)	0.26 ± 0.06	ND	0.31 ± 0.06[2]	0.03 ± 0.03	<0.001	NS	<0.01
Plasma insulin (pmol/L)	53 ± 44[2]	326 ± 102	26 ± 14[2]	230 ± 97	<0.001	NS	<0.01
Plasma cortisol (nmol/L)	443 ± 190	306 ± 126	396 ± 121	379 ± 237	NS	NS	NS

[1] $\bar{x}$ ± SD. ND, not detectable (<0.02 mmol/L).
[2] Significantly different from glucose infusion, $P < 0.05$ (ANOVA followed by Bonferroni correction).
[3] Significantly different from control subjects, $P < 0.05$ (ANOVA followed by Bonferroni correction).

Statistical analysis

The results are presented as means ± SDs, except for leg exercise endurance time, which are presented as absolute values. 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 (triacylglycerol compared with glucose infusion) 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 control subjects. SPSS for WINDOWS (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, oxygen consumption, and substrate utilization

In control subjects exercising at 7 W, plasma glucose, plasma insulin, and blood pyruvate concentrations were significantly higher during the glucose infusion than during the triacylglycerol infusion, as expected (**Table 2**). Plasma fatty acid, triacylglycerol, and glycerol concentrations were significantly higher during the triacylglycerol infusion than during the glucose infusion. Plasma lactate, plasma cortisol, blood β-hydroxybutyrate, and blood acetoacetate concentrations and lactate-pyruvate ratios were not significantly different between the 2 infusion conditions.

Plasma glucose, insulin, and lactate concentrations and lactate-pyruvate ratios were significantly lower during the glucose infusion in control subjects exercising at 15% *W*max than in control subjects exercising at 7 W (data not shown); however, plasma substrate concentrations during the triacylglycerol infusion were not significantly different between the 2 exercise conditions.

Plasma glucose and insulin concentrations were not significantly different between the CID patients and the control subjects exercising at either 7 W or 15% *W*max during infusion of either substrate. Plasma lactate concentrations were significantly higher in the patients than in the control subjects during the triacylglycerol and glucose infusions. Blood pyruvate concentrations and lactate-pyruvate ratios were significantly higher in the patients than in the control subjects during infusion of both substrates. Plasma fatty acid concentrations were significantly lower in the patients than in control subjects during the triacylglycerol infusion, but were not significantly different between the 2 subject groups during the glucose infusion. During the triacylglycerol infusion, the ratio of β-hydroxybutyrate to acetoacetate in blood was significantly higher in the patients than in the control subjects exercising at 15% *W*max (data not shown for control subjects).

In control subjects, whole-body $\dot{V}O_2$ was not significantly different between the 2 infusion conditions, but the respiratory exchange ratio and the total carbohydrate oxidation rate were significantly lower during the triacylglycerol infusion than during the glucose infusion at exercise intensities of 7 W and 15% *W*max (data not shown for control subjects at 15% *W*max) (**Table 3**). In the 6 control subjects who received no lactate tracer, the background enrichment of lactate was not significantly different during the triacylglycerol and glucose infusions (data not shown).

Although there was no significant main effect of infusion condition, group, or condition × group interaction on whole-body $\dot{V}O_2$ rates (Table 3), whole-body $\dot{V}O_2$ values in the CID patients were higher during the triacylglycerol infusion than during the glucose infusion (**Table 4**). During the triacylglycerol infusion, respiratory exchange ratios were significantly higher in the patients than in the control subjects at exercise intensities of 7 W and 15% *W*max (data not shown for control subjects at 15% *W*max in Table 3); total carbohydrate oxidation rates were significantly higher in the patients than in the control subjects at the exercise intensity of 7 W. Plasma glucose turnover rates in the patients were not significantly different from those in the control subjects during infusion of either substrate.

TABLE 3
Respiratory calorimetry and the rate of appearance (Ra) of whole-body glucose during cycling exercise and infusion of triacylglycerol or glucose in patients with complex I deficiency (CID) and healthy control subjects[1]

	CID patients exercising at 15% *W*max (7–9 W) (*n* = 4)		Control subjects exercising at 7 W (*n* = 8)		*P* (ANOVA)		
	Triacylglycerol infusion	Glucose infusion	Triacylglycerol infusion	Glucose infusion	Infusion condition	Group	Interaction
$\dot{V}O_2$ (mL·kg^{-1}·min^{-1})	7.98 ± 2.24	6.72 ± 0.46	6.57 ± 0.82	6.43 ± 0.71	NS	NS	NS
$\dot{V}CO_2$ (mL·kg^{-1}·min^{-1})	6.61 ± 1.66[2]	6.53 ± 0.46	5.08 ± 0.62[3]	5.89 ± 0.62	NS	<0.05	<0.05
RER	0.83 ± 0.04[2,3]	0.97 ± 0.06	0.77 ± 0.03[3]	0.92 ± 0.03	<0.001	<0.01	<0.01
CHOx (μmol·kg^{-1}·min^{-1})	24.2 ± 7.0[2]	41.7 ± 22.4	9.9 ± 3.1[3]	32.7 ± 5.9	<0.001	<0.05	<0.05
Glucose Ra (μmol·kg^{-1}·min^{-1})	17.9 ± 2.8[3]	54.1 ± 2.0	13.8 ± 2.5[3]	53.3 ± 7.9	<0.001	NS	<0.01

[1] $\bar{x}$ ± SD. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; CHOx, carbohydrate oxidation in glucose units.
[2] Significantly different from control subjects, $P < 0.05$ (ANOVA followed by Bonferroni correction).
[3] Significantly different from glucose infusion, $P < 0.05$ (ANOVA followed by Bonferroni correction).

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

Whole-body oxygen consumption, plasma lactate, and lactate+pyruvate Ra

Changes in $\dot{V}O_2$, plasma lactate, and lactate+pyruvate Ra in the individual patients and the respective 95% CIs in the control subjects are shown in Table 4 and **Figure 1**. The triacylglycerol infusion was associated with substantially higher $\dot{V}O_2$ rates than was the glucose infusion in all 4 CID patients. In each of the patients, this effect of triacylglycerol infusion on $\dot{V}O_2$ was additional to that in the control group. In comparison with control subjects exercising at an exercise intensity of 7 W, absolute rates of whole-body $\dot{V}O_2$ were lower (on average by 14%) in patients 1 and 2 but were higher (on average by 40%) in patients 3 and 4.

Additional effects of the triacylglycerol infusion on plasma lactate concentrations and lactate+pyruvate Ra were shown in 2 and 3 of the 4 patients, respectively. In patient 2, the triacylglycerol infusion had an additional effect on plasma lactate concentrations and lactate+pyruvate Ra.

Leg exercise endurance time

The control subjects completed the leg exercise protocol with ease, irrespective of the substrate being infused (**Figure 2**). Patients 1, 2, and 3 had to stop exercising prematurely (between 48 and 65 min) during the glucose infusion because of muscle fatigue and exhaustion, whereas patient 4 completed the nominal 90-min study period. All patients completed the 90-min cycling exercise during the triacylglycerol infusion.

^{31}P MRS measurements of PCr-Pi ratios at rest and during exercise

PCr-Pi ratios measured by ^{31}P-MRS at rest and during exercise in one typical CID patient and in one typical control subject are shown in **Figure 3**. Relative exercise levels in the forearm exercise ramp protocol during the triacylglycerol infusion were not significantly different from those during the glucose infusion in both the patients and control subjects. At rest, PCr-P_i ratios were lower in all patients than in the control subjects during infusion of both triacylglycerol and glucose. During exercise, PCr-P_i ratios were not significantly different during the

TABLE 4
Whole-body oxygen consumption ($\dot{V}O_2$), plasma lactate concentrations, and rate of appearance (Ra) of lactate+pyruvate during cycling exercise and infusion of triacylglycerol or glucose in individual patients with complex I deficiency (CID) and healthy control subjects[1]

	CID patients exercising at 15% *W*max (7–9 W)				Control subjects[2]	
	1	2	3	4	Exercising at 7 W (*n* = 8)	Exercising at 15% *W*max (*n* = 7)
Whole-body $\dot{V}O_2$ (mL·kg^{-1}·min^{-1})						
Triacylglycerol infusion	5.66	6.87	8.56	10.82	6.57 (5.88, 7.26)	11.95 (11.19, 12.71)
Glucose infusion	4.86	4.99	7.87	9.16	6.43 (5.84, 7.02)	11.44 (10.95, 11.93)
$\Delta\dot{V}O_2$[3]	0.80	1.88	0.69	1.66	0.14 (−0.21, 0.49)	0.51 (−0.49, 1.51)
Plasma lactate (mmol/L)						
Triacylglycerol infusion	6.93	7.27	12.3	2.85	0.90 (0.59, 1.21)	0.83 (0.63, 1.03)
Glucose infusion	6.58	4.15	15.0	3.48	1.18 (0.90, 1.46)	0.78 (0.68, 0.88)
ΔPlasma lactate[3]	0.35	3.12	−2.7	−0.63	−0.28 (−0.63, 0.07)	0.05 (−0.10, 0.20)
Whole-body lactate+pyruvate Ra (μmol·kg^{-1}·min^{-1})						
Triacylglycerol infusion	57	77	106	52	33.0 (28.6, 37.4)[4]	—
Glucose infusion	87	55	162	72	44.0 (36.4, 51.6)[4]	—
ΔLactate+pyruvate Ra[3]	−30	22	−56	−20	−11.0 (−16.5, −5.5)[4]	—

[1] *W*max, maximal workload.
[2] $\bar{x}$; 95% CI in parentheses.
[3] Difference between the triacylglycerol and glucose infusion studies.
[4] Pooled data for 9 control subjects.

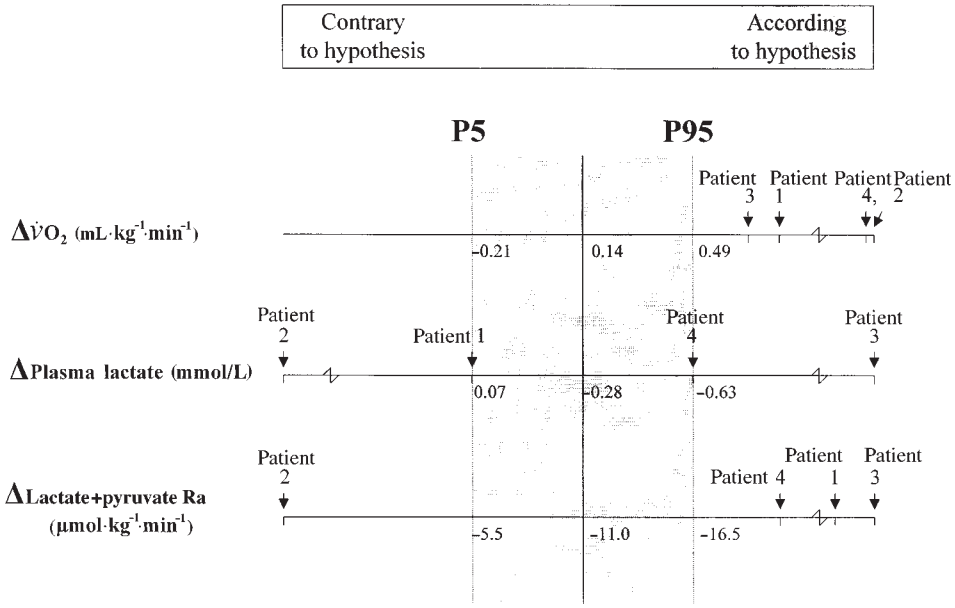

FIGURE 1. Mean differences (Δ) in oxygen consumption ($\dot{V}O_2$), plasma lactate, and rate of appearance (Ra) of lactate+pyruvate between the triacylglycerol and glucose infusion studies in the individual patients and the respective mean 95% CIs for differences in the control subjects (gray-shaded area). P5, lower limit of 95% CI; P95, upper limit of 95% CI. Values greater than the P95 in the individual patients indicate an effect of triacylglycerol additional to that in the control subjects according to the following hypothesis: $\Delta\dot{V}O_2$ in patients > $\Delta\dot{V}O_2$ in control subjects, and Δplasma lactate and Δlactate+pyruvate Ra in patients < Δplasma lactate and Δlactate+pyruvate Ra in control subjects.

triacylglycerol infusion than during the glucose infusion in either the patients or control subjects.

DISCUSSION

Oxidative phosphorylation in the muscle of patients with mitochondrial CID is stimulated during exercise, whereas the ability of the muscle cells' mitochondria to oxidize NADH is limited. We hypothesized that fatty acid oxidation, by providing more $FADH_2$-linked reducing equivalents to the respiratory chain distal of complex I, would enable the affected cells to bypass the metabolic defect. The finding of beneficial effects of triacylglycerol infusion in 4 myopathic patients with isolated CID provides new insights into the nutritional metabolism and cellular pathophysiology of CID patients. Results were not unequivocal in all patients however; therefore, the limitations of the study need to be discussed.

Whole-body oxygen consumption

Our assumption that substrate oxidation rates are impaired during exercise was true in only 2 of the 4 patients exercising at 7 W on the basis that absolute whole-body $\dot{V}O_2$ rates during the triacylglycerol infusion were lower in patients 1 and 2 but higher in patients 3 and 4 than in control subjects. In our previous study (1), we suggested that the higher whole-body $\dot{V}O_2$ rates after infusion of both substrates in all 4 CID patients is explained by a compensatory mechanism necessary to maintain the resting ATP synthetic rate when mitochondrial oxidative phosphorylation is less efficient because of the respiratory chain deficit (1). The increased $\dot{V}O_2$ rates during exercise in the present study suggest that this putative mechanism may also partly compensate for the lower efficacy of ATP synthesis during exercise in 2 of the patients.

Plasma lactate and lactate+pyruvate Ra

In 3 of the 4 CID patients, our finding of a significant additional diminishing effect of triacylglycerol infusion on plasma lactate concentrations and the lactate+pyruvate Ra was as expected, ie, supported our hypothesis. In the fourth patient (patient 2), however, our finding was contrary to our hypothesis; this patient had a higher plasma lactate concentration and higher lactate+pyruvate Ra during the triacylglycerol infusion than during the glucose infusion. Because Steele's equation was used to calculate lactate+pyruvate Ra (*see* Methods), the lactate concentration and Ra in this patient were not independently measured. We have no explanation for this contrary finding, which is even more remarkable because this patient's leg exercise endurance time during triacylglycerol infusion was significantly better than that of the other 3 patients.

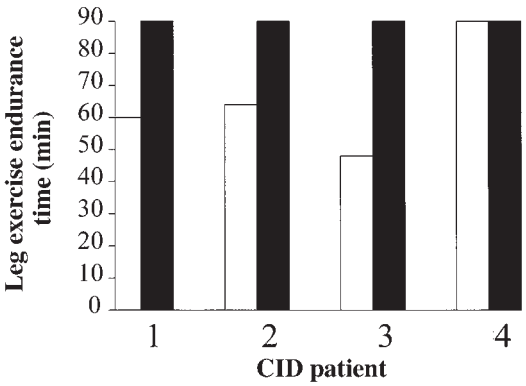

FIGURE 2. Leg exercise endurance time during stationary cycling exercise in 4 patients with complex I deficiency (CID) during infusion of glucose (□) or triacylglycerol (■).

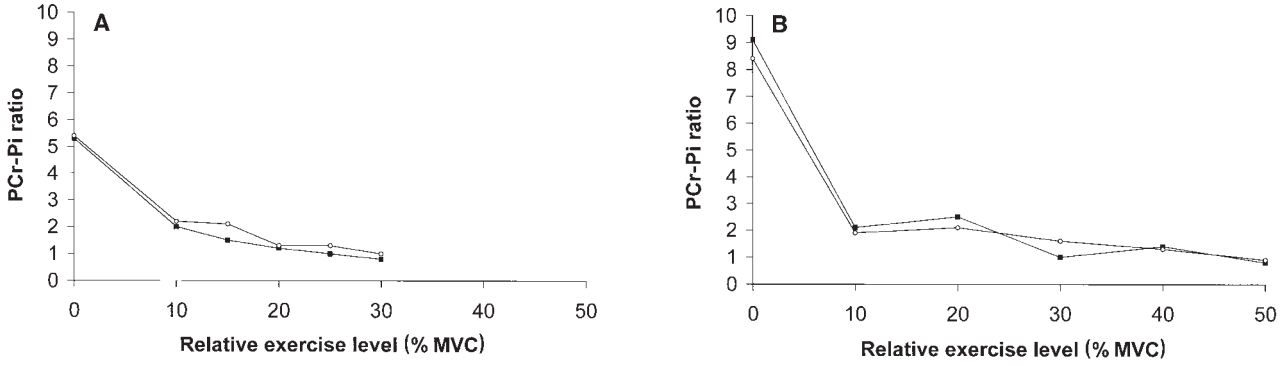

FIGURE 3. Ratios of phosphocreatine (PCr) to inorganic *o*-phosphate (P_i) at rest and during forearm bulb-squeeze exercise in a typical patient with complex I deficiency (A) and in a typical control subject (B) during the triacylglycerol (■) and glucose (○) infusions. MVC, maximal voluntary contraction.

Leg exercise endurance time and muscle bioenergetics

The leg exercise endurance time was clearly lower during the glucose infusion than during the triacylglycerol infusion in 3 of the 4 CID patients because these patients terminated their low-intensity cycling exercise prematurely during the glucose infusion. The remaining patient (patient 4) completed the 90-min low-intensity exercise trial irrespective of the substrate infused and had the lowest plasma lactate concentration of the 4 patients during infusion of both substrates. These findings suggest that leg exercise endurance time may be related to circulating plasma lactate concentrations. Unfortunately, patient 4 was not available for the ^{31}P-MRS studies. The finding that leg exercise endurance time in patient 2 was lower during the glucose infusion, despite lower plasma lactate concentrations during the glucose infusion than during the triacylglycerol infusion, suggests that leg exercise endurance time is not just a simple function of plasma lactate concentrations (13).

The question arises as to why 3 of the 4 patients were unable to complete the 90-min exercise trial. Although our infusion experiments were not double-blind, we believe that the patients stopped exercising because of total exhaustion. Therefore, we are confident that the observed difference in leg exercise endurance time between the 2 infusion periods has a physiologic rather than a psychological basis. Because lactate production in muscle is usually accompanied by release of [H^+], differences in the release of [H^+] could explain the observed differences in leg exercise endurance time. However, none of the patients became acidotic during infusion of either substrate during exercise at this low intensity level, ie, 15% *W*max (data not shown), suggesting that these patients can effectively compensate for an excess release of [H^+] from their exercising muscles.

Blood bicarbonate concentrations decreased only mildly and to a comparable extent during exercise and infusion of either substrate (data not shown). These findings do not rule out a relation between exercise endurance and a decrease in intramuscular pH. The MRS data obtained from forearm muscle during exercise showed no significant differences in chemical shift of Pi resonance (reflecting differences in intramuscular pH; data not shown) between the glucose and triacylglycerol infusions, however. Thus, we can only speculate that the mechanism responsible for the early termination of leg exercise during the glucose infusion in these patients was the early depletion of muscle glycogen stores. In healthy subjects, carbohydrate administration during exercise does not spare muscle glycogen stores (14–16); in contrast, triacylglycerol infusion is associated with significant sparing of muscle glycogen during exercise (17–21). Most of these studies, however, involved exercise at moderate-to-intense exercise levels (≈70% $\dot{V}O_2$max, corresponding to ≈60% *W*max in healthy individuals). Although we considered the exercise intensity of 15% *W*max to be low and sustainable for a prolonged period in our myopathic patients, their relatively high $\dot{V}O_2$ rates (55% of $\dot{V}O_2$max on average) and respiratory exchange ratios during exercise suggest that this exercise level was relatively intense for our patients. (In the control subjects, exercise intensity levels correlated with carbohydrate oxidation rates, and thus with respiratory exchange ratios). If we then assume, on the basis of observations in healthy subjects at higher intensity levels (70% $\dot{V}O_2$max), that the effects of glucose and triacylglycerol infusion on muscle glycogen stores during exercise were similar in our patients, the possibility that glycogen stores are depleted faster during glucose infusion than during triacylglycerol infusion seems likely.

^{31}P MRS measurements of PCr-Pi ratios during forearm exercise

The finding in CID patients of no significant differences in PCr-P_i ratios during exercise between the triacylglycerol and glucose infusion studies—despite improvements in endurance time, $\dot{V}O_2$, and lactate metabolism in the patients during the triacylglycerol infusion (compared with glucose infusion)—is explained by the different exercise protocols used. The tracer infusion and respiratory calorimetry studies involved steady state cycling at a low intensity level chosen to be endurable for a prolonged period of time (>0.5 h). The ^{31}P-MRS measurements were made in forearm muscle during submaximal exercise at increasing workloads designed to fatigue the muscle in a relatively short period of time.

Conclusion

In the present study, the triacylglycerol infusion was associated with higher mitochondrial substrate oxidation rates and lower plasma lactate concentrations and lactate+pyruvate Ra in myopathic CID patients during exercise than was the glucose infusion. These findings were associated with improved exercise endurance times during triacylglycerol infusion in most of the patients. However, PCr-P_i ratios measured in a forearm muscle during submaximal exercise were not significantly different in the patients during the 2 infusion studies. Thus, despite the beneficial effect of triacylglycerol on mitochondrial oxidative phosphorylation and muscle function during exercise, this macronutrient does not seem to change the muscle's energy state.

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