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Fatty Acid Oxidation in Peripheral Blood Cells: Characterization and Use for the Diagnosis of Defects of Fatty Acid Oxidation

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ABSTRACT

Disorders of mitochondrial fatty acid oxidation are increasingly recognized as an important group of inborn errors of metabolism that are associated with a significant, but easily preventable, morbidity and mortality in children. However, diagnosis is often delayed because there is no easily applied method that detects all defects. Therefore, we have characterized the acylcarnitine intermediates of fatty acid oxidation in peripheral blood cells from healthy control volunteers and patients with four different defects. After selective permeabilization with histone II AS, a novel permeabilizing agent, the cells were incubated with [U-^{14}C]hexadecanoate and β-oxidation flux and the acylcarnitine esters formed were measured. Blood cells from the control population produced large amounts of 3-hydroxyacylcarnitines and 2-enoylcarnitine esters, in addition to saturated acylcarnitine esters. This result is different from that found in other tissues (fibroblasts and muscle), where only saturated acylcarnitine esters could be detected. In blood cells from patients with defects of enzymes involved in long-chain fatty acid oxidation, flux was significantly reduced at 15 to 20% of control values (7.1 ± 2.3 nmol C_2 units formed per minute per International Unit of citrate synthase activity). There was a characteristic accumulation of acylcarnitines that was pathognomonic for the site of the defect. Thus, analysis of β-oxidation intermediates from blood cells allows unequivocal identification of the four most common β-oxidation defects. (***Pediatr Res* 37: 354–360, 1995**)

Abbreviations

ETF, electron-transfer flavoprotein
VLCAD, very long-chain acyl-CoA dehydrogenase
MCAD, medium-chain acyl-coA dehydrogenase
CPT, carnitine palmitoyltransferase

Fatty acids are essential metabolic fuels in humans, especially at times of fasting or stress. They are predominantly oxidized in mitochondria by a series of repetitive enzymatic steps that generate acetyl-CoA and a chain-shortened fatty acyl-CoA ester until the acyl-CoA ester is completely cleaved into acetyl-CoA (1). In recent years a number of genetically distinct inborn errors of mitochondrial fatty acid oxidation have been described. These include abnormalities of short-chain acyl-coA dehydrogenase, MCAD, and VCLAD (1); ETF and ETF-oxidoreductase (2); trifunctional protein (3, 4); and CPTI and II (5). Inherited disorders of mitochondrial β-oxidation are life threatening and relatively common diseases predominantly affecting infants and children. The clinical features include sudden infant death, hypoketotic hypoglycemic coma, Reye-like episodes, encephalopathy, myopathy, or cardiomyopathy (6–8). The acute metabolic decompensation associated with these defects is generally induced by prolonged fasting or intercurrent infections; the patients are usually free of symptoms between episodes, which often delays the diagnosis. Although they represent a major and very significant group of inborn errors of metabolism, β-oxidation defects remain difficult to diagnose biochemically, and the development of new methodologies is therefore important. Measurement of intermediary metabolites in plasma or an abnormal pattern of urinary organic acids can be helpful, but they may only be present intermittently. More recently, molecular genetic techniques have been applied successfully, but only a limited number of patients can be identified by these methods (9). Therefore, in many cases measurement of the individual enzyme activities is still required, which is a difficult process because of the lack of commercially available substrates.

In view of the need to use easily accessible cells to identify a β-oxidation defect, we have characterized β-oxidation in peripheral blood cells. We have shown that mitochondrial fatty acid oxidation occurs in permeabilized blood cells and that characteristic acylcarnitine esters accumulate in cells from

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control subjects. In addition, in blood cells from patients with defects of β-oxidation, different amounts of specific acylcarnitines are found when compared with controls, and the pattern is pathognomonic for the site of the defect.

METHODS

Materials. BSA (fraction V, fatty-acid free), CoA, ATP, ADP, L-carnitine, cytochrome *c*, and histone II-AS were obtained from Sigma Chemical Co. (Poole, Dorset, UK). DEAE-Sephacel was from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). Acetonitrile (S-grade) was purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Dowex 50W-X8 was supplied by Aldrich (Gillingham, UK). [U-^{14}C]hexadecanoic acid (30.6 GBq/mmol) was purchased from Amersham International (Little Chalfont, UK). For the experiments it was complexed to albumin in a 5:1 molar ratio (10), and the sp act was adjusted to 3.7 MBq/mmol. All other chemicals were obtained from BDH Chemicals Ltd. (Lutterworth, Leicestershire, UK).

Patients. Blood samples were taken from two patients with proven β-oxidation defects involving either VLCAD (11) or MCAD (12). Two other patients were referred for investigation of muscle pain. Using the technique described in this article, a diagnosis of trifunctional enzyme deficiency and CPT deficiency was made. It was confirmed subsequently by direct enzyme measurement in fibroblast homogenates that the first patient had a deficiency of the long-chain 3-hydroxyacyl-CoA dehydrogenase component of the trifunctional enzyme (patient: 10.6 nmol NADH reduced per minute per milligram of protein; mean ± SD for six controls: 36.9 ± 6.8 nmol NADH reduced per minute per milligram of protein). The CPT-II deficiency was proven in the second patient by measurement of CPT activity in fibroblasts using the radiochemical forward assay (13).

Synthesis of internal standards. Heptadecanoyl-CoA and undecanoylcarnitine were synthesized as described previously (14, 15).

Preparation of peripheral blood cells. Heparinized blood (10–30 mL) was collected in conical plastic tubes and centrifuged at 150 × *g* for 15 min at 4°C. The supernatant, buffy coat, and top erythrocyte layer were pipetted into 50-mL conical plastic tubes and diluted with 4 volumes of lysis medium [0.155 M ammonium chloride, 0.1 mM EDTA, and 10 mM potassium hydrogen carbonate (pH 7.4)] and incubated on ice until hemolysis occurred. The leukocytes and platelets were then pelleted by centrifugation at 700 × *g* for 10 min (4°C). The supernatant was discarded, the cell pellet resuspended in 50 mL of lysis medium, and the lysis procedure repeated. The final cell pellet, which was free of contaminating red cells, was washed once with PBS. It consisted of a leukocyte-platelet mixture and contained more than 90% viable cells as shown by the exclusion of Trypan blue. The cell pellet was resuspended in 1.5 mL of PBS and aliquoted in a 1:2 ratio for measurement of flux and intermediates. The fractions were incubated for 10 min at room temperature with an aqueous solution of histone II-AS at a final concentration of 3 mg/mL. The cells were then sedimented by centrifugation at 11 000 × *g* for 1 min and washed with PBS. After recentrifugation at 11 000 × *g* for 1 min, the final cell pellets were resuspended in 1 mL (for intermediates) and 0.5 mL (for fluxes) of incubation medium (pH 7.2) containing 110 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer, 5 mM $MgCl_2$, 10 mM potassium phosphate, 1 mM EGTA, and 1 mM ADP. Immediately before the incubation, 5 mM ATP, 0.1 mM CoA, 0.2 mg/mL cytochrome *c*, and 2.5 mM L-carnitine were added. Citrate synthase and lactate dehydrogenase activities were measured by standard methods (16, 17).

Incubations with [U-^{14}C]hexadecanoate. Incubations were performed at 37°C in a shaking water bath (170 strokes per minute). After a preincubation period of 10 min, the incubations for the determination of intermediates were started by addition of [U-^{14}C]hexadecanoate (50 μM) complexed to fatty acid-free albumin in a 5:1 molar ratio (sp act 3.7 MBq/mmol). At the appropiate time the incubations were terminated by the addition of 0.1 mL of glacial acetic acid; 50 nmol of heptadecanoyl-CoA and undecanoylcarnitine were added as internal standards. The acyl-CoA and acylcarnitine ester fractions were prepared and analyzed by radio-HPLC as described previously (14, 15). For the flux measurements the incubations were initiated by adding [U-^{14}C]hexadecanoate (40 μM) complexed to albumin. Aliquots (50 μL) were quenched at timed intervals with an equal volume of glacial acetic acid. Unreacted substrate was removed by addition of fatty acid-free albumin (0.1 mL of a 25% (wt/vol) aequous solution) followed by precipitation with 0.1 mL of 2 M perchloric acid. After centrifugation (11 000 × *g* for 5 min) radioactivity was measured in an aliquot of the supernatant by scintillation counting. For quantitation of $^{14}CO_2$ production, incubations were performed in glass vials, fitted with rubber caps, containing a 0.4-mL microcentrifuge tube inside a 1.5-mL microcentifuge tube. After quenching with 100 μL of 2 M perchloric acid, 400 μL of hyamine hydroxide (40% in methanol) was added to the central 0.4-mL microcentrifuge tube and left overnight to ensure complete trapping of $^{14}CO_2$ (18).

RESULTS

Characterization of cell permeabilization. The aim of the permeabilization protocol was to allow access of the substrates, [U-^{14}C]hexadecanoate and L-carnitine, to the mitochondrial compartment while preserving intact mitochondria. Commonly used permeabilizing agents such as digitonin (10–100 μg/mL) (19) or saponin (30–100 μg/mL) (20) caused immediate degranulation of the platelets with subsequent extensive cell aggregation. In contrast, cell aggregation did not occur with histone II-AS. The degree of permeabilization was assessed by monitoring the release of marker enzymes from the cytosol (lactate dehydrogenase) and mitochondrial matrix (citrate synthase). Approximately 75% of lactate dehydrogenase was released with histone at 3 mg/mL after 10 min (Fig. 1*A*). Higher concentrations did not produce significantly more permeabilization, whereas even at maximum histone concentration virtually all citrate synthase activity remained in the pellet, indicating that the mitochondria were intact. There was no further release of lactate dehydrogenase or citrate synthase after 10

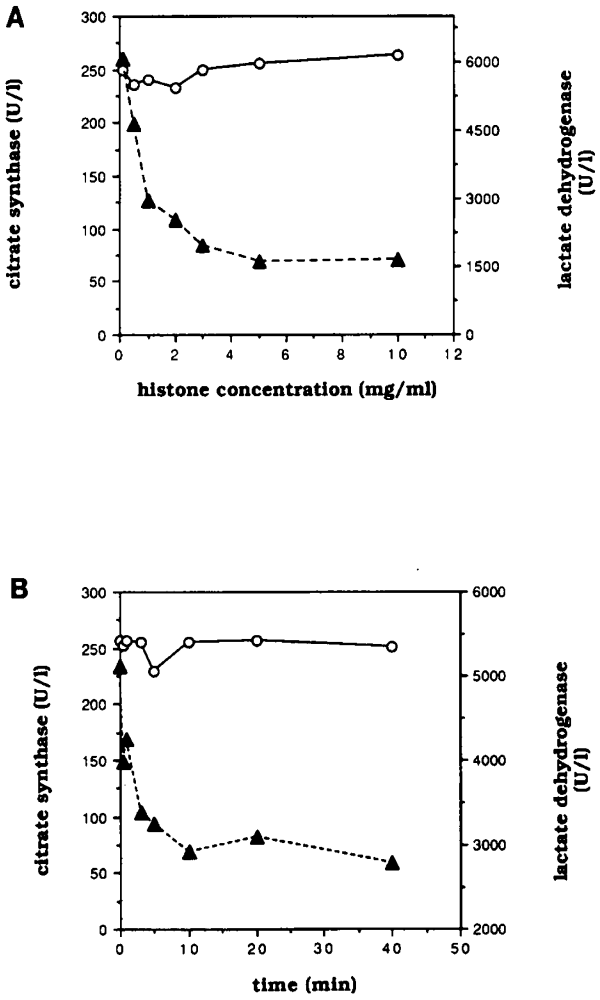

Figure 1. Release of citrate synthase and lactate dehydrogenase during histone permeabilization of blood cells. Cells were isolated from 200 mL of peripheral blood, as described, resuspended in isotonic buffer solution (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer 20 mM, EDTA 3 mM, sucrose 250 mM, pH 7.4), and divided into equal portions. Histone concentrations (*A*) and incubation time (*B*) were as indicated. After washing the cells with PBS, citrate synthase (○) and lactate dehydrogenase (▲) activities were measured in the remaining cell pellet. Complete disruption of the cells was achieved by sonication (three 5-s bursts) and incubation with Triton X-100 (5%).

Table 1. *β-Oxidation fluxes in histone-permeabilized peripheral blood cells*

Leukocytes from	β-Oxidation flux*
Controls (n = 14)	7.1 ± 2.3
Controls in the presence of etomoxir (50 μM) (n = 3)	0.58 ± 0.18
Controls in the presence of rotenone (2.5 mg/mL), antimycin (2.5 mg/mL), cyanide (1 mM) (n = 3)	0.50 ± 0.25
VLCAD deficiency	1.0
Trifunctional enzyme deficiency	1.1
MCAD deficiency	4.0
CPT deficiency	1.2

* Values are expressed as nmol C_2 units formed per minute per International Unit of citrate synthase activity; expressed as mean ± SD.

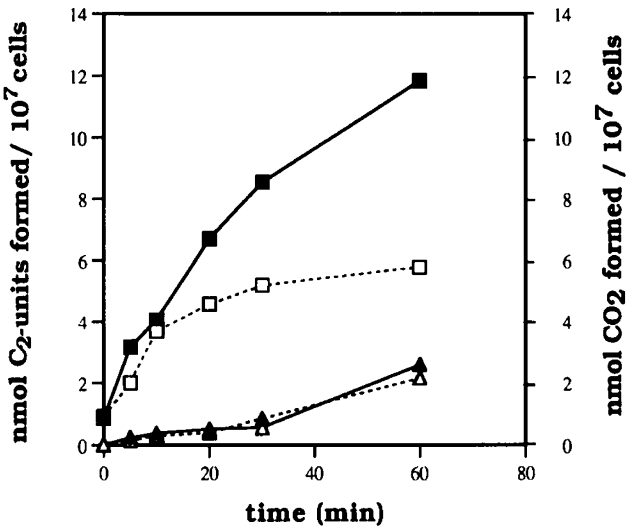

Figure 2. β-Oxidation flux in intact and permeabilized blood cells. β-Oxidation flux, expressed as acid-soluble metabolites (■/□) and $^{14}CO_2$ (▲/△), formed in histone-permeabilized (■/▲) and untreated (□/△) blood cells. Cell isolation, permeabilization, and measurements of acid-soluble radioactivity and $^{14}CO_2$ production were performed as described in Methods.

min at a histone concentration of 3 mg/mL (Fig. 1*B*). A standard permeabilization procedure (histone 3 mg/mL for 10 min) was used for all further experiments.

Flux through β-oxidation. The rate of β-oxidation flux, measured as total acid-soluble radioactivity, was 7.1 ± 2.3 nmol C_2 units formed per minute per International Unit of citrate synthase activity (mean ± SD for 14 controls) (Table 1). The increased availability of hexadecanoate and L-carnitine in the permeabilized system compared with intact cells leads to a higher production of acid-soluble radioactive metabolites, whereas the amounts of $^{14}CO_2$ produced are similar in both systems (Fig. 2). β-Oxidation of hexadecanoate was markedly inhibited by the absence of L-carnitine, the presence of respiratory chain inhibitors, or etomoxir (R,S-2[-6(4-chlorophenoxy)hexyl]oxirane-2-carboxylate), an inhibitor of CPT-I (Table 1, Fig. 3). The accumulation of acid-soluble metabolites was linear for 60–90 min (Fig. 3).

Intermediates of β-oxidation in peripheral blood cells. In both intact and permeabilized blood cells, either no acyl-CoA ester intermediates or only a small peak corresponding to hexadecanoyl-CoA could be detected (results not shown). In intact whole cells no acylcarnitines could be detected, whereas in histone-permeabilized cells acylcarnitine esters can be identified easily. During a time course for the formation of acylcarnitine ester intermediates, a gradual decrease in the amount of the long-chain acylcarnitines was associated with an increase in the amounts of the shorter chain length acylcarnitines (Fig. 4, Table 2). This change in the proportion of acylcarnitines means that steady state concentrations of acylcarnitines were not seen despite incubating the permeabilized cells for up to 4 h. Saturated carnitine esters were the major intermediates, but, in addition, substantial amounts of chain-shortened 3-hydroxyacylcarnitines and 2-enoylcarnitines were formed (Fig. 4, Table 2). Because of the limited chromatographic resolution of C_n-3-hydroxyacylcarnitines and C_{n-2}-2-enoylcarnitines, the amount of each compound could not be calculated separately (Table 2). To determine whether the cell preparation or methodology was responsible for this unexpected formation of 3-hydroxyacyl- and 2-enoylcarnitine esters, we isolated mito-

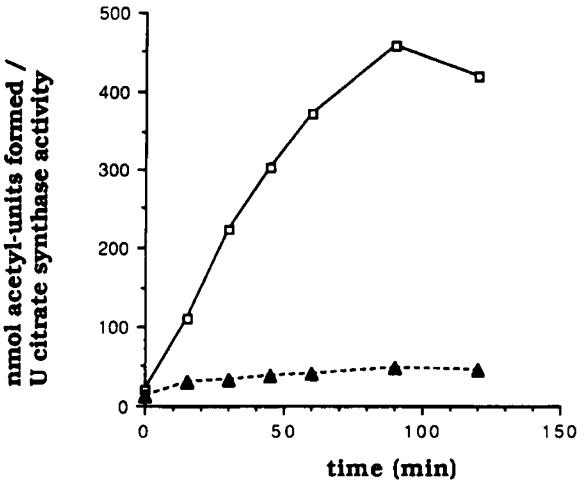

Figure 3. β-Oxidation of hexadecanoate in blood cells as a function of time. Blood cells were isolated and incubated as described in Methods. Acid-soluble material was quantitated as described in the presence (□) and the absence (▲) of L-carnitine (2.5 mM).

chondria from leukocytes and platelets from 150 mL of blood using the method described by Singh-Kler *et al.* (15) for the preparation of fibroblast mitochondria. After incubation of these mitochondria with [U-^{14}C]hexadecanoate, 3-hydroxyacyl- and 2-enoylcarnitine esters were formed in quantities similar to those seen in the permeabilized cells (results not shown).

Mitochondrial fatty acid oxidation flux and intermediates in blood cells from patients. The rate of β-oxidation was measured in four patients with defects of VLCAD, MCAD, CPT-II, and the trifunctional protein. The oxidation of [U-^{14}C]hexadecanoate was significantly slower in blood cells from the patients with long-chain defects, whereas flux was normal in cells from the patient with MCAD deficiency (Table 1).

For studies comparing the acylcarnitines formed in permeabilized cells from controls (Fig. 5*A*) and patients, the reaction was quenched at 1 h, because flux was linear with time during this period. In CPT-II deficiency only hexadecanoylcarnitine was found (Fig. 5*B*, Table 3). In blood cells from the patient with VLCAD deficiency, only hexadecanoyl-, tetradecanoyl-, and a small amount of 3-hydroxyhexadecanoyl-carnitine were detected (Fig. 5*C*, Table 3). In blood cells from a patient with a trifunctional enzyme deficiency, there was a greater amount of 3-hydroxyhexadecanoylcarnitine compared with hexadecanoylcarnitine (Fig. 5*D*, Table 3). The intermediates seen in MCAD deficiency are also characteristic, showing increased amounts of medium-chain acylcarnitines, mainly decanoyl- and octanoyl-carnitine. The concentrations of hexadecanoyl- and tetradecanoyl-carnitine were normal (Fig. 5*E*, Table 3).

DISCUSSION

Permeabilization of blood cells by histone. To avoid preparation of a mitochondrial fraction from peripheral blood cells, which is impractical if small amounts of blood are used, we selectively permeabilized the cells while preserving mitochondrial intactness. With platelets, commonly used permeabilizing agents such as digitonin (19) or saponin (20) lead to immediate

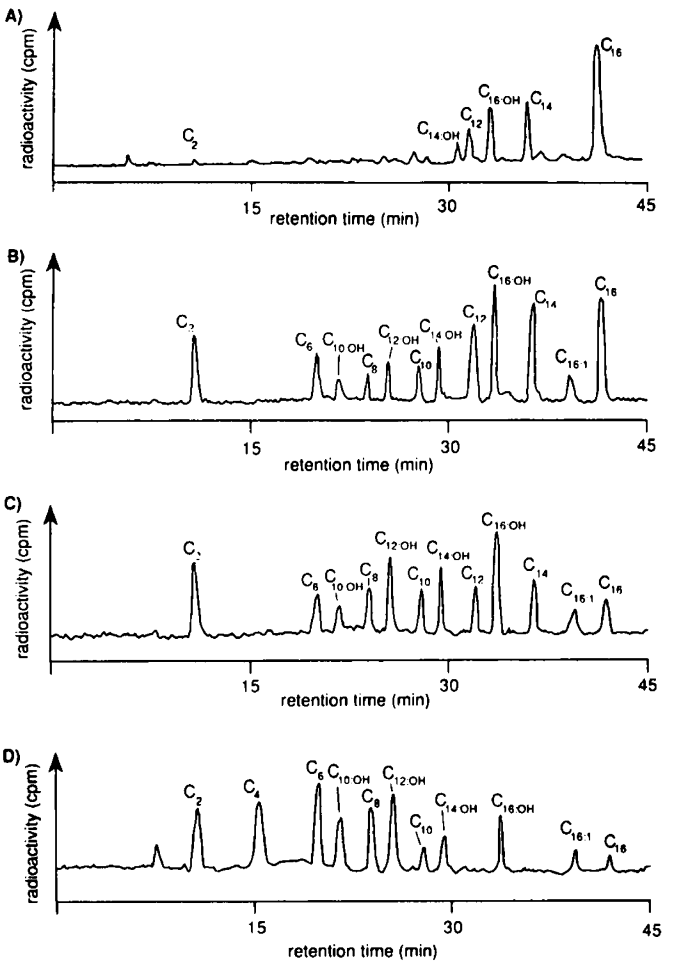

Figure 4. Formation of acylcarnitine intermediates from [U-^{14}C]hexadecanoate by blood cells as a function of time. Incubations were carried out as described and were quenched after 0.5 h (*A*), 1 h (*B*), 2 h (*C*), and 4 h (*D*). The identities of the peaks are: C_{16}, hexadecanoylcarnitine; $C_{16:1}$, hexadec-2-enoylcarnitine; C_{14}, tetradecanoylcarnitine; $C_{16:OH}$, 3-hydroxy-hexadecanoylcarnitine + tetradec-2-enoylcarnitine; C_{12}, dodecanoylcarnitine; $C_{14:OH}$, 3-hydroxytetradecanoylcarnitine + dodec-2-enoylcarnitine; C_{10}, decanoylcarnitine; $C_{12:OH}$, 3-hydroxydodecanoylcarnitine + dec-2-enoylcarnitine; C_8, octanoylcarnitine; $C_{10:OH}$, 3-hydroxydecanoylcarnitine + oct-2-enoylcarnitine; C_6, hexanoylcarnitine; C_4, butyrylcarnitine; and C_2, acetylcarnitine.

degranulation and aggregation unless aggregation inhibitors are applied. Therefore, we used the polycationic protein histone II-AS as a novel permeabilizing agent. Histone effectively permeabilized the cell membranes without disrupting the mitochondria, as shown by cytosolic (lactate dehydrogenase) and mitochondrial (citrate synthase) marker enzymes. Mitochondrial integrity was preserved over a wide range of histone concentrations, thus avoiding the need for adjustments to the protein concentration of the sample before permeabilization. Furthermore, no platelet aggregation occurred, presumably because the platelets became positively charged and repelled each other when the polycationic histone molecules bound to the platelet membrane.

Measurement of flux through β-oxidation. Because $^{14}CO_2$ release from radiolabeled fatty acids represents only a minor product of β-oxidation (21, 22), we determined β-oxidation flux as the amount of acid-soluble metabolites formed. In both intact and permeabilized blood cells, the majority of β-oxida-

Table 2. *Time course of acylcarnitine ester formation in peripheral blood cells from a control*

Time (h)	C_{16}	$C_{16:1}$	$C_{16:OH}$ + $C_{14:1}$	C_{14}	$C_{14:OH}$ + $C_{12:1}$	C_{12}	$C_{12:OH}$ + $C_{10:1}$	C_{10}	C_8	C_6	C_2
0.5	16.3	0.94	4.9	6.1	1.0	4.0	0.1	1.4	0.8	1.4	6.2
1	12.1	3.6	12.1	10.8	5.8	9.4	4.8	5.5	5.1	16.2	60.1
2	4.1	3.4	9.7	5.0	6.2	5.0	11.7	5.8	6.6	12.8	73.5
4	0.9	1.9	2.7	1.2	2.9	1.2	7.3	2.2	8.1	14.5	45.1

All values are expressed as nmol acetyl units formed per International Unit of citrate synthase activity.

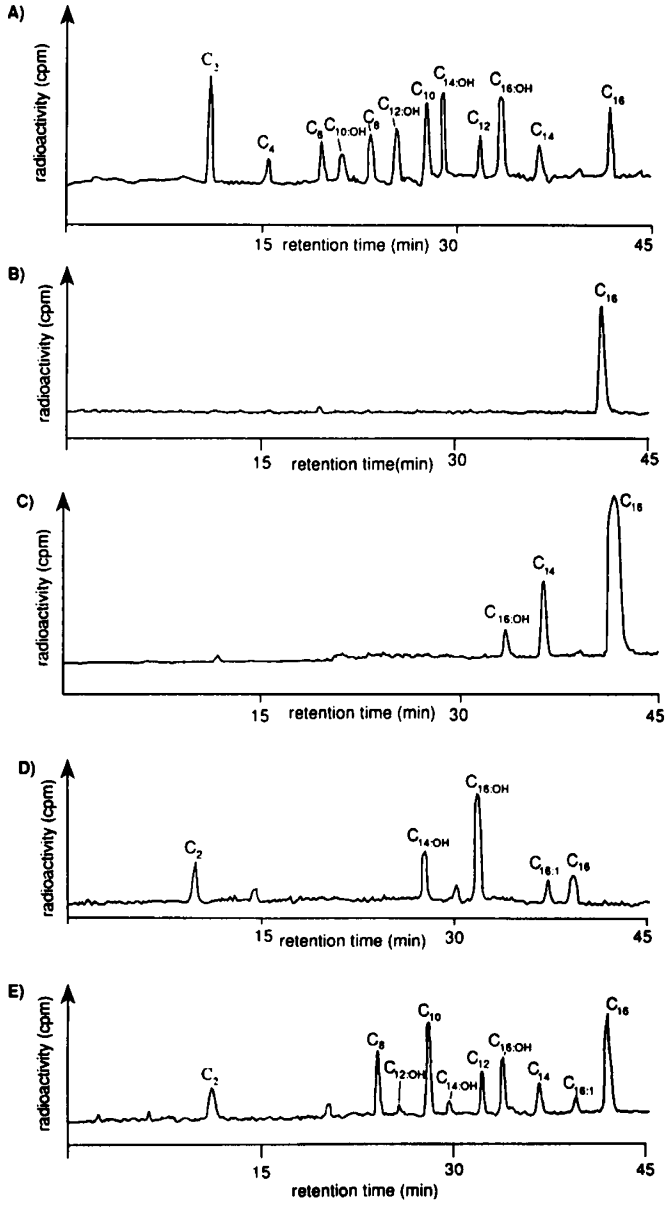

Figure 5. Radiochromatograms of acylcarnitine esters resulting from the incubation of permeabilized blood cells from patients and controls with [U-^{14}C]hexadecanoate. *A*, Healthy control; *B*, CPT deficiency; *C*, VLCAD deficiency; *D*, trifunctional enzyme deficiency; and *E*) MCAD deficiency. The peak identities were the same as for Figure 4.

tion products were acid soluble (Fig. 2). Similar results have also been found recently in a whole-cell leukocyte preparation (23). Interestingly, whereas histone permeabilization stimulated the formation of acid-soluble metabolites, the amount of $^{14}CO_2$ formed was similar to that in intact cells (Fig. 2). Presumably, in the permeabilized cells there is an increased amount of L-carnitine available, thus promoting the formation of acylcarnitine esters. These acylcarnitines are able to diffuse out of the cells when they are permeabilized. Thus, proportionally fewer acetyl units are available for oxidation by the tricarboxylic acid cycle in permeabilized cells compared with intact cells.

Intermediates of β-oxidation. By incubating histone-permeabilized blood cells in the presence of carnitine, chain-shortened acyl-CoA esters are converted to the corresponding acylcarnitine esters by the action of the carnitine acyltransferases. In contrast to acyl-CoA esters, which are restricted to the mitochondrial matrix, acylcarnitine esters are distributed via carnitine-acylcarnitine translocase throughout the incubation mixture. This explains why acylcarnitine esters, but not acyl-CoA esters, can be detected in our permeabilized cell system.

The only intermediates seen in incubations with human fibroblast (15) and muscle (4, 24) mitochondria from healthy subjects were saturated acyl-CoA and acylcarnitine esters. Therefore, the production of 3-hydroxyacylcarnitine and 2-enoylcarnitine esters in peripheral blood cells was a surprising finding. Disturbance of the mitochondrial architecture or inhibition of β-oxidation enzymes by the permeabilization procedure was unlikely to be responsible for the accumulation of these unusual metabolites, because untreated isolated mitochondria from peripheral blood cells gave a similar pattern of intermediates. Moreover, the incubations were performed under conditions identical to those described for muscle (4, 24) and fibroblast (15) mitochondria, in which only saturated intermediates are generated. Our preparation of peripheral blood cells contained a mixture of platelets and leukocytes. In incubations with permeabilized lymphocytes, only saturated acylcarnitine esters were detected (results not shown), suggesting that the 3-hydroxyacyl- and 2-enoylcarnitine esters are formed in the platelets. This may occur because complex I activity in platelet mitochondria is low compared with the other respiratory chain complexes (25), which would result in a low NAD^+/NADH ratio and thus inhibit the 3-hydroxyacyl-CoA dehydrogenases. Alternatively, because platelets do not possess the ability to synthesize new proteins, their mitochondria may selectively become depleted of enzymes with a high turnover rate, resulting in an apparent partial deficiency of enzymes involved in β-oxidation or NADH reoxidation.

β-Oxidation in peripheral blood cells from patients. The potential value of measuring β-oxidation flux and intermediates in blood cells was demonstrated in four patients with proven or suspected defects of β-oxidation. In the blood cells

Table 3. *Acylcarnitines in peripheral blood cells from patients and controls*

	C_{16}	$C_{16:1}$	$C_{16:OH}$ + $C_{14:1}$	C_{14}	$C_{14:OH}$ + $C_{12:1}$	C_{12}	$C_{12:OH}$ + $C_{10:1}$	C_{10}	C_8	C_6	C_2
Controls											
1	33.3	5.4	22.5	16.7	6.2	8.3	5.7	2.9	2.7	2.4	43.5
2	33.1	7.7	34.0	19.4	10.2	11.9	6.6	3.9		4.8	30.0
3	12.1	3.6	12.1	10.8	5.8	9.4	4.8	5.5	5.1	10.2	60.1
4	7.4	2.2	8.0	5.8	2.2	3.4	2.6	2.5	2.7	3.3	67.6
5	17.9	3.7	16.7	10.5	4.7	6.9	3.4	2.7	5.0	7.1	131.3
Mean	20.7	4.5	18.7	12.6	5.8	8.0	4.6	3.5	3.9	5.6	66.5
(SD)	(11.9)	(2.1)	(10.1)	(5.4)	(2.9)	(3.2)	(1.6)	(1.2)	(1.3)	(3.0)	(39.0)
Patients											
CPT	22.7										
VLCAD	62.5		4.1	11.3							
TFP	1.2	2.4	8.0	1.0	2.4		1.4				44.6
MCAD	30.7	4.6	14.4	8.6	3.2	11.0	3.4	30.1	25.9	8.7	111.6

All values are expressed as nmol acetyl units formed per International Unit of citrate synthase activity and represent the amount of acylcarnitine esters formed after 1 h of incubation. TFP, trifunctional enzyme deficiency.

from three patients with long-chain fatty acid oxidation defects, there was slow flux through β-oxidation. In MCAD deficiency, β-oxidation flux was within normal range because medium-chain acyl-CoA and acylcarnitine esters are acid soluble. Thus, although slow β-oxidation flux is an important indicator of a defect of long-chain fatty acid oxidation, defects of the medium-chain and short-chain specific enzymes are not detectable by simple flux measurements.

The intermediates of β-oxidation observed in permeabilized cells from patients proved to be specific for the site of the defect. Indeed, two of these patients (trifunctional enzyme deficiency and CPT-II deficiency) were diagnosed by this method. The diagnosis of trifunctional enzyme deficiency can be made on the basis of slow overall flux, lack of significant chain-shortening, and an abnormal ratio of saturated to 3-hydroxyacylcarnitines. In VLCAD deficiency there was again no significant chain shortening, but the ratio of saturated to 3-hydroxyacylcarnitines was far greater than in control subjects or in the patient with trifunctional enzyme deficiency. This pattern of acylcarnitines is consistent with a block at the site of VLCAD, ETF, or ETF-dehydrogenase. In CPT-II deficiency the only acylcarnitine detected was hexadecanoylcarnitine due to impaired conversion of this compound to the corresponding acyl-CoA. The absence of 3-hydroxyhexadecanoylcarnitine and tetradecanoylcarnitine allows differentiation between this deficiency and that involving VLCAD. In MCAD deficiency the characteristic features were increased amounts of decanoyl- and octanoylcarnitine, with analysis showing marginally higher concentrations of C_{10}-products than C_8-products (Table 3, Fig. 5*E*). This finding is consistent with observations in fibroblast mitochondria from MCAD-deficient patients (15). An additional interesting finding is the normal amount of acetylcarnitine in both the patient with MCAD deficiency and trifunctional protein deficiency. This result is similar to that observed in fibroblast mitochondria from patients with these defects (15). We believe that this is caused by inhibition of either the tricarboxylic acid cycle or the respiratory chain by the accumulating acyl-CoA and acylcarnitine esters.

In conclusion we have shown that permeabilized blood cells oxidize fatty acids but produce a different pattern of intermediates to other tissues. However, the measurement of fatty acid oxidation flux and intermediates permits the diagnosis of the four most common β-oxidation defects using peripheral blood cells. This technique avoids the need for invasive procedures such as a muscle biopsy or the time and expense involved in preparing cultured cells.

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