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ingestion (by hand-to-mouth activity) of powder residues (3,4).

In conclusion, this case illustrates how drug testing not only in conventional matrices, which account for acute exposure, but also in nonconventional matrices can shed light on past and possibly chronic exposure and can disclose the situation of an infant living in an unsafe and high-risk environment in which exposure to drugs of abuse takes place. The hair is always available and gives information on the past exposure as it grows by approximately 1 cm per month and accumulates drugs (5). It is particularly useful if the results of urine analysis are negative during acute investigation. The accurate assessment of both acute and chronic exposure of a young child to drugs of abuse through the objective use of a biomarker is of major importance because it provides the basis for appropriate immediate treatment, adequate medical follow-up and social intervention. In case of any acute exposure to drugs of abuse or of postnatal withdrawal syndrome, we suggest to investigate further the possibility of chronic exposure.

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A case of glycogen storage disease type Ib presenting with prolonged neonatal hypoglycaemia and minimal metabolic abnormalities

Sir,

The symptoms of hypoglycaemia in glycogen storage disease (GSD) patients usually do not manifest during the newborn period, because frequent feeding obviates the need for the gluconeogenic process. We herein report a case of neonatal hypoglycaemia with GSD type Ib that showed minimal metabolic abnormalities, thus making it difficult to make a definitive diagnosis.

A boy, the first child of healthy unrelated parents, was born at 38 weeks gestation and his birth weight was 2,686 g. At day 1, the patient showed tachypnea and hypoglycaemia and the intravenous infusion of glucose was started. On day 4, repeated episodes of hypoglycaemia were noted and he was transferred to our hospital. On physical examination, only the palpable edge of the liver was noted. Blood chemistry showed an elevated level of triglyceride (504 mg/dL) and lactate (36.3 mg/dL). Total cholesterol, free fatty acid, uric acid, carnitine profile and blood gas analysis were all within the normal level. A serum sample at hypoglycaemia showed a measurable level of insulin (insulin and glucose were 2.4 IU/mL and 34 mg/dL respectively), and ketoacidosis was not noted.

To differentiate between hyperinsulinemic hypoglycaemia and defects in glycogenolysis, a glucagon stimulation test was performed, showing no glycaemic response (Figure 1). The glucose administration (2 g/kg) showed a decrease in the lactate level (from 32.8 to 19.6 mg/dL). These results suggested a defect in glycogenolysis, and we started to give the patient formula milk under a tentative diagnosis of GSD. However, the patient could not show a stable blood glucose level. Furthermore, a gene analysis of GSD-Ia/b focused on hot spot mutations (1) revealed no mutation. Although the serum insulin level was not so high, diazoxide was added to suppress insulin secretion, which resulted in a poor glycaemic response.

From 3 months of age, diarrhoea and repeated episodes of bronchitis were noted. Simultaneously, the blood count showed a decreased neutrophil level. A complete gene analysis of *glucose-6-phosphate transporter 1* was performed, and a compound heterozygous mutation IVS1 + 1G>A and c.1016G>A (Gly339Asp) in exon 7 was found.

The median age of the presenting signs is an age from 4 to 6 months in GSD-I (2), while some neonatal cases have also been reported (3). GSD-Ib is a rare but important cause of neonatal hypoglycaemia, and it may be present with minimal accompanying metabolic abnormalities.

The authors thank Dr S. Kure (Tohoku University, Sendai) for performing gene analysis.

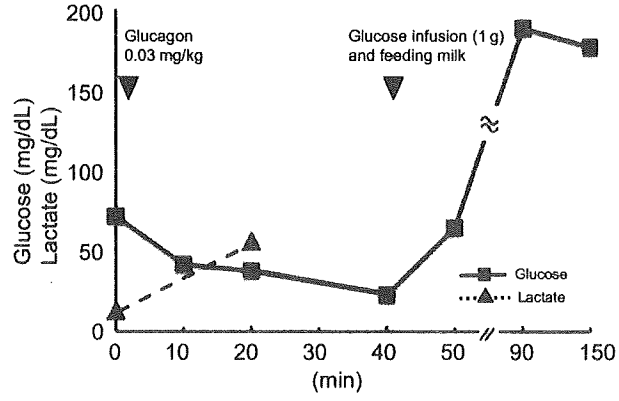

Figure 1 Glucagon stimulation test showed the absence of a glycaemic response and a rise in lactate level.

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G6PC mutations in two patients with glycogen storage disease type Ia in Thailand

Dear Sir,

Glycogen storage disease type Ia (GSD Ia) is characterized by hepatomegaly and hypoglycaemia and caused by mutations in the glucose-6-phosphatase gene (*G6PC*). Mutations unique to several ethnic groups have been described (1). However, no reports on Thai patients have been published. Here, we present the first description of *G6PC* mutations in Thailand.

Two Thai patients with GSD Ia, described elsewhere (2), were studied. In brief, patient 1 was aged 11 years and patient 2 was aged 9 years. Both showed hepatomegaly and treated with uncooked cornstarch to prevent hypoglycaemia. Parents of patient 1 had a consanguineous marriage. Sequence analysis of *G6PC*, as described previously (3), showed that patient 1 was a homozygote for p.R83H (c.248 G>A) and that patient 2 was a compound heterozygote for p.R83H and c.648G>T, previously described as G727T mutation. Two mutations were verified by PCR–restriction fragment length polymorphism (RFLP) analyses: RFLP with restriction enzyme *Hga* I for detection of p.R83H and RFLP with *Bst*GI for c.648G>T as described (3). In patient 2, c.648G>T allele had 1176C polymorphism as well as in Japanese and Chinese patients (4,5).

R83H is frequently detected in Chinese patients (26% of alleles studied) and c.648G>T is prevalent in Japanese (91%), Korean (75%) and Chinese (54%) patients (1). Our results suggest that Thai GSD Ia patients share the same mutations as Asian patients. Other examples of founder mutations in Asians include phenylketonuria (6) and complement component C9 deficiency (7).

Clinically, instead of performing an invasive liver biopsy, a rapid and non-invasive DNA testing using PCR-RFLP enables us to make a precise diagnosis of GSD Ia in Thailand. Our report expands the spectrum of Asian-specific *G6PC* mutations to Thailand.

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Correction was added on 14 October 2009: the first author's name was amended.

Increased levels of Monocyte Chemoattractant Protein-1 in cerebrospinal fluid with gamma globulin induced meningitis

Dear Sir,

Intravenous immunoglobulin G (IVIG) is widely used in the treatment of severe infectious diseases, immune thrombocytopenic purpura (ITP) in children. Complications of IVIG therapy include acute generalized reactions and aseptic meningitis (1). Although IVIG-induced meningitis is recognized, the mechanisms remain unknown. We applied this technique to investigate multiple cytokines and chemokines in gamma globulin-induced meningitis, as compared with mumps meningitis.

An 11-year-old boy was admitted because of bleeding tendency. We diagnosed him as having ITP and initiated gamma globulin for wet purpura. The day after the start of IVIG therapy, he complained of severe headache and exhibited vomiting with nuchal stiffness. Cerebrospinal fluid findings (CSF) revealed mild pleocytosis and we diagnosed IVIG-induced meningitis.

All specimens were collected for diagnostic tests and the remainder of these specimens were used for cytokine investigation. The Institutional Review Board approved the collection and investigation of samples, and written informed consent was obtained from all subjects. Cytokine measurement in CSF was performed simultaneously for 17 different cytokines (Interlekin-1β, -2, -4, -5, -6, -7, -8, -10, -12, -13, -17, Granulocyte-colony stimulating factor, Granulocyte-Monocyte colony stimulating factor, interferon-γ, Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage inflammatory protein-1β), and Tumour Necrosis Factor-α using BioPlex Cytokine Assay System (Bio-Rad Laboratories, Tokyo, Japan).

ORIGINAL ARTICLE

Living Donor Liver Transplantation for Glycogen Storage Disease Type Ib

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Glycogen storage disease type 1b (GSD-1b) is due to an autosomal recessive inborn error of carbohydrate metabolism caused by defects in glucose-6-phosphatase translocase. Patients with GSD-1b have severe hypoglycemia with several clinical manifestations of hepatomegaly, obesity, a doll-like face, and neutropenia. Liver transplantation has been indicated for severe glucose intolerance. This study retrospectively reviewed 4 children with a diagnosis of GSD-1b who underwent living-donor liver transplantation (LDLT). Between November 2005 and June 2008, 96 children underwent LDLT with overall patient and graft survival of 92.3%. Of these, 4 (4.2%) were indicated for GSD-1b. All patients are doing well with an excellent quality of life because of the stabilization of glucose intolerance, decreased hospital admission, and normalized neutrophil count. LDLT appears to be a feasible option and is associated with a better quality of life for patients with GSD-1b. Long-term observation may be necessary to collect sufficient data to confirm the efficacy of this treatment modality. ***Liver Transpl 15:1867-1871, 2009.*** © **2009 AASLD.**

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Glycogen storage disease type 1 (GSD-1) is an autosomal recessive inborn error of carbohydrate metabolism caused by defects of the glucose-6-phosphatase (G6Pase) complex, which is encoded on chromosome 11q23.[1] GSD-1 affects approximately 1 in 100,000 live births.[2] Two major types of the disease have been reported: GSD-1a, which is caused by a deficiency of microsomal G6Pase, and GSD-1b, which is caused by a glucose-6-phosphate translocase (G6PT) deficiency.[3] A patient with GSD-1 presents severe hypoglycemia, hepatomegaly, kidney enlargement, truncal obesity, a rounded doll-like face, wasted muscles, and a bleeding tendency.[4] GSD-1b shows the added features of neutropenia and neutrophil dysfunction.[5]

Major progress has been made in patient survival and the prevention of neurological sequelae secondary to hypoglycemia in affected children with early diagnosis and meticulous treatment. The aim of treatment is to prevent hypoglycemia and neutropenia, which predispose the patients to developing both neurological sequelae and severe infections. The usual medical treatment for this disease consists of frequent meals, continuous nocturnal gastric drip feeding, the administration of uncooked cornstarch for hypoglycemia, and the regular administration of recombinant human granulocyte colony stimulating factor (G-CSF) for neutropenia.[6,7] This treatment, however, is not always sufficient for avoiding hypoglycemia and recurrent infections. Moreover, such treatments may not prevent poor metabolic control and/or growth retardation.

Liver transplantation (LT) may offer a complete cure for genetically acquired errors of metabolism in the liver.[8] The main rationale of LT for congenital metabolic disorders is to supply the missing enzymes by sacrificing the native liver, which is an otherwise normally functioning entity. Recent case studies have reported

Abbreviations: ALT, alanine aminotransferase; DQ, developmental quotient; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; G6PC, glucose-6-phosphate catalytic subunit; G6PT; glucose-6-phosphate translocase; G-CSF, granulocyte stimulating factor; GSD, glycogen storage disease; LDLT, living-donor liver transplantation; LLS, left lateral segment; LT, liver transplantation; PG, poor growth; PMC, poor metabolic control; SD, standard deviation.

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TABLE 1. Living Donor Liver Transplantation for Glycogen Storage Type 1b

Case	Age	Sex	Height/Weight (SD)	Mutation	Donor	Blood-Type Combination	Graft Type	Complication
1	8 years 4 months	Female	115.2 cm (−2.2)/ 26.3 kg (−0.1)	W118R	Father	Identical	LLS	Biliary stricture
2	3 years 6 months	Female	94.1 cm (−0.4)/ 16.2 kg (+1.2)	W118R	Father	Identical	LLS	None
3	3 years 11 months	Female	95.2 cm (−0.9)/ 16.9 kg (+0.9)	W118R/IVS1 +1G>A	Father	Incompatible	LLS	Tacrolimus encephalopathy
4	1 year 1 month	Male	71.2 cm (−2.4)/ 10.1 kg (−0.2)	G339D/IVS1 +1G>A	Mother	Incompatible	LLS	None

Abbreviations: LLS, left lateral segment; SD, standard deviation.

the benefits of LT in GSD-1b, demonstrating that correcting the hepatic enzyme deficiency by LT leads to clinical improvements, including a normal feeding regimen and fewer intercurrent infections.[9] The present report describes the recent experience with living-donor liver transplantation (LDLT) in 4 GSD-1b patients.

PATIENTS AND METHODS

Between November 2005 and June 2008, 96 children underwent LDLT. Of these, 4 (4.2%) were indicated for GSD-1b. The diagnosis of GSD-1b was made by a mutation analysis of the glucose-6-phosphate (G6P) transporter gene in all case. Medical records were reviewed for the following: the personal and family history, physical findings, laboratory data, histological reports, operation records, and special findings obtained by cardiologists, nephrologists, gastroenterologists, and radiologists. A developmental delay was measured with the developmental quotient (DQ), which is a norm used to express aspects of a child's development similar to the intelligence quotient.[10]

Eight donor candidates were evaluated by standard liver function tests, blood group combination, anatomical variation, graft size matching, and mutation analysis. The mutation analysis revealed heterozygotes among all of the potential donors; however, laboratory data showed normal liver function and blood sugar and serum insulin levels. Table 1 shows profiles of the recipients and donors involved in this study.

All patients underwent LDLT by a standard procedure.[11] Gross hepatomegaly was observed in all cases. No venovenous bypass was used because total clamping of the inferior vena cava could be avoided in all cases. Immunosuppression was administered with tacrolimus and low-dose steroids. Tacrolimus administration was started on the day before transplantation. The target whole blood trough level of tacrolimus was 10 to 12 ng/mL for the first 2 weeks, approximately 10 ng/mL for the following 2 weeks, and 8 to 10 ng/mL thereafter. Treatment with steroids was initiated at the time of graft reperfusion at a dose of 10 mg/kg; the dose was tapered from 1 to 0.3 mg/kg/day during the first month, and the treatment was withdrawn within the first 3 months. Patient 3, who received an ABO-incompatible transplant, underwent preoperative plasma exchange in order to reduce the anti-ABH antibody titer, and prostaglandin E1, nafamostat mesilate, and steroids were administered postoperatively via a portal vein catheter.[12] Patient 4 was less than 2 years old and, with an ABO-incompatible transplant, had no preoperative treatment for ABO incompatibility.[13] This study was approved by the institutional review board, and informed consent was obtained in all the cases.

RESULTS

The patients presented with hepatomegaly (cases 1-4), hypoglycemia (cases 1-4), neutropenia (cases 1-4), recurrent respiratory (cases 1 and 3)/skin (cases 1-3) infections, sinusitis (cases 2-4), and failure to thrive (cases 1 and 4). Recurrent infection caused 20, 12, 40, and 8 casualty hospital attendances per year in the 4 patients, respectively. No patient demonstrated coagulopathy, inflammatory bowel disease, or renal insufficiency. Case 1 had experienced frequent hypoglycemic comas and had low mental development. The pretransplant DQs were 32, 92, 62, and 90, respectively. Antiepileptics were administered preoperatively in cases 1 and 3. The frequent daytime administration of uncooked cornstarch, continuous nighttime feeding (case 1), and 24-hour continuous nasogastric tube feeding (cases 2-4) did not provide satisfactory metabolic control. G-CSF was initiated for severe neutropenia 2 to 3 times per week at 5 years, 3 years, 6 months, and 8 months of age, respectively. Aspiration cytology of the bone marrow before transplantation showed hypercellular marrow in all cases. The indications for LDLT were poor metabolic control (cases 1-4) and poor growth (cases 1 and 4).

LDLT was performed at 8.3, 3.5, 3.8, and 1.1 years of age, respectively. The duration and blood loss of the recipient operation ranged from 436 to 557 minutes and from 365 to 1330 g, respectively. Cold and warm ischemic times ranged from 24 to 53 minutes and from 22 to 41 minutes, respectively. The histopathological examination of the explanted livers revealed gross hepatomegaly with microvesicular steatosis and mild fibrosis. The explanted native livers weighed 1399, 1065, 903, and 1200 g, that is, 219.3%, 237.0%, 195.7%, and

TABLE 2. Laboratory Data Before and After Liver Transplantation

	Case 1		Case 2		Case 3		Case 4		Reference
	Before	After	Before	After	Before	After	Before	After	Range
Neutrophil count (c/mm^3)	329	640	170	1095	86	880	138	1289	1500–5000
G-CSF	On	Off	On	Off	On	Off	On	Off	
Platelet count ($10^4/\mu L$)	66.3	20.0	61.0	44.2	73.1	40.8	56.2	42.3	15.0–35.0
ALT (IU/L)	21	21	26	10	16	25	31	14	4–24
Glucose (mg/dL)	81	100	93	102	66	110	91	89	70–109
Total protein (g/dL)	8.4	7.1	8.0	7.0	7.3	6.4	7.4	6.1	6.4–8.1
Uric acid (mg/dL)	5.7	2.7	5.8	3.2	5.8	3.5	11.8	3.8	2.3–5.7
Triglycerides (mg/dL)	275	150	697	159	97	132	676	66	30–218
Cholesterol (mg/dL)	212	155	192	150	136	138	179	115	111–222
Lactate (mmol/L)	6.90	1.00	5.70	0.70	2.70	0.90	6.00	0.80	0.44–1.78

Abbreviations: ALT, alanine aminotransferase; G-CSF, granulocyte stimulating factor.

398.5% of the estimated standard liver volume, respectively. No malignant lesion or solid occupied lesion was identified in any of the patients. Histological examinations of the grafts from the heterozygous donors showed 5%, 10%, 10%, and 30% microvesicular steatosis, respectively. All the donors were discharged from the hospital within 9 days after the operation, and they are currently doing well without any complications. The post-LDLT course was uneventful in case 2. Case 1 underwent duct-to-duct biliary reconstruction and showed a biliary stricture, which was successfully managed with radiological intervention. Case 3 experienced tacrolimus-related leukoencephalopathy on postoperative day 6, which was successfully managed with cyclosporine conversion. Cases 1 and 4 showed methicillin-resistant *Staphylococcus aureus* catheter sepsis on postoperative days 6 and 5, respectively. Cases 1 and 3 had cytomegalovirus infections on postoperative days 42 and 38, respectively, which were successfully treated with the administration of ganciclovir. All the children are doing well, with normal graft function at follow-up 3.5, 2.5, 1.5, and 1.0 years after LDLT, respectively (Table 2). There has been marked improvement in the patients' quality of life. All the patients achieved resolution of their metabolic derangement, including correction of hypoglycemia on a normal feeding regimen without a nasogastric tube. The posttransplant DQs were 36, 95, 75, and 95, respectively, which were not significantly different from the pretransplant DQs. The neutropenia improved during the follow-up period without the use of G-CSF; despite this, the absolute neutrophil count remained over 500 per cubic millimeter after LDLT in all cases. The patients have experienced no significant infectious episodes necessitating hospitalization after the successful LDLT.

DISCUSSION

The aim of this study was to evaluate the outcome of patients who underwent LT for GSD-1b. As a result of early diagnosis and a radical treatment with nocturnal nasogastric feeding and uncooked cornstarch, the prognosis of patients with GSD-1b has improved dramatically. After the initiation of a radical dietary treatment, however, the development of neurological impairment as a result of metabolic derangement has been reported in 40% of GSD-1b patients.[6] Three patients in the current series (75%) showed neurological disability at the time of LT. Early LT might be recommended from this point of view because it could reduce the magnitude of the progressive neurological disability resulting from poor metabolic control.

There have been 13 cases of LT for GSD-1b reported in the literature, including the present cases (Table 3). The indications for LT were poor metabolic control in 13 patients and poor growth in 4 patients. The median age of the recipients was 8.3 years (range, 1.1-34 years). Twelve of the 13 patients (92.3%) were alive with excellent graft function at the time of publication. If a patient's quality of life is impaired by the strict feeding schedule to avoid hypoglycemia and by recurrent infections, the patient should be listed for LT.

Improvements in neutropenia after LT were reported in 6 cases (46.2%), 5 of whom received a graft from a parental living donor. Inflammatory bowel disease was not seen in these 6 cases, and this is at variance with previously reported series in which it was associated with neutropenia.[6] Because no donor-derived leukocytes could be detected in the recipient's peripheral blood mononuclear cells by analysis for XY chromosomes in the 4 present cases (data not shown), it appears that the donor-derived leukocytes were not cotransplanted or migrated with the liver graft in the recipient's bone marrow and did not restore its cellularity.[19] G6P is transported to the endoplasmic reticulum by G6PT.[20] Recently, the stoichiometry and topological relationship between the catalytic subunits of G6P and G6PT revealed that the complex forming between G6PT and glucose-6-phosphate catalytic subunit 1/2 (G6PC1/2) appears to maintain normoglycemia and that G6PC3 is needed for neutrophil viability; this suggests an important role for glucose in the homeostasis of human neutrophils.[21] An impairment of this function may cause glucose and neutrophil abnormalities. Neutrophils have been reported to express the

TABLE 3. Worldwide Experience in Liver Transplantation for Glycogen Storage Type 1b

Case	Age (Years)	Sex	Donor	Indications	Improved Neutropenia	Follow-Up (Years)	Reference
1	7	Female	Deceased	PMC	No	2	13
2	7.4	Female	Deceased	PMC	No	6.2	14
3	13.8	Female	Deceased	PMC	No	4.4	14
4	8	Male	Deceased	PG, PMC	No	4	15
5	11.1	Male	Deceased	PG, PMC	No	—	15
6	32	Male	Deceased	PMC	Yes	4	16
7	34	Female	Deceased*	PMC	No	0.7	17
8	13.2	Male	Living	PMC	—	0.1†	18
9	18	Male	Living	PMC	Yes	4	19
10	8.3	Female	Living	PG, PMC	Yes	3.5	This report
11	3.5	Female	Living	PMC	Yes	2.5	This report
12	3.8	Female	Living	PMC	Yes	1.5	This report
13	1.1	Male	Living	PG, PMC	Yes	1	This report

Abbreviations: PG, poor growth; PMC, poor metabolic control.
*Staged kidney and liver transplantation.
†The patient died from systemic candidiasis.

ubiquitously expressed G6PT and G6Pase-beta, which together transport G6P into the endoplasmic reticulum lumen and hydrolyze it into glucose. G6PT-deficient neutrophils, which are unable to produce glucose, may have endoplasmic reticulum stress and increased apoptosis.[22] Overall, a hypothesis explaining why neutropenia improves after transplantation is still not very clear here.

Although LT remains an option in patients with GSD-1b, it does not necessarily address neutropenia, and it thus remains an open question whether LT improves neutropenia in patients with GSD-1b. The mechanisms of G6PT and G6PC3 expression and endoplasmic reticulum stress and the role of G6P-beta are now under investigation in order to clarify the exact role of improved neutropenia in this study population.

In summary, LDLT appears to be a feasible option and is associated with a better quality of life for patients with GSD-1b. Long-term observation may, however, be necessary to obtain sufficient data and establish a clear protocol for this treatment modality.

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Liver transplantation in a patient with propionic acidemia requiring extra corporeal membrane oxygenation during severe metabolic decompensation

Sato S, Kasahara M, Fukuda A, Mizuguchi K, Nakagawa S, Muguruma T, Saito O, Karaki C, Nakagawa A, Yoshii K, Horikawa R. Liver transplantation in a patient with propionic acidemia requiring extra corporeal membrane oxygenation during severe metabolic decompensation.
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Abstract: LDLT is an effective treatment modality in patients with congenial metabolic liver disease. PA is a rare autosomal recessive disorder caused by deficiency in propionyl-CoA carboxylase. The present study demonstrates a two-yr-old girl with PA who was admitted for metabolic decompensation and immediately treated with CHD and protein intake restriction at 46 days of age. Two yr later, the patient was readmitted for severe metabolic decompensation with complete atrioventricular block and ventricular fibrillation. CHDF and ECMO were indicated because of progressive metabolic and cardiac deterioration. After full recovery of the ejection fraction, planned LDLT was performed to prevent further metabolic decompensation and fatal cardiac insufficiency. No significant events occurred after the operation and the condition of the patient is stable with continued protein restriction and carnitine supplementation.

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Key words: extra corporeal membrane oxygenation – living-donor liver transplantation – propionic acidemia

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PA, an organic acidemia, is a rare autosomal recessive disorder caused by a deficiency in PCC, a mitochondrial matrix enzyme involved in catabolism, which converts propionyl-CoA to methylmalonyl-CoA before entering the TCA cycle (1). PA is estimated to occur in every 465 000 births in Japan (2). Deficiency in PCC leads to accumulation of toxic substances in the body, that results in severe metabolic decompensation. In two subunits of the PCC gene (PCCA and PCCB), several genetic mutations were reported, that give rise to the varying activity of PCC (3–5). PA is categorized into two forms: an early (neonatal) onset form, in which clinical symptoms are presented within the first 90 days of life, and a late onset form. Clinical features of the early onset form are metabolic decompensation and fatal cardiac events, including cardiomyopathy, whereas those of the late onset form consist of encephalopathy, episodic ketoacidosis, as well as developmental retardation without metabolic decompensation (3). The survival

Abbreviations: CHD, continuous hemodialysis; CHDF, continuous hemo-diafiltration; ECMO, extra corporeal membrane oxygenation; EF, ejection fraction; LDLT, living-donor liver transplantation; LT, liver transplantation; PA, propionic acidemia; PCC, propionyl-CoA carboxylase; TCA cycle, tricarboxylic acid cycle; UCG, ultrasonic cardiography.

period is significantly shorter for the early compared with the late onset form because of metabolic decompensation and fatal cardiac events (4, 6, 7). A study in 17 patients reported a median survival period of eight months in early onset patients and death at age 2.8 and four yr in two late onset patients (4).

Here, we report a LDLT in a patient with early onset PA presenting with severe metabolic decompensation and potentially fatal cardiac insufficiency, which required CHDF and ECMO support.

Case report

A two-yr-old girl, born by normal delivery after a full-term pregnancy and having no significant family disease history, was referred at 46 days of age for hyperammonemia and metabolic acidosis treatment. The patient showed elevated serum ammonia levels at 1123 μg/dL and decreased base excess at −13.2 mmol/L. Treatment by CHD was immediately initiated after admission, and protein administration was restricted to reduce the load on amino acid catabolism. After CHD initiation, serum ammonia levels were gradually corrected. Diagnosis of PA was determined based on urinary organic acid analysis, which revealed elevated levels of 3-hydroxypropionate. In addition, no cardiomyopathy, a life-threatening complication of PA, was detected.

Protein administration was restricted at 0.9 g/kg/day and L-carnitine supplements, which enhance renal excretion of propionyl CoA as propionylcarnitine, were provided. The general condition of the patient appeared stable with no metabolic decompensation, albeit showing a mild developmental delay.

Two yr later, the patient was readmitted for decreased oral intake and general malaise. Serum ammonia and lactate levels were 116 μg/dL and 3.20 mmol/L, respectively, whereas blood gas analysis showed a base excess of −2.1 mmol/L on admission. Despite fluid resuscitation, deterioration of the metabolic acidosis condition was observed (pH 7.078, HCO_3 8.7 mmol/L, Lac 18 mmol/L, B.E. −22.1 mmol/L, NH_3 62 μg/dL). In addition, electrocardiography showed a rapid deterioration of circulation due to complete atrioventicular block and ventricular fibrillation. Cardiopulmonary resuscitation was immediately performed, and recovery of the sinus rhythm followed. However, blood pressure could not be sufficiently maintained and UCG revealed a decrease in EF to 50%. Despite administration of high-dose inotropic agents and CHDF, UCG showed an EF decrease to 10–20% with diffuse hypokinesis. ECMO (veno-arterial ECMO) was initiated based on diagnosis of cardiac insufficiency secondary to severe metabolic decompensation, and a gradual improvement in blood pressure, EF, and oxygenation followed (Fig. 1). Seven days later, EF recovered to 60% and the patient was successfully weaned from ECMO while maintaining optimal blood pressure

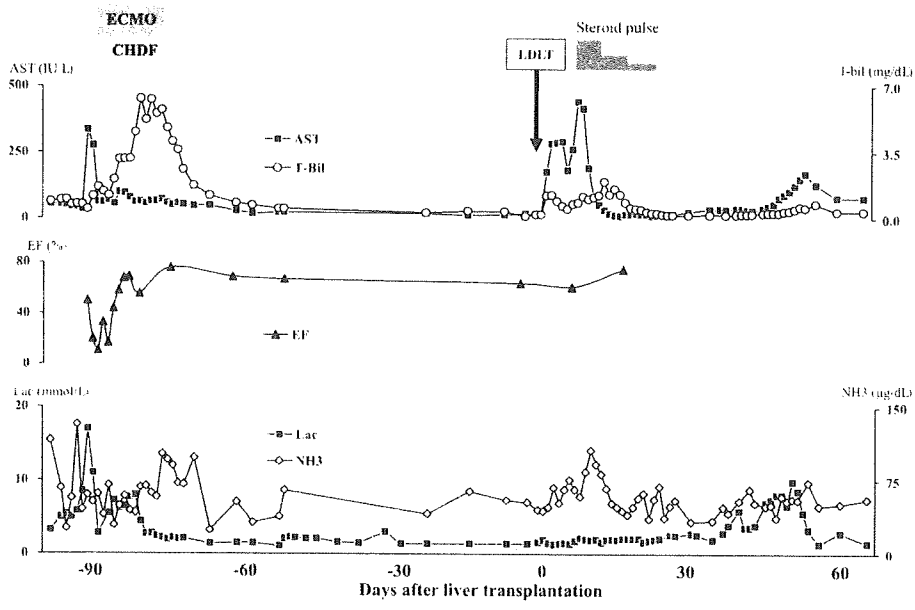

Fig. 1. Clinical course of the patient with propionic acidemia.

and P/F ratio (PaO_2/FiO_2 ratio: oxygenation index). In addition, the patient was weaned from CHDF because of metabolic status improvement.

To avoid further metabolic decompensation and fatal cardiac events, LT was considered and performed three months after cardiac decompensation using the left lateral segment from the father of the patient. Although mild acute rejection was observed after the operation, no metabolic decompensation or cardiac events occurred. Immunosuppressive therapy consisted of tacrolimus and corticosteroid administration. Seven months after LT, metabolic/neurological decompensation and cardiac insufficiency were avoided using mild protein restriction (1.8 g/kg/day) and L-carnitine administration.

Discussion

The medical treatment of PA, consisting of protein restriction, as well as L-carnitine and metronidazole administration, which facilitates the decrease in propionate production by the gut flora, has contributed to survival prolongation (3, 5). Despite medical treatment, life-threatening complications during early-onset PA, namely fatal cardiac events, have frequently occurred. In a study of 19 patients with PA, six (31.6%) were reported to show cardiomyopathy, of which three (50%) died from cardiac insufficiency (6). Electrophysiological investigation by Baumgartner et al. (7) reported a prolonged QTc interval, an independent risk factor for sudden cardiac death, and reduced left ventricular function, detected by UCG, in 70% and 40% of patients with PA, respectively. Given these findings, regular cardiological evaluation of PA patients was recommended. Although still unclear, the causes of cardiomyopathy and QTc prolongation are suggested involvement carnitine deficiency, which possibly induces electromyocardial changes, direct toxic effects of metabolites, which cause prolonged myocardium repolarization, inhibition of oxidative phosphorylation in mitochondria by propionyl CoA, as well as a genetic abnormality (7, 8).

Although LT is indicated for liver-related metabolic disorders, its use in organic acidemias, which are non-liver-related metabolic diseases, is still controversial (9–12). To the best of our knowledge, a total of 11 patients with PA were reported to have undergone LT (Table 1) (5, 10, 13–18). During the neonatal period, metabolic decompensation episodes were observed in all patients, and the median age at LT was two yr, ranging from eight months to nine yr. Livers from seven deceased and four living donors were

Table 1. Worldwide reports of liver transplantation for propionic acidemia

Case	Age at LT	Gender	Graft type	Indication of LT	Post-LT metabolic decompensation	Post-LT protein restriction	Outcome	Reference
1	2 yr	F	Auxiliary	Metabolic decompensation	–	No restriction	Alive, 10 yr	(13, 14)
2	7 yr	M	Deceased (whole)	Metabolic decompensation	+Chronic hyperammonemia	Continued	Died due to PTLD, 15 months	(15)
3	9 yr	F	Deceased (whole)	Metabolic decompensation	–	No restriction	Alive, 5 yr	(15)
4	3 yr	–	Deceased (split-liver)	Metabolic decompensation	Unknown	Unknown	Died, 3 months	(16)
5	2 yr	F	Living	Metabolic decompensation Failure to thrive	+three yr after LT	Unrestricted, then re-initiated because of metabolic decompensation after LT	Alive, 59 months	(10, 17)
6	5 yr	M	Living	Metabolic decompensation	–	Mild	Alive, 30 months	(10, 17)
7	1 yr	M	Living	Metabolic decompensation	–	Mild	Alive, 21 months	(10, 17)
8	8 months	F	Deceased (split-liver)	Metabolic decompensation	–	No restriction	Alive, 12 months	(18)
9	1 yr	M	Deceased (split-liver)	Metabolic decompensation Failure to thrive	–	Mild	Alive, 44 months	(5)
10	2 yr	–	Deceased (whole) Deceased (split-liver)	Metabolic decompensation Failure to thrive Developmental delay	–	Continued	Retransplanted for HAT, Alive, 6 months after initial LT	(5)
11	2 yr 2 months	F	Living	Metabolic decompensation Cardiac insufficiency	–	Mild	Alive, 7 months	Present case

LT: liver transplantation, PTLD: post-transplantation lymphoproliferative disorders, HAT: hepatic artery thrombosis

used for transplantation. It was reported that LDLT using a graft from a heterozygote donor is an effective treatment modality for PA (10). Indications to perform LT included refractory metabolic decompensation (n = 11), failure to thrive (n = 3), developmental delay (n = 1), and cardiac insufficiency (n = 1). In the present study, LDLT was performed after overcoming severe metabolic decompensation and cardiac insufficiency, which required ECMO. However, from Table 1, previous studies report that case 5 presented with metabolic decompensation after LT (10, 17).

For post-LT protein intake, restriction was partly alleviated in eight patients (72.7%), of which four (36%) returned to normal or near normal protein intake. However, protein restriction was re-initiated in one patient because of metabolic decompensation after transplantation (17). These observations demonstrate the importance of protein restriction and L-carnitine administration after LT. In addition, based on post-transplant data from previous reports, LT for PA patients is considered an effective method to avoid further metabolic decompensation and cardiac insufficiency.

In conclusion, we suggest indicating LT in PA patients with frequent hospitalization for metabolic decompensation and failure to thrive despite conventional medical treatment. Further, LT is a potentially important option in avoiding further fatal cardiac events.

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Original Article

Wide Range of Biotin (Vitamin H) Content in Foodstuffs and Powdered Milks as Assessed by High-performance Affinity Chromatography

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Abstract. The biotin (vitamin H) contents of various foodstuffs were determined by using a newly developed high-performance affinity chromatography with a trypsin-treated avidin-bound column. Biotin was derivatized with 9-anthryldiazomethane (ADAM) to fluorescent biotin-ADAM ester. A wide range of biotin contents were found in various foodstuffs depending upon the species (strain), season, organ (of plants and animals), geography, freshness, preparation method and storage method. Among the foodstuffs and fermented foods tested, it was found that wide distributions of biotin content were observed in powdered milk, natto, sake (rice wine), beer, edible oil and sea weed. Since powdered milk is important for child health and development, 14 kinds of powdered and special milks for use in children's diseases were intensively measured. We found that several special milk powders for children with allergies contained low levels of free biotin. Use of these powdered milks caused skin diseases and alopecia in some patients possessing thermolabile serum biotinidase, and administration of free biotin improved their symptoms dramatically. Therefore, it is essential to estimate the total and free biotin contents on each foodstuff in order to improve effective biotin intake and support better health and quality of life for people.

Key words: total biotin, free biotin, wide distribution, foodstuffs, powdered milk

Introduction

Determination of biotin, especially free-form biotin, in foodstuffs is important, because appropriate biotin intake is beneficial in attaining a good quality of life (QOL), better health and development of children and adults, improved physical mechanisms that combat aging and disease and efficient mental capacity.

Recently, we developed a new high-performance affinity chromatographic (HPAC) determination method for biotin using a trypsin-

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Dedication: In memory of the kind encouragement of my beloved daughter, Reiko Hayakawa (21 November 1979 – 1 February 2007).

treated avidin-bound column (1). In this new method, biotin is derivatized by 9-anthryldiazomethane (ADAM) to an ester of fluorimetric biotin-ADAM and detected fluorimetrically at an excitation wavelength of 365 nm and emission wavelength of 412 nm (1–3). This is a simple chromatographic method using the affinity of avidin for biotin. We recently found that avidin is a bifunctional binding protein; i.e., avidin (a well-known biotin-binding protein) can also strongly recognize lipoic acid (4). However, biotin and lipoic acid can be separated and measured safely using this new chromatographic technology. It is a rapid (analysis requires one day per sample), reliable and sensitive fluorometric detection method that makes use of the linear calibration line through the origin. Furthermore, other nutrients and antibiotics do not interfere with this chemical method; i.e., other biological biotin assay methods are sensitive to nutrients and antibiotics in serum samples.

Herein, we describe the wide ranges of biotin contents detected among various foodstuffs depending on the species (strain), season, organ (of plants and of animals), geographical area, freshness and preparation and storage methods. The importance of the free biotin content in powdered milk in relation to babies, who have unstable biotinidase and exhibit biotin deficiency symptoms, is also discussed.

Materials and Methods

Chemicals and reagents

Highly pure form of methanol, acetonitrile, ethanol and ethyl acetate (>99.8%), D-biotin, activated charcoal (acid washed; for column chromatography; P/N 035-18081), 2-propanol (HPLC grade), ethylene glycol (amino acid analysis grade), 25% ammonia water (metal analysis grade), sulfuric acid, sodium chloride, lithium chloride (anhydrous; amino acid analysis grade; >97%) and sodium dihydrogen phosphate dihydrate were purchased from Wako Pure Chemical Industries (Osaka, Japan). D-Desthiobiotin (5-methyl-2-oxo-4-imidazolidine hexanoic acid; D 1411), biocytin (ε-N-biotinyl-L-lysine, Mr 372.5; B 4261) and biotin methyl ester (B 7883) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 9-anthryldiazomethane (ADAM) was purchased from Funakoshi Pharmaceutical (Tokyo, Japan). A 0.25% (w/v) trypsin-EDTA solution was purchased from Invitrogen Corporation (Grand Island, NY, USA).

A light-intercepting microtube with a cap (2 ml; P/N 72.693.018) and a microtube with cap (2 mL; P/N 72.694.007) were obtained from Sarstedt Aktiengesellschaft & Co. (Nümbrecht, Germany). Microcentrifuge tubes (1.5 mL, polypropylene, lock-cap; P/N 96.8668.9.01) were obtained from Treff AG (Degersheim, Switzerland). Ekicrodisc 13 CR (0.2 μm; PTFE; P/N E135), Ekicrodisc 13 (0.2 μm; Versapor; P/N E134) and Ekicrodisc 25 membrane filters (0.2 μm; Versapor; P/N E254) were obtained from Nihon Pall Ltd. (Tokyo, Japan). Paper pH indicator (pH 6.4–8.0, narrow range) were obtained from Whatman Ltd. (Maidstone, Kent, England). Blades and disposable scalpels were obtained from Feather Safety Razor Co. (Osaka, Japan).

An affinity column, Bioptic AV-1 (250 × 4.6 mm I.D.; with chicken egg-white avidin bound to a 5 μm diameter silica gel), was purchased from GL Sciences Inc. (Tokyo, Japan). The contents of the column were removed using an HPLC pump. Bioptic AV-1 affinity gels (5 μm diameter silica gel) are now available (1 g and/or 10 g) from GL Sciences Inc.

Trypsin-treated avidin-bound gel was prepared as described previously (1). A trypsin-treated avidin-bound column (33 × 4.6 mm I.D.) was then prepared.

Ten types of natto (a Japanese food made from fermented soybeans), thirteen sakes (rice wines), ten beers, four coffees, three red wines, four breads, four cheeses, three vinegars, four bananas (three from the Philippines and one

from Formosa), two peanut butters, four edible oils (salad oils including soybean and rapeseed, rice bran, olive, and sesame oils), seven sea weeds, ("Aosa [*Ulva pertusa*], Me-hijiki [the sporophylls of Hijiki seaweed [*Sargassum fujifome*], Hijiki [*Sargassum fujifome*], Kinumozuku [*Nemacystis decipiens*], Ne-Kombu [root of the Sea Tangle; *Laminaria japonica*], Ao-nori [green laver; *Enteromorpha compressa*], and Nori [laver; *Porphyra tenera*], three bovine milks (purchased in February [winter] and May [summer]), four flours (buckwheat, potato and weak and strong wheat flours), five root crops (onion, carrot, scallion, bamboo shoot and garlic), sauerkraut (Hengstenberg, Esslingen, Germany), shiitake (mushroom), soy sauce (Kikkoman Corporation, Noda City, Chiba, Japan), miso (soybean paste), chicken eggs, sujiko (salmon roe), sea urchin roe, black pepper, rice bran, Yakult (purchased in February and May, Yakult Honsha Co. Ltd., Tokyo, Japan), peanuts (parched), soybeans (parched), soy milk, pickles, Nukamiso-zuke (vegetables pickled in fermented rice bran, Lactobacillus and yeast), tofu (bean curd), honey, komatsuna (*Brassica rapa* var. *pervidis*), spinach, Japanese pepper (*Zanthoxylum piperitum*), pork (thigh), corned beef and chocolate were purchased from grocery stores. Dried yeast (The Japan Pharmacopoeia; Ebios; Tanabe Pharmaceutical Co., Osaka, Japan) was purchased from a drugstore. Royal jellies were purchased from apiaries (Yamada Apiary Corp., Kagamino-cho, Okayama, Japan; Bushu Apirary Co., Kumagaya, Saitama, Japan; and San Ken Co., Tokyo, Japan). Bee pollen (imported from Spain) was purchased from Kano Apiary Co., Yame, Fukuoka, Japan. An anemone flower (*Anemone coronaria*) was purchased from a flower shop.

Human breast milk, milk powders and special milk powders for diseases were kindly donated by our institution. Human serum and urine were kindly donated by volunteers. LEW rats (9 wk of age; male) were purchased from Sankyo Labo Service Corporation (Tokyo, Japan).

Lactobacillus casei (*Shirota*) and *Bacillus natto* cells were prepared as described in a previous study (5).

High-performance liquid chromatography

The HPLC system used was as described previously (1). A six-bored high-pressure valve (GL Sciences Inc.) was used with a 0.1 mL sample-loading loop. Biotin-ADAM was detected with a fluorescence detector (Shimadzu Model RF-10Axl with a Cell Temperature Controller) at an excitation wavelength of 365 nm, emission wavelength of 412 nm and flow-through cell temperature of 28°C. The parameters used for the fluorescence detector were gain of 1, sensitivity of 1 and range of 4. One analysis cycle took 80 min using the program shown in Table 1.

Determination of total biotin

Hydrolysis treatment was performed as follows. First, 0.35 mL of sample solution (dispersed in distilled water) and 0.05 mL of concentrated sulphuric acid were mixed together (final concentration of sulphuric acid of 2.25 M). The mixture was placed in a light-intercepting microtube with a cap and autoclaved at 120°C for 1 h (1). Normally, 0.05–0.2 mL of liquid samples were adjusted to 0.35 mL with distilled water, and 5–50 mg of powdered or wet solid samples were dispersed in 0.35 mL of distilled water; the resulting samples were then hydrolyzed after adding 0.05 mL of concentrated sulphuric acid. After hydrolysis, the samples were treated and derivatized as described previously (1).

Determination of free biotin

Free-form biotin was measured as follows. The samples (0.2 mL of milk, 0.1 mL of serum and 10–100 mg of powdered dry and/or minced wet foodstuffs were suspended in 95% methanol and ultrasonicated for 5 min. After filtration through Ekicrodisc 13CR or 25 fiter, the filtrate was dried under a stream of nitrogen gas. The dried methanol extract was dissolved in 1 mL of

Table 1 Typical elution program for the trypsin-treated avidin-affinity column used for the biotin analysis with an analysis time of 80 min*

Time (min)	Function	Value (%)	Value (mL/min)
0.01	B conc	8.0	
0.01	T flow		0.38
0.01	B conc	8.0	
1.0	T flow		0.38
16.0	T flow		0.38
16.01	T flow		1.0
31.99	T flow		1.0
32.0	T flow		0.38
32.0	B conc	8.0	
32.0	B curv	0	
32.0	T flow		0.38
66.0	T flow		0.38
66.0	B conc	47.0	
66.01	B conc	99.0	
66.01	T flow		1.40
72.00	B conc	99.0	
72.00	T flow		1.40
72.01	B conc	8.0	
74.00	B conc	8.0	
74.00	T flow		1.40
74.01	T flow		0.38
74.02	Stop (of programme)		

*A Shimadzu LC-10AD two-pump system (system controller: SCL-10A) was used. The injector was a high-pressure 6-bored valve with a sample loop of 0.10 mL (internal volume). The column temperature was 17°C. The initial conditions were a flow rate of 0.38 mL/min and an 8.0% concentration of solvent B. B conc: concentration (%) of solvent B. B curve: curve mode. 0 was linear. T flow: flow rate (mL/min). Stop: end of program. One analysis cycle takes 80 min. The other conditions are as described in the Materials and Methods section.

distilled water, and 0.015 mL of 2.25 M NaOH was added to the dissolved free biotin in water. After 0.06 mL of 1.40 M phosphoric acid was added and mixed with the extract (pH of approximately 5.4), activated charcoal (ca. 4 mg) was added to adsorb the biotin onto the surface of activated charcoal. The charcoal was then washed by centrifugation with PBS (phosphate-buffered saline) and distilled water 3 times each. The free biotin adsorbed by the charcoal was extracted with 1 mL of 5% ammonia-ethanol, and the extracted solution was dried under a stream of nitrogen gas. The dried free biotin was dissolved by adding 0.1 mL of methanol and was derivatized by adding 0.08 mL of 0.1% ADAM solution as described previously (1).

Statistics

Since the numbers of foodstuff and biological samples were insufficient for estimating the distribution pattern, non-parametric analysis was applied in this text. Therefore, values are indicated as the median and range.

Results and Discussion

Commercially available foodstuffs in Tokyo were analyzed using a short column (3.3 cm long). Using the improved timetable shown in Table 1, the time required for analysis of biotin was reduced to 80 min from the previously reported analysis time of 92 min (1). A representative example of analysis of foodstuffs (carrot, onion and natural cheese) is shown in Fig. 1.

The distributions of total biotin in foodstuffs are summarized in Table 2 in descending order. The biotin concentration, as assessed by the median value, was highest in the royal jelly product from Okayama. The difference in the values for royal jelly may be due to the production methods of the different prducers. Natto (a Japanese food made from soybeans fermented with *Bacillus natto*) also possessed a high median value of biotin and a wide range in distribution. This wide range may be due to strain differences in *Bacillus natto*. Plant species may be important, since the ranges of peanut butter, root crops, banana and coffee were relatively narrow. Edible seeweeds, cheese and bread also showed wide ranges in biotin content. Geographical differences may also be observed; i.e., biotin content is higher

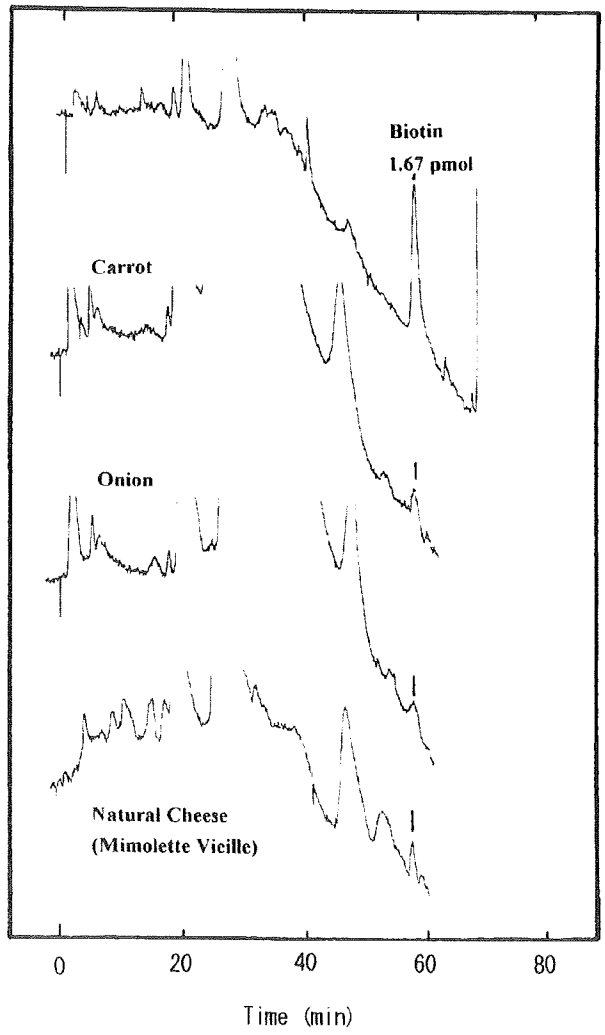

Fig. 1 Typical examples of total biotin analyses of foodstuffs. Upper chromatogram: standard biotin (1.67 pmol). Second chromatogram from the top: 0.002 mL of 10-fold diluted carrot sample (from 34.4 mg wet weight) was injected. The total biotin content of the carrot was 4.03 μg/g wet weight. Third chromatogram from the top: 0.002 mL of 10-fold diluted onion sample (from 33.3 mg wet weight) was injected. The total biotin content of the onion was 3.60 μg/g wet weight. Bottom chromatogram: 0.002 mL of 10-fold diluted natural cheese sample (from 5.1 mg of weight) was injected. The total biotin content of the natural cheese (Mimolette Vieille) was 28.8 μg/g.

Table 2 Total biotin distributions in various foodstuffs available in Tokyo*

Foodstuff	Total biotin (μg/g)**	
	Median	Range
Royal jelly$^{\#\#\#.\ 1}$ (n=3)	180	(1120–20.6)
Natto$^{\#.\ 2}$ (n=4)	40.3	(558–22.4)
Natto$^{\#\#.\ 3}$ (n=9)	13.2	(49.7–4.80)
Coffee$^{\#.\ 4}$ (n=4)	13.1	(37.5–10.4)
Peanut butter$^{\#\#\#.\ 5}$ (n=2)	12.7	(13.5–9.95)
See weed$^{\#\#.\ 6}$ (n=7)	9.27	(12.2–1.52)
Cheese$^{\#\#\#.\ 7}$ (n=4)	9.16	(28.8–1.71)
Bread$^{\#\#\#.\ 8}$ (n=4)	3.52	(12.3–1.31)
Yogurt$^{\#\#\ 9}$ (n=3)	2.86	(9.60–2.10)
Root crops$^{\#\#.\ 10}$ (n=5)	2.50	(4.03–2.19)
Meat$^{\#\#\#.\ 11}$ (n=3)	1.81	(3.45–0.0827)
Flour$^{\#.\ 12}$ (n=4)	1.62	(2.00–0.398)
Banana$^{\#\#\#\ 13}$ (n=4)	1.18	(1.27–0.511)

*Median, distribution, and the top product name and producer are indicated. ** $^{\#}$: Value per gram dry weight. $^{\#\#}$: Value per g wet weight. $^{\#\#\#}$: Foodstuff was weighed as is. [1]Ounyuu-no-hana (Yamada Apiary Corp., Kagamino-cho, Okayama, Japan), [2]Okame Hikiwari Natto (Takano Foods Co. Ltd., Omitama, Ibaragi, Japan), [3]Kizami Natto (Yamada Foods Co. Ltd., Misato-cho, Akita, Japan), [4]Instant coffee; Saty Cafe (Brazil) (Ryoennet Co., Fukutsu-city, Fukuoka, Japan), [5]Skippy (Unilever, Englewood Cliffs, NJ, USA), [6]Aosa (Ulva pertusa) (Ohmoriya Co. Ltd., Osaka, Japan), [7]Mimolette Vieille (Nihon Maisera Co., Kanagawa, Japan; imported from France), [8]Pain Traditionnel (Yamazaki Baking Co. Ltd., Tokyo, Japan), [9]Bulgaria Yogurt (Meiji Milk Products Co. Ltd., Tokyo, Japan), [10]Carrot (Daucus carota), [11]Nozaki Corned Beef (Kawasho Foods Corp., Tokyo, Japan), [12]Buckwheat flour, [13]Mindanao (product of highland), Philippines.

in the Philippines than in Formosa for bananas and is higher in Brazil than in Indonesia and Columbia for coffee. The distributions of biotin in various drinks and beverages are summarized in Table 3. It is apparent that fermented and fermented drinks have wider ranges in their distributions (Tables 2 and 3). This may be due to differences in the microbe strains used in their fermentation and production methods. In our