# Carnitine-Acylcarnitine Translocase Deficiency with Severe Hypoglycemia and Auriculo Ventricular Block

## Translocase Assay in Permeabilized Fibroblasts

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## Abstract

**Deficiency of the enzymes of mitochondrial fatty acid oxidation and related carnitine dependent steps have been shown to be one of the causes of the fasting-induced hypoketotic hypoglycemia. We describe here carnitine–acylcarnitine translocase deficiency in a neonate who died eight days after birth. The proband showed severe fasting-induced hypoketotic hypoglycemia, high plasma creatine kinase, heartbeat disorder, hypothermia, and hyperammonemia. The plasma-free carnitine on day three was only 3 $\mu$M, and 92% of the total carnitine (37 $\mu$M) was present as acylcarnitine. Treatments with intravenous glucose, carnitine, and medium-chain triglycerides had been tried without improvements. Measurements in fibroblasts confirmed deficient oxidation of palmitate and showed normal activities of the carnitine palmitoyltransferases I and II and of the three acyl-CoA dehydrogenases. A total deficiency of the carnitine–acylcarnitine translocase was found in fibroblasts using the carnitine acetylation assay (1986. *Biochem. J.* 236:143–148). This assay has been further simplified by seeking conditions permitting application to permeabilized fibroblasts and lymphocytes. (*J. Clin. Invest.* 1993. 91:1247–1252.) Key words: carnitine–acylcarnitine translocase • permeabilized fibroblasts • hypoketotic hypoglycemia • ventricular arrhythmia • fatty acid oxidation defect**

## Introduction

Long-chain fatty acid oxidation contributes to energy homeostasis, especially in heart, liver, and skeletal muscle. In the myocardium, long-chain fatty acids are the preferred substrates in the resting state (1). In the liver, oxidation of long-chain fatty acid produces ketone bodies, enhances gluconeogenesis, and thereby allows the maintenance of normoglycemia during fasting (2). In the skeletal muscle, oxidation of long-chain fatty acid is the major source of energy both in the resting state (3) and during prolonged exercise. Inborn errors of mitochondrial long-chain fatty acid oxidation give rise to a number of symptoms, mostly hypoketotic hypoglycemia, cardiac injury, and skeletal myopathy. These symptoms result from an abnormal metabolite buildup proximal to the enzyme defect and from deficient formation of energy-yielding substrates after the block (4). 10 hereditary defects of mitochondrial long-chain fatty acid catabolism have been described with various clinical phenotypes, ranging from malformations and sudden infant death to nearly asymptomatic adult. Six of these involve enzymes of intramitochondrial beta-oxidation itself, namely long-chain acylCoA dehydrogenase (5), 3-hydroxy long-chain acylCoA dehydrogenase (6), electron transfer flavoprotein (7), electron transfer flavoprotein dehydrogenase (7), trifunctional enzyme (8) and 2-4 dienoylCoA reductase (9). Four others affect the carnitine shuttle for the transport of long-chain fatty acids into the mitochondria; these involve either intracellular uptake of carnitine (10) or the enzymes carnitine palmitoyl transferase (CPT) I and II[1] (11–13). The possibility that a deficiency of the carnitine–acylcarnitine translocase might occur has been speculated for some time, but direct evidence has been obtained in only one case recently (14). The carnitine–acylcarnitine translocase is an inner mitochondrial membrane protein that allows the passage of carnitine and acylcarnitines across the inner mitochondrial membrane (15). Herein, we report a neonatal case of carnitine–acylcarnitine translocase deficiency with severe hypoketotic hypoglycemia, hyperammonemia, and auriculo-ventricular block. In all cases of long-chain fatty acid oxidation disorders, it is interesting to correlate the clinical expression of the different defects to the metabolic profile and to the residual enzyme activity. In this regard, we note that the symptomatology of the present near-total translocase-deficient patient closely matches that seen in the infantile form of CPT II deficiency where a severe loss of the CPT II activity occurs (13).

## Methods

*Case report.* C.O. was a male infant, weighing 2.6 kg, 51 cm long, with a 33 cm head circumference, born after an uncomplicated full-term

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1. *Abbreviation used in this paper:* CPT, carnitine palmitoyltransferase.

pregnancy and normal delivery to healthy Turkish parents who are first cousins. Two living brothers born in 1975 and in 1990 are healthy. Two other sibs, a boy and a girl, died within the first six months of life in Turkey; their clinical and medical data were unavailable.

Breast-feeding was started immediately after birth. At 36 h of life, the infant was found hypothermic (temperature 34.5°C), pale, without reactivity, and severely hypoglycemic (dextrostix nil). After intravenous administration of glucose was started, he was transferred to the intensive care unit. At admission, temperature was 35.4°C. The general condition was very serious, with diaphanous gray, mottled skin, profuse sweating, moderate general hypotonia with fluctuating muscle, hypo- and hypertonicity but normal consciousness, normal provoked reactivity, normal cry, normal primitive reflexes, and no abnormal movements. Heart rate was regular (140/min) with normal peripheral pulses; blood pressure was 40 mmHg. There was no hepatosplenomegaly. Initial laboratory data, at admission, (normal values in parentheses) were: plasma glucose 2.24 mmol/liter (3.5–6 mmol/liter), calcium 2 mmol/liter (2–2.6), normal blood electrolytes, arterial pH 7.40, $PCO_2$ 34 mmHg, bicarbonate 22 meq/liter, normal blood cell count. After a 1-h initial improvement while receiving continuously 10% glucose i.v., 15 ml 20% albumin solution i.v. and 40 ml vamine (10% glucose and 7% amino acid solution), hypoglycemia fell again to 1 mmol/liter with gray-colored skin, generalized hypotonia, bradycardia at 65/min, first degree auriculo-ventricular block (PR 200 ms) and left bundle branch block (see Fig. 1). After increasing glucose infusion rate, hypoglycemia normalized, clinical condition improved, and EKG was normalized within 1 h. Chest x-ray and cardiac echography were found normal without cardiomyopathy. Cardiologists concluded that there was a paroxysmal heartbeat disorder, secondary-to-severe hypoglycemia, and hypothermia.

At 48 h of life, alimentation was started with a special infantile formula, delivered in eight meals 20 ml each and providing in total 4 g protein, 12 g maltodextrins, 3 g long-chain triglycerides, and 3 g medium-chain triglycerides. In addition, he continued to receive 250 ml 5% serum glucose i.v. and 50 ml of vamine (10% glucose and 7% amino acid solution) providing in total 65 usable calories per kg.

At day 3, a new episode of hypoglycemia (0.7 mmol/liter) with hypotonia, sweating, and gray skin was noted with negative ketone in urine. Increasing glucose infusion from 250 to 350 ml allowed a rapid and definitive correction of hypoglycemia. Despite this normalization, the patient's clinical state deteriorated. Hyperpnea, lethargy and then coma, truncal and limbs hypertonia, and absence of reactivity progressively developed. Biological data included (normal in parentheses): pH 7.5, $PCO_2$ 24 mm Hg, bicarbonate 19 meq/liter, ammonia 491 μmol/liter (< 50), blood lactate 2.6 mmol/liter (< 2), normal blood glucose, electrolytes, fibrinogen and clotting factors, alanine amino transferase 196 IU/liter (5–25). Creatine kinase was 4,595 IU/liter (15–100) with isozyme MB being 6.7% of total (< 3%) indicating muscle damage. Total plasma carnitine level was 37 μmol/liter (28–71) with esterified/total ratio 92% (< 30%). Plasma and urine amino acid chromatography was normal, and so was the organic acid profile determined by GC-MS in analyses performed at the time patient was hyperammonemic. Orotic acid excretion was 2.7 μmol/mmol creatinine (0.5–3). Acetest was negative. At this time, urea cycle defect was considered, and patient began treatment with arginine, sodium benzoate, carnitine, and protein withdrawal.

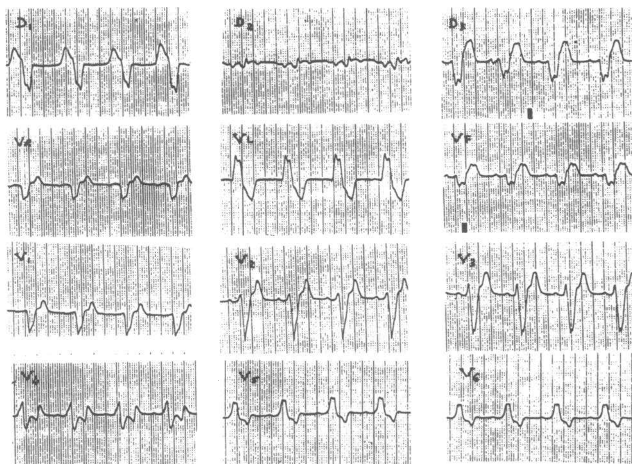

*Figure 1.* EKG: First degree auriculoventricular block (PR 200 ms) and left bundle branch block.

Despite supportive measures and progressively decreasing ammonemia (from 491 to 73 μmol/liter) clinical conditioned worsened. Hepatomegaly and bilateral nephromegaly (with normal echostructure and without cysts) appeared at day 7 with severe anemia and uncontrolled metabolic acidosis (pH 7.15, bicarbonates 8 meq, $PCO_2$ 26 mm Hg), whereas cardiac function remained normal.

Patient died at 8 d of age with pulmonary hemorrhage. 6 h before death, lactate was 1.6 mmol/liter and ammonia 73 μmol/liter, and organic acid profile was normal. A postmortem needle biopsy of muscle and liver was performed. Liver displayed a massive macrovesicular steatosis. Muscle was normal. Parents refused necropsy.

*Cell lines.* Cultured skin fibroblasts were obtained from controls and patients with deficiencies of CPT I, CPT II, long chain acyl-CoA dehydrogenase, and medium chain acyl-CoA dehydrogenase. The cells were cultured in RPMI 1640 ($^{14}C$ fatty acid oxidation) or in HAM F10 ([9,10(n)-$^3H$]palmitate oxidation and translocase assay). Both media were supplemented with antibiotics, 2.5 mM glutamine and 10% fetal calf serum.

*Fatty acid oxidation in fibroblasts.* $^{14}CO_2$ production from 1-$^{14}C$-fatty acids was measured with 100 μM [1-$^{14}C$]palmitate, and 1 mM [1-$^{14}C$]octanoate as previously described (16). Incubation time was 4 h. Tritiated water release experiments from [9,10(n)-$^3H$]palmitate were performed in 24 well microtiter plates, as described in (17). Briefly, 50,000 cells per well were plated in HAM F10 medium. After 48 h, the cells were washed, and the monolayers were incubated with 100 μM [9,10(n)-$^3H$]palmitate (specific activity 75 Ci/mol) bound to albumin (0.5 mg/ml) in 0.2 ml of Dulbecco's phosphate buffered saline with 0.9 mM $Ca^{2+}$ and 0.5 mM $Mg^{2+}$. Blank wells without cells were run in parallel. Tritiated water was recovered by ion exchange treatment on Dowex-1 columns (17). The activity showed linearity with 10 to 50 μg protein per well for up to at least 4 h of incubation.

*Carnitine–acylcarnitine translocase assay.* The method as described in (18) was used with modifications to allow application to permeabilized fibroblasts and to further simplify the procedure (see Results). Digitonin was used as the permeabilizing agent. To offer protection against protease interference during assay incubations, the fibroblasts were harvested with only 200 μg of trypsin and 100 μg EDTA per ml. The cells were washed twice with phosphate buffered saline and once with 50 mM Tris–150 mM KCl, pH 7.4, the cells were suspended in medium (225 mM mannitol–25 mM Hepes, pH 7.4–50 μM EDTA) containing aprotinin (0.2 mg/ml), and, during assays, bovine serum albumin (0.2 mg/ml) was included. In brief, the assay system at 30°C in a final volume of 200 μl contained 225 mM mannitol, 25 mM Hepes, pH 7.4, 50 μM EDTA, 3 mM ADP, 5 mM potassium phosphate, pH 7.4, 2 mM dichloroacetate, 2 mM malonate, 200 μM [2-$^{14}C$]pyruvate (0.1 μCi), 0.5 mM, or as shown, carnitine, 4 μg digitonin, and 0.4 mg fatty acid free bovine serum albumin. Reactions were started by the addition of fibroblast suspension. Incubations were for 60 min or as shown. Reactions were stopped by the addition of 200 μl of a mixture of methanol (195 μl) and of 1 M sulfobetaine$_8$ (5 μl, pH near 7.4). Control tubes differed in having sulfobetaine$_8$ present from the beginning of incubations and the reactions in these tubes were stopped with 200 μl of methanol. After mixing and brief centrifugation, 350 μl of the supernatant was applied on a Pasteur pipette column (5 mm × 40 mm, in water) of AG 2-X 8, 200–400 mesh, $Cl^-$ exchanger

resin. 60% methanol (0.5 ml four times) was passed through the column, and all the eluents were collected in a scintillation vial to which was added 8 ml of ACS II scintillant of Amersham and radioactivity measured.

*Measurement of pyruvate oxidase activity.* Incubation system was the same as that for the translocase above except that carnitine and malonate were replaced by 3 mM malate. Controls were without fibroblasts. Reactions were stopped by the addition 50 μl of a freshly prepared chilled equal volume mixture of 10% Triton X-100 and ~ 240 mM hydrogen peroxide. After vortexing and incubation for at least 5 min, 200 μl were transferred to glass vials containing 30 μl of a mixture of 6 M HCl and 2% acetic acid. After being mixed, the vials were left on a block heater at moderate temperature and the contents dried under a stream of nitrogen. The dried residue was dissolved in 500 μl water and its radioactivity was determined. This pyruvate oxidation monitoring procedure, validity of which was checked, is a simplification of the procedure described earlier (18).

*Carnitine acetyltransferase activity.* The assay was as in (18) with minor changes. In brief, the incubation system, in a final volume of 100 μl contained: 50 mM Hepes, pH 7.4, 5 mM carnitine, 300 μM [$^{14}$C]-acetyl-CoA, 0.5% (vol/vol) Triton X-100, and 4–10 μg of fibroblast protein (none in controls). Incubations were for 60 min at 30°C. Linearity was observed for up to about 10% acetyl-CoA used up. Reactions were stopped by adding 800 μl of chilled water and about 300±20 mg of AG 2-X 8 resin ($Cl^-$ form, 200–400 mesh); for convenience, resin addition was made using a 1-ml tuberculin syringe as in (19). The tubes were vortexed twice, left for about 10 min, briefly centrifuged; the radioactivity of the supernatant was then determined.

*Protein estimation.* In the fatty acid oxidation experiments, protein content of each well was determined (20) after solubilization of the cell material in 1 M NaOH. During assay of the translocase, protein was estimated using the BCA reagent of Pierce Chemical Co. (Rockford, IL); the supplier's protocol was followed with the following modification. The fibroblast suspension in mannitol-Hepes was supplemented with Triton X-100 (1%, vol/vol), and after vigorous vortexing the tubes were left at room temperature for at least 10 min to solubilize protein prior to the addition of the BCA reagent. Mannitol-Hepes and Triton X-100 were included both in the reagent blanks and the bovine serum albumin standards.

*Chemicals.* Carnitine was a gift from Sigma-Tau (Pomezia, Italy); [2-$^{14}$C]pyruvate, [1-$^{14}$C]palmitic acid, [1-$^{14}$C]octanoate were from New England Nuclear Co (FRG). [9,10(n)-$^{3}$H]palmitic acid was from Amersham (France). Most other chemicals, including digitonin (catalog No. D-5628, lot 16F-01191), fatty acid-free fraction V bovine serum albumin, and Dowex-1-anion exchange resin, were from Sigma Chemical Co. (St. Louis, MO).

## Results

The severe hypoketotic hypoglycemia in the proband pointed to a likely defect in fatty acid oxidation. Measurements of fatty acid oxidation in the patient's fibroblasts confirmed this; the production of $^{14}CO_2$ from [1-$^{14}$C]palmitate and $^{3}H_2O$ release from [9,10(n)-$^{3}$H]palmitate were decreased by more than 95% of the mean control value (Table I). The oxidation of [1-$^{14}$C]octanoate, which does not require carnitine-dependent steps, was normal. The impaired palmitate oxidation was not corrected by the inclusion of 1 mM carnitine (data not shown). The possibility of systemic or severe carnitine deficiency in the proband was further excluded by the observation that the total plasma carnitine level was 37 μM. However, an unusually high fraction (92%) of the total carnitine was present as acylcarnitine (see case report). Measurements of the CPT activities in the fibroblasts of the proband showed that the activities of both CPT I and II, assayed as described (13), were normal (data not shown). The possibility of acyl-CoA dehydrogenase deficiency was excluded as analyses showed normal activities of the long chain, the medium chain, and the short chain acyl-CoA dehydrogenases. We examined the possibility of carnitine–acylcarnitine translocase deficiency as Stanley et al. (14) have identified such a deficiency recently.

*Table I. Fatty Acid Oxidation in the Fibroblasts of the Patient and Controls*

| | Monitored as the production of | | |
|---|---|---|---|
| | $^{14}CO_2$ from [1-$^{14}$C]palmitate | $^{14}CO_2$ from [1-$^{14}$C]octanoate | $^{3}H_2O$ from 9,10 *n*[$^{3}$H]palmitate |
| | *nmol product formed/mg protein per h* | | |
| Patient | 0.03; 0.03 | 1.61 | 0.08 |
| Control | 2.11±0.73 | 1.72±0.78 | 9.39±1.70 |
| | (1.24–3.18) | (1.04–3.33) | (6.75–13.40) |
| | (*n* = 12) | (*n* = 12) | (*n* = 12) |

Results are means of triplicate determinations in the patient's cells. Control values are mean ± SD with the number of different cell lines shown in parentheses.

For the assay of carnitine–acylcarnitine translocase clinically, measurement of pyruvate-dependent acetylation of added carnitine is presently the only available method (18). In applying this method to fibroblasts, we felt it necessary to ascertain its reliability with fibroblasts, as this method was initially elaborated and tested with muscles. We began by assaying the carnitine–acylcarnitine translocase in homogenates of fibroblasts but found that cell permeabilization with digitonin simplified the procedure, the latter lessened both the time and the quantity of fibroblasts needed. Controlled cell permeabilization, by not damaging mitochondria, likely enabled participation of most mitochondria of the cell in the assay, which is not feasible in procedures entailing homogenization. The optimum digitonin concentration found for carnitine acetylation was 3–6 μg per tube with 10 to 50 μg fibroblast protein. Inhibition by excess digitonin became noticeable at > 9 μg digitonin with 50% inhibition resulting at ~ 25 μg digitonin. The translocase activity measured in the absence of digitonin was ~ 8% of that found with the optimal digitonin; it showed complete dependence on added carnitine and full inhibition by sulfobetaine$_8$. This slight activity without digitonin likely arose from the partial permeabilization of fibroblasts that the use of trypsin for cell harvesting is known to cause (21).

In earlier experiments, we allowed a 15-min prior incubation of the fibroblast with digitonin at 30°C to allow complete cell permeabilization before the commencement of the translocase assay by pyruvate addition. Subsequent work showed that identical results were obtained regardless of whether prior incubations were allowed or not. The assay procedure was therefore simplified by adding fibroblasts directly to an otherwise complete incubation system. Good linearity was obtained with respect to the quantity of the fibroblast protein and the assay time under these conditions (Fig. 2).

The following additional observations helped establish that the carnitine acetylation approach could reliably be used for the assay of translocase in fibroblasts (*a*) the activity was totally

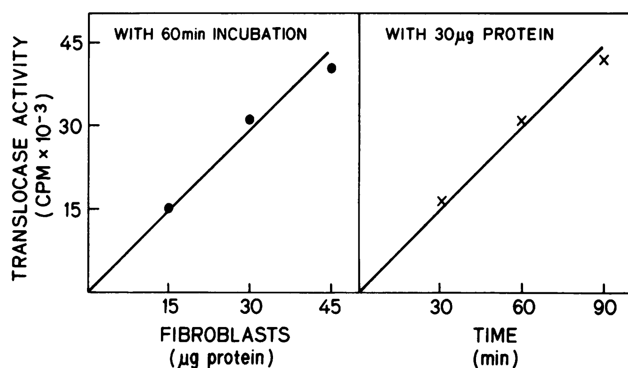


*Figure 2.* Linearity during the assay of carnitine–acylcarnitine translocase in permeabilized fibroblasts. Incubation conditions were as described in Methods. Reactions were started by the addition of fibroblasts.

dependent on added carnitine, (*b*) the 0.5 mM carnitine that was recommended as a below saturating concentration during assay with human muscle homogenates to ensure that the translocase activity itself limited the rates of carnitine acetylation (18) was also found subsaturating with fibroblasts (activity was much higher with 10 mM carnitine, Table II), (*c*) the activity was fully inhibited by sulfobetaine$_8$, a translocase inhibitor (22), (*d*) the rates of carnitine acetyltransferase and pyruvate oxidation reactions with the different cell lines were adequate so as not to limit the translocase activity observed with 0.5 mM carnitine (Table II).

The carnitine–acylcarnitine translocase activity was measured in fibroblasts of normal controls and patients with known and unknown β-oxidation defects. The results showed that the activity of the translocase was normal in patients with defects of CPT I, CPT II, long-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, and in a patient with still unidentified mitochondrial fatty acid oxidation defect (Table II). No translocase activity was found in the fibroblasts of the proband (Table II), and this remained so even after inclusion of the dithiothreitol during assay (data not shown). The effect of dithiothreitol was tested in view of the report that dithiothreitol restores to normal the decreased translocase activity seen in ischemic heart (23).

The present translocase assay is based on the oxidation of [2-$^{14}$C]pyruvate to [$^{14}$C]acetyl-CoA and the conversion of the latter to [$^{14}$C]acetylcarnitine. This process includes participation of the pyruvate oxidase system and the carnitine acetyltransferase. Any deficiency in the overall acetylation of carnitine can therefore be ascribed to translocase deficiency only if the other participating activities are normal. Table II shows that this was the case as in the proband the activity of carnitine acetyltransferase and pyruvate oxidase system were normal. Thus, with 0.5 mM carnitine in the incubation system, the carnitine acetylation procedure proved reliable for the assay of the carnitine–acylcarnitine translocase in fibroblasts as shown earlier for the human muscle (18).

## Discussion

We have identified here a case of carnitine–acylcarnitine translocase deficiency in a neonate who died a week after birth. This disorder was recognized later by analyses in fibroblasts, and the diagnosis explains why attempts to manage the recurrent severe hypoglycemia by providing triglycerides and carnitine and the persistent hyperammonemia by providing benzoate had proved futile.

The occurrence of carnitine–acylcarnitine translocase deficiency as a clinical entity has been speculated earlier based on indirect evidences (24, reviewed in 25). Only recently has this activity been assayed directly by the carnitine acetylation procedure (18) to support the conclusion that its deficiency indeed occurs (14). Our present work, where the reliability of the carnitine acetylation procedure for translocase assay in fibroblast

*Table II. Carnitine-Acylcarnitine Translocase Activity in Patients with Mitochondrial Fatty Acid Oxidation Defects Measured in Permeabilized Fibroblasts*

| Fibroblasts from | Translocase activity at 0.5 mM carnitine | Translocase activity at 10 mM carnitine | Carnitine acetyl transferase | Pyruvate oxidase system |
|---|---|---|---|---|
| Controls | *1.6±0.31 | 2.9±0.59 | 11.1±2.2 | 3.3±0.48 |
| $n$ = (5 or 6) | (1.3–2.0) | (2.0–3.6) | (8.4–13.4) | (2.6–3.9) |
| CPT I deficient | 2.5, 1.7 | 4.2, 3.4 | 16.0 | |
| CPT II deficient | 1.5, 1.9 | 3.3, 4.4 | 10.2 | 4.8 |
| LCAD‡ deficient | 1.8 | 3.0 | 13.7 | |
| MCAD§ deficient | 1.9 | 2.6 | — | |
| ‖Long chain oxidation defect | 1.1 | 2.3 | — | 1.7 |
| Patient (C.O.) | 0.0 | 0.0 | 9.3 | 3.4 |
| | 0.0 | 0.0 | 7.4 | 2.8 |
| | 0.0 | 0.0 | | |

All activities are expressed as nmol/min/mg.
* Mean±SD, with range shown in parentheses. Other individual values shown are for fibroblasts of the same person analyzed on different days.
‡ Long-chain acyl-CoA dehydrogenase.
§ Medium-chain acyl-CoA dehydrogenase.
‖ Still unidentified.

has been rigorously verified, clearly establishes translocase deficiency in our patient. We have emphasized previously that the conventional assays, based on the uptake or efflux of metabolites as frequently used, are not suitable for clinical work because of the misleading results that they can give (18, 26). For the carnitine–acylcarnitine translocase an approach that overcame the limitations of the conventional assays was developed and validated for muscles (18). In the present work, we have markedly simplified this assay particularly by extending its use to permeabilized fibroblasts. In preliminary experiments, we found that the same digitonin permeabilization procedure enables the translocase assay also in lymphocytes. Availability of these methods should now enormously simplify the clinical screening for translocase deficiency in suspected patients and their pedigree.

The present findings have helped resolve some questions concerning this translocase. Thus, whether the transport of short- and long-chain acylcarnitines involves one translocase protein with broad chain-length specificity or more than one translocase with narrower chain-length specificity has remained uncertain. Here, as described previously (14), the deficiency of the translocase was found using an assay based on the measurement of the transmitochondrial transport of carnitine and acetylcarnitine, whereas the defect in fatty acid oxidation was identified as that for long-chain fatty acid, palmitate. In our patient, as in the case of Stanley et al. (14), acylcarnitines accumulated to an abnormally high level (as 92% of the total plasma carnitine in our patient); moreover, the data of Stanley et al. (14) showed that this increase occurred in both the short- and the long-chain acylcarnitines. These findings show that the same transporter in vivo catalyzes the transport of both the short- and the long-chain acylcarnitines. This is consistent with the observation that a purified translocase preparation from rat liver mitochondria shows activity with short- as well as long-chain acylcarnitines (27). Moreover, as the translocase deficiency in fibroblasts has been found in patients showing signs of hepatic (lack of ketone bodies) cardiac (cardiomyopathy, heartbeat disorder) and muscular (creatine kinase) defects, the disorder identified seems to involve defect of the same protein in the different tissues mentioned.

Whereas the relationship of the translocase deficiency to severe hypoglycemia, seen in our patient, is understandable in view of the known role of fatty acid oxidation in supporting gluconeogenesis and in sparing glucose oxidation, its relationship to hyperammonemia, seen in our patient, as in (14), remains intriguing. Hyperammonemia has also been frequently observed in long-chain fatty acid oxidation defects presenting as Reye's syndrome (28, 29). It is possible that a severely curtailed fatty acid oxidation in liver causes an energy deficit intramitochondrially resulting in impaired ureagenesis and hyperammonia.

The striking cardiac abnormalities seen in our translocase deficient patient (Fig. 1) were similar to those identified earlier in the infantile form of (severe) CPT II deficiency (13). In both these disorders a marked buildup of acylcarnitines is to be expected, and the cardiac abnormalities could have arisen from the known toxicity of long-chain acylcarnitines (30). Long-chain fatty acid oxidation defects with such an accumulation of acylcarnitine (not only in CPT II and translocase but also in long-chain and 3-hydroxy long-chain acyl-CoA dehydrogenases) should be therefore systematically considered in neonates and infants presenting cardiac arrest, unexpected collapse, or heart trouble, symptoms that otherwise can easily lead to the misdiagnosis of toxic shock, sudden death, or malignant hyperthermia.

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