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A critical reappraisal of dietary practices in methylmalonic acidemia raises concerns about the safety of medical foods. Part 1: Isolated methylmalonic acidemias (MMA)

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Abstract

PURPOSE—Medical foods for methylmalonic and propionic acidemias (MMA/PA) contain minimal valine, isoleucine, methionine and threonine, but have been formulated with increased leucine. We aimed to assess the effects of imbalanced branched-chain amino acid intake on metabolic and growth parameters in a cohort of MMA patients ascertained via a natural history study.

METHODS—Cross-sectional anthropometric and body composition measurements were correlated with diet content and disease-related biomarkers in 61 patients with isolated MMA (46 *mut,* 9 *cblA* and 6 *cblB*).

RESULTS—Patients with MMA tolerated close to the recommended daily allowance (RDA) of complete protein (mut^0: 99.45 ± 32.05% RDA). However, 85% received medical foods, the protein-equivalent in which often exceeded complete protein intake (35%). Medical food consumption resulted in low plasma valine and isoleucine concentrations, prompting paradoxical supplementation with these propiogenic amino acids. Weight and height–for age *Z*-scores correlated negatively with the leucine/valine intake ratio (r=−0.453, P=0.014, R^2=0.209 and r=−0.341, P=0.05, R^2=0.123, respectively).

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Author contributions:

I.M. designed the study, performed data collection and statistical analyses and wrote the paper.
J.M. collected and analyzed dietary data and edited the paper.
J.L.S. coordinated the clinical research and edited the paper.
O.S. contributed patient clinical data and edited the paper.
C.P.V. designed the clinical research studies and wrote the paper.

CONCLUSION—Increased leucine intake in patients with MMA resulted in iatrogenic amino acid deficiencies and was associated with adverse growth outcomes. Medical foods for propionate oxidation disorders need to be redesigned and studied prospectively, to ensure efficacy and safety.

TRIAL REGISTRATION—This clinical study is registered in www.clinicaltrials.gov with the ID: NCT00078078. Study URL: http://clinicaltrials.gov/ct2/show/NCT00078078

Keywords

Methylmalonic acidemia; medical foods; branched-chain amino acids; leucine; dietary guidelines

INTRODUCTION

Medical foods are specially-formulated products intended to provide safe alternatives to a regular diet in patients with inborn errors of metabolism[1]. These products are designed to abrogate the accumulation of toxic metabolites by limiting the amounts of precursors metabolized through a disease-specific enzymatic block. However, when the underlying defect involves the metabolism of essential nutrients, such as branched chain amino acids (BCAA), harmful deficiencies can arise if medical foods are used as a primary dietary source. Despite the inherent risk of inducing iatrogenic side effects such products are currently classified as “foods for special dietary use” and therefore excluded from the regulatory requirements that apply to drugs (37 FR 18229-30, 1972 and 21 U.S.C.360ee (b) (3)). It is recognized that except for phenylketonuria, where medical foods have proven critical in improving disease outcomes, studies on composition and efficacy of other special formulas remain sparse[2,3], yet, their implementation has emerged as a cornerstone of therapy for many inborn errors of metabolism detected through newborn screening, including the hereditary isolated methylmalonic acidemias (MMA)[1,4,5].

This group of IEMs results from deficiency of the methylmalonyl-CoA mutase (MUT) enzyme (*mut* ^{0} and *mut* $^{-}$, caused by complete or partial MUT deficiency) or related disorders (*cblA, cblB, cblD* variant 2) that affect the synthesis and transport of the cofactor of the MUT enzyme, 5′-deoxyadenosyl-cobalamin[6]. MUT deficiency leads to impaired metabolism of BCAA valine and isoleucine, as well as methionine and threonine, odd-chain fatty acids and cholesterol, all of which depend on MUT activity to isomerize succinyl-CoA at the terminal step of propionyl-CoA metabolism into the Krebs cycle[7,8]. The management of this group of disorders remains particularly challenging and controversial because, despite early diagnosis by newborn screening and strict adherence to a protein restricted diet, the patients still experience high mortality and morbidity, including recurrent metabolic ketoacidosis and hyperammonemia, growth failure, chronic kidney disease, pancreatitis, and neurologic complications[6,9–12].

The management of MMA includes restriction of dietary protein, which is challenging because sufficient essential amino acid (including valine and isoleucine) intake is needed for optimal growth and must be balanced against the production of “toxic” metabolites derived mainly from propiogenic amino acid oxidation[1,4]. The use of specialized amino acid formulations containing minimal to no valine, isoleucine, methionine and threonine in the treatment for MMA has become widely implemented[13,14], despite conflicting results about

their efficacy over the past thirty years[11,15,16]. The early studies by Nyhan et al. conducted with a carefully characterized patient, convincingly demonstrated that protein restriction significantly improved somatic development, head growth and intellectual outcome, but the supplementation with non-offending amino acids offered no additional benefit[17]. A subsequent study showed slightly improved nitrogen retention and plasma protein indices when patients were administered medical foods, but no improvement in growth[18]. The multicenter trial that provided the basis for the current practice guidelines[1,5] employed a 6-month non-randomized, non placebo-controlled study of an amino acid-modified medical food (Propimex-1, Ross Products Division, Abbott Laboratories, Columbus, OH) that enrolled 16 infants (3 with MMA) and recorded a non-significant improvement in growth parameters, primarily body weight, in 7/16, 43% of the patients[15]. No genotypes or enzyme activity data were provided and the wide range of amino acid intakes and plasma values were not correlated with the growth outcomes.

The lack of strong supporting evidence for a specific medical nutrition therapy and the continued poor growth outcomes for MMA patients reported in multiple studies[11,13] prompted a critical reappraisal of medical food use in the management of MMA. In this single center natural history study, the dietary parameters along with detailed phenotyping data were directly assessed in a large cohort of isolated MMA patients. We demonstrate that a skewed BCAA intake resulting from generous medical foods consumption was associated with disturbed plasma amino acid ratios and suboptimal growth and body habitus, likely because of dynamic BCAA metabolic rechanneling induced by the high leucine content in the medical foods[19]. Although a cause and effect relationship between medical foods and growth outcomes cannot be established without a randomized-controlled or cross-over study, our study raises concerns about the liberal use of medical foods in MMA and related disorders of propionate oxidation, which has implications not only for the current management of MMA, but a large number of IEMs.

METHODS

Study population

Patient studies were approved by the NHGRI Institutional Review Board and performed in compliance with the Helsinki Declaration (clinicaltrials.gov identifier: NCT00078078). Subjects were enrolled between 2004 and 2014 from regional centers across the US, with a small number of international patients (UK, Belgium, Argentina, Canada, Taiwan and China) also assessed. Long-term medical care and dietary management were provided by regional metabolic centers. Study participants were evaluated at steady state and were without clinical symptoms or laboratory markers of metabolic instability.

The diagnosis of MMA in a total of 61 patients was made using cellular enzymology (laboratory of Dr. David S. Rosenblatt, Division of Medical Genetics, McGill University, Canada) and/or molecular genetic analysis (GeneDx; Gaithersburg, MD)[6]. Ten patients were evaluated following isolated or combined organ transplantation: 2 with isolated liver, 3 with kidney and 5 with combined liver and kidney transplantation. Data from transplanted patients were used for the analysis of the dietary effects on plasma amino acid concentrations, but were excluded from long-term outcome analyses, due to the

liberalization of protein intake in several transplanted patients[20]. Five *mut*^{0} patients were not able to provide detailed diet data and were not included in the individual amino acid intake calculations, but contributed to the dietary data with their daily natural and deficient protein and caloric intake prescription information. Thirty-four of 61isolated MMA (55%) and 30 of 39 *mut*^{0} (76%) patients had a gastrostomy tube receiving part or all their daily nutrition through bolus or continuous feeds. This allowed for accurate calculations of food intake in the majority of our patient population.

Laboratory studies

Routine laboratory investigations included complete blood count, serum electrolytes, protein, albumin and prealbumin levels, liver and pancreatic enzymes, thyroid function tests, lipid panel, free and total carnitine and acyl/free carnitine ratios, IGF-1, as well as a battery of research measurements. Metabolites (plasma and urine methylmalonic acid, plasma amino acids) were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS).

Samples were obtained in the fasting state, or 2–4 hours after a meal; a smaller number of patients had blood drawn in both states. The NIH Biomedical Translational Research Information System (BTRIS) system was used to retrieve patient clinical research data.

Dietary regimens and daily amino acid intake calculations

Diet analysis was performed using formula recipes, a three-day food record collected prior to their admission to the NIH and a detailed dietary history obtained by a research dietitian during their inpatient evaluation. Diet analyses and calculations were performed using Nutrition Data System for Research (NDS-R) software versions 2007–2012 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN[21]. Formula composition information were obtained from the respective manufacturers. Protein intake was recorded as complete protein from natural protein sources and incomplete/deficient protein equivalent (g/kg/day) intake in the form of specialized MMA formulas, such as Propimex1/2, OA1/2, XMTVI Analog, Maxamaid or Maxamum and MMA/PA Express (Table S2). Recommended daily allowance (RDA) for protein and amino acids were based on the Dietary Reference Intakes for Protein and Amino acids, NAP, 2005[22].

Anthropometry and body composition analysis

Anthropometric measurements were expressed as age- and gender-specific *Z*-scores, using the epidemiological software package Epi Info™, Version 3.5.1., based on the CDC 2002 reference database (Centers for Disease Control and Prevention, Atlanta, GA, USA). Whole body composition in grams of fat or fat mass (FM), and fat free (or lean) body mass (FFM) and bone density were measured using dual energy X-ray absorptiometry (DXA, Hologic Delphi A; Hologic, Bedford, MA). Values were compared to the mean and standard deviation of reference child and adolescent models of body composition, matched for age, gender and ethnicity.

Statistical analysis

The results are presented as means ± SD. Significance was set at $P<0.05$. Statistical manipulations were performed using the IBM SPSS version 21.0 (Chicago, IL, USA), or GraphPad Prism version 6.0 software (San Diego, CA, USA). Independent Student's *t*-test was used to compare values between patients that had or had not been receiving medical foods, patients with incomplete amino acid equivalent/complete protein intake ratio over and less than 1, and for comparisons between males and females. One-way ANOVA, with Bonferroni correction for multiple comparisons was employed for comparisons among different MMA subtypes (*mut, cblA* and *cblB*). Pearson's correlation coefficient and linear or multiple logistic regression analyses were used to evaluate correlations between independent variables. Independent variables used in a multiple-regression equation included MMA subtype, gender, height-for-age *Z*-score, serum creatinine, leucine/valine intake ratio, IGF1 and other serum biomarkers (prealbumin, hemoglobin, white cell and platelet count, protein, albumin, eGFR).

RESULTS

Patient cohort and anthropometric characteristics

Sixty-one patients with isolated MMA (46 *mut,* 9 *cblA* and 6 *cblB*; 36 males, 25 females; age range 2.5y to 35y, mean ± SD: 13.3 ± 9.1y) were studied. In the *mut* cohort, 31/46 patients (67%) presented in the newborn period with hyperammonemia, 26 individuals (56%) harbored at least one nonsense or frameshift mutation (38 of 86 alleles), of whom 12 (26%) were compound heterozygotes or homozygotes for these classes of alleles (Table S1).

Height, weight and BMI-for-age are presented for patients ≤ 20y of age, as *Z*-scores (mean ± SD) per MMA subtype in Figure 1A, and on gender specific growth charts in Figure S1, to allow comparisons to a cohort of 51 MMA patients from Paris, France[11]. For the non-transplanted mut^0 patients ($N=28$), the mean height *Z*-score was −2.0725 ± 1.71, the weight *Z*-score was −0.817 ± 1.46 and the BMI *Z*-score was 0.726 ± 0.67, while the mean head circumference *Z*-score was −1.61 ± 1.75 ($N=21$, Figure 1B). The clinically less severe mut^- and B12-responsive *cblA* patients had values closer to normal, while *cblB* patients' growth outcomes were similar to the mut^0 patients. No significant differences were observed in growth outcomes between males or females, or between patients with the mut^0 subtype who presented in the newborn period with hyperammonemia ($N=18$), vs those without ($N=7$) or those diagnosed by newborn screening ($N=3$), (one-way-ANOVA, $P=0.275$). A trend for a lower OFC-*Z*-score was observed in the young subgroup, 2–9y old patients, with hyperammonemia as newborns ($P=0.088$).

Whole body composition was measured by DXA imaging in 39 *mut,* 7 *cblA* and 5 *cblB* patients. mut^0 class patients had significantly higher percent fat mass (36.7 ± 9.3%) and, thus, lower percent fat-free or lean mass (61.09 ± 9.25%), compared to those with *cblA* (24.9 ± 10.94 and 72.7 ± 10.6, respectively. $P=0.031$), but not to *cblB* ($P=0.08$) patients (Figure 1C). Moreover, mean %fat mass in patients aged <18y were higher compared with the highest values observed in gender-, and ethnicity-matched control reference data [27.6 ± 6.1% ($N=46$) for females, $P=0.0001$ and 22.2 ± 10.3 ($N=51$) for males, $P=0.004$][4].

Protein needs and medical foods utilization

We aimed to investigate whether low protein intake or unbalanced BCAA composition of MMA medical foods could contribute to the poor growth outcomes and low muscle mass observed in our MMA patient cohort. Complete and incomplete protein intakes in g/kg/d are presented for each of the patients per MMA subtype and age (Figure 2A). To our surprise, the mean daily complete protein intake (Table 1) was close to the recommended daily allowance for protein for healthy children: mean for all the *mut* patients was 102.6 ± 30.3% RDA[22], while similar intakes were observed even in the fragile *mut*^{0} patients, 99.45 ± 32.05% for all non-transplanted patients (Figure 2B), especially in the younger age groups – mean complete protein %RDA was 105.4 ± 25.7 for the 2–9y olds and 99.8 ± 47.5 for the 10–18y old *mut*^{0} patients. Values are presented per patients' actual weight (kg), hence, would appear even more generous if expressed per ideal weight or adjusted for the decreased lean mass of the patients.

65% of the patients and 85% of those with *mut* MMA were consuming various amounts of special MMA formulas in addition to a natural protein intake approaching the RDA. The protein content of the special MMA formulas often exceeded the amount of natural protein intake (in 13/37 or 35.0% of the *mut* patients). This is presented as a ratio of incomplete/complete protein intake (Figure 2C). A number of *mut* patients (N=7) were prescribed additional valine and/or isoleucine by their home metabolic clinics because of persistently low plasma levels (labeled with a star in Figure 2A). Interestingly, a number of the patients requiring valine and/or isoleucine supplementation had a natural protein daily intake at or above RDA and a total protein intake between 2–2.5g/kg/d (N=4; filled stars in Figure 2A).

Individual BCAA intake and plasma concentrations

In order to better understand the individual amino acid requirements of MMA patients and analyze the effects of their daily intakes on various biochemical parameters, we estimated daily intakes for each of the BCAA, leucine, valine and isoleucine (Leu, Val, Ile) from dietary records. In Table 2, we show the range of recommended individual amino acid intakes by age from reference nutrition books that are used to guide dietary management in MMA[1,4] and the ranges we observed in our MMA cohort. The mean intake of Leu, Val, Ile in mg/kg/day by age group is shown in Figures 2D and E.

Leu content is higher than Val and Ile in all food sources[19,22]. In human breast milk relative ratios of leucine:valine:isoleucine are about 2.0:1.2:1(Table S2). Hence, normal BCAA intake ratio was observed in our patients who did not consume medical foods (Figure 3A). These patients were mostly international with limited access to medical foods or from US centers that did not prescribe medical foods. Medical foods for MMA and PA contain minimal to no Val or Ile, but have an increased content of Leu (Table S2). Therefore, patients consuming medical foods had a significantly higher intake ratio for Leu/Val or Leu/Ile (P< 0.001 for both, compared to patients using no medical foods, Figure 3A). As expected, this difference was also translated to their measured plasma amino acid ratios, obtained 2–4 hours after last meal/feed (Figure 3B). Moreover, these abnormal plasma BCAA ratios were more pronounced the closer to the feeds a blood sample was taken, as

illustrated with a case example (Figure S2A), while they returned to the normal ratios immediately following the discontinuation of medical foods (Figure S2B and C).

The temporal association of depressed plasma isoleucine and valine concentrations in the setting of medical food ingestion suggested that the high leucine content of medical foods was distorting the BCAA ratios. A negative, statistically significant correlation was observed between amount of incomplete daily protein intake (g/kg/d) and plasma Val and Ile concentrations, taken 2–4h after feeds, across all patients (Val: r=−0.569, P<0.001, R^2=0.324; Ile: r=−0.469, P=0.001, R^2=0.22) (Figure 3C) and for *mut*^{0} patients separately (Val: r=−0.538, P=0.002, R^2=0.289; Ile: r=−0.417, P=0.022, R^2=0.174). Importantly, the correlation persisted in the subset of patients consuming >100% RDA complete protein intake (Val: r=−0.602, P=0.001, R^2=0.363; Ile: r=−0.413, P=0.032, R^2=0.171). A similar correlation to incomplete protein intake was observed for methionine and threonine plasma levels (in *mut*^{0} patients r=−0.523, P=0.003, R^2=0.273 for methionine and r=−0.458, P=0.011, R^2=0.210 for threonine) (data not shown).

Correlation to growth and other disease-related outcomes

Recognizing that dietary prescriptions can change significantly in a patient's life-span and that data on patient-years on a higher than recommended medical foods/natural protein intake (leucine/valine, 1:1) ratio would be the preferred variable for the associations presented, we attempted to evaluate whether patients' protein intakes at the time of our study were correlated to their growth parameters. We looked for such associations in the subgroup of patients with genotype-confirmed *mut*^{0} MMA subtype, prior to any transplantation procedure.

A negative correlation was observed between the ratio of dietary leucine/valine intake and height, weight and BMD Z-scores in our *mut*^{0} patients: height-for-age Z-score: r=−0.341, P=0.05, R^2= 0.123, N=23; weight-for-age Z-scores: r=−0.453, P=0.014, R^2=0.209 (Figure 3D), and BMD: r=−0.406, P=0.049, R^2=0.165 (data not shown). However, when the absolute amount of deficient protein/kg/day was correlated with the growth indices these correlations were not statistically significant. In aggregate, our data suggest that diets containing a high content of Val- and Ile-deficient protein provided by medical foods, especially in the context of reduced natural protein intake, are associated with poor growth parameters. Stepwise regression modeling revealed that the combination of serum creatinine (β coefficient=−0.66, P=0.003), leucine/valine intake ratio (β=−0.482, P=0.008) and serum IGF-1 values (β=0.471, P=0.025) best predicted height Z-score, with a model R^2 of 0.478. The R^2 of the regression model improved significantly from 0.123 (only dietary leucine/valine intake included) to 0.296 (creatinine added to the model) and 0.478 (IGF-1 added), showing the effect of incorporating the well-described effects of renal function and growth hormone axis on growth. The significant correlations of renal function with height Z-score and plasma MMA values are provided separately on Figure S3 and expand on previous observations in a subset of this patient cohort[13,23,24].

Protein intake and biochemical indices of protein status correlated with lean body mass and platelet counts (S3A and D), while on the other hand, indices of renal function showed a negative correlation with height Z-score and, similarly, with prealbumin (transthyretin)

concentrations, a lab test used by many clinics for monitoring of protein status in patients with MMA and other IEMs (r=−0.659, P=0.002, R^2=0.434, Figure S3C), while a positive correlation was observed with the plasma MMA values, as shown previously in a subset of this cohort[13,23,24].

DISCUSSION

This study presents a critical reappraisal of medical food use in MMA, derived from inpatient measurements performed as part of a dedicated, single center natural history protocol (NCT00078078). Because we enrolled patients from national and international metabolic clinics with varied management approaches to dietary therapy, our study has revealed unique insights into the current management of MMA as well as iatrogenic effects of medical food use. Over ten years we encountered a number of patients with MMA, who, despite an apparently adequate natural protein intake, had persistently low plasma BCAA levels, prompting their clinics to increase the complete protein or supplement with individual amino acids, i.e. valine and/or isoleucine, to avoid essential amino acid deficiencies (Figure 1A; stars)[13]. The supplementation with valine and/or isoleucine was paradoxical because these are the propiogenic amino acids targeted for dietary minimization because they are the main contributors to the “toxic” metabolite pool[25].

Growth outcomes in our MMA cohort were poor (Ht, Wt and OFC-for-age Z-scores), and body composition showed significantly increased percent fat mass (Figure 1). We have previously shown that *mut* MMA patients receive nearly two times more calories than their measured resting energy expenditure[13], which predisposes them to obesity. In this study, we sought to explore whether severe protein restriction could contribute to the short stature and decreased lean body mass in MMA patients, as well as the paradoxical requirement for BCAA supplementation observed in a subset of patients.

Surprisingly, the complete protein intake and individual essential BCAA intake consumed by patients in our study were higher than the recommended amounts for other inborn errors, like phenylketonuria (PKU) or maple syrup urine disease (MSUD)[1,4,26], where protein restriction is an essential part of their management, and higher than the FAO/WHO/UNU (2007) safe protein intake levels[5] proposed in the European guidelines or metabolic textbooks for MMA (Table 2). It has been argued that RDA is inadequate in organic acidemias because 1) most of the dietary protein is plant-derived, which may not contain complementary AA’s or can be less digestible; 2) complete protein when given as free AA formula (Splash, Neocate, Elecare, etc) should be increased by about 20% to account for altered absorption and oxidation rates; 3) patients have frequent catabolic episodes resulting in need for catch up growth[1]. However, the argument for increased natural/complete protein is often translated to a higher deficient protein prescription, resulting in a higher than recommended ratio of deficient/natural protein. Many patients in this study indeed consumed significant quantities of medical foods in addition to an apparently sufficient amount of complete protein. Of note, total protein intake reached 2–3 g/kg/day in patients up to 11y of age, a significant amount of total nitrogen load, especially for a patient cohort with chronic kidney disease and at risk to develop hyperammonemia. It was therefore even more perplexing that some patients required valine and isoleucine supplementation, which

prompted us to search for alternative explanations for possible deficiencies and re-evaluate the amino-acid composition of dietary and medical food-derived protein equivalents.

Compared to formulas composed for the treatment of MSUD, which are devoid of all BCAAs, those prepared for disorders of propionate oxidation contain little to no valine, isoleucine, methionine and threonine because these amino acids are oxidized to propionyl-CoA and methylmalonic acid. However, upon analysis it became evident that MMA formulas contain a normal to increased amount of leucine (Table S2). Hence, they represent the only formulas with an imbalanced BCAA composition, and their administration results in a significantly increased and non-physiologic leucine vs. valine and isoleucine intake, especially when prescribed in significant quantities. As proof of this unbalanced amino acid composition, we found that leucine intake in our MMA patient cohort was indeed four to five times higher than the daily recommended intake (DRI) based on the 2007 FAO/WHO guidelines (mean 195.1mg/kg/day, range 30–510, compared to the DRI of 44–50mg/kg/d, Figure 2D). In some of the MMA patients, leucine intake reached levels shown to cause hyperammonemia and other side effects, when administered to healthy volunteers[27] (Table 2). Of note, target amounts for leucine consumption are not considered in the proposed guidelines for the dietary management of MMA[1,4,5].

Hence, the present study raises the following intriguing question: Can we assume that, just because leucine is not oxidized into MMA, any amount of leucine is completely safe for MMA patients? From the work of Nyhan et al. on the originally reported case of ketotic hyperglycinemia, it was noted that leucine administration resulted in a decrease of valine and isoleucine plasma concentrations in the patient, as well as in healthy controls[28]. These observations have been further extended in animal models (rats [29,30], chicks[31], pigs[32] among others) that demonstrate when leucine is ingested, especially to animals receiving a low-protein diet, it causes depletion of valine/KIV (α-ketoisovaleric acid) and isoleucine/KMV (α-keto-β-methylvaleric) concentrations in the plasma and tissue pools, and is associated with marked growth depression that can be overcome with Val or Ile supplementation. Furthermore, in numerous studies performed with human subjects, administration of a leucine load (but not of valine or isoleucine) by mouth or intravenously results in dramatic drops in plasma concentrations of valine, isoleucine, phenylalanine, tyrosine, threonine and methionine within 1–3 hours from administration[19,27,33–35]. While the mechanism(s) behind the lowering effect of leucine on plasma amino acids concentrations are not fully understood, it has been proposed that the inhibitory role of leucine-derived α-ketoisocaproate (KIC) on the BCKDH-kinase (branched chain ketoacid dehydrogenase kinase) results in activation of BCKDH and increased BCAA oxidation[19].

Additionally, leucine displays a multitude of effects that have not been carefully considered in the management of patients with MMA. Leucine enhances protein synthesis [36], inhibits muscle protein breakdown[37,38], stimulates insulin secretion[39] and plays a role in central nervous system food intake regulatory circuits and feeding behavior[40]. Because leucine is primarily transported via the large neutral amino acid transporter, LAT1, at the blood brain barrier (BBB)[41], it can compete with other large neutral amino acids for uptake/transport and subsequently neurotransmitter biosynthesis, as detailed in studies on MSUD[42], PKU[43] and GA-1[44]. The high leucine:valine, isoleucine and methionine plasma ratios observed in

the current study would be predicted to impair the uptake of Val, Ile and Met through the BBB and result in depletion of these amino acids in the brain with potentially detrimental consequences, especially during periods of brain growth.

The main caveat of the study is the cross sectional representation of dietary composition and growth outcomes, which precludes causal claims, especially because dietary prescriptions can change significantly over time. Furthermore, growth failure is a well-recognized complication of MMA and a result of multiple factors, including frequent catabolic events, protein restriction, chronic renal disease, growth or thyroid hormone deficiency, and possibly factors intrinsic to the disease pathophysiology, such as mitochondrial dysfunction. However, our observations highlight a previously unrecognized iatrogenic amino acid deficiency with use of medical foods, a concept reinforced by the fact that removing medical foods from the diet restored BCAA homeostasis in two patients in our cohort (Figure S2), and raise questions about the current lack of consideration of the effects of leucine supplementation in the dietary management of MMA.

In conclusion, the excessive use of medical foods, especially in the setting of reduced natural protein intake, resulted in iatrogenic amino acid deficiencies and was associated with poor growth outcomes in a large cohort of isolated MMA patients. The unbalanced intake of leucine carries theoretical risks, especially with respect to brain amino acid uptake and whole body metabolism, and unexplored long-term sequelae. Medical foods and dietary guidelines for MMA should be revised based on well-controlled and sufficiently powered clinical studies to support their efficacy and safety. The questions raised with this work are relevant to a collectively large number of inborn errors of metabolism detected by newborn screening.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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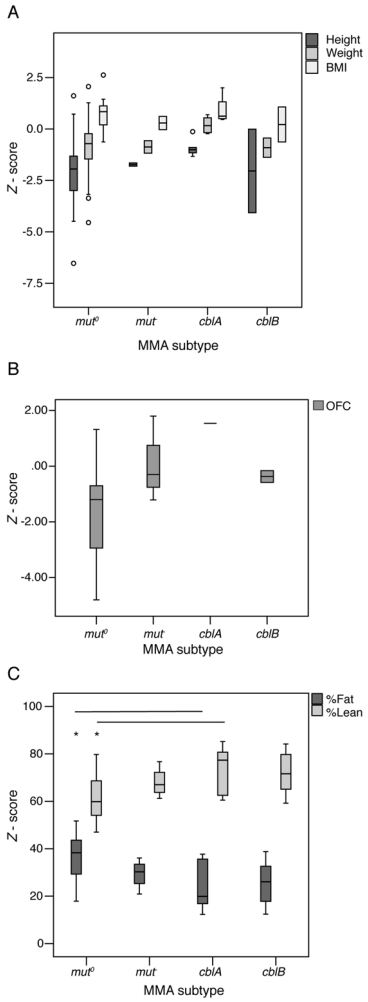

Figure 1.

A. Height-, weight- and BMI-for-age *Z*-scores for patients 20 y and under (*N*=28 *mut*^{0}, 3 *mut*$^{-}$, 5 *cblA*, 2 *cblB*) are depicted in a box plot. The box represents the middle 50% of all cases per variable, while the remaining 50% is contained between the box and whiskers on each side. The single line inside the box represents the median of the entire data set. The location of this line suggests the skewness in the distribution, when noticeably shifted away from the center, as is the case for the BMI-for-age *Z*-score in the *mut*^{0} subgroup. As evidenced by the Z-scores, *mut*^{0} patients were short and overweight or obese. **B**. Head circumference *Z*-score was lower in the *mut*^{0} group, −1.66 ± 1.63 (mean ± SD, *N*=22). **C.** Percent fat and fat-free (lean) mass are depicted by MMA subtype for non-transplanted patients in a box plot (*N*=28 *mut*^{0}, 3 *mut*$^{-}$, 7 *cblA* and 5 *cblB).* Percent fat mass was significantly higher in the *mut*^{0} subtype compared to the milder cblA subtype (one-way ANOVA between groups *P*=0.01, Bonferroni post-hoc correction between *mut*^{0} and *cblA*, $^{*}P$=0.042 for %fat and $^{*}P$=0.037 for %lean).

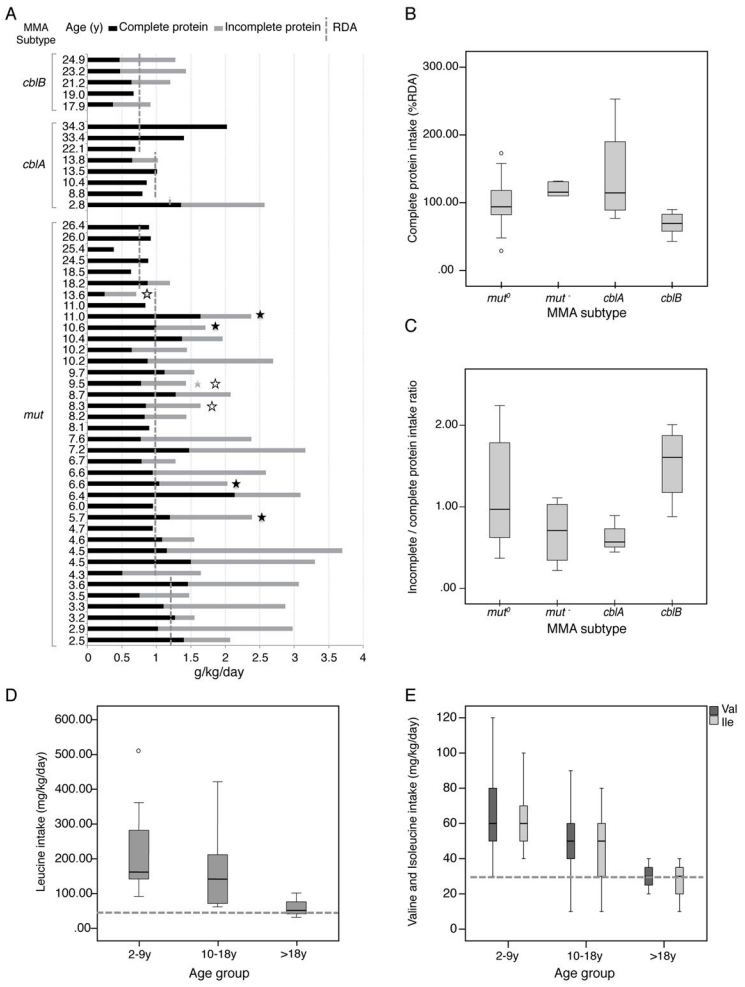

Figure 2.

A. Daily protein intake (g/kg/day) is provided per patient sorted by age and MMA subtype (*mut, cblA* and *cblB*). A number of *mut* patients (N=7) required additional valine and/or isoleucine supplementation because of persistently low plasma amino acid levels during their follow-up monitoring by their home metabolic clinics (labeled with a star). Four of these patients had a complete protein intake at or above the RDA (recommended dietary allowance) for age (solid stars), while three were on a low complete protein diet (clear stars). Age-adjusted RDA is depicted as a dotted line. **B.** The mean daily complete protein intake is depicted as %RDA for protein for healthy children in a box plot. The box represents the middle 50% of all cases per variable, while the remaining 50% is contained between the box and whiskers on each side. The single line inside the box represents the median of the entire data set. Patients with the *mut*^{0} subtype consumed 99.45 ± 32.05% RDA complete protein (mean ± SD), *mut*$^{-}$ 119.0 ± 10.00%, *cblA* 139.6 ± 66.37 and *cblB* 68.83 ± 18.19% (*N*=31, 6, 8, and 6, respectively). Transplant recipients were excluded. Although adjusting for the high versus low biological value of dietary protein source might decrease slightly the aforementioned percentage, this analysis was not feasible based on existing dietary records. On the other hand, calculations are provided for the actual and not ideal weight of the patients, suggesting that protein intake would be more generous if expressed per gram of

their decreased lean mass. **C.** The ratios of incomplete/complete protein intakes in the subset of patients consuming medical foods are provided per MMA subtype in a box-plot. Patients with *mut*^{0} MMA had a ratio of 1.16 ± 0.13 (mean ± SD), *mut*$^{-}$ 0.68 ± 0.20, *cblA* 0.63 ± 0.13 and *cblB* 1.53 ± 0.24 (*N*=24, 4, 3, and 4, respectively). 13/37 or 35.0% of *mut* patients on medical foods exceeded the current treatment guidelines of 1:1 ratio of complete to deficient protein intake. **D and E.** Daily intake of leucine, valine and isoleucine (mg/kg/day by age group) is provided for patients with the *mut* subtype of MMA in a box plot. Leucine intake was 222.0 ± 24.9 in the 2–9y olds, 173.33 ± 55.6 in the 10–18y olds, and 60.0 ± 20.8 in the >18y olds. The younger patients consumed amounts four to five times higher than the recommended DRI based on the 2007 FAO/WHO guidelines (DRI of 44–50 mg/kg/day, dotted line). High daily consumption was recorded even for the two propiogenic amino acids, valine and isoleucine, in the younger age groups.

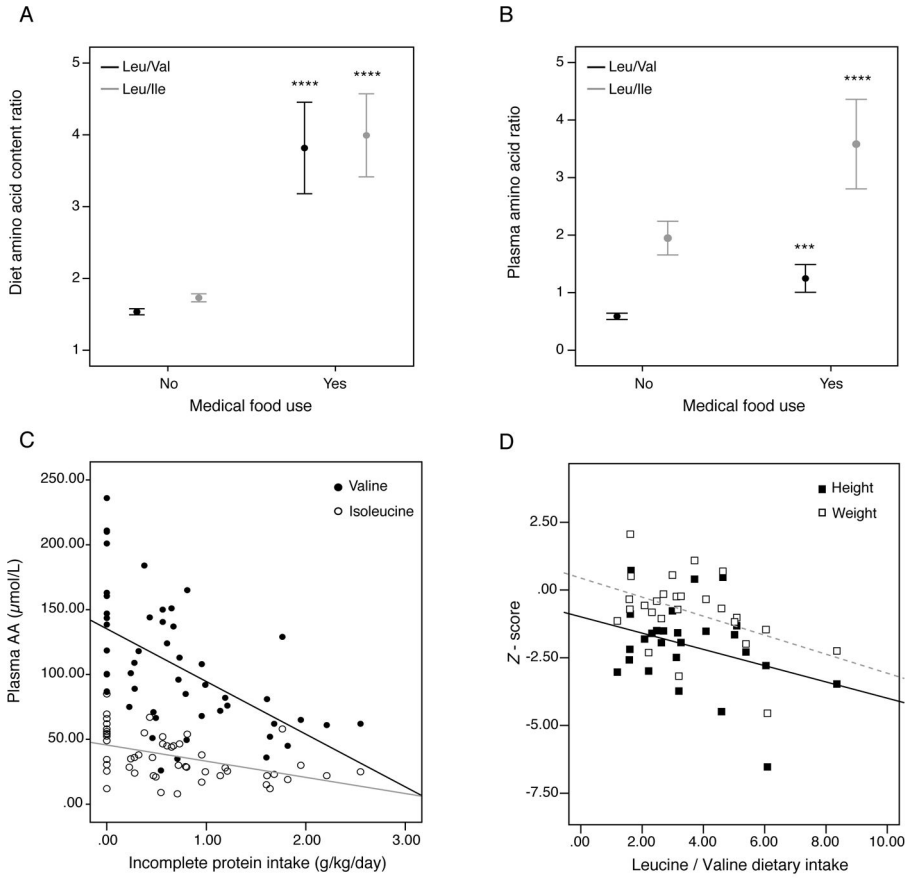

Figure 3.

A. Ratios of leucine over valine or isoleucine dietary intake are compared between patients on and off medical foods. Normal BCAA ratios with very narrow distribution were observed in patients who consumed no medical foods, (Leu/Val mean intake ratio was 1.54 ± 0.07, while Leu/Ile intake ratio was 1.73 ± 0.10, N=16, bars represent mean with 95%CI), in contrast to significantly higher ratios recorded in patients taking medical foods (Leu/Val of 3.82 ± 1.82 and Leu/Ile of 3.99 ± 1.65, N=34, independent t-test $^{****}P<0.001$ for both Leu/Val and Leu/Ile) as a result of the high leucine content in these formulations. **B.** Higher leucine over valine and isoleucine dietary intake ratios translated in reversed or higher ratios, respectively, in their relative plasma amino acids concentrations. Patients on medical foods had a reversed plasma Leu/Val ratio of 1.25 ± 0.74 and a close to two-fold increased ratio of Leu/Ile 3.58 ± 2.4, compared to patients on no medical foods ($^{***}P=0.003$ and $^{****}P<0.001$ for Leu/Val and Leu/Ile). **C.** Amount of deficient protein intake (g/kg/day) was inversely related to the plasma valine (solid circles) and isoleucine (clear circles) concentrations. Although a range of plasma concentrations were observed in patients without medical food intake – depicted on the left aspect of the graph, the lowest plasma Val and Ile values were observed in patients consuming the highest amounts of medical food. **D.** Dietary leucine/valine intake showed a negative correlation to height-for-age (solid squares) and weight-for-age Z-scores (clear squares) in the subgroup of mut^0 MMA patients, supporting that increased consumption of deficient protein administered at the expense of complete protein may adversely affect the growth parameters. Patients of comparable severity (age of onset,

frequency and severity of metabolic crises/hospitalizations, renal disease, among other disease complications) are present at each end of the regression curve.

Table 1

Demographic and dietary characteristics of 61 patients with isolated MMA.

Subgroups	(N)	Total Protein (g/kg/d)	Complete Protein (g/kg/d)	Incomplete Protein-equivalent (g/kg/d)	Incomplete/Complete Protein ratio	Complete Protein %RDA
mut						
All patients	(37)	1.77 ± 0.85	0.99 ± 0.32	0.78 ± 0.68	1.09 ± 0.63	102.6 ± 30.3
Age groups						
2–9y	(24)	2.04 ± 0.81	1.06 ± 0.29	0.98 ± 0.68	1.11 ± 0.62	105.4 ± 25.7
10–18y	(7)	1.67 ± 0.73	0.94 ± 0.45	0.72 ± 0.55	1.14 ± 0.72	99.8 ± 47.5
>18y	(6)	0.81 ± 0.28	0.76 ± 0.21	0.05 ± 0.13	0.37 ± 0.10	94.6 ± 27.8
cblA						
All patients	(8)	1.58 ± 0.89	1.26 ± 0.56	0.31 ± 0.49	0.63 ± 0.23	139.6 ± 66.3
cblB						
All patients	(6)	1.04 ± 0.29	0.56 ± 0.15	0.47 ± 0.40	1.52 ± 0.48	68.8 ± 18.2
Transplanted						
All patients	(10)	0.92 ± 0.44	0.73 ± 0.44	0.59 ± 0.18	0.68 ± 0.32	87.1 ± 55.7

61 patients are presented by MMA class and by age for patients in the *mut* group. Transplanted patients include 9 *mut* and 1 *cblA* patient. Data are presented as mean ± SD.

Recommended daily allowance for protein intake was calculated based on the *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients).*Washington, DC: The National Academies Press, 2005.

Table 2

Individual amino acid intake in *mut* MMA patient cohort vs. recommended daily nutrient intakes.

Age (years)	Valine	Isoleucine	Threonine (mg/day)	Methionine	Leucine
Recommended					
1–4	500 – 800	480 – 730	400 – 600	180 – 390	***None provided***
4–7	700 – 1100	600 – 1000	500 – 750	250 – 500	
7–11	800 – 1250	700 – 1100	600 – 900	290 – 550	
11–15	1000 – 1600	750 – 1300	800 – 1200	300 – 800	
15–19	1100 – 2000	800 – 1500	800 – 1400	300 – 900	
>19	900 – 2000	900 – 1500	800 – 1500	250 – 1000	
Observed					
1–4	675 – 1360	755 – 1017	468 – 1114	233 – 495	2285 – 5045
4–7	607 – 2290	674 – 2059	424 – 1780	231 – 1227	2273 – 7104
7–11	776 – 2583	888 – 1732	609 – 1489	269 – 1653	2332 – 9732
11–15	1920	1720	1430	730	6274
15–19	1729	1618	1363	1241	2764
>19	756 – 2258	963 – 1953	501 – 1713	219 – 1009	2553 – 3507

Sources:

Acosta P. Nutrition Management of Patients with Inherited Metabolic Disorders: Burlington, MA: Jones & Bartlett Publishers; 2010; and Blau N. et al. Physician’s Guide to the Treatment and follow-up of Metabolic Diseases.