CLINICO-PATHOLOGICAL CONFERENCE

A case of impairment of mitochondrial fatty acid β-oxidation

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Abstract. We describe a patient with impairment of mitochondrial fatty acid β-oxidation. A Japanese baby boy was delivered in the 38th week of gestation by emergency cesarean section due to fetal asphyxia. His birth weight was 1,985 g (<10th percentile), length 44.8 cm (<10th percentile), and head circumference 31.0 cm (10th percentile). His Apgar scores were 3 and 5 at 1 min and 5 min, respectively. Blood glucose was 12 mg/dl at 1 hour after birth, requiring glucose administration. On day 1 his serum CK was 20,780 IU/l, which was thought to be due to asphyxia. His serum CK levels gradually began to decrease. At 3 months of age, he sucked poorly, had poor body weight gain, and muscle hypotonia was observed. On day 117 his general condition was impaired, and marked hepatomegaly was observed. The blood glucose level was 43 mg/dl. The patient's urine was negative for ketone bodies. His serum triglyceride level was 3,670 mg/dl. Abdominal CT scan revealed a fatty liver. Serum levels of acyl carnitine from very-long chain fatty acid increased. On day 118 he died due to ventricular fibrillation. On necropsy, massive lipid deposition was observed in the liver, cardiac muscle, kidney, skeletal muscle, and intestinal mucosa. The ratio of very-long chain acyl-CoA dehydrogenase (VLCAD) activity for C16/C8 fatty acid was 0.50 (normal control 1.29), suggesting abnormal VLCAD. He was diagnosed as having impairment of mitochondrial fatty acid β-oxidation, presumably due to the VLCAD deficiency. (Keio J Med 51 (2): 100–106, June 2002)

Key words: mitochondria, fatty acid β-oxidation, very-long chain acyl-CoA dehydrogenase(VLCAD), hypoglycemia

Dr. Hasegawa (Moderator): I now announce the opening of the 1040th Clinico-pathological Conference (CPC).

Today's CPC is being conducted with two main goals in mind. The first is to understand the function of mitochondrial fatty acid β-oxidation in terms of energy production in the living body or cells. The second is to understand when, *i.e.*, with what symptoms or laboratory data, clinicians must suspect the impairment of mitochondrial fatty acid β-oxidation.

By way of introduction, I would like to mention three points. The first point is that, as we are all well aware, glucose is the most important source of energy production in the cells. Now, let me ask you, Mr. Kanasaka – The energy, namely ATP, is critical for the cells to survive. The most important source of energy or ATP production is glucose. Ingested carbohydrates are broken down in the intestine, and absorbed in the form of glucose. Blood glucose is then taken up by the various cells of the body to serve as the source of energy. After prolonged fasting, *i.e.* when the supply of dietary glucose stops, what would the cells obtain energy from?

Mr. Kanasaka (5th year student): Under such circumstances, fatty acids are β-oxidized to acetyl-CoA, and acetyl-CoA enters the TCA cycle to produce energy.

Dr. Hasegawa: I shall ask you the same question again, as you have perhaps not understood my question clearly. If there is no supply of glucose from the diet, where can glucose be obtained from before the period when energy is produced by mitochondrial fatty acid β-oxidation?

If the dietary supply of glucose is stopped, the cells still try to procure energy from glucose before relying on mitochondrial fatty acid β-oxidation. This is because ATP production through the glycolytic pathway, the TCA cycle, and the electron transfer system are extremely efficient. Thus, the cells still attempt to

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use glucose rather than relying on mitochondrial fatty acid β-oxidation.

Under conditions in which dietary glucose is no longer supplied or after prolonged fasting, glucose is derived from the degradation of glycogen in the liver. Glycogen is a storage form of glucose. The glucose that is stored in the liver in the form of glycogen, like money in a bank, is thus used during periods of starvation. At these times of necessity, the glucose derived from the degradation of glycogen is released into the blood, and used for energy production. When the glycogen in the liver is exhausted, just as when there is no money left in the bank, then the source of energy is changed from glucose to fatty acids.

Therefore, the liver can be compared to a bank, where money is deposited. When you have no income today, but need money, you withdraw some money from the bank to cater to your needs of the day. When the money in the bank is used up, you ask a moneylender for a loan. Fatty acids can be compared to moneylenders.

The second introductory point I would like to mention is, ''when is the source of energy production is changed from glucose to fatty acids''. The source of energy production is changed from glucose to fatty acids when there is no dietary glucose supply and the glycogen in the liver is also exhausted. In other words, cells use fatty acids as the source of energy under the following conditions: 1. prolonged fasting; 2. a newborn whose glycogen in the liver is very poor; and, 3. increased energy demands of the body for any reason. During exercise or ill health, the energy demands of the body is high. The source of energy, therefore, is changing from glucose to fatty acids.

The third introductory point I wish to mention is that the heart relies on fatty acids rather than glucose as its source of energy. As I told you previously, most cells produce ATP, initially using glucose as the source of energy. Fatty acids are not used until all of the glucose is used up. However, as an exception, the heart overwhelmingly relies on fatty acids rather than glucose as its source of energy. If there is some problem in the production of energy from fatty acids, the heart may be the first organ to be affected when the energy source is changed from glucose to fatty acids.

That was by way of introduction. Does anybody have any questions?

So, now, Dr. Hori, the pediatrician-in-charge of today's case, would you please present the case of the day?

Dr. Hori (Pediatrics): The patient was a 3-month-old boy who presented with hypoglycemia. This boy was delivered by emergency caesarian section because of fetal asphyxia at 38 weeks and 2 days of gestation. The weight, length and head circumference at birth were 1,985 g (below the 10th percentile), 44.8 cm (below the 10th percentile), and 31.0 cm (10th percentile), respectively, indicative of intrauterine growth retardation. The one-min and 5-min APGAR scores were 3 and 5, respectively, indicative of neonatal asphyxia. The baby was admitted to the NICU. Although the blood glucose level was 12 mg/dL 1 h after birth, it improved to 67 mg/dL following administration of 20% glucose.

The serum CK level was as high as 20,780 IU/L the day after birth (Day 1), although it decreased to 214 IU/L on Day 6; the initial high CK level was thought to be attributed to asphyxia. The body temperature was 35.5 °C on Day 9, with abnormally high serum CK, LDH, AST and ALT levels. The cause for these abnormal elevations, however, remained unclear. Thereafter, the serum CK level varied between 800 and 1,500 IU/L. Since the feeding status and weight gain were favorable, the infant was discharged on Day 43. His weight was 3,200 g at discharge.

The patient's feeding status worsened gradually from Day 90, and the boy was brought to the same hospital on Day 111. Poor weight gain, hypotonia and hepatomegaly were found at that time. The feeding status worsened further on Day 116, and the child was admitted to the hospital with vomiting on Day 117. Physical examination on admission revealed low temperature (35.9 °C), tachypnea, a decreased level of consciousness, generalized perspiration, and nasal flaring. The blood glucose level was 43 mg/dL, and urinary ketone bodies were negative. Cardiac ultrasonography revealed pericardial effusion, and abdominal CT showed a prominent fatty liver. The patient was referred to our service for further evaluation on Day 118.

With regard to the developmental status, head control and eye contact were yet negative. The baby laughed for the first time at 3 months of age.

There was no family history of consanguineous marriage or muscular disease.

Are there any questions so far?

Mr. Kanasaka: Could you give us more details of the family history? For instance, was there any history of SIDS, especially in the child's siblings?

Dr. Hori: This patient was the first child. There were no siblings. There was no family history of sudden death.

Mr. Kanasaka: Was the course of the pregnancy in the mother normal?

Dr. Hori: Yes, absolutely normal.

Mr. Kamata (5th year student): The CK level varied between 800 IU/L and 1,500 IU/L before the patient was discharged on Day 43. Were the isozymes of CK examined?

Dr. Hori: The MM isoenzyme from the skeletal muscle was always the predominant.

Ms. Kawade (5th year student): Hepatomegaly was

noted when the patient was brought to the hospital on Day 111. Was it found by palpation or by abdominal ultrasonography?

Dr. Hori: By palpation.

Ms. Kawade: Poor weight gain was noted on Day 111. Do you have any precise data on the rate of weight gain?

Dr. Hori: No. I do not have any numerical data.

Mr. Gatayama (5th year student): Urinary ketone bodies were negative when the blood glucose level was 43 mg/dL on Day 117. Is it common for infants under 1 year of age to be negative for urinary ketone bodies even when the blood glucose level is so low, like 43 mg/dL?

Dr. Hori: No. It is not common for even infants of this age to be negative for urinary ketone bodies when the blood glucose level is low.

Now, I shall proceed with the presentation.

Physical examination on admission revealed the following findings: length, 59.5 cm; weight, 4,363 g; head circumference, 38.5 cm; temperature, 36.2 °C; heart rate, 150/min; respiratory rate, 46/min; systolic pressure, 96 mmHg. The child showed poor movement of the extremities, and cried weakly. Prominent edema of the eyelids and extremities was noted. The chest was clear to auscultation, and there was no cardiac murmur. There was abdominal distension, and the liver, with a sharp edge, was palpable 9 cm below the right costal margin. The spleen was not palpable. Moro's reflex was positive, with bilateral symmetry.

I shall now give the laboratory data on admission. Peripheral blood examination revealed a total WBC count of 5,600/μL, Hb of 10.4 g/dL, and platelet count of 30.8×10^4/μL.

The serum was chylous. I will refer to some of the biochemical data (Table 1). Serum UA was 12 mg/dL, Na 120.8 mEq/L, Cl 85 mEq/L, Ca 7.6 mg/dL, AST 185 IU/L, ALT 69 IU/L, LDH 830 IU/L, CK 1,133 IU/L (MM isoenzyme predominant), aldolase 13 IU/L, Glu 84 mg/dL, TC 371 mg/dL, TG 3,670 mg/dL, FFA 2.09 mEq/L, lactate 22.5 mg/dL, pyruvate 0.95 mg/dL, total ketone bodies 521 μmol/L, acetoacetate 127 μmol/L, and 3-hydroxybutyrate 394 μmol/L.

Blood gas analysis did not reveal acidosis.

On chest X-ray, the cardiothoracic ratio was calculated to be 63%.

Electrocardiography showed non-specific T-wave changes.

Cardiac ultrasonography revealed an ejection fraction of 64.1%, and there was a moderate pericardial effusion from the lateral to posterior wall.

Head CT revealed no abnormalities.

Serum acyl carnitine analysis revealed that the level of acyl carnitine derived from long chain fatty acids was elevated.

Table 1 Laboratory Data and Imaging Studies

(Biochemistry) TP 4.2 g/dL, Alb 3.0 g/dL, BUN 31.3 mg/dL, Cr 0.4 mg/dL, UA 12.0 mg/dL, Na 120.8 mEq/L, K 4.7 mEq/L, Cl 85 mEq/L, Ca 7.6 mg/dL, IP 5.7 mg/dL, AST 185 IU/L, ALT 69 IU/L, LDH 830 IU/L, CK 1,133 (MM 99%, MB 1%) IU/L, Aldolase 13 IU/L, Glu 84 mg/dL, CRP 0.02 mg/dL, NH_3 50 μmol/L, TC 371 mg/dL, TG 3,670 mg/dL, HDL-C 23 mg/dL, FFA 2.09 mEq/L, Lactate 22.5 mg/dL, Pyruvate 0.95 mg/dL, Total ketone body 521 μmol/L (N = 26–122), Acetoacetate 127 μmol/L (N = 13–69), 3-hydroxy lactate 394 μmol/L (N = 76 or less), Total carnitine 52.8 μmol/L (N = 45–91), Free carnitine 18.0 μmol/L (N = 36–74), Acyl carnitine 34.8 μmol/L (N = 6–23).
(Venous blood gas analysis) pH 7.403, PCO_2 44.5 Torr, HCO_3 27.1 mEq/L, BE 2.0 mEq/L.
(Urinalysis) S.G. 1.015, Ketone body (−), Protein (−), Glucose (−), Blood (−).
(Chest X-ray) Cardiothoracic ratio 63%.
(Electrocardiography) Sinus rhythm, Axis +10, nonspecific T wave changes (+).
(Cardiac ultrasonography) Ejection fraction 64.1%, prominent pericardial effusion collection (+) from the lateral to posterior wall.
(Head CT) No abnormal findings such as bleeding, edema, or hydrocephalus.
(Urinary organic acid analysis) High level of non-ketone dicarboxylate.
(Serum acyl carnitine analysis) High level of acyl carnitine derived from long-chain fatty acids.

Abdominal CT findings were suggestive of a fatty liver.

Mr. Kimura (6th year student): On physical examination, did he have any odd looking face or congenital anomaly?

Dr. Hori: No. – there were no such abnormalities.

Now, I shall describe the clinical course of the patient after admission. On Day 119 (the second hospital day), nystagmus was observed, which persisted for 1 h. Soon after, hypoglycemia (48 mg/dL) was noted, but the urinary ketone bodies were negative. The child then abruptly developed ventricular fibrillation with cardiopulmonary arrest, and eventually died, without any response to resuscitation.

If you have any questions or comments about the clinical course of the patient, or any other questions so far, I shall be happy to answer them.

Mr. Kanasaka: What race did the patient and his family belong to?

Dr. Hori: The family is Japanese.

Mr. Kanasaka: So, they are not Caucasian.

Dr. Hori: No.

Mr. Oda (5th year student): The patient showed intrauterine growth retardation. What did that suggest?

Dr. Hori: Your point is well taken. I shall come to that point later.

Dr. Hasegawa: No definite diagnosis has yet been established at this moment. Now, I would like to ask

Table 2 Postmortem Pathological Diagnosis

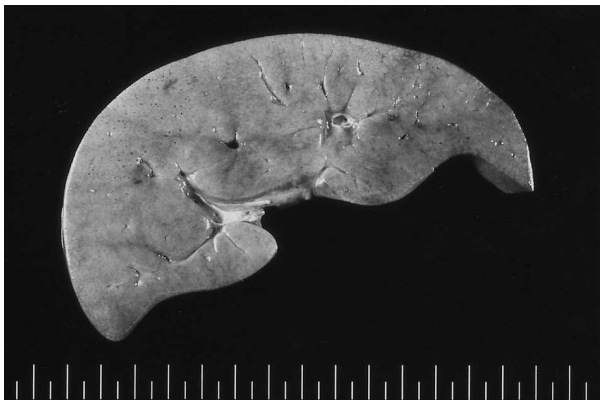
Congenital defects in fatty acid beta-oxidation
[strongly suggesting very-long-chain-acyl-CoA dehydorogenase deficiency]
a. Weight at birth low (gestational age 38 weeks and 2 days, weight 1,985 g)
At autopsy, length 58 cm, weight 4.6 kg, 3 month old
b. Decreased activity of very-long-chain-acyl-CoA dehydorogenase (Table 2)
c. Severe fatty changes in systemic organs (heart, liver, kidney, stomach, systemic skeletal muscles)
d. Pericardial effusion (50 ml)

Dr. Hori to briefly summarize the tentative diagnosis from the clinical viewpoint.

Dr. Hori: I clinically suspected the impairment of mitochondrial fatty acid β-oxidation because, 1) the urinary ketone bodies were negative in association with hypoglycemia, 2) there was prominent hepatomegaly, 3) the blood triglyceride level was high, and 4) CT suggested a fatty liver. However, since the patient died too soon, no definitive diagnosis could be made.

Dr. Hasegawa: Then, I should ask the pathologist to solve the riddle.

Dr. Du (pathology): The postmortem pathological diagnosis is shown in Table 2. The weight of the patient at birth was low, but without any malformations. The length of the patient (3 month old) was 58 cm which was above the 10th percentile. The body weight was 4.6 kg that was below the 10th percentile. Mild-retardation of the body growth was observed. Marked hepatomegaly is shown in Fig. 1a and the weight of the liver (460 g) was over twice that of normal infants. The liver was yellowish and histologically severe fatty changes were revealed (Fig. 1b). The nuclei were located in the peripheral spaces of liver cells and vacuoles were stained with oil red-O (Fig. 1c). Hypertrophy of the heart (26 g) and a diffuse fatty degenerartion stained with the oil red-O, was revealed in the heart muscle (Fig. 2a and b). Positive staining with oil red-O in heart muscle cells of normal infants should not be shown. These fatty changes in systemic organs have been seen in some disorders with lipid metabolism including the abnormality of fatty acid oxidation. The fat degeneration was also seen in the kidneys (in the cytoplasm of the uriniferous tubular epithlia), stomach (in the cytoplasm of the foveolar cells), intestine and systemic skeletal muscles. Very-long-chain-acyl-CoA dehydrogenase activity was examined. Mitochondria fractions were obtained from the liver specimens. Very-long-chain-acyl-CoA dehydrogenase activity against C8, a medium chain fatty acid, and C16, a very long chain fatty acid, were examined by the ferrocemoium reduction assay (Table 3).



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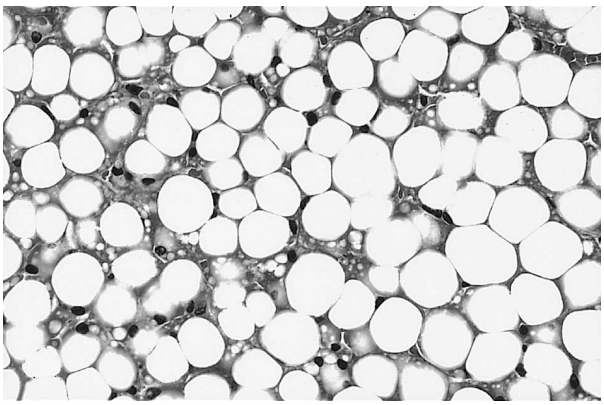

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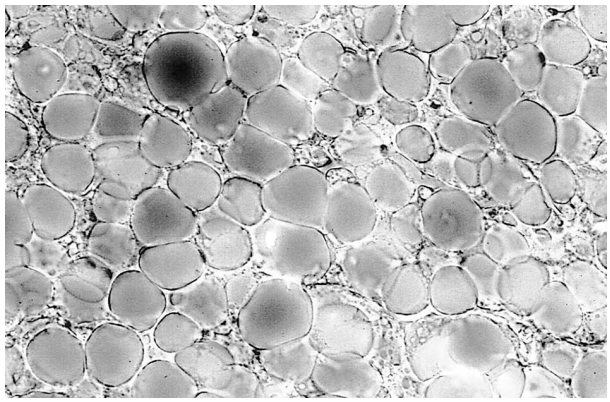

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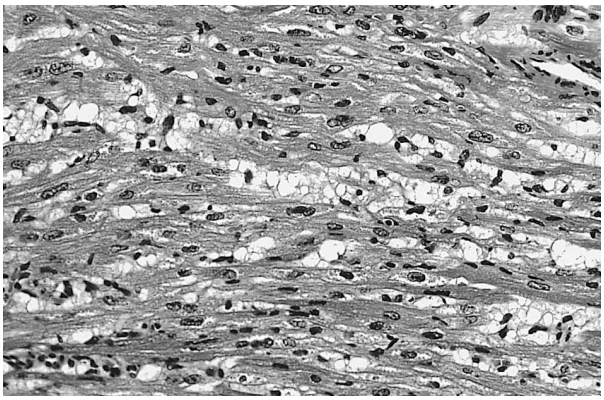
Fig. 1 Fatty liver. (a) Gross appearance showing marked hepatomegaly (460 g) with a yellow colour. (b) HE staining. Hepatocytes with vacuolar degeneration are shown. The hepatocyte nuclei are located in the peripheral spaces. (c) Oil-red O staining. The vacuoles in the cytoplasm are stained orange-red.

Normal infant liver and human skin fibroblasts derived from a normal donor were used as negative controls. The enzymatic activity, which was expressed



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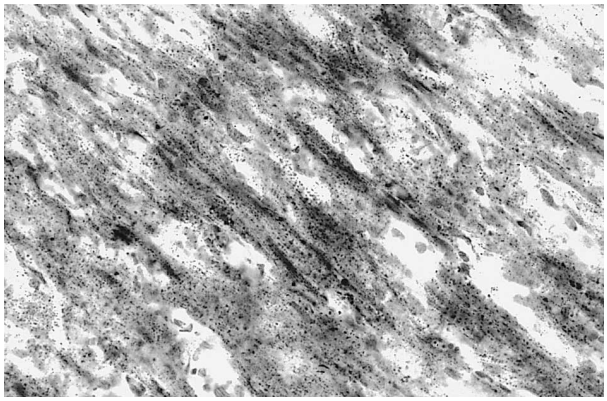

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Fig. 2 Heart muscle specimens with fatty changes. (a) HE staining. (b) Oil-red O staining. Small fatty droplets stained with oil-red O exist diffusely in the heart muscle.

Table 3 Very-long-chain-acyl-CoA Dehydrogenase Activity (Ferricenium Reduction Assay)

		Protein (mg/ml)	C8*	C16*	C16/C8
Patient liver	exp-1	13.7	35.6	17.8	0.50
	exp-2		35.6	19.7	0.56
Normal infant liver		16.8	11.3	14.5	1.29
Human fibroblast		14.2	1.67	2.15	1.29

C8: octanoyl-CoA,
C16: palmitoyl-CoA,
*: nmol reduction of ferricenium/min/mg protein.
exp-1: the first experiment.
exp-2: the second experiment using the same specimen.

as the C16/C8 ratio, was 0.5–0.56 in this patient's liver tissue compared with the control ratio of 1.29. This result showed decreased activity of the very long chain acyl-CoA dehydrogenase. As a result, it was suggested that the disorder in this case was caused by the impairment of beta-fatty acid oxidation.

Mr. Kanasaka: Did you observe any changes in the brain?

Dr. Du: Autopsy of the brain was not permitted by the bereaved family.

Dr. Hori: I shall now speak a little about the significance and mechanism of mitochondrial fatty acid β-oxidation. The living body normally uses glucose as the energy source. Under conditions in which no glucose is available, energy is produced by mitochondrial fatty acid β-oxidation.

Fatty acids cannot enter the mitochondria without undergoing biochemical changes. First, they are converted to acyl-CoA, and later to acyl carnitine, which then enters the mitochondria, where it is reconverted to acyl-CoA. I shall not discuss in detail the mechanisms of entry of fatty acids into the mitochondria.

In the mitochondria, acyl-CoA is subjected to β-oxidation. β-oxidation is a reaction in which two carbons are removed in each step. The first enzyme in β-oxidation is acyl-CoA dehydrogenase, which consists of three types: acyl-CoA dehydrogenase for very-long-chain fatty acids (VLCAD), acyl-CoA dehydrogenase for medium-chain fatty acids (MCAD), and acyl-CoA dehydrogenase for short-chain fatty acids (SCAD).

I will come back the question about the race of the child. Most of reported patients with MCAD deficiency are Caucasians. When the impairment of mitochondrial fatty acid β-oxidation is suspected, it is important to confirm the race of the patient.

Only seven cases of VLCAD defixiency have been reported so far in Japanese patients. In fact, only about 60 cases have been reported world-wide.

Based on the clinical findings and the enzyme activity in the liver, a diagnosis of VLCAD deficiency was suspected, as mentioned by Dr. Du.

At present, a mutation analysis of the VLCAD gene is under way to reach a definitive diagnosis.

Does anyone have any questions?

Mr. Hasegawa (6th year student): Does VLCAD deficiency fall under the category of peroxisomal diseases, because it is a disorder of very-long-chain fatty acid metabolism?

Dr. Hori: No. VLCAD deficiency does not belong to the category of peroxisomal diseases. VLCAD is an enzyme localized in the mitochondria, and it mainly catalyzes the β-oxidation of 18-carbon fatty acids. On the other hand, oxidation of fatty acids in peroxisomes usually involves 24- or 26-carbon fatty acid substrates.

Mr. Hasegawa: The patient did not have peroxisomal disease. Didn't he exhibit peroxisomal-disease-specific symptoms, such as central nervous system symptoms?

Dr. Hori: No. A question was asked some time ago regarding intrauterine growth retardation (IUGR) in this child; to tell you the truth, I can't explain why the patient had intrauterine growth retardation. IUGR can

be symmetrical or asymmetrical. In symmetrical IUGR, length, weight, and head circumference were appropriately small. Conversely, in asymmetrical IUGR, while the length and weight are small, the head is relatively normal in size. When the mother herself or the placenta is under stress, the fetus tries to protect the most important part of the body, namely, the head. That is, blood flow to the head is maintained, so that the head grows normally, while the length and weight are small; this is asymmetrical IUGR. On the other hand, in the case of symmetrical IUGR, as in our case, fetal factors must exist without (or in addition to) maternal and placental factors. The patient showed symmetrical IUGR, which could be due to the inborn error of fatty acid metabolism.

Mr. Sato (6th year student): I have two questions; 1) The child had nystagmus. Why did that happen? 2) What are the reasons for the clinical differences between VLCAD deficiency and peroxisomal diseases. In my understanding, the difference between the two is the number of carbons in the substrate, while both are ultimately defective of fatty acid metabolism? Clinically malformations are characteristic of peroxisomal diseases. Does that suggest that organogenesis is influenced differently according to the number of carbons in the fatty acid chain?

Dr. Hori: In response to your first question, I should admit that the cause of the nystagmus remains unclear.

Dr. Hasegawa: I will answer the second question. Peroxisomal diseases comprise several different types. In the representative form, the peroxisomes themselves are absent. Each cell in our body is composed of various factories. The absence of peroxisomes means the absence of one entire factory. On the other hand, in VLCAD deficiency, in which an abnormality of fatty acid β-oxidation occurs, the mitochondria (one of the factories) are normal in structure, however, the process of β-oxidation, *i.e.*, one of the various processes in the factory, is out of order. In other words, the factory itself is present, but a part of the machinery is unserviceable. Thus, in VLCAD deficiency, all machines other than VLCAD are in working condition. On the other hand, in peroxisomal diseases, the factory itself is absent, so that not only fatty acid β-oxidation but also all the other reactions in the peroxisomes are defective. These features may explain why there are marked clinical differences between the two conditions.

Mr. Sato: Is there any disease in which only the metabolism of fatty acids that takes place in the peroxisomes is defective?

Dr. Hasegawa: Yes. Other than the representative form of peroxisomal diseases which I told you, there are some forms with peroxisome being present but functionally defective. For instance, 3-ketothiolase deficiency has defective fatty acid metabolism that takes place in the peroxisomes, while other metabolic processes in the peroxisome are normal. Needless to say, 3-ketothiolase deficiency has normal mitochondrial fatty acid β-oxidation.

Mr. Hasegawa: I can understand why pathological abnormalities in the impairment of mitochondrial fatty acid β-oxidation are prominent in organs such as the liver, myocardium, and skeletal muscle, where mitochondria are abundant. On the other hand, I guess that the pathological abnormalities in peroxisomal diseases are systemic. Is that right?

Dr. Hasegawa: As you have pointed out, in diseases like VLCAD deficiency, which are associated with mitochondrial failure, lesions are mainly found in organs which originally have abundant mitochondria. On the other hand, in peroxisomal diseases, the organ showing the most severe pathological abnormalities varies according to the type of the disease.

Mr. Kiuchi (5th year student): I would like to ask about the laboratory findings on admission. What is the reason for the rather high total ketone body levels in the blood?

Dr. Hori: I will change your question as follows. Why was the blood ketone body level increased, while urinary ketone bodies were negative? In VLCAD deficiency, β-oxidation by MCAD and SCAD are normal. Even in VLCAD deficiency, mitochondrial fatty acid β-oxidation by MCAD and SCAD occurs leading to the production of ketone bodies. The blood ketone body level in the child was rather high, although it was not high enough to be excreted into the urine.

Mr. Kiuchi: I have a question regarding the enzyme activity. You mentioned that the ratio of C16/C8 β-oxidation activity was low. It appeared like there was an increase in the oxidation activity of C8, rather than a decrease in that of C16. Was there an increased oxidation of short- and middle-chain fatty acids?

Dr. Hasegawa: You misunderstood the experiment to measure the enzyme activity. I would like to point out that the enzyme activity measured was the activity of VLCAD. The activity of VLCAD was measured by two different substrates, C16- and C8-fatty acid. The activity of MCAD and SCAD was not measured. The normal VLCAD mainly oxidizes C16-fatty acid, while it acts little on C8, because the enzyme is substrate-specific. In this patient, the data show that the substrate specificity of VLCAD may be altered.

Since we are running out of time, I would like to briefly summarize the discussion. Under conditions in which the body or cells cannot use glucose as the energy source, fatty acids are oxidized to produce ATP for survival. This is the essence of mitochondrial fatty acid β-oxidation. The patient with the impairment of mitochondrial fatty acid β-oxidation showed signs and symptoms in the organ in which the cells depend more

on fatty acids rather than glucose, when the source of energy is changing from glucose to fatty acid.

Are there any questions or comments?

Ms. Kim (5th year student): I have two questions. 1) How did you tell the results of the autopsy to the parents? 2) Is prenatal diagnosis of VLCAD deficiency possible?

Dr. Hasegawa: We told the parents that the child had an impairment of mitochondrial fatty acid β-oxidation. We did not tell them that the diagnosis of VLCAD deficiency was convincing.

Prenatal diagnosis of VLCAD deficiency is theoretically possible once the gene mutation is identified. We, however, should bear in mind that prenatal diagnosis includes a lot of ethical issues.

Mr. Kanasaka: What is the prognosis of children with VLCAD deficiency? Has any case of VLCAD deficiency been reported to survive into childhood or adult?

Dr. Hasegawa: All patients with VLCAD deficiency do not have a poor prognosis. Let us think about the treatment of a patient with VLCAD deficiency if an early diagnosis has been established. Glucose dependency as the source of energy is critical such as 1) taking meals frequently to avoid fasting, 2) intake of a diet that promotes accumulation of glycogen in the liver, and 3) glucose supplementation when the energy demands are increased, *e.g.*, during exercise or illness.

Mr. Kanasaka: Some patients with VLCAD deficiency have a poor prognosis, while others survive for a long period. What is the reason for this difference?

Dr. Hasegawa: The clinical picture varies from very mild to very severe in patients with VLCAD deficiency. One of the reasons for the varied clinical picture is the differences in the residual enzymatic activity. Patients with VLCAD deficiency who have some residual enzymatic activity may not have any symptoms at all until they reach adulthood – some may not have any symptoms until they have run a marathon.

Mr. Kamata: Patients with VLCAD deficiency have skeletal muscle symptoms, while MCAD-deficient patients have no such symptoms. How can you explain this difference?

Dr. Hasegawa: That is because in skeletal muscles, mainly VLCAD is functional, *i.e.*, fatty acids that have long carbon chains are oxidized to provide energy.

I would now like to thank the participants for the lively question-answer session and discussions. I now declare today's CPC closed.