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Original article

High protein prescription in methylmalonic and propionic acidemia patients and its negative association with long-term outcome

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SUMMARY

Background and objective: Methylmalonic acidemia (MMA) and propionic acidemia (PA) are inborn errors of metabolism. While survival of MMA and PA patients has improved in recent decades, long-term outcome is still unsatisfactory. A protein restricted diet is the mainstay for treatment. Additional amino acid mixtures (AAM) can be prescribed if natural protein is insufficient. It is unknown if dietary treatment can have an impact on outcome.

Design: We performed a nationwide retrospective cohort study and evaluated both longitudinal dietary treatment and clinical course of Dutch MMA and PA patients. Protein prescription was compared to the recommended daily allowances (RDA); the safe level of protein intake as provided by the World Health Organization. The association of longitudinal dietary treatment with long-term outcome was evaluated.

Results: The cohort included 76 patients with a median retrospective follow-up period of 15 years (min −max: 0–48 years) and a total of 1063 patient years on a protein restricted diet. Natural protein prescription exceeded the RDA in 37% (470/1287) of all prescriptions and due to AAM prescription, the total protein prescription exceeded RDA in 84% (1070/1277). Higher protein prescriptions were associated with adverse outcomes in severely affected patients. In PA early onset patients a higher natural protein prescription was associated with more frequent AMD. In MMA vitamin B12 unresponsive patients, both a higher total protein prescription and AAM protein prescription were associated with more mitochondrial complications. A higher AAM protein prescription was associated with an increased frequency of cognitive impairment in the entire.

Conclusion: Protein intake in excess of recommendations is frequent and is associated with poor outcome.

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Abbreviations

AAM	Amino acid mixtures
AAM total protein ratio	The amount of protein from AAM versus the total protein prescription
AMD	Acute metabolic decompensations
AUC	Area under the curve
BCAA	Branched chain amino acids
E-IMD	European registry and network for Intoxication type Metabolic Diseases
EO	early onset
kcal	kilocalorie
LAT	large-neutral amino acid transporter
LO	late onset
MMA	Methylmalonic acidemia
natural:total protein ratio	The amount of natural protein versus the total protein prescription
PA	Propionic acidemia
P:E ratio	Protein-to-energy ratio
RDA	Recommended daily allowances
vitB12	Vitamin B12
WHO	World Health Organization

1. Introduction

Methylmalonic acidemia (MMA, OMIM #251000, #251100, #251110 and #251120) and propionic acidemia (PA, OMIM #606054) are inborn errors of metabolism caused by deficiencies of enzymes or cofactors that contribute to the breakdown of the branched-chain amino acids (BCAA) L-isoleucine and L-valine.

While survival of MMA and PA patients has greatly improved in recent decades, overall outcome remains poor. Despite currently available treatment, patients continue to have frequent acute metabolic decompensations (AMD) (often requiring hospitalization or even intensive care unit admission), they frequently develop severe (mitochondrial) long-term complications such as cardiomyopathy and renal failure and numerous patients have impaired cognitive function and impaired height [1–6]. Newborn screening in MMA and PA patients is not expected to improve patient outcome regarding these complications substantially [3], and therefore evaluation and potential improvement of current applied dietary treatment is essential.

For several decades, a protein-restricted diet has been the mainstay of treatment for patients suffering from MMA and PA. The current guideline for MMA and PA advises a natural protein requirement, i.e. an intake of 100% of the safe levels of protein defined by the World Health Organization (WHO) [7], and to supplement amino acid mixtures (AAM) when the tolerated natural protein intake is below 100% [8]. However, the current guideline advices are not yet based on studies that evaluate the effect of dietary treatment on specific outcome measures. Only the effect of dietary treatment on height has previously been studied and minimum and maximum values for the prescribed protein-to-energy-ratio (P:E ratio) for gaining optimal height (>1.5 to <2.9 g protein/100 kcal/day) have been provided [9]. Without this crucial knowledge, clinicians continue to compose their own approach to optimize the protein-restricted diet for MMA and PA patients. This is illustrated by a recent study evaluating dietary treatment among patients included in the European registry and network for Intoxication type Metabolic Diseases (E-IMD), that revealed that several patients with a natural protein prescription of 100% of the recommended daily allowances (RDA) received additional AAM, which is against guideline advices and may be harmful [8–13]. In order to determine the influence of currently prescribed dietary treatment on the long-term outcome of MMA and PA patients, this study will evaluate the association of longitudinal dietary treatment −natural protein, AAM protein and total protein− with patient outcome, defined as: 1) AMD episodes, 2) long-term mitochondrial complications, 3) cognitive development and 4) height [8,14].

2. Methods

2.1. Patient inclusion, data collection and outcome parameters

This study is a second publication based on the extensive retrospective data of 76 MMA and PA patients. Consequently, patient inclusion, informed consent, data collection and definition of outcome parameters of this study cohort have been described before [3]. By the time of collecting the data for this manuscript and writing this manuscript newborn screening for MMA and PA was not introduced in the Netherlands yet. Patients were grouped according to disease severity: PA early onset (EO) (disease onset before day 28 of life) (n = 20), PA late onset (LO) (disease onset beyond day 28 of life) (n = 10), MMA vitamin B12 (vitB12) unresponsive (n = 24) and MMA vitB12 responsive (n = 21) (Table 1 in Haijes et al. [3] and Supplementary Table 1). PA patients identified by family testing were grouped as either EO or LO based on the disease course of their index sibling [3]. For each of the included MMA patients, we carefully checked on what grounds the assumption of being vitamin B12 responsiveness was based. In some cases, this was based on the *in vitro* assay on cultured fibroblasts as described by Hörster et al., 2007 and Baumgartner et al., 1982, but in most cases, this was based on the *in vivo*, clinical assessment as described by Fowler, Leonard and Baumgartner 2008, or the conclusion was based on both observations [3]. For this study, data collection also included dietary prescriptions and plasma BCAA levels at outpatient visits. Plasma BCAA levels were available for patients from the University Medical Center Utrecht and the Erasmus Medical Center (n = 41 patients). Renal disease was defined as chronic kidney disease beyond stage I, which is based on an estimated or measured GFR <90 mL/min/1.73m^2. Cognitive function was classified in three categories (IQ > 90, IQ 60–90, IQ < 60) based on neuropsychological test results, or in the absence of neuropsychological test results on educational level or professional employment [3,15]. Adolescence was defined according to the WHO as patient age between 10 and 19 years [16]. The mitochondrial complications as defined by Haijes et al., 2019 (Supplementary Table 7 and Supplementary notes 2 in Haijes et al. [3]) were cumulated and the total count of mitochondrial complications was used as outcome parameter.

2.2. Data analysis

Prescription of natural protein in gram/kg and protein from AAM supplementation in gram/kg were compared to the protein requirement as mentioned in the MMA and PA guideline based on the safe levels of protein intake (according to gender and age) defined in the report: “Protein and amino acid requirements in human nutrition” [7,8]. This protein requirement has been defined as recommended daily allowances (RDA). Total protein prescription in gram/kg was calculated by combining natural protein intake and protein from AAM supplementation. The natural:total protein ratio was calculated by dividing natural protein over the total amount of protein prescribed. The AAM:total protein ratio was calculated by dividing protein from AAM supplementation (AAM protein) over

Table 1
Dietary prescription and risk on certain outcome in disease severity groups. In each subgroup the association between age, natural/AAM/total protein RDA, natural:total protein ratio, AAM:total protein ratio, kcal RDA, total P:E ratio and the four outcome parameters (acute metabolic decompensations, mitochondrial complications, cognitive function and height z-score) was tested. All empty boxes concern tests not performed; all boxes with a dash sign concern non statistically significant test results.

	Outcome parameter	Age	Natural protein RDA	AAM protein RDA	Total protein RDA	Natural:total protein ratio	AAM:total protein ratio	kcal RDA	Total P:E ratio
MMA vitB12 UR	Metabolic decomp.		–	–	–	–	–	–	–
	Mitochondrial compl.		–	Exp (coef) = 1.02 p < 0.001	Exp (coef) = 1.01 p < 0.001	–	–	–	–
	Cognitive function	–	–	–	–	–	–	–	–
	Height z-score	β-coef = −0.13 p < 0.001		β-coef = −0.02 p < 0.001					β-coef = 0.76 p = 0.002
MMA vitB12 R	Metabolic decomp.		–	–	–	–	–	–	–
	Mitochondrial compl.		–	Exp (coef) = 1.01 p = 0.003	Exp (coef) = 1.01 P = 0.011	Exp (coef) = 0.08 P < 0.001	Exp (coef) = 12.0 p < 0.001	Exp (coef) = 1.01 p = 0.001	–
	Cognitive function		–	–	–	–	–	–	–
	Height z-score	β-coef = −0.17 p < 0.001		β-coef = −0.01 p = 0.003					–
PA EO	Metabolic decomp.		–	–	–	Exp (coef) = 77.4 P = 0.027	Exp (coef) = 0.01 p = 0.027	–	–
	Mitochondrial compl.		–	–	–	–	–	–	Exp (coef) = 0.57 p = 0.010
	Cognitive function		–	–	–	–	–	–	–
	Height z-score	β-coef = −0.11 p < 0.001		–					–
PA LO	Metabolic decomp.		–	–	–	–	–	–	–
	Mitochondrial compl.		–	–	–	–	–	–	Exp (coef) = 0.14 p = 0.002
	Cognitive function		–	–	–	–	–	–	–
	Height z-score	β-coef = −0.08 p = 0.023		β-coef = −0.01 p = 0.010					–
Entire cohort	Metabolic decomp.		–	–	–	–	–	–	–
	Mitochondrial compl.		Exp (coef) = 0.99 p < 0.001	Exp (coef) = 1.01 p < 0.001	Exp (coef) = 1.01 p < 0.001	Exp (coef) = 0.10 p < 0.001	Exp (coef) = 9.62 p < 0.001	Exp (coef) = 1.01 p < 0.001	Exp (coef) = 0.73 p = 0.003
	Cognitive function		–	Exp (coef) = 1.03 p = 0.012	–	Exp (coef) = 0.01 p = 0.022	Exp (coef) = 75.43 p = 0.022	–	–
Entire cohort without CblA	Cognitive function		–	Exp (coef) = 1.01 p = 0.028	–	–	–	–	–
						Exp (coef) = 5.39 p = 0.058	Exp (coef) = 5.39 p = 0.058		

Abbreviations: coef: coefficient; compl.: complications; decomp.: decompensations; EO: early onset; exp: exponential; kcal: kilocalorie; LO: late onset; MMA: methylmalonic acidemia; NS = non-significant; ND = not determined; PA: propionic acidemia; P:E ratio: protein: energy ratio; R: responsive; RDA: recommended daily allowance; UR: unresponsive; vitB12: vitamin B12.

the total amount of protein prescribed. Energy prescription in kilocalories (kcal) per day was compared to the RDA [7]. The total P:E ratio was defined as the total amount of protein prescribed in grams per prescription of 100 kcal per day. Standard deviation scores of height, indicated by height z-scores, were calculated according to the LMS method [17], using reference data [18,19]. Plasma BCAA levels were compared with reference values as provided by the "Laboratory Guide to the Methods in Biochemical Genetics" [20]. A ratio of 1:2:4 for L-isoleucine: L-leucine: L-valine was considered normal [20,21].

2.3. Statistical analysis

To determine the association of protein and energy prescription with the occurrence of AMD a recurrent event analysis using a Cox regression model was performed using the R package survival. Poisson regression analysis with age of follow-up as offset (to correct for follow-up time) was performed using the R glm() function to determine the association of protein, energy prescription, and plasma valine/isoleucine/leucine with the total number of mitochondrial complications. Ordinal regression analysis was performed using the R polr() function to determine the association of protein and energy prescription with the category of cognitive functioning. For both Poisson and ordinal regression, a mean area under the curve (AUC) was calculated using the AUC() function of the R DescTools package, to calculate a weighted mean of protein and energy prescription during a patient's follow-up time. The association of natural protein, AAM protein, total protein prescription and AAM:total protein with their outcome variables were tested in each patient subgroup. A linear mixed-effects model analysis was performed using SPSS to determine the association of protein prescription with the patient's height. In particular, we used this model first to study the longitudinal evolutions of the height z-score, and then to investigate the association of dietary treatment with height. In our model the three fixed effects were 1) the total P:E ratio; 2) prescription of synthetic protein as % RDA; 3) age at each visit. For the random-effects structure we used a build-up approach, starting from random intercepts, and including linear and nonlinear random slopes for the age variable. The appropriate random-effects structure was selected using the Akaike Information Criterion.

Pearson correlation tests were performed to assess correlations between the mean AUC of natural protein RDA as well as the mean AUC of the AAM:total protein ratio and the mean AUC of the plasma levels of valine, isoleucine and leucine throughout life. In addition, Pearson correlation tests were performed to assess the correlation between the frequency of AMD per patient year during the first four years of life and the mean AUC of the plasma levels of valine, isoleucine and leucine during the first four years of life. A Kendall-tau correlation test was performed to assess the association between the mean AUC AAM:total protein intake and the mean AUC natural protein intake. The Holm-Bonferoni method was used to correct for familywise error rates for multiple testing. All statistical analyses were discussed with a statistician (prof. D. Rizopoulos).

3. Results

3.1. Baseline characteristics

The nationwide cohort consisted of 76 MMA and PA patients (24 MMA vitB12 unresponsive, 21 MMA vitB12 responsive, 20 PA LO, 10 PA EO patients and one PA patient for whom presentation type was unknown) [3] (Supplementary Table 1). A mean of 6.7 mitochondrial complications per patient was noted in the entire cohort, 30% of the patients had an IQ < 60 (19/63) and the majority of the patients had impaired height (Supplementary Table 1 and Supplementary Fig. 1). As expected based on disease severity AMD were frequent in MMA vitB12 unresponsive and PA EO patients (Supplementary Fig. 2).

Of these patients, 75 were prescribed a protein-restricted diet at some point in life. In 78% (59/76) a protein-restricted diet was prescribed at all evaluated time points during outpatient visits. During a median retrospective follow-up period of 15 years (min–max: 0–48 years) details of protein prescription were available at 1287 time points (Supplementary Table 1), describing a total of 1063 patient years on diet (median: 14.7 years, min–max: 0.0–36.6 years).

3.2. Dietary protein prescription and outcome

3.2.1. Entire cohort

In 37% (470/1287 measurements in 75 patients) of all prescriptions, natural protein prescription exceeded RDA. Natural protein prescription mainly exceeded RDA in patients from 1 year of age till adolescence and after the age of 20 years, in all subgroups (Fig. 1a–c). AAM were prescribed in 84% of patients (n = 64/76) (Fig. 1d–f, Supplementary Table 1). 32% (343/1087) of the AAM prescriptions occurred despite a prescribed natural protein prescription already above the RDA. The additional AAM protein supplementation resulted in a total protein prescription exceeding RDA in 84% of the measurements (1070/1277), with a mean (±SD) of 150 ± 52 %RDA (Fig. 1g–i). Overall, the AAM:total protein ratio differed widely (mean ± SD AAM:total protein: 0.36 ± 0.20) (Fig. 1j–l). A higher AAM protein prescription, as well as a higher amount of AAM:total protein prescription were associated with a higher frequency of impaired cognition in the entire cohort (Table 1, Supplementary Fig. 3a–b). This association between a higher AAM protein prescription and a higher frequency of impaired cognition persists in the cohort even when CblA patients are excluded (Table 1, Supplementary Fig. 3c). Unexpectedly, a higher AAM protein prescription was negatively associated with the height z-score in the entire cohort, except for PA EO patients (Table 1).

3.2.2. MMA VitB12 unresponsive patients

Natural protein prescription (mean %RDA ± SD: 87 ± 30) was above RDA in 34% (174/518) and total protein (mean %RDA ± SD: 149 ± 48) was above RDA in 80% (416/517) of all prescriptions. Of all the time points that AAM was prescribed, natural protein was already above RDA in 34% (167/498) and total protein prescription was above RDA in 82% (410/498). Both a higher total protein and a higher AAM protein prescription were associated with more frequent mitochondrial complications (Fig. 3a–b, Table 1).

3.2.3. MMA VitB12 responsive patients

In 53% (120/227) of all prescriptions natural protein (mean % RDA ± SD: 109 ± 35) was above RDA and total protein (median % RDA, min–max: 141, 56–432) was above RDA in 79% (179/226). In 45% (56/125) of the time points that AAM was prescribed, natural protein prescription was already above RDA. A higher amount of AAM protein, a higher AAM:total protein prescription and a higher total protein prescription were associated with more frequent mitochondrial complications (Supplementary Fig. 3d–f, Table 1).

3.2.4. PA EO patients

Natural protein prescription (mean %RDA ± SD: 78 ± 30) was above RDA in 26% (109/423), and total protein prescription (mean %

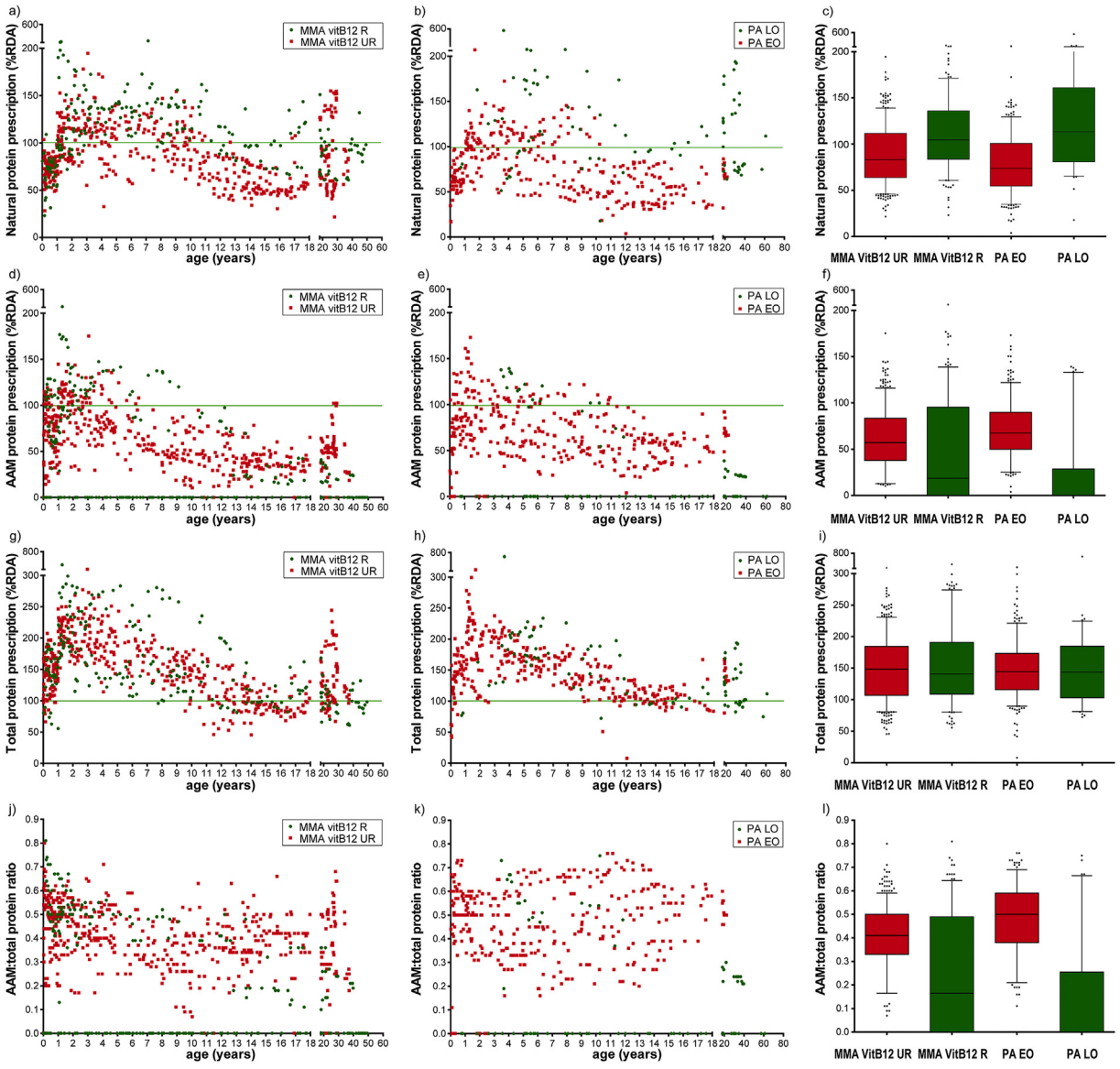

Fig. 1. Natural, amino acid mixtures and total protein and amino acid mixture: total protein ratio prescription according to patient's age (years) (a–h), and according to disease subgroup (i–l). a–h: dots/squares represent one measurement per patient. Red squares indicate the more severely affected patients, namely MMA vitB12 unresponsive and PA EO patients, and green dots indicate the less severely affected patients, namely MMA vitB12 responsive and PA LO patients. Horizontal green lines in a–c and e–g indicate a RDA of 100%. i–l: boxplots based on all measurements per patient, indicating median, 25th – 75th percentile and 95% CI. Black dots indicate outliers. Abbreviations: AAM: amino acid mixtures; EO: early onset; LO: late onset; MMA: methylmalonic acidemia; PA: propionic acidemia; R: responsive; RDA: recommended daily allowances; UR: unresponsive; vitB12: vitamin B12.

RDA ± SD: 154 ± 49) was above RDA in 91% (383/421) of all prescriptions. Of all the time points that AAM was prescribed, natural protein prescription was already above RDA in 25% (105/413) and total protein prescription was above RDA in 92% (397/413). A higher natural: total protein prescription (especially when exceeding RDA) was associated with more frequent AMD and a higher AAM:total protein intake was associated with less frequent AMD (Fig. 3c, Table 1).

3.2.5. PA LO patients

In 44% (52/119) of all prescriptions the natural protein prescription (median %RDA, min–max: 110, 18–558) was above RDA and total protein (median %RDA, min–max: 142, 65–689) was above RDA in 81% (92/113). No significant associations were found between dietary prescription and the outcome parameters.

3.3. Plasma BCAA levels

Plasma BCAA levels were low in almost all assessed patients (n = 41) (Supplementary Fig. 4). This included the valine:leucine ratio (mean: 1.2, reference value: 2.0) and the isoleucine:leucine ratio (mean: 0.37, reference value: 0.50) (Supplementary Fig. 4). No associations were found between the natural protein prescription and plasma BCAA levels in any subgroup. In PA EO patients, a higher amount of AAM:total protein prescription was associated with lower plasma valine levels ($r = -0.89$, $p < 0.001$). In PA LO patients, a higher mean AUC plasma valine, isoleucine, and valine:isoleucine

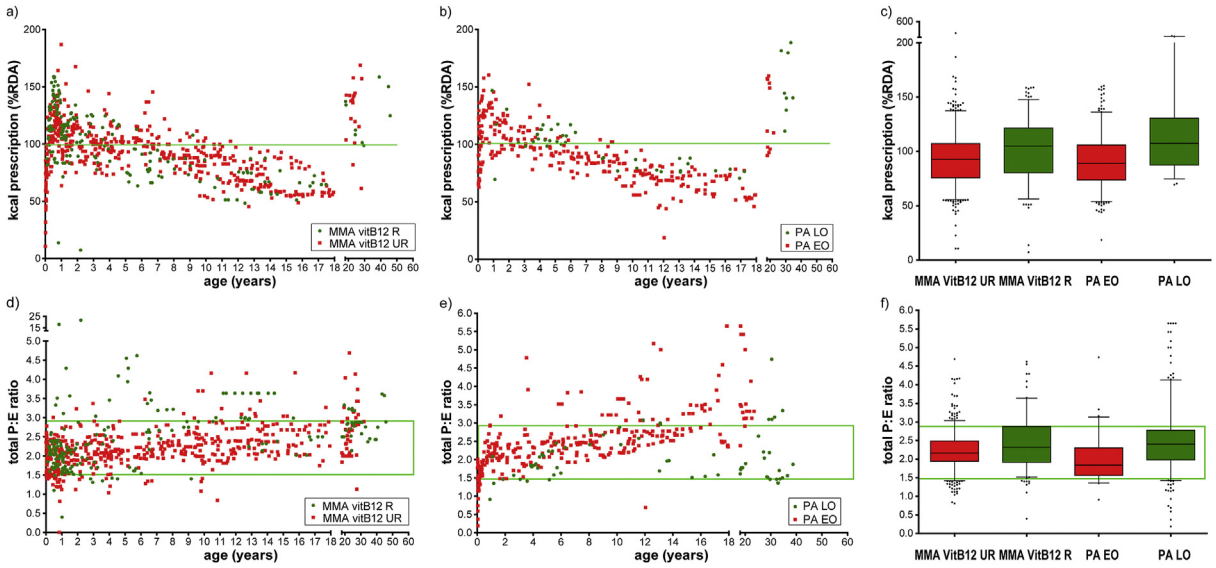

Fig. 2. Kilocalorie and total P:E ratio prescription according to patient's age (years) (a–d), and according to disease subgroup (e–f). a–b: dots/squares represent one measurement per patient. Red squares indicate the more severely affected patients, namely MMA vitB12 unresponsive and PA EO patients, and green dots indicate the less severely affected patients, namely MMA vitB12 responsive and PA LO patients. c–d: boxplots based on all measurements per patient, indicating median, 25th – 75th percentile and 95% CI. Black dots indicate outliers. Horizontal green lines in a and c indicate a RDA of 100%, the horizontal green square in b and d indicates the dispersion of the recommended total P:E ratio. Abbreviations: EO: early onset; kcal: kilocalorie; LO: late onset; MMA: methylmalonic acidemia; P:E ratio: protein-to-energy ratio; PA: propionic acidemia; R: responsive; RDA: recommended daily allowances; UR: unresponsive; vitB12: vitamin B12.

ratio were associated with less frequent mitochondrial complications (Supplementary Table 2). In MMA vitB12 responsive patients, a higher mean AUC plasma valine level and a higher mean AUC isoleucine:leucine ratio were associated with less frequent mitochondrial complications (Supplementary Table 2). No clear associations between mean AUC valine, isoleucine, and leucine (first four years) and AMD (per patient year in first four years) were found.

3.4. Dietary energy prescription and outcome

Energy prescription decreased with age and was below RDA in all subgroups during adolescence, while increasing thereafter (Fig. 2a–c). Total P:E ratio increased with age and was prescribed according to recommendations in the majority of patients (Fig. 2d–f) [9].

The higher the energy (kcal) prescription, the higher the frequency of mitochondrial complications in MMA VitB12 responsive patients (Supplementary Fig. 3g, Table 1). A higher total P:E ratio was associated with increased height z-score in MMA VitB12 unresponsive patients and with decreased mitochondrial complications in PA EO and LO patients (Fig. 3d and Supplementary Fig. 3h, Table 1).

4. Discussion

The aim of this study was to evaluate longitudinal dietary treatment in MMA and PA patients. Through a concise retrospective study we demonstrated that the prescribed amount of protein in many MMA and PA patients exceeded the RDA. Importantly, we revealed that this high (natural, AAM and total) protein prescription is strongly associated with adverse patient outcome.

Our observation that more than one fourth of patients received a natural protein prescription exceeding RDA aligns with previous studies [22–24]. Prescription of (excessive) AAM protein resulted in a total protein prescription above RDA in almost all patients, as also observed in the E-IMD cohort [10]. Intriguingly, additional AAM was often applied when natural protein was already according to RDA, despite the fact that guidelines suggest to only apply AAM when natural protein is below RDA [8]. This total protein largely exceeding RDA, is likely very harmful regarding patient outcome. Reasons for providing patients that are on a protein-restricted diet with a protein prescription above RDA might be 1) adjusting protein prescription due to impaired height, 2) compensating for the inferior quality of the protein that patients consume, 3) adjusting protein prescription based on low BCAA levels or 4) the use of other guidelines/protein calculation methods (g/kg) than the RDA of the WHO.

The negative association between protein prescription and patient outcome was most evident in the more severely affected patients: PA EO and MMA vitB12 unresponsive patients [3]. PA EO patients who receive a high natural protein intake are at risk of AMD, especially when natural protein is above RDA. MMA patients who receive a high total protein (vitB12 unresponsive patients) and a high AAM protein prescription (vitB12 unresponsive as well as vitB12 responsive patients) are at risk of mitochondrial complications. Therefore, we recommend for PA EO patients and MMA vitB12 unresponsive patients that total protein should not exceed the RDA. If the tolerated amount of natural protein is below the RDA, additional AAM should be applied in amounts that result in a total protein intake which approximates the RDA, but not exceeds it. Recently, two major pathophysiologic principles have been reported that could play an important role in (ongoing) mitochondrial dysfunction: both disturbed autophagy/mitophagy as well as enhanced protein acylation [25]. The role of protein intake, including resulting abnormalities in BCAA and energy intake on both mechanisms needs further study.

We here report a negative association of AAM prescription with mitochondrial complications in MMA patients, and with impaired height in PA LO and MMA patients. In addition, we also observe a trend towards a negative association of AAM prescription with impaired cognitive function, which warrants further study. Importantly, the frequent application of AAM, inconsistency in practice in Europe [26] and the potential harmful effects of AAM prescription have been reported previously. AAM can, due to the

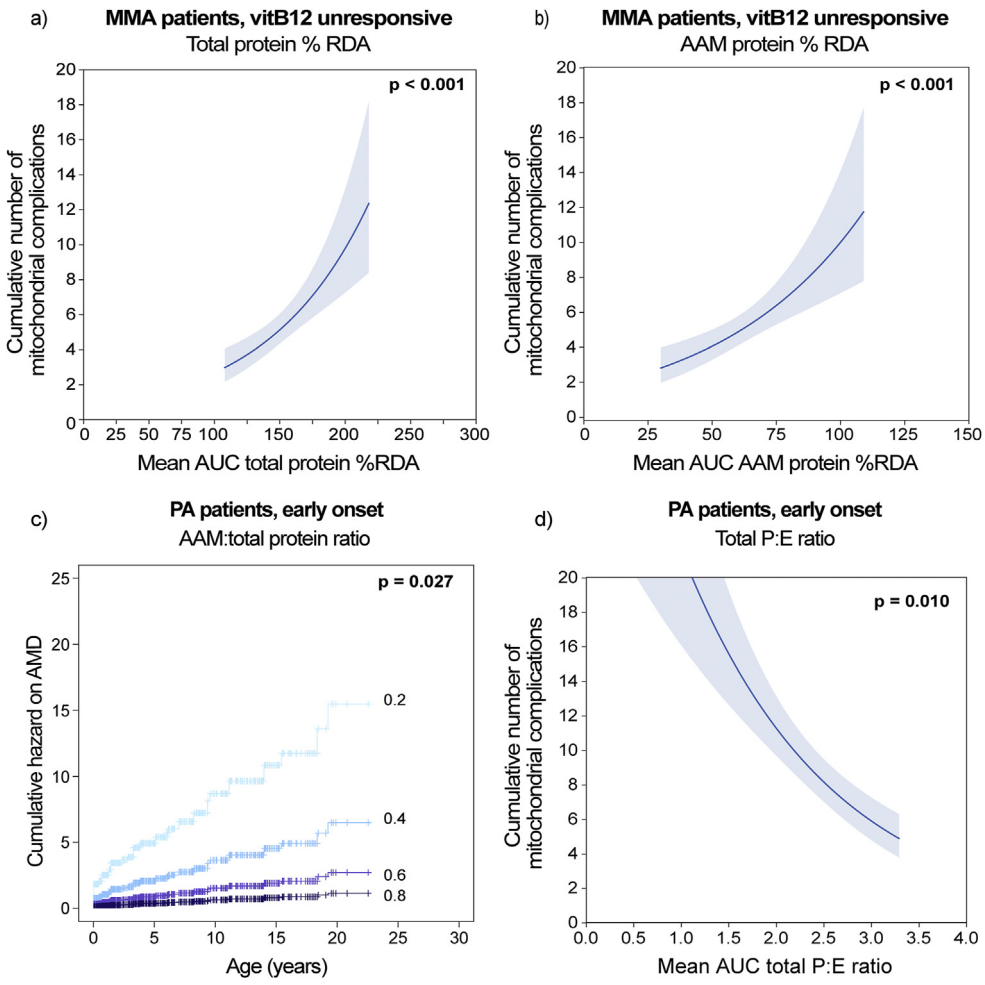

Fig. 3. Protein prescription (AAM, total, natural:total and AAM:total) and outcome (mitochondrial complications and acute metabolic decompensations). a,b and d show the association between total protein prescription, AAM protein prescription and total P:E ratio prescription, respectively, and the risk on mitochondrial complications. We analyzed the association of the cumulative count of mitochondrial complications with the mean area under the curve of these parameters using a poisson regression. Per patient we calculated the cumulative count of mitochondrial complications and we calculated for each patient – using each time point when the dietary prescription was noted – what the mean area under the curve was, for total and AAM protein and for the total P:E ratio. As all these parameters are dependent of the follow-up time, we corrected for follow-up time using age at follow-up as offset in the regression (see: Methods). The regression line is depicted in dark blue, the 95% confidence interval is depicted in light blue. c shows the association between AAM:total protein ratio prescription and cumulative hazard on an AMD. We analyzed this association using a – survival-like – recurrent event analysis using a Cox regression model. Hereby, we calculate the cumulative hazard on AMD in relation to the patient's age and the prescribed AAM:total protein ratio at that age (see: Methods). Abbreviations: AAM: amino acid mixtures; AMD: acute metabolic decompensations; AUC: area under the curve; MMA: methylmalonic acidemia; PA: propionic acidemia; RDA: recommended daily allowances; vitB12: vitamin B12.

relatively high leucine content, be a major cause of disturbed BCAA ratios as well as of decreased valine and isoleucine plasma levels [9,11–13]. This is due to interaction of BCAA at the large neutral amino acid transporter (LAT). We indeed observed low plasma BCAA levels and disturbed BCAA ratios, and in PA EO patients, a higher AAM:total protein ratio prescription was associated with lower plasma valine concentrations. It is known that low BCAA levels can increase the risk of an AMD [27] and that normal BCAA levels are essential for growth and development [28–30]. In order to define and study the associations of BCAA levels and patient outcome (including the effect on pathophysiological processes, such as mitochondrial function, autophagy and protein acylation) a prospective study would be necessary. We strongly encourage clinicians to be cautious with AAM prescription, and to only prescribe them when use is inevitable to achieve a total protein prescription meeting the RDA.

Next to a protein prescribed diet, current best practice is to prescribe a high energy intake to prevent catabolism, especially in infants. Intriguingly, we observed that a high energy prescription can be harmful as we showed that high energy was associated with increased risk of mitochondrial complications in MMA vitB12 responsive patients. Moreover, the relatively high kilo caloric intake prescribed, results in a lower P:E ratio. We observed that a lower total P:E ratio in PA EO patients was associated with a higher frequency of mitochondrial complications and with decreased height, as has also been reported by others [9,31]. Aging studies demonstrate that indeed, high energy prescription can be harmful [32], and it has been demonstrated that caloric restriction can potentially improve mitochondrial function [33–35]. Energy supply is also essential for optimal protein utilization. It is hard to determine the optimal P:E ratio in MMA and PA patients, due to four factors: 1) energy intake not always equals energy uptake due to impaired gut mobility [2], 2) energy requirement is highly dependent on physical activity, which is often impaired in MMA and PA patients, 3) children require more energy for optimal growth, and 4) the energy balance can be disturbed by mitochondrial dysfunction in MMA and PA patients [7]. Furthermore, the P:E ratio can appear optimal when both a high protein and a high energy intake are prescribed,

despite the fact that a high energy intake could result in a deleterious effect on the compromised mitochondria. Thus, in summary, before P:E ratios in MMA and PA patients can accurately be optimized, further studies are required to determine the optimal ratio, taking into account all outcome measures.

5. Limitations and strengths

The limitations of our study are mainly due to the retrospective design. First of all, other factors than dietary treatment, such as genetic variation within subgroups, hyperammonemia during the first AMD or the frequency and severity of infections, are associated with the studied outcome parameters. It would be expected that more severely affected patients would be prescribed less protein, but instead they are prescribed more natural, AAM and total protein, corroborating the veracity of our results. Infections could be a confounder for the frequency of AMD, but this cannot explain the negative association between a higher protein intake and AMD, as protein intake is often decreased during infectious episodes. Regarding the potential effect of hyperammonemia during the first AMD on cognitive development, we have shown that siblings identified through family testing – in whom severe hyperammonemia could be prevented – still have a comparable cognitive development as their sibling that presented with a severe AMD [3]. This suggests that not the first presentation, but more likely our clinical management during follow-up, plays a greater role in the outcome of cognitive development especially in PA. It remains uncertain weather, adjusted dietary treatment (meeting guideline advises), as described, could improve this outcome. Outcome could be just disease related.

Secondly, theoretically new therapies could have influenced patient outcome as well, given the long follow-up time in our study. Though, we expect this effect to be limited, as for example, N-carbamylglutamate was prescribed only in a few patients. In the third place, we recorded dietary treatment prescription rather than actual intake, which might be different due to suboptimal therapy compliance. This is likely to affect size, but unlikely to affect the direction of the here reported findings. Fourthly, we did not include AMD that were managed at home, as these were often not noted in the patient's medical records. This could give an underestimation of the AMD frequencies, although the more severe AMD are likely accounted for. In the fifth place, more severely affected patients could be overrepresented, as they are followed more closely. Though, since regular follow-up visits of all patients were included, we expect this overrepresentation to be limited. Lastly, sampling for BCAA plasma levels was not performed at the same time point as prescription of dietary treatment and height were recorded, and we therefore could not correlate these measures directly, potentially affecting the observed correlations.

Also due to the retrospective design of our study, it is important to notice that we here discuss associations, rather than causation. For example, AAM prescription seems to be associated with impaired cognitive outcome in this cohort, but we explicitly do not claim that AAM intake directly affects cognitive development in PA and MMA patients.

An important strength of our study is that, while over the past decades no clear effects of dietary treatment on patient outcome have been reported, we describe clear correlations between dietary treatment prescriptions and patient outcomes. This was possible since we extensively described an entire national cohort, with a large follow-up time, and very detailed information on dietary prescriptions and patient outcome parameters [3]. Prospective studies are needed to confirm our observations and to substantiate our suggestions, in order to ultimately attain more personalized dietary treatment for MMA and PA patients that could lead to a more satisfying long-term outcome. A promising option is determination of individual patient protein and energy requirement. This could be achieved by amino acid oxidation method or nitrogen balance studies and determination of individual resting energy expenditure [36,37].

6. Conclusion

Natural protein prescription exceeded RDA in one fourth of MMA and PA patients. Numerous patients received additional AAM protein prescription, even when natural protein was already according to RDA, resulting in a very high total protein prescription. A high protein prescription was negatively associated with MMA and PA patient outcome (AMD, mitochondrial complications, cognition and height). We therefore advise to reduce protein prescription in (mainly severely affected) patients receiving protein above RDA and to be cautious with the prescription of AAM.

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Short running head

Despite a protein restricted diet, protein prescribed to methylmalonic and propionic acidemia patients often exceeds WHO recommended daily allowances, and this is negatively associated with patient outcome.

Details of ethics approval

All procedures followed were in accordance with the ethical standards of the responsible committee of the University Medical Centers Utrecht (17–490/C) and Rotterdam (MEC-2018-1312) and with the Helsinki Declaration of 1975, as revised in 2000. Written informed consent was obtained from all included patients or their legal guardians.

Animal rights

This article contains no studies with animal subjects performed by any of the authors.

Details of individual authors' contributions

F.M. and M.W. conceived the research. H.A.H., F.M., M.W. and P.M.v.H. designed the study protocol and gained approval of the responsible ethics committees. J.J.M.J., N.M.V.-D., M.W. and P.M.v.H supervised the research. M.W. and J.G.L. gave advice as experts of the Dutch expertise center on organic acidemias. M.C.J., A.M.B., F.J.v.S., M.F.M., S.F., A.T.v.d.P., M.A.W., M.E.R.G., M.C.G.J., M.C.d.V. and M.W. coordinated patient inclusion and data collection in the six Dutch metabolic centers. H.A.H. and F.M. obtained written informed consent from all patients or their legal guardians. H.A.H. and F.M. designed the electronic clinical record forms in OpenClinica, collected the data and performed the data analyses. F.M. wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the final version.

Conflict of interest

All authors state that they have no competing interests to declare. None of the authors accepted any reimbursements, fees or funds from any organization that may in any way gain or lose financially from the results of this study. The authors have not been employed by such an organization. The authors do not have any other competing interest.

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Appendix A. Supplementary data

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