Original Article

# Plasma and Urine Amino Acid Profiles in a Healthy Adult Population of Singapore

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## Abstract

**Introduction: The analysis of amino acids in plasma and urine was introduced in Singapore when a laboratory for the investigation of inherited metabolic disorders was established by the Ministry of Health. Reference ranges are required for interpreting test results and making diagnoses. Initially, reference ranges established for Caucasians were used as there were no local data and we were unable to find data obtained by the same analytical method for Asian populations. This was not considered an ideal and long-term solution, as Singaporeans may have amino acid concentrations quite different from those of Caucasians due to genetic factors, dietary difference, environment, and other influences. This study was therefore undertaken when a number of healthy laboratory personnel volunteered to provide specimens for the study. Materials and Methods: Sixty healthy male and female laboratory workers not on any form of medication were recruited. They consisted of 24 males (range, 23 to 58 years) and 36 females (range, 20 to 60 years), with a mean age of 38.7 years. Non-fasting random blood and urine specimens were collected on ice. Removal of protein and peptides from heparinised plasma and urine was achieved by ultrafiltration through protein-exclusion membrane. Amino acid analysis on the ultrafiltrate was performed by a dedicated Beckman 6300 Amino Acid Analyzer using a cation exchange resin column and post-column colour reaction with ninhydrin reagent. Urine creatinine was measured by a Beckman LX 20 PRO Analyzer. Results for urine amino acids were expressed as μmol/mmol of creatinine. Results: Reference ranges for 32 amino acids in blood plasma and 36 amino acids in urine were calculated by a non-parametric method using the SPSS statistical calculation software. The ranges cover 95% of the population and the low and high limits of each reference range represent the 2.5th percentile and 97.5th percentile of the frequency distribution respectively. Conclusions: We observed differences in the reference ranges of several plasma and urine amino acids between Singaporean and Caucasian populations. Moreover, the list of urine amino acids for Caucasian population is incomplete. We have therefore discontinued the use of reference values established for Caucasians and adopted the results of this study for our patient diagnostic work.**

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**Key words: Blood amino acid, Normal ranges, Reference values, Urine amino acids**

## Introduction

Analysis of blood plasma and urinary amino acids is required for the diagnosis and management of inherited metabolic disorders (IMDs) affecting one or more amino acids, such as phenylketonuria, tyrosinaemia, citrullinaemia, cystine-lysinuria, hyperglycinaemia, and maple syrup urine disease. It may also be useful for monitoring patients requiring long-term nutritional support, formulating enteral feeds, and studying other diseases that affect amino acid metabolism. While many IMDs result in acute and severe clinical symptoms during infancy and early childhood, some disorders manifest symptoms only during adolescence and adulthood. Thus, patients with citrullinaemia,[1-7] cystine-lysinuria,[1,8] hyperornithinaemia,[9-12] and lysinuric protein intolerance[2,13,14] may not have clinical symptoms that are sufficiently severe for them to be referred to the laboratory for investigations during childhood.

Quantitative measurement of a complete range of amino acids in biological fluids is one of the most difficult tasks in clinical biochemistry. Unlike the wide range of biochemical investigations provided by hospital clinical laboratories, the methods for amino acid analysis are tedious, technically demanding, and costly. For this reason, most clinical laboratories do not offer amino acid analysis

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as a service and leave such analysis to highly specialised national reference laboratories and centres specifically established for the diagnosis and treatment of IMDs. Our laboratory was able to offer amino acid analysis as a diagnostic service only after a substantial research grant was made available for the purchase of appropriate equipment for the investigation of IMDs.

The National Advisory Council of the Disabled has been concerned with the problems and welfare of the physical and mental handicapped as they are a serious burden and cause of severe stress not only to their families but also to society at large. The Council would like to know the cause of congenital disabilities and whether early diagnosis and treatment can be effective in preventing the development of disabilities. Once the diagnosis is known, prenatal investigations and counseling can be provided for future pregnancies. At the recommendation of the Council, the Ministry of Health provided the necessary funds for the establishment of a national laboratory for the study of inherited metabolic disorders other than G6PD deficiency, thalassaemias and hypothyroidism, which already have their respective screening programmes. The primary objective was to determine the incidence and diversity of other metabolic disorders, especially those leading to mental retardation, physical disability and other severe clinical consequences. The strategy for the prevention of IMDs lies in the definitive identification of these diseases, genetic counseling for the affected families and prenatal diagnosis where possible. One of the instruments acquired for this nationwide study was a dedicated amino acid analyser.

Upon the establishment of the laboratory for IMDs, an important task was to obtain reference ranges for amino acids for making diagnostic decisions. Although a number of neonatologists and paediatricians from major hospitals were nominated to serve in the national committee for the first study of IMDs in Singapore, it was not possible to obtain blood or urine specimens from normal healthy infants and children for the study and establishment of reference values.

As amino acid concentration is known to be affected by age,[15-18] especially from infancy to adolescence, we attempted to source for published or unpublished reference values for similar Asian populations. Unfortunately, such information was not available. Due to the lack of financial and manpower resources, priority for diagnosis and treatment of common diseases that affect much larger populations, clinical laboratories in Asia, with the exception of Japan, do not perform amino acid analysis for the diagnosis of IMDs. Visits to several major centres for the diagnosis of IMDs proved fruitful. As infants and young children worldwide are generally on a diet of milk and milk products, variations in amino acid concentrations in body fluids due to dietary differences do not appear to be a major concern. Therefore, we adopted the age-related reference ranges kindly provided by a reference laboratory in Australia (Personal communications 1992 – Hammond J, Wilken B. Oliver Latham Laboratory, New South Wales Biochemical Genetics Service, Royal Alexandra Hospital for Children, Parramatta, NSW, Australia). Separate ranges for female and male children were not available and not necessary as their values are similar.[18]

During the course of the national study, some adult patients who presented with symptoms suggestive of an IMD were referred to us for investigations. Our laboratory was also requested to assist with various studies of nutritional and metabolic changes in adult patients with diseases other than IMDs, as well as studies on experimental animals. As it was not known whether the Caucasian reference values were applicable to the local population, we were requested to provide local reference values. This prompted us to conduct the present study on adult Singaporeans. Much as we would have liked to have a large study population, which would have enabled us to establish comprehensive reference ranges according to gender, age and race, we had serious constraints in obtaining a sufficiently large number of healthy subjects willing to donate blood and urine specimens for this purpose. Unlike highly publicised national studies on common diseases of current public concern, such as hypertension, diabetes mellitus, hyperlipidaemia, metabolic syndrome, breast cancer and prostate cancer, a study on amino acids does not have the same appeal to participants even if the investigations are free and they are paid for their time and effort, as they do not see any immediate benefits. After many attempts and much effort, we eventually managed to persuade 60 laboratory staff of our department to contribute specimens for our study.

## Materials and Methods

Patients with IMDs often present with acute and severe symptoms triggered by high protein intake, stress or infection. This means that the patients are likely to seek medical treatment at any time of the day and under non-fasting conditions. Blood and urine specimens taken at the time of acute presentation are the most appropriate for laboratory diagnosis as they are most likely to contain high concentration of the abnormal metabolites which confirm a specific IMD. A delay in obtaining specimens is likely to cause false-negative test results. When patients are fasted, placed on low-protein diets, given vitamin supplements, or receive intravenous fluid infusion, or various drug therapies, the concentration of abnormal metabolites associated with an IMD may be reduced to normal, or even undetectable levels. Therefore, the most appropriate reference values should be those which take into consideration the maximum

variables affecting the test subject. In practice, at the time of blood specimen collection, most patients are in a non-fasting state and may be on a wide diversity of diets. A spot urine specimen is easily obtained and best serves diagnostic purposes. It is hardly practical to obtain 24-hour urine collection for diagnosing IMDs. Furthermore, the high concentration of metabolites in a random specimen taken at an acute phase of the disease can be reduced by more dilute urine excreted subsequently, rendering the test less sensitive. The current practice of IMD laboratories is to use random urine corrected for creatinine level, expressing test results in micromole amino acid per millimole of creatinine. For these reasons, random non-fasting blood and urine were collected.

Sixty male and female staff of the Department of Pathology, Singapore General Hospital, in good health and not on any form of medication, volunteered for the study. They consisted of 24 males aged between 23 and 58 years and 36 females aged between 20 and 60 years. The mean age of the subjects was 38.7 years. Subjects were not on any special diet so that any variation due to dietary differences would be included in the reference values, which should be representative of the ethnic dietary diversity of Singaporeans. All non-fasting blood and random fresh urine specimens were collected on ice. Plasma was separated from heparinised blood specimens within 30 to 45 minutes of blood collection. When haemolysis in a specimen was observed, it was rejected and a fresh specimen collected. Removal of proteins from the plasma and urine specimens was achieved by ultrafiltration through a protein-exclusion membrane filter device at high centrifugal force at 4°C on the same day. An aliquot of 200 µL of plasma or urine was transferred to a Millipore Ultrafree-MC filter unit (10,000 NMWL) and centrifuged at 8800 × g for 2 h for plasma and 1 h for urine, using a refrigerated Heraeus centrifuge. The resulting ultrafiltrate was stored at -70°C until analysis.

Prior to analysis, the ultrafiltrate was diluted 10 times with a buffer containing the internal standard, (S)-2-aminoethyl-L-cysteineHCl. Amino acid analysis was performed by a dedicated high-performance Amino Acid Analyzer model 6300 (Beckman, USA) using a cation exchange resin column and post-column colour reaction with ninhydrin reagent for the quantitative measurement of individual amino acids (Beckman Instruments Inc, Palo Alto, California, USA). Amino acids were separated on a cation exchange resin column and eluted with a gradient of acidic citrate buffers applied stepwise. There was a programmed increase in column temperature. The column effluent was mixed with ninhydrin reagent for colourimetric detection. Amino acids were identified and measured quantitatively by relating the peaks to an internal standard and by comparison with a standard mixture chromatographed in parallel. Results were calculated using the software package (System Gold) provided with the personal computer system attached to the analyzer. Analytical coefficient of variation (CV) for various amino acids calculated from 10 replicate measurements was 2.3% or less. All urine amino acid values were corrected for creatinine concentration and expressed in micromole amino acid per millimole creatinine. Assay for urine creatinine was performed by a Beckman LX20 PRO automated analyser.

The 95% reference intervals for 32 amino acids in blood plasma (60 adults: 24 males, 36 females; 42 Chinese, 9 Indians, 8 Malays and 1 Eurasian) and 36 amino acids in urine (51 adults: 17 males, 34 females; 39 Chinese, 8 Indians, 4 Malays) were calculated by a non-parametric method using SPSS statistical calculation software (SPSS 10.1.3 for Windows released 16 March 2001).[19] The lower and upper limits for each reference range represent the 2.5th and 97.5th centiles of the frequency distribution respectively.

## Results and Discussion

The results of our study on the reference ranges for plasma and urine amino acids in healthy Singaporeans are listed in Tables 1 and 2, respectively, along with those reported for the Caucasian populations, which we used to interpret patients' test results prior to this study.

Due to technical constraints, tryptophan is not measured by the amino acid analyser. Tryptophan is one of a number of amino acids excreted in high concentrations 5 to 20 times that of normal levels in Hartnup disorder.[20] Renal transport of neutral amino acids, which include alanine, serine, threonine, valine, leucine, isoleucine phenylalanine, tyrosine, histidine as well as glutamine and asparagines, is impaired. However, an inability to measure tryptophan does not affect diagnosis as many other amino acids are increased and indican, a product of intestinal bacterial metabolism of tryptophan, can be measured by a colorimetric method. Another amino acid which presents a problem with the method used in this study is homocystine, as some 70% is protein-bound and 20% to 30% circulates as mixed disulphide in blood.[18] Pretreatment of plasma is necessary for accurate measurement but this would render the specimen unsuitable for analysis of other amino acids. Therefore, plasma homocystine is determined separately using a different method.

Some amino acids are not present in the blood and urine of normal subjects and only appear as abnormal metabolites in patients with IMDs. For example, sarcosine is not found in the urine of normal subjects but is excreted in excess in cases of sarcosinaemia, glutaric aciduria type II and folate deficiency.[2,21] Sulfocysteine is not present in normal urine

Table 1. Reference Ranges of Plasma Amino Acids in Healthy Adult Singaporean and Caucasian Populations

| Amino acids | Reference range in μmol/L | | |
|---|---|---|---|
| | Singaporeans n = 60 (24 M, 36 F) Age: 20-60 y | Caucasians (A)[15] n = not given Age: >18 y | Caucasians (B)[16] n = 280 (140 M, 140 F) Age: 17-65 y |
| | Range (median) | Range (median/mean value not given) | Range (mean) |
| Alanine | 260-585 (396) | 177-583 | 191-531 (350) |
| β-alanine | nd | nd-12 | – |
| α-aminoadipic acid | nd-12 (6) | nd-6 | 0-19 (5) |
| α-aminobutyric acid | 7-51 (28) | 5-41 | 8-29 (16) |
| Arginine | 61-132 (94) | 15-128 | 48-146 (91) |
| Asparagine | 39-83 (53) | 35-74 | 18-106 (62) |
| Aspartic acid | 2-18 (6) | 1-25 | 2-11 (5) |
| Citrulline | 14-61 (32) | 12-55 | 17-40 (28) |
| Cystathionine | nd-7 (3) | nd-3 | – |
| Cystine | 23-71 (52) | 5-82 | 48-111 (82) |
| Ethanolamine | nd | nd-153 | – |
| Glutamic acid | 4-68 (32) | 10-131 | 2-88 (37) |
| Glutamine | 259-687 (561) | 205-756 | 352-689 (527) |
| Glycine | 135-342 (216) | 151-490 | 142-297 (211) |
| Histidine | 56-113 (77) | 72-129 | 58-104 (78) |
| Hydroxyproline | 5-43 (18) | nd-53 | 6-32 (14) |
| Isoleucine | 50-111 (68) | 30-108 | 26-95 (54) |
| Leucine | 96-203 (125) | 72-201 | 56-189 (111) |
| Lysine | 108-234 (173) | 116-298 | 112-271 (175) |
| Methionine | 26-48 (34) | 10-42 | 13-43 (25) |
| 1-Methylhistidine | nd-29 (3) | nd-39 | 5-36 (15) |
| 3-Methylhistidine | nd-16 (7) | nd-8 | 2-6 (4) |
| Ornithine | 36-92 (59) | 48-195 | 43-109 (70) |
| Phenylalanine | 48-73 (58) | 35-85 | 36-88 (55) |
| Phosphoethanolamine | 3-15 (7) | nd-40 | – |
| Phosphoserine | 2-13 (7) | 2-14 | – |
| Proline | 82-301 (164) | 97-329 | 89-361 (194) |
| Serine | 77-167 (120) | 58-181 | 71-165 (111) |
| Taurine | 31-61 (49) | 54-210 | 26-130 (70) |
| Threonine | 97-221 (134) | 60-225 | 69-182 (120) |
| Tyrosine | 48-96 (67) | 34-112 | 30-97 (58) |
| Valine | 169-354 (226) | 119-336 | 109-300 (197) |

Note: 1. Reference ranges were defined as the 2.5th to 97.5th centile of the distribution;
2. nd: not detected;
3. amino acids not measured in the study on Caucasians are indicated by the symbol – ;
4. analysis for Singaporeans and Caucasians (A) and Caucasians (B) was performed by the same method using the Beckman 6300 Amino Acid Analyzer.

but is found in patients with sulfite oxidase-xanthine oxidase deficiency and molybdenum cofactor disorder.[1,22] Pipecolic acid is excreted by patients with pipecolic acidaemia,[21,23,24] Zellweger's syndrome[21] and normal subjects who have consumed durian, a popular tropical fruit in Southeast Asia (Personal communication – External Proficiency Testing Programme organised by the Human Genetics Society of Australasia, Australia). In one of the urine specimens received from the proficiency testing programme organised by the Human Genetics Society of Australasia (HGSA website: www.hgsa.com.au) for laboratories providing IMD investigations, we found a high concentration of pipecolic acid which was not expected in urine specimens of healthy subjects. We were informed that the specimen was obtained from a normal subject who had consumed durian and excreted pipecolic acid as a metabolite. Homocitrulline is only present in patients with hyperammonaemia, hyperornithinaemia with homocitrullinaemia (HHH) syndrome.[1,2] β-alanine is usually not detected or only present at very low concentrations in blood and urine. Primary hyper-β-alaninaemia is a very rare disorder. High levels of β-alanine are more often associated with inherited disorders of uracil and thymine metabolism, and are observed in patients treated with drugs such as isoniazid and aminooxyacetic acid.[25] Anserine and carnosine are also not detectable in normal fasting plasma or in significant amounts in the urine of adults. Patients with carnosinase deficiency have measureable amounts of carnosine in serum and anserine in urine after consuming food containing these 2 dipeptides.[25] However, the consumption of large quantitities of meat rich in imidazole dipeptides, such as poultry and rabbit, can also lead to their excretion in urine. Normal persons excrete 1-methylhistidine after ingesting anserine but patients with serum carnosinase deficiency excrete little or no 1-methylhistidine.[25]

Several abnormal metabolites were identified in our patients and specimens received for a proficiency testing programme. These did not match any of the 39 amino acids used for instrument calibration and were initially noted as "unknown peaks". Their identities were revealed to us by organisers of the proficiency testing programme: hawkinsin in a case of hawkinsinuria,[26] alloisoleucine in patients with maple syrup urine disease,[27] argininosuccic acid and its anhydrides in several patients with argininosuccinase deficiency,[2,28] and saccharopin in a case of hyperlysinaemia.[29]

Three of 54 individuals were found to have urine concentrations of cystine (27, 29, 26 μmol/mmol creatinine) and lysine (137, 135, 133 μmol/mmol creatinine) significantly higher than the rest of the reference population, making them distinct outliers of the data distribution.

Table 2. Reference Ranges of Urine Amino Acids in Healthy Adult Singaporean and Caucasian Populations

| Amino acids | Reference range in μmol/mmol of creatinine | | | |
|---|---|---|---|---|
| | Singaporeans<br>n = 51 (17M, 34F)<br>Age: 20-60 y<br>Random sample | Caucasians (C)[6]<br>n = not given<br>Age: adult<br>Random sample | Caucasians (D)[17]<br>n = 40 (M and F)<br>Age: >13 y<br>First morning | Caucasians (E)[18]<br>n = 40 (M and F)<br>Age: >21 y<br>Random sample |
| | Range (median) | Range (median or mean not given) | Range (mean) | Range (median or mean not given) |
| Alanine | 14-89 (37) | 0-75 | 16-68 (30) | 8-41 |
| β-alanine | nd-6 (0) | – | – | – |
| α-aminobutyric acid | nd-31 (5) | – | nd-4 (–) | – |
| α-aminoadipic acid | nd-14 (5) | 0-14 | – | – |
| β-aminoisobutyric acid | nd-136 (17) | – | nd-91 (–) | – |
| Arginine | 2-13 (3) | 0 | nd-5 (–) | nd-3 |
| Asparagine | 6-50 (15) | 0-35 | nd-23 (–) | – |
| Aspartic acid | 5-11 (7) | – | 2-7 (4) | – |
| Carnosine | nd -5 (0) | – | – | – |
| Citrulline | nd -3 (0) | – | nd-4 (–) | – |
| Cystathionine | nd -9 (0) | – | – | – |
| Cystine | 2-17 (5) | 0-10 | 3-17 (7) | 2-11 |
| Ethanolamine | nd-64 (42) | – | – | – |
| Glutamic acid | nd-7 (2) | 0-40 | nd-12 (–) | – |
| Glutamine | 19-179 (63) | 0-85 | 20-76 (36) | 11-68 |
| Glycine | 37-690 (145) | 0-380 | 43-173 (107) | 31-382 |
| Histidine | 7-281 (94) | 0-220 | 26-153 (79) | – |
| Homocystine | 0-9(2) | – | – | – |
| Hydroxyproline | nd-15 (8) | – | nd-13 (–) | – |
| Isoleucine | 3-9 (5) | 0-20 | nd-4 (–) | – |
| Hydroxylysine | nd-23 (0) | – | – | – |
| Leucine | 2-10 (4) | 5-15 | 2-11 (5) | 1-9 |
| Lysine | nd-87 (22) | 0-50 | 7-58 (17) | 2-17 |
| Methionine | 4-27 (6) | 0-10 | 2-16 (6) | – |
| 1-Methylhistidine | nd-141 (26) | – | – | – |
| 3-Methylhistidine | 5-40 (22) | 19-47 | 19-47 (32) | – |
| Ornithine | nd-11 (2) | 0-15 | nd-5 (–) | nd-6 |
| Phenylalanine | 1-22 (7) | 5-15 | 2-19 (7) | 2-10 |
| Phosphoethanolamine | 2-13 (5) | – | – | – |
| Phosphoserine | 5-29 (9) | – | – | – |
| Proline | nd-9 (0) | – | nd-9 (–) | nd |
| Serine | 21-133 (45) | 5-100 | 21-50 (30) | 14-65 |
| Taurine | 21-244 (103) | – | 16-180 (72) | – |
| Threonine | 8-58 (21) | nd-50 | 7-29 (13) | – |
| Tyrosine | 6-38 (15) | nd-35 | 2-23 (10) | – |
| Valine | nd-18 (4) | nd-30 | 3-13 (5) | – |

Notes: 1. Reference ranges were defined as the 2.5th to 97.5th centile of the distribution;
2. nd: not detected;
3. Amino acids not measured in the study on Caucasians are indicated by the symbol – ;
4. (–): mean value not calculated in the study;
5. Analysis for Singaporeans and Caucasians (C) performed by the Beckman 6300 Amino Acid Analyzer;
Caucasian (D) by a Technicon NC 3M Analyzer and a Chromakon 500 Analyzer (results between the 2 instruments were similar);
and Caucasians (E) by a pre-1980 method.
6. Caucasians (C): Hammond J, Wilken B. Oliver Latham Laboratory, Royal Alexandra Hospital for Children, NSW, Australia.
Personal communications 1992.

Although these concentrations are not sufficiently high for a diagnosis of homozygous cystine-lysinuria, they suggest a possible heterozygous state. We were not certain whether the higher concentrations were due to genetic factors or dietary influence. It will be necessary to perform repeat analyses on these subjects over a period of time to see if the increases are transient or persistent. The test results of these 3 subjects were therefore omitted from the calculation of urine reference ranges. We were surprised by this unexpected finding. However, when we checked the literature for the incidence of cystine-lysinuria, we found that there are populations where homozygous cystinuria (high lysine excretion is associated with cystinuria) is a frequently inherited disorder.[8] Its prevalence in Israeli Jews of Libyan origin has been estimated to be 1 in 2500.[8] Newborn screening programmes revealed a prevalence of 1 in 2000 in England, 1 in 4000 in Australia and 1 in 15,000 in the United States. Summarising the results of newborn screening programmes, the overall prevalence is 1 in 7000, making cystinuria one of the most common inherited disorders. In our study of 3650 patients suspected of having an IMD, 8 were found to have cystine-lysinuria, making the prevalence in this IMD high-risk group 1 in 456. The prevalence in the general population should be lower. While older children and adult patients presented with haematuria, urinary tract infection and renal calculali, young patients did not have obvious symptoms and would not have been diagnosed had they not been referred for investigations because of manifestations suggestive of an IMD other than cystine-lysinuria. Prior to the availability of amino acid analysis for routine diagnosis purposes, patients were mostly diagnosed during adulthood and/or when they presented with renal colic, urinary tract obstruction or infection.

β-aminoisobutyric aciduria is a benign metabolic polymorphism. It has been reported to be more common in Asian than Caucasian populations.[25,30,31] In our current study, although the upper limit of reference range of 136 μmol/mmol creatinine for urine β-aminoisobutyric acid was 1.5 times higher than that observed in Caucasians, we did not find any outlier values (test results of high excreters) which needed to be excluded when calculating its reference range. This is in contrast to our study in 448 young children, which found 14.9% of high excretors of β-aminoisobutyric acid (exceeding double the upper limit of the reference range). β-aminoisobutyric acid is known to be increased by catabolism and during somatic growth. This may explain the high incidence of increased excretion in children, as they were often in distress, severely ill, and in a catabolic state at the time of specimen collection. It would be interesting to perform repeat analyses on these subjects when they become adults to see if the high excretion persists.

Our experience showed that a surprisingly high proportion of 28.3% of patients referred to us for investigation manifested clinical symptoms between puberty and the fifth decade of life. This means that a significant number of patients have been undiagnosed or misdiagnosed as their presenting symptoms may have been suggestive of a variety of diseases other than IMDs. The presence of an IMD in a patient presenting with severe neurological symptoms and coma may not be on the list of possible diagnoses in the mind of an attending physician because of low awareness and limited experience in the area of IMD in adult medicine. This relatively low level of awareness can be partly attributed to the general misconception that the incidence of IMD in Singapore is very low and the absence of highly specialised laboratory investigations for IMD diagnosis in Singapore for a very long time. Although neonatologists and paediatricians in Singapore and neighbouring countries have been referring patients to our laboratory, the availability of specialised tests for disorders of amino acids, organic acids and mucopolysaccharides in our reference laboratory is still not widely known to many physicians practicing adult medicine. Thus, adult patients presenting with hyperammonaemia, stroke-like episodes, vomiting, diarrhoea, cirrhosis, osteoporosis and fractures, Reye-like syndrome, pancreatitis, leucopenia with or without thrombopenia and anaemia, corneal opacity, cardiomyopathy seizure and coma may not be recognised as part of the manifestations of some IMDs.[1,2] Thus hyperammonaemia, a common presentation of amino acid disorders of the urea cycle and several organic acidurias, could be mistaken as a result of liver pathology and failure.

When amino acid analysis was first offered for patient investigation, we did not have reference values for local or Asian populations. As a temporary measure, we had to depend on published or unpublished data on Caucasians so that we could carry out interpretations and make diagnostic decisions. As diagnosis is largely based on an increase in amino acids due to an enzyme deficiency causing a block in one or more metabolic pathways, the upper limit of a reference range is most frequently used. The lower limit of reference range is usually not diagnostic and only helpful in certain disorders. In many IMDs, accumulation of the diagnostic amino acids is so marked that concentrations can reach 10 to 20 times the upper limit of reference ranges or even higher, during a severe acute episode of the disease. However, for some IMDs, only a slight or moderate increase may be observed, especially when a patient is not having acute metabolic decompensation or is under treatment, or when taken off regular meals and placed on dextrose-saline infusion. In such situations, the diagnostic metabolites may be as low as 1.5 to 2.5 times the upper limit of normal. We were advised by the organiser of the Australasian proficiency testing programme that sometimes, the branched

chain amino acids in maple syrup disease may not increase remarkably. We should not readily dismiss results that are slightly above normal as insignificant. (Personal communications 1992 – Hammond J, Wilken B. Oliver Latham Laboratory, New South Wales Biochemical Genetics Service, Royal Alexandra Hospital for Children, Parramatta, NSW, Australia).

Initially, we used the 2 ranges for Caucasians listed in Table 1 as references for plasma amino acids. While about 50% of the amino acid ranges listed under Caucasian (A) and Caucasian (B) are similar, there are significant differences in the upper limit values in the remaining 50%. For those amino acids where there are significant differences in upper limit values, we selected the higher values for reference so that we would not inadvertently interpret a laboratory test result as "elevated".

Urine amino acid ranges listed in Table 2 under Caucasians (D) were initially used for our reference. However, a different method of analysis was used for subjects in Caucasians (D) and this is likely to give results not comparable with our method. The age range for the 40 control subjects (sex and ethnic origin not indicated) was given as over 13 years. The subjects were hospitalised patients who did not have kidney disease, liver failure, known metabolic diseases, or other disorders for which variations of aminoacidurias have been described: e.g., muscle, bone, skin, and eye diseases. Patients whose therapy was known to induce abnormal aciduria or interfere with the method of measurement were excluded. All patients were in good nutritional states with normal protein intake. First-morning urine specimens were collected for analysis. This study was conducted in a paediatric hospital in France and involved 9 groups of 40 subjects whose aged ranged from the day of birth to over 13 years. The highest age in the group of subjects of over 13 years was unlikely to exceed 18. Therefore, while we were able to use the reference ranges for paediatric patients, they were inappropriate for our adult patients.

The published reference ranges listed under Caucasians (E) are incomplete and the data were obtained by a pre-1980 analytical method, at a time when automated high-performance liquid chromatography was less well developed. When the unpublished data listed under Caucasians (C) were offered to us by a reference centre for the study of genetic disorders in Australia, we gladly adopted them for reference as these had been were obtained by analytical method identical to ours (Personal communications — Hammond J, Wilken B. Royal Alexandra Hospital for Children, Parramatta, NSW, Australia). Even so, these data are not ideal as we do not know the extent to which amino acid concentrations would be influenced by diet, ethnic differences or other genetic factors, environmental factors, and physiological statuses of the subjects.[18] Furthermore, the reference ranges for 16 amino acids were not provided in this list. Thus, in spite of various constraints, we undertook this study to establish reference ranges which are more appropriate for the Singaporean population. This will, hopefully, satisfy the many enquiries and requests to provide local reference values for a variety of studies other than the diagnosis of IMDs. However, as indicated earlier, rather than establishing narrower ranges which cater to each potential variable factor separately, it is more practical and useful to have values which cover the common variables, so that the upper limit of the reference ranges would include any increase in amino acid concentrations that is not due to an IMD. For this reason, our reference ranges may not be the most appropriate for those who wish to evaluate the nutritional status of subjects. Fasting blood specimens and first-morning urine specimens may be preferable, as the nature of different diets and the time of specimen collection after food intake can have significant effects on amino acid concentrations. The results of this study showed that some of the ranges listed under Caucasians (C) are quite different from those obtained for Singaporeans. We have therefore replaced the Caucasian reference values for plasma and urine amino acids with those obtained in this study.

In interpretating the results of amino acid analyses, it is necessary to obtain a dietary history from the patient when an increase in certain amino acids does not appear to be consistent with the clinical symptoms or possible diagnosis indicated by laboratory findings. Increase in plasma and urine citrulline concentration is observed in the urea cycle defects, argininosuccinate synthetase deficiency and argininosuccinate lyase deficiency while elevated arginine is found in argininaemia. Results of a recent report on watermelon-induced citrullinaemia highlighted the importance of obtaining a comprehensive history of food intake from patients presented for diagnosis of a suspected IMD. Watermelon, a common fruit in Singapore and other countries, has been found to be a natural and rich source of citrulline.[32] Citrulline content between seeded and seedless types was similar, but was lower in red-fleshed watermelons than the yellow and orange varieties. Rind contained more citrulline than flesh. A 10-month-old girl and 6 healthy adults developed elevated plasma citrulline and moderately elevated arginine after consuming large quantities of watermelon.[33]

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