European Journal of Clinical Nutrition (2016) **70,** 1123–1126

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ORIGINAL ARTICLE

Polyunsaturated fatty acid status in treated isovaleric acidemia patients

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BACKGROUND/OBJECTIVES: Nutritional deficiencies are frequently observed when treating patients with inborn errors of metabolism due to an unbalanced diet. Thus far, patients with isovaleric acidemia (IVA) who adhere to a restricted protein diet have not been investigated in this respect. We hypothesize that these patients may have a polyunsaturated fatty acid (PUFA) deficiency, leading to potential clinical complications.

SUBJECTS/METHODS: We examined the nutritional status by reporting on potential deficiencies in PUFAs in treated IVA patients. A general clinical chemistry work-up as well as gas chromatography flame ionization detector analysis was performed to determine PUFAs in the plasma of 10 IVA patients.

RESULTS: The general clinical chemistry tests did not indicate severe hematological abnormalities or nutritional insufficiencies. We identified a significant reduction in plasma PUFA levels, especially in omega-3 (all acids, $P < 0.001$) and omega-6 (in particular 20:3n-6 $P < 0.0001$ and 20:4n-6 $P = 0.0005$) fatty acids. In addition, an elevation in omega-9 fatty acids, with the exception of 20:3n-9 and C22:1n-9, was not suggestive of complete essential fatty acid deficiency but rather indicative of isolated and/or combined omega-3 and omega-6 fatty acid depletion.

CONCLUSIONS: This study emphasizes the potential nutritional insufficiencies that may occur because of therapeutic intervention in IVA.

European Journal of Clinical Nutrition (2016) **70,** 1123–1126; doi:10.1038/ejcn.2016.100; published online 22 June 2016

INTRODUCTION

The conventional management and intervention of patients with inborn errors of amino acid catabolism encompass a specific dietary regimen. Isovaleric acidemia (IVA), a genetic defect of leucine catabolism as a result of deficient isovaleryl-CoA dehydrogenase (E.C.1.3.99.10), is a treatable condition, which requires a diet low in protein or more specifically leucine. Affected patients may present with hyperglycemia or hypoglycemia, ketoacidosis, hyperammonia, as well as immunological/hematological aberrations including pancytopenia and isolated neutropenia and thrombocytopenia.[1] Prognosis is generally good; however, several patients may display with mild motor dysfunction and/or cognitive deficits. The extent of the neurological dysfunction appeared to be independent of the number and severity of the episodes of metabolic decompensation.[2]

The practice of dietary treatment includes the limited intake of animal products (for example, meat, fish, milk, eggs), which may subsequently impose a potential risk of acquiring deficiencies in vitamins, trace elements and essential fatty acids (EFAs) in patients with treatable inborn errors of metabolism.[1,3] Related deficiency, more specifically in EFAs and consequently polyunsaturated fatty acid (PUFAs), has been described in patients with phenylketonuria, urea cycle defects, maple syrup urine disease, methylmalonic acidemia and homocystinuria.[3–6] The latter may therefore be considered as candidate-causing factors for the development of neurocognitive disturbances in IVA patients following a low-protein diet, without notable nutrient supplementation.

The group of PUFAs includes the omega-3 and omega-6 fatty acids, which are derived from the EFAs alpha-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), respectively, as well as omega-7 and omega-9 fatty acids, which are non-essential and can be synthesized from saturated fatty acids.[7] The production of PUFAs includes processes of elongation (via elongases), desaturation (via desaturases) and β-oxidation. These modifying reactions are mainly regulated by substrate availability.[8–10] Numerous PUFAs including dihomo-gamma-linolenic acid (20:3n-6), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), docosapentaenoic acid (C22:5n-3) and docosahexaenoic acid (C22:6n-3) have important clinical functions in the cells, of virtually all organs, including the central nervous system.[10–12]

PUFAs have an intricate role in membrane fluidity, as they are incorporated into phospholipids such as phosphatidylcholine and cardiolipin, thereby contributing to the structure and function of cell membranes. They are directly or indirectly involved in signal transduction processes, immunological, gestational, blood pressure regulation, as well as in neurological development and maintenance including myelination.[12–15] There is furthermore a body of evidence indicating the role of PUFA deficiency in the occurrence of cognitive dysfunction, as well as in the progress of a variety of psychiatric disorders.[16,17] For this reason, an adequate intake of EFAs and a fine balance in the regulatory production of PUFAs are required.

Our recent clinical and metabolomics studies of a genetically homogenous IVA patient group have shown a considerable variability with regards to outcome after therapeutic intervention.

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Received 10 September 2015; revised 15 May 2016; accepted 17 May 2016; published online 22 June 2016

The latter correlates with previous findings reported in a large German IVA patient group.[2,18,19] This prompted us to investigate the nutritional status of the patient group in analogy to previously published studies.[5] A general clinical chemistry work-up was carried out to investigate their basic clinical and nutritional status and PUFA levels in plasma were measured in each IVA patient.

PATIENTS AND METHODS

Collection of samples from IVA patients

Ten patients with IVA as a result of a homozygous c.367 G > A (p.G123R) mutation of the isovaleryl-CoA dehydrogenase gene, with ages ranging from 2 to 24 years, were initially diagnosed by the Potchefstroom Laboratory for Inborn Errors of Metabolism, South Africa.[19] The patients and their families attended an informative 1-day session in which all aspects of the investigation were discussed. Informed consent was given by all participating individuals or their legal guardians. A questionnaire completed by the patient and/or families indicated that all patients but one (a 24-year-old male) complied with a daily low-protein diet (varying between 0.9 g/kg/day and 1.04 g/kg/day) prescribed by a registered dietician in accordance to age and weight. Non-fasting blood specimens and random urine samples were collected on the same day, following the consumption of a meal consisting mainly of carbohydrates with limited protein content. None of the patients suffered from any metabolic decompensation at the time of sample collection. Anonymized plasma samples collected after a self-chosen simple meal from 54 healthy young adults were used in this study as controls.

Analysis of PUFAs in plasma

We analyzed the PUFAs in plasma samples using a modification of the method initially described by Muskiet *et al.*[20] and validated by Duran and Wanders[9] routinely performed in the laboratory of genetic metabolic disease, Amsterdam medical center, the Netherlands. A volume of 50 µl of plasma was pipetted into a 2 ml glass tube. Subsequently, an internal standard (100 µl), containing 72 µmol/l of 18-methyl-C19:0-methylester dissolved in chloroform, was added to the samples. Simultaneous hydrolysis and methylation of the fatty acids was achieved with 1 ml methanolic hydrochloric acid (3 M). The samples were vortexed and subsequently incubated at 90 °C for 4 h. After cooling, 2 ml hexane was added and vortexed for 10 s. The hexane layer was collected and dried under nitrogen at room temperature. The dried residue was resuspended in 100 µl hexane. The derivatized PUFAs were analyzed on a GC-FID (HP GC5890 series II, Agilent, Palo Alto, CA, USA) equipped with a combined capillary-free fatty acid pre-column (Hewlett-Packard FFAP, Agilent) and a DB17 column (J & W Scientific, Agilent). All concentrations were expressed as µmol/l, and significance was given by *P*-value calculation.

Statistical analysis

The data were analyzed using the STATISTICA data analysis software system, version 10 (StatSoft, Inc, Tulsa, OK, USA). Two sample *t*-tests (equal variances not assumed) were utilized to compare the means of the indicated groups in each analysis. Normal probability plots on the residuals in the models were carried out to assure that the data were normally distributed, which is an assumption when using a parametric statistical approach.[21] All *P*-values < 0.05 were considered to represent significant differences between groups.

RESULTS

A general clinical chemistry work-up was performed as routine for all IVA patients to obtain information on their general health and condition including nutritional and infectious status. The results of the hematological tests did not reveal any obvious aberrations, including the absence of pancytopenia and isolated neutropenia and thrombocytopenia, commonly observed in patients with IVA.[1]

PUFA status of IVA patients

To ascertain possible insufficiencies in PUFAs, we first measured individual monounsaturated fatty acids and PUFAs in plasma of the IVA patients. All plasma omega-3 fatty acids were significantly decreased compared with the levels in control subjects, a somewhat worrying finding in the light of the proven neurological and anti-inflammatory roles of the omega-3 fatty acids.[14,22,23] Some omega-6 fatty acids (C20:3n-6, C20:4n-6) were significantly lower than controls, whereas C22:5n-6 was higher. We unexpectedly found a significant reduction in individual omega-7 and omega-9 fatty acids—namely, C18:1n-7, C20:3n-9 and C22:1n-9. This is in contrast with previous descriptions of an EFA deficiency in patients with fat malabsorption. It may therefore theoretically be associated with the dietary differences between various patient groups.[7] Furthermore, traditional essential fatty acid deficiency (EFAD) markers, including significantly lowered C18:2n-6, increased C20:3n-9 and increased triene-tetraene (20:3n-9/20:4n-9) ratio, were not observed.[24,25]

As can be seen from Table 1, individual PUFA values (some of these only marginally decreased in the IVA patient group) did not give a clear indication of PUFA status. Therefore, the sum of the fatty acids belonging to each PUFA group was used to achieve a more discriminative profile for the evaluation of the PUFA status of IVA patients. We observed a statistically significant decline in total omega-3 ($P < 0.001$) and total omega-6 ($P < 0.005$) fatty acids with a non-significant ($P = 0.34$) accumulation of total omega-9 fatty acids, which may be suggestive of mild EFAD (Figure 1).[7] In addition, the omega-6/omega-3 ratio value in IVA patients was more than twice the ratio value observed in the control group ($P < 0.001$), a proposed indicator of EFAD.[22]

Table 1. Individual monounsaturated fatty acids and polyunsaturated fatty acids and related ratios of control and IVA groups

Fatty acid and ratios	*IVA (n = 10)*	*Control subjects (n = 54)*	P-*value (t-test)*
Omega-3 fatty acids			
C18:3n-3	24.8 ± 13.3	73.0 ± 45.6	0.0016[a]
C20:5n-3	13.9 ± 17.9	67.6 ± 50.2	0.0015[a]
C22:5n-3	18.7 ± 10.4	34.8 ± 12.2	0.0002[a]
C22:6n-3	64.3 ± 28.5	128.5 ± 46.1	0.0001[a]
Omega-6 fatty acids			
C18:2n-6	2796.7 ± 493.9	3217.0 ± 630.1	0.0510
C18:3n-6	35.0 ± 17.7	46.1 ± 21.7	0.1332
C20:2n-6	16.9 ± 4.8	20.3 ± 6.8	0.1361
C20:3n-6	84.8 ± 30.8	157.0 ± 44.1	< 0.0001[a]
C20:4n-6	415.6 ± 114.3	594.5 ± 144.9	0.0005[a]
C22:4n-6	16.1 ± 6.5	14.5 ± 4.6	0.3549
C22:5n-6	13.9 ± 5.2	10.0 ± 4.1	0.0107[a]
Omega-7 fatty acids			
C16:1n-7	206.0 ± 92.7	244.4 ± 108.9	0.2997
C18:1n-7	115.3 ± 25.6	186.5 ± 45.8	< 0.0001[a]
Omega-9 fatty acids			
C16:1n-9	67.3 ± 75.2	50.6 ± 15.8	0.1353
C18:1n-9	2365.1 ± 844.5	2135.4 ± 665.5	0.3403
C20:3n-9	6.3 ± 3.8	10.7 ± 4.4	0.0050[a]
C22:1n-9	3.4 ± 2.2	20.2 ± 6.8	< 0.0001[a]
C24:1n-9	77.5 ± 21.6	68.5 ± 18.2	0.1692
Omega-6/omega-3 ratio	32.7 ± 13.2	14.75 ± 4.3	< 0.0001[a,b]
C20:3n-9/C20:4n-6	0.018 ± 0.018	0.017 ± 0.006	0.8838[b]

Abbreviation: IVA, isovaleric acidemia. EFA values are in µmol/l and represent mean ± standard deviation observed in patients and control groups. [a]Significant differences ($P < 0.05$) between IVA patient and control groups. [b]Omega-6 (sum)/omega-3 (sum) ratio and C20:3n-9/C20:4n-6 may be used as indicators of EFAD deficiency.[21]

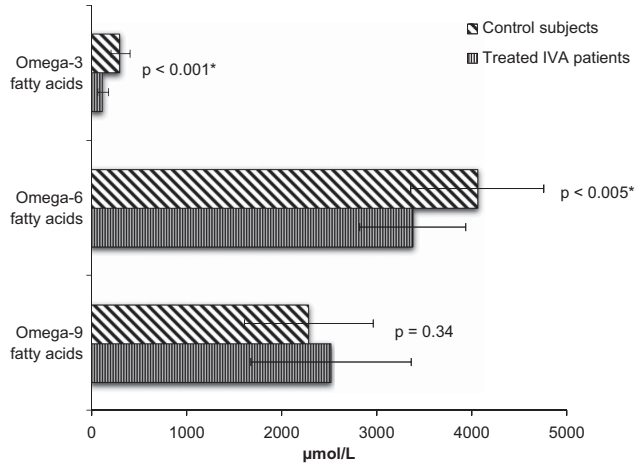

Figure 1. The sum of omega-3 (C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3), omega-6 (C18:2n-6, C18:3n-6, C20:2n-5, C20:3n-6, C20:4n-6, C22:4n-6. C22:5n-6) and omega-9 fatty (C16:1n-9, C18:1n-9, C20:3n-9, C22:1n-9, C24:1n-9) acids in control (indicated by the diagonal line bar) and treated IVA patients (indicated by vertical line bar). A significant decline in omega-3 and omega-6 fatty acids ($P < 0.001$ and $P < 0.005$, respectively) was observed with a concomitant moderate (non-significant) increase ($P = 0.34$) of the omega-9 fatty acids in the IVA patient group. The bars represent mean ± standard deviation observed in patients and control groups. Significant differences between IVA patient and control are indicated by $P < 0.05$.

DISCUSSION

The main purpose of this study was to investigate whether there are PUFA deficiency in metabolically stable, treated IVA patients. Individuals with similar amino acid disorders have shown nutritional deficiencies.[6] We compared the PUFA profiles of 10 genetically homogeneous IVA patients with control subjects and found a significant reduction in all the individual omega-3 fatty acids in the IVA patient group. Significant deficiencies in individual PUFAs were found in the omega-6 and omega-9 fatty acid classes, with the exception of C18:2n-6, C18:3n-6, C20:2n-6, C22:4n-6, C16:1n-9, C18:1n-9 and 24:1n-9. The significantly reduced 22:1n-9 found in IVA patients is noteworthy. This has also been reported in adrenoleukodystrophy patients with aberrant neurological function.[26] EFA and PUFA levels in plasma are, however, a reflection of their intake during a limited period before blood sampling, as well as of their interconversion in the liver, which is an ongoing process.[27] Normal plasma levels of C18:2n-6 (linoleic acid) and C18:3n-6 (linolenic acid) as observed in our patients may therefore indicate that their recent dietary intake was not necessarily insufficient.

The current data presented in this study do not fully support EFAD as originally defined by Fulco and Mead[24] and Holman.[25] Recent literature, however, emphasizes the need for a novel look at EFAD, which includes omega-3 fatty acid inclusion.[28] We therefore support the use of the total omega-3/total omega-6 ratio as suggested by Simopoulos[22] as a valued marker for isolated and/or combined omega-3 and omega-6 deficiency. The increased omega-6/omega-3 ratio in combination with the summation of the groups of omega-3 (significantly lower), omega-6 (significantly lower) and omega-9 fatty acids, depicted in Figure 1, rather points to an isolated and/or combined depletion of omega-3 and omega-6 fatty acids.[7,22]

Furthermore, the striking shortage of eicosapentaenoic acid 20:5n-3 ($P = 0.0015$), docosahexaenoic acid 22:6n-3 ($P = 0.0001$) and arachidonic acid 20:4n-6 ($P = 0.0005$) is a worrying observation that needs careful attention, as it may contribute to the already observed neurological aberrations present in IVA patients. These three PUFAs are generally considered to be the most crucial for a number of central nervous system-related functions. Their dietary content is quite low and, consequently, their bioavailability is virtually totally dependent on biosynthesis from exogenous unsaturated fatty acids such as linoleic acid and alpha-linolenic acid.[27] It is important to note that the measurement of PUFAs in erythrocytes, which was unfortunately not feasible in our study, may provide even more detailed information with regard to the PUFA status of the IVA patients. Nevertheless, our data are in good agreement with those in previous reports, which studied patients with various defects of amino acid metabolism, including the urea cycle defects.[5,6]

CONCLUSIONS

The proposed PUFA deficiency (indicated by a significantly increased individual PUFAS as well as the omega-6/omega-3 fatty acid ratio) was evident in treated IVA patients. We suggest that dietary supplementation of docosahexaenoic acid and arachidonic acid may be required to overcome nutritional deficiencies imposed by the protein-restricted diet prescribed to IVA patients. Such a supplementation may theoretically contribute to a more favorable neurological outcome of treatment in the long run.[23] The monitoring of nutritional markers in plasma and erythrocytes should be included to optimize the treatment regimen of IVA patients and provide continuous individualized health care.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was financially supported as a Carolina MacGillavry PhD Fellowship awarded by 'Koninklijke Nederlandse Akademie van Wetenschappen' to assist in research in Isovaleric acidemia. We thank the staff at the Laboratory Genetic Metabolic Diseases (GMDs) at the Academic Medical Center, University of Amsterdam, for their expert advice and assistance. The study was also made possible with the logistical, scientific and statistical contribution of the staff of Human Metabolomics and Wilma Breytenbach, the statistical consultant at the North-West University in Potchefstroom, South Africa. We are grateful to the patients and their families for participation in this study. Data presented at ICIEM 2013 and Submitted for publication.

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