Long-term effects of medical management on growth and weight in individuals with urea cycle disorders

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Low protein diet and sodium or glycerol phenylbutyrate, two pillars of recommended long-term therapy of individuals with urea cycle disorders (UCDs), involve the risk of iatrogenic growth failure. Limited evidence-based studies hamper our knowledge on the long-term effects of the proposed medical management in individuals with UCDs. We studied the impact of medical management on growth and weight development in 307 individuals longitudinally followed by the Urea Cycle Disorders Consortium (UCDC) and the European registry and network for Intoxication type Metabolic Diseases (E-IMD). Intrauterine growth of all investigated UCDs and postnatal linear growth of asymptomatic individuals remained unaffected. Symptomatic individuals were at risk of progressive growth retardation independent from the underlying disease and the degree of natural protein restriction. Growth impairment was determined by disease severity and associated with reduced or borderline plasma branched-chain amino acid (BCAA) concentrations. Liver transplantation appeared to have a beneficial effect on growth. Weight development remained unaffected both in asymptomatic and symptomatic individuals. Progressive growth impairment depends on disease severity and plasma BCAA concentrations, but cannot be predicted by the amount of natural protein intake alone. Future clinical trials are necessary to evaluate whether supplementation with BCAAs might improve growth in UCDs.

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Urea cycle disorders (UCDs) are rare inherited metabolic diseases, consisting of 5 enzymopathies, 2 transporters and 2 associated cofactor-producing enzymes, with an estimated overall prevalence of 1 in 35,000 to 52,000 newborns. The phenotypic spectrum is wide ranging from severe life-threatening hyperammonemic decompensations within the first 28 days of life (EO, early onset) to mild or moderate chronic hyperammonemic conditions reflected by a heterogeneous clinical spectrum such as lethargy, headache, hepatological, gastrointestinal and neurological or psychiatric symptoms any time after the neonatal period (LO, late onset). Long-term dietary management is challenging and consists of a low protein diet with or without supplementation of essential amino acids (EAAs), vitamins, trace elements and/or single amino acids, while pharmacological long-term treatment reflected by a heterogeneous clinical spectrum such as lethargy, headache, hepatological, gastrointestinal and neurological or psychiatric symptoms any time after the neonatal period (LO, late onset). Long-term dietary management is challenging and consists of a low protein diet with or without supplementation of essential amino acids (EAAs), vitamins, trace elements and/or single amino acids, while pharmacological long-term treatment reflected by a heterogeneous clinical spectrum such as lethargy, headache, hepatological, gastrointestinal and neurological or psychiatric symptoms any time after the neonatal period (LO, late onset).

Results

Anthropometrical parameters at birth are within normal range. Overall, 205 individuals with UCDs [male ornithine transcarbamylase deficiency (mOTC-D): n = 54, 26.4%; female OTC-D (fOTC-D): n = 39, 19.0%; argininosuccinate lyase deficiency (ASL-D): n = 91, 32.1%] have a mean z-score within the normal range for birth weight, length, and head circumference [mean z-score (birth weight): −0.42; mean z-score (birth length): 0.14; mean z-score (birth head circumference): −0.13] (Fig. 1A–C). Neither disease (mOTC-D, fOTC-D, ASS1-D, ASL-D) nor sex (male, female) showed a specific impact on birth weight (disease: p = 0.15; sex: p = 0.15; ANOVA), birth length (disease: p = 0.30, sex: p = 0.57; ANOVA), or birth head circumference (disease: p = 0.74, sex: p = 0.09; ANOVA).

Asymptomatic individuals have a normal postnatal growth and weight development. Next, we studied the postnatal anthropometrical development of asymptomatic and untreated individuals with UCDs.
Overall 11 individuals (mOTC-D: n = 2, 18.2%; fOTC-D: n = 3, 27.3%; ASS1-D: n = 4, 36.3%; ASL-D: n = 2, 18.2%) with a mean individual observation period of 2.95 years (min: 1.59 years; max: 4.54 years) were investigated. Mean age at first visit was 1.56 years (min: 0.01 years; max: 8.71 years) and mean age at last visit corresponded to 4.51 years (min: 1.96 years; max: 12.27 years), reflecting a preschool population of UCDs. Neither disease nor age showed an impact on postnatal weight development (disease: $p = 0.33$, age: $p = 0.27$; ANOVA) and linear growth (disease: $p = 0.72$, age: $p = 0.16$; ANOVA) within the observation period (Fig. 2A,B), highlighting that asymptomatic individuals with UCDs have a normal postnatal anthropometrical development.

Symptomatic individuals have a risk of postnatal growth retardation. To assess whether symptomatic individuals had a risk of postnatal weight and growth retardation, we evaluated 130 symptomatic individuals (mOTC-D: n = 33, 25.4%; fOTC-D: n = 42, 32.3%; ASS1-D: n = 25, 19.2%; ASL-D: n = 30, 23.1%) receiving conservative management during a mean individual observation period of 4.81 years (min: 1.00 years; max: 12.44 years). Overall, the observation period spanned a time frame within prepubertal childhood from mean age at first visit of 6.33 years (min: 0.03 years; max: 15.52 years) to mean age at last visit corresponded to 11.14 years (min: 1.27 years; max: 17.99 years). Since early disease onset was associated with higher disease severity10, we investigated whether specific diseases were associated with higher initial peak plasma ammonium concentrations ($NH_4^+$) in our study population. Interaction between disease onset (EO vs. LO) and a specific disease (mOTC-D, fOTC-D, ASS1-D, ASL-D) was not significant ($p = 0.86$; LME ANOVA). Furthermore, neither disease onset ($p = 0.77$; LME ANOVA), nor a specific disease ($p = 0.71$; LME ANOVA) or age ($p = 0.41$; LME ANOVA) had a measurable impact on weight gain during the observation period, demonstrating that current conservative management does not impair weight development of symptomatic individuals with UCDs (Fig. 3A). In contrast, symptomatic individuals suffered from postnatal growth retardation. This was not associated with a specific UCD ($p = 0.45$; LME ANOVA), while disease onset ($p = 0.03$; LME ANOVA), age ($p < 0.001$; LME ANOVA),...
and interaction between disease onset and age \((p < 0.001; \text{LME ANOVA})\) had a significant impact on linear growth indicating that particularly EO patients of any UCD studied suffered from postnatal growth retardation (Fig. 3B). Accordingly, body mass index (BMI) of affected individuals increased over time for the EO but not LO group as indicated by a significant interaction between disease onset and age \((p = 0.02; \text{LME ANOVA}; \text{Fig. 3C})\).

Postnatal growth retardation in symptomatic individuals with UCDs is not associated with a protein restricted diet. To evaluate whether the observed postnatal growth retardation in symptomatic individuals was due to the protein restricted diet or the caloric intake as part of the conservative management, we studied 46 severity-adjusted UCD individuals (mOTC-D: \(n = 9, 19.6\%\); fOTC-D: \(n = 11, 23.9\%\); ASS1-D: \(n = 11, 23.9\%\); ASL-D: \(n = 15, 32.6\%\)) with sufficient information on biochemical and therapy-related longitudinal data, comprising a mean individual observation period of 3.13 years (min: 1.01 years; max: 9.94 years) within the preschool age. Overall, half of the patients received a protein restricted diet (mOTC-D: \(n = 3, 13.0\%\); fOTC-D: \(n = 5, 21.7\%\); ASS1-D: \(n = 4, 17.5\%\); ASL-D: \(n = 6, 26.1\%\); fOTC-D: \(n = 6, 26.1\%\); ASS1-D: \(n = 7, 30.4\%\); ASL-D: \(n = 4, 17.4\%\)). Individuals with and without a protein restricted diet did not differ with regard to their initial \(\text{NH}_4^+\) \((p = 0.20; \text{t-test})\). In addition, the degree of protein restriction was not disease-dependent in individuals receiving a protein restricted diet \((p = 0.28; \text{LME ANOVA})\), but showed an overlapping mean natural protein intake ranging from 62.95% to 77.45% WHO in mOTC-D, fOTC-D, ASS1-D, and ASL-D (Fig. 4A). In analogy, a protein restricted diet had no impact on weight gain. Neither age \((p = 0.48; \text{LME ANOVA})\) nor application of a protein restricted diet \((p = 0.43; \text{LME ANOVA})\) or interaction of both \((p = 0.98; \text{LME ANOVA})\) affected weight development (Fig. 4B). Regardless of this, symptomatic individuals receiving conservative medical management developed a progressive growth retardation \((p = 0.008; \text{LME ANOVA})\). However, this was not explained by the use of a protein restricted diet \((p = 0.61; \text{LME ANOVA})\), showing that both symptomatic individuals with or without a protein restricted diet \((p = 0.27; \text{LME ANOVA})\) experienced postnatal growth retardation (Fig. 4C). Based on 39.1\% (\(n = 18/46\)) of individuals in this sample with sufficient data density, mean caloric intake does not appear to be associated with growth retardation \((p = 0.32; \text{LME ANOVA})\).

Symptomatic UCDs have reduced to low normal plasma BCAA concentrations. Since growth retardation was independent from the use of a protein restricted diet, we wondered whether BCAA concentrations (L-valine, L-leucine, L-isoleucine) might contribute to impaired growth in symptomatic individuals with or without a protein restricted diet. Therefore, we investigated the weighted arithmetic mean values of plasma BCAAs along with the weighted arithmetic mean dosage of EAA supplements during the individual observation periods in both individuals with and without a protein restricted diet. Although individuals with a protein restricted diet received a higher weighted arithmetic mean dosage of EAA supplements \((p = 0.03; \text{t-test})\), concentrations of L-valine \((p = 0.25; \text{t-test})\), L-leucine \((p = 0.06; \text{t-test})\), and L-isoleucine \((p = 0.24; \text{t-test})\) did not differ between groups (Table 1), but were found to be reduced or at the lower end of the reference range\(^{13}\). Weighted arithmetic mean values of plasma L-arginine did not differ between both groups \((p = 0.78; \text{t-test})\), but were, in contrast to plasma BCAA concentrations, within the normal or upper end of the reference range\(^{13}\). Moreover, application of nitrogen scavengers (mono- or bicavenger therapy) showed a similar distribution between groups.
Liver transplantation rescues postnatal growth retardation in symptomatic UCDs. To investigate whether LTx might exert a positive effect on growth and weight in severely affected individuals, we analyzed both endpoints of liver-transplanted individuals at three different time points, i.e. at first observation (first time point), at last observation prior to LTx (second time point), and at last observation after LTx (third time point). Overall, 19 individuals (mOTC-D: n = 7, 36.8%; ASS1-D: n = 6, 31.6%; ASL-D: n = 6, 31.6%) receiving LTx at a mean age of 2.15 years (min: 0.42 years; max: 7.76 years) were included into this analysis. Disease severity, as mirrored by initial NH$_4^+$max, did not differ between EO ($\beta$ = −0.01) and LO ($\beta$ = 0.02; $p$ = 0.23, LME) individuals. Height (B) however, was affected by age ($p < 0.001$; LME ANOVA), disease onset ($p = 0.03$; LME ANOVA) and interaction of both ($p < 0.001$; LME ANOVA), suggesting that disease severity—as reflected by EO individuals—is associated with impaired growth over time. In line, $\beta$-coefficients differed between EO ($\beta$ = −0.11) and LO ($\beta$ = −0.02; $p < 0.001$, LME) individuals. Accordingly, BMI (C) was determined by age ($p < 0.001$; LME ANOVA) and interaction between age and disease onset ($p = 0.02$; LME ANOVA), indicating that BMI increases with age for the EO but not the LO group. Gray lines are fitted weight, height, and BMI values from the LME model; the gray shaded area corresponds to 95% confidence interval. Descriptive characteristics are presented separately in Supplementary Table S1. BMI, body mass index; EO, early onset; LO, late onset.

Figure 3. Symptomatic individuals (n = 130) have normal weight development but abnormal linear growth. Prepubertal children were observed during a period of approximately 5 years. Weight (A) was not affected neither by age ($p = 0.41$; LME ANOVA), nor by disease onset ($p = 0.77$; LME ANOVA) or specific diseases ($p = 0.71$; LME ANOVA). $\beta$-Coefficients did not differ between EO ($\beta$ = −0.01) and LO ($\beta$ = 0.02; $p = 0.23$, LME) individuals. Height (B) however, was affected by age ($p < 0.001$; LME ANOVA), disease onset ($p = 0.03$; LME ANOVA) and interaction of both ($p < 0.001$; LME ANOVA), suggesting that disease severity—as reflected by EO individuals—is associated with impaired growth over time. In line, $\beta$-coefficients differed between EO ($\beta$ = −0.11) and LO ($\beta$ = −0.02; $p < 0.001$, LME) individuals. Accordingly, BMI (C) was determined by age ($p < 0.001$; LME ANOVA) and interaction between age and disease onset ($p = 0.02$; LME ANOVA), indicating that BMI increases with age for the EO but not the LO group. Gray lines are fitted weight, height, and BMI values from the LME model; the gray shaded area corresponds to 95% confidence interval. Descriptive characteristics are presented separately in Supplementary Table S1. BMI, body mass index; EO, early onset; LO, late onset.
to LTx: −0.75, mean z-score at last observation after LTx: −0.17, p = 0.13; contrast t-test) (Fig. 5B). Interestingly, liver transplantation led to the elevation of all plasma BCAAs concentrations from reduced or low normal values before LTx to values well within the normal range after LTx [L-valine (p = 0.001; t-test), L-leucine (p = 0.048; t-test), L-isoleucine (p = 0.001; t-test); Table 1].

Discussion
This study aimed at characterizing growth and weight gain in individuals with UCDs and elucidating risk factors for potentially impaired anthropometrical development. The study revealed five major results: (1) Intrauterine development in all investigated UCDs and postnatal linear growth of asymptomatic and untreated individuals were normal. (2) Symptomatic individuals were at risk of developing progressive growth impairment regardless of the underlying UCD or degree of natural protein intake. (3) Growth impairment was determined by disease severity and associated with reduced or low normal plasma BCAA concentrations. (4) LTx appeared to have a beneficial effect on growth. (5) Weight development was normal in both asymptomatic and symptomatic individuals with UCDs, regardless of the medical management.

Intrauterine growth of the investigated study sample was unaffected as indicated by anthropometrical parameters at birth which were within the normal range, thereby confirming previous data from a European cohort.
of individuals with UCDs. In asymptomatic UCD individuals not receiving long-term management with low protein diet and nitrogen scavengers, linear growth and weight gain remained unaffected until preschool age.

In contrast, symptomatic individuals of any UCD studied receiving conservative management, particularly EO individuals, showed progressive growth impairment over time, which is in line with previous studies highlighting that disease severity rather than the type of UCD is a major determinant of linear growth. Since treatment intensity was shown to reflect disease severity and protein restriction has been hypothesized to be causally related to growth failure in UCDs, we further investigated the impact of current treatment modalities on linear growth, particularly with regard to natural protein intake as underlying cause of potential growth retardation. Unexpectedly however, both symptomatic UCD individuals with or without protein restricted diet exhibited a progressive growth deficit, indicating that natural protein intake per se does not explain this finding. Analogously, caloric intake appeared not to affect neither weight development nor linear growth in the studied cohort. However, due to the small study sample for caloric intake, these results should be considered somewhat

Table 1. Weighted arithmetic mean values of plasma BCAAs and L-arginine in the study sample. Reference ranges were defined according to (20) for children in the age range of 2–10 years and are shown in µmol/l. P values were calculated using a two-sided t-test, p values < 0.05 were considered significant, n refers to number of patients included in each group. Of note, 65% (n = 15/23) of individuals with a protein restricted diet and 59% (n = 13/22) of individuals without a protein restricted diet received (at least temporarily) supplementation with L-arginine within the observation period. BCAAs, branched-chain amino acid(s); LTx, liver transplantation; n/a, not available.

|                  | L-valine | L-leucine | L-isoleucine | L-arginine |
|------------------|----------|-----------|--------------|------------|
|                  | Mean, SD; n | Mean, SD; n | Mean, SD; n | Mean, SD; n |
| Reference range  | 133–273  | 64–164  | 31–83  | 38–98  |
| Conservative management |          |          |          |          |
| Protein restricted diet | 145, 54; 23 | 68, 25; 23 | 38, 16; 23 | 73, 33, 23 |
| p value           | 0.25     | 0.06     | 0.24     | 0.78     |
| No protein restricted diet | 166, 72; 22 | 92, 45; 22 | 45, 23; 22 | 74, 32, 22 |
| Liver transplantation |          |          |          |          |
| Prior to LTx      | 144, 58; 18 | 86, 63; 18 | 40, 23; 18 | n/a     |
| After LTx         | 224, 70; 15 | 123, 32; 15 | 67, 21; 16 | n/a     |
| p value           | 0.001    | 0.048    | 0.001    | n/a     |

Figure 5. Liver transplantation appears to have a beneficial effect on growth of 19 individuals with UCDs. Individuals with UCDs (n = 19) have normal weight gain (A) over time pre- and post-transplantation (p = 0.48; LME ANOVA), but suffer from impaired growth (B) prior to transplantation (mean z-score at first observation: 0.53, mean z-score at last observation prior to LTx: −0.75; p < 0.001; contrast t-test). However, growth retardation does not further aggravate after transplantation (mean z-score at last observation prior to LTx: −0.75, mean z-score at last observation after LTx: −0.17; p = 0.13; contrast t-test). Data are shown as median (black line) and mean (triangle), length of the box corresponds to IQR, upper and lower whiskers correspond to max. 1.5 × IQR, each point represents an outlier. Descriptive characteristics are presented separately in Supplementary Table S1. LTx, liver transplantation.
exploratory. Moreover, although weight development was normal in both asymptomatic and symptomatic indi-
viduals with UCDs, it remains to be elucidated whether the composition of body weight in terms of fat and lean
mass might be affected by medical management.

Recently, it has been shown that supplementation of L-arginine restored plasma IGF-1 and IGF-BP3 levels in
seven male individuals with LO TSC-D aged 3–5 years and gradually improved linear growth\(^8\). However, UCD
individuals in our study cohort exhibited progressive growth failure despite plasma L-arginine levels well within
the normal range independent from protein restriction or supplementation with EAAs, suggesting alternative
factors causing reduced linear growth in UCDs.

Intriguingly, UCD individuals in both groups (restricted vs. non-restricted) exhibited similarly reduced or
low normal plasma BCAA concentrations as indicated by the weighted arithmetic mean values for plasma
L-valine, L-leucine and L-isoleucine concentrations in our study. Maintaining stable concentrations of essential
and functional BCAAs is crucial to stimulate growth\(^17\), and deficiencies in the amino acids L-valine, L-leucine
and L-isoleucine have been shown to be associated with growth failure in children\(^18\). Reduced BCAA concen-
trations are a biochemical hallmark in acute and chronic hyperammonemic conditions. Enhanced consump-
tion of BCAAs via increased propionate oxidation as major compensatory mechanism to prevent bioenergetic
impairment under acute and chronic hyperammonemic conditions has recently been elucidated\(^19\)–\(^25\), highligh-
ting the role of the deamination of BCAAs for the generation of succinyl-CoA (deamination of isoleucine and
valine), which can supply the TCA cycle with important carbon backbones. Consistently, individuals with UCDs
exhibited decreased plasma BCAA concentrations correlating with hyperammonemia\(^20\). Moreover, L-isoleucine
and L-valine have been shown to be the only amino acids with significant cerebral uptake in patients with ful-
minant hepatic failure\(^26\), and cerebral BCAA transaminases (BCATs) are stimulated under hyperammonemic
conditions, thereby enhancing the consumption of L-isoleucine and L-valine for anaplerotic reactions as well as
transamination processes for the generation of L-glutamate and L-glutamine (consumption of L-valine, L-leucine,
and L-isoleucine) via activities of BCAT1 and BCAT2, respectively\(^27\). Notably, male mice hemizygous for the
OTC\(_{p^t-ash}\) mutation, which is characterized by chronically elevated NH\(_{4}^+\) concentrations without spontaneously
occurring acute hyperammonemic decompensations, exhibited growth failure with significantly reduced body
height under regular diet when compared to their wildtype littermates at 4–6 months\(^28\)–\(^30\), indicating that growth
failure in UCD might be causatively linked to the chronically elevated NH\(_{4}^+\) concentrations and subsequent
depletion of BCAAs due to enhanced propionate oxidation. Moreover, sodium or glycero1 PBA prevents the
phosphorylation of the E1α subunit of the branched-chain a-keto acid dehydrogenase complex (BCKDHc)
via inhibition of the BCKDHc kinase, resulting in activation of BCKDHc and increased breakdown of BCAA,
resulting in synergistic reduction of plasma BCAA concentrations\(^7\).

In line, height z-scores in UCD patients were positively associated with patient's plasma L-leucine (CPS1-D,
mOTC-D and HHHh-syndrome) and L-valine (ASS1-D and ASL-D) concentrations\(^14\), clearly supporting our
findings. However, while the latter observation was based on a cross-sectional analysis, our results further sub-
stantiate the relevance of the identified association between BCAA depletion and growth failure by providing
longitudinal data in UCDs. Of note, plasma BCAA concentrations were within the lower normal range or reduced
in UCD individuals in our study, independent from the administration of EAA supplements, which has already
been reported by Molema et al.\(^14\), indicating that the current clinical practice of prescribing EAA or BCAA sup-
plements is not sufficiently compensating for BCAA depletion in UCD individuals. Since LTx appeared to be
beneficial for the cognitive outcome in UCD individuals in a cross-sectional analysis\(^14\)–\(^17\), we further investigated
the impact of LTx on linear growth. Intriguingly, UCD individuals who exhibited growth impairment with
decreasing height z-scores prior to LTx, did show a stable linear growth within a mean individual observation
period of 4 years after LTx, suggesting that LTx might also prove beneficial for linear growth in UCDs. Further
systematic prospective long-term follow-up investigation and increased number of analyzed individuals will be
crucial to reliably determine the effect of LTx on linear growth. However, our findings are not unexpected, since
LTx is an effective measure to correct the enzymatic defect thereby preventing further hyperammonemic episodes
and subsequent consumption of BCAAs induced by elevated NH\(_{4}^+\) concentrations as well as the necessity of
protein restriction and long-term nitrogen scavenger therapy. Of note, this is substantiated by our finding that
plasma BCAA concentrations normalized after LTx in the study sample investigated.

This analysis has several inherent limitations. While both, the E-IMD and UCDC registry contain detailed
information on dietary prescriptions, they do not verify and describe the actual daily intake by a participating
UCD individual or adequately control for patient compliance. Furthermore, there was no information on the
quality of natural protein consumed. Practice of dietary management may vary considerably between different
study centers of both consortia. Moreover, the first data entry used for different analyses might not reflect the
exact time period between diagnosis and implementation of a specific treatment, which might have introduced
some noise into the data due to inadequate or varying treatment modalities prior to first assessment. Next, bio-
chemical values (plasma concentrations of NH\(_{4}^+\), BCAAs and L-arginine) were not assessed in a central labora-
tory using a standardized protocol and therefore reference values differ slightly between participating study sites.
There was no assessment neither of the quality of the preanalytical process nor the correctness of measurements
and potential differences between contributing laboratories. Due to small sample sizes, a comparative analysis
regarding the effect of a specific nitrogen scavenger (BZA vs. PBA) on plasma BCAA concentrations could not
be performed and thus remains subject to future studies. Study visits in both registries usually are several months
apart, therefore it has to be implied that the status recorded at any given point is representative for the preceding
interval. Given the observational nature of this international, multicenter registry study, data sets can have miss-
ing values. Thus, the prospective study was performed using solely available data, which is only valid under the
missing completely at random (MCAR) assumption. No methods of imputation of missing data were applied.
Further intraindividual long-term follow up studies are needed, to substantiate our findings and to systematically
assess linear growth in UCD individuals beyond the preschool age.
Conclusions

Our longitudinal study reveals that intrauterine development in all investigated UCDs as well as postnatal linear growth and weight progress of asymptomatic individuals in the absence of hyperammonemic episodes and/or conservative treatment is unaffected. In contrast, symptomatic individuals are at risk of developing progressive growth impairment over time independent from the underlying disease and the degree of natural protein restriction. Growth impairment is associated with reduced or borderline plasma BCAA concentrations. Moreover, LTx appeared to have a beneficial effect on linear growth. Future prospective clinical trials are indispensable to unequivocally prove the pathomechanistic role of BCAA depletion on impaired linear growth and potential beneficial effects of adequate EAA supplementation in UCDs.

Materials and methods

Eligibility criteria and overview of the UCDC and E-IMD databases. In brief, the UCDC database is registered in the U.S. National Library of Medicine (https://clinicaltrials.gov), whereas the E-IMD registry is recorded on the German Clinical Trials Register (https://www.drks.de). Requirements set forth by the ICMJE (International Committee of Medical Journal Editors) were met. All procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2013. The E-IMD and UCDC study protocols have received approval by the Institutional Review Board of the Medical Faculty of Heidelberg University (Ethikkommission der Medizinischen Fakultät Heidelberg, Germany; lead institution for this study, permit S-525/2010 and S-198/2011) and were also approved by all ethics committees of the participating North-American and European study sites. Written informed consent was obtained from all participants prior to inclusion in both databases. Both registries use remote data entry via electronic case report forms comprising clinical, biochemical and therapy-related data from baseline, scheduled regular follow-up investigations, and unscheduled (emergency) visits to the hospital. A detailed description of the combined and comparative research approach is provided separately.

Cornerstones and strategy for data analysis. Cut-off date for data analysis was February 25, 2019. Since the scope of this analysis comprised the effects of dietary long-term management, only data from baseline and regular follow-up visits were considered eligible. All subsequent analyses focus on individuals with OTC-D (MIM #311250), ASS1-D (MIM #215700), and ASL-D (MIM #207900) forming the study sample for this analysis, and investigating the majority of individuals with UCDs. (11) Ultra-rare UCDs with a disease-specific incidence of equal to or below 1:1,000,000, such as N-acetylglutamate synthase deficiency, carboxylmethylphosphate synthetase 1 deficiency, carboanhydrase VA deficiency, arginase 1 deficiency, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, and citrin deficiency are subject to future studies. We analyzed the effect of dietary long-term management on the anthropometrical endpoints weight, height and, if available, head circumference. Since we showed recently that therapeutic intensity depends on the phenotypic severity of individuals with UCDs, our study sample (individuals with mOTC-D, fOTC-D, ASS1-D, and ASL-D) was severity-adjusted. To this end, individual observation periods were defined for each patient within the study sample, reflecting a period of specific therapy intensity, and thus specific phenotypic severity. The regular visit prior to therapy escalation or therapy de-escalation corresponded to the last observation timepoint within the individuals’ observation periods. Therapy escalation was defined as newly introduced application of mono- or combined scavengers, therapy de-escalation was considered as discontinuation of mono- or bescavenger therapy. Each individual within the study sample had at least two successive visits with a maximum interval of 18 months. The minimum duration of an individual observation period for each individual corresponded to 12 months. Visits within the individual observation periods provided information on biochemical values, dietary and pharmacological management, and anthropometrical endpoints.

European individuals with UCDs were compared to growth charts from the UK, since the ethnic background of the European UCD sample corresponds well to the UK, while for North American individuals the Center for Disease Control and Prevention growth charts (https://www.cdc.gov/growthcharts/cdc_charts.htm) were used. Z-scores for height, weight, BMI and head circumference were calculated at baseline and each regular follow-up visit. Preterm infants (< 37th pregnancy week), and z-scores < -3 or > 3 were excluded from data analysis due to probably erroneous entries into the databases. Mean natural protein intake (g/kg/d), mean EAA intake (g/kg/d), and mean caloric intake (kcal/kg/d) were calculated for the patients’ individual observation periods (using time weighted averages based on data from each available visit) and were indicated as percentage of WHO safe values recommendations for mean natural protein and mean EAA intake, and in percentage of Food and Agriculture Organizations of the United Nations (FAO) for mean caloric intake. Individuals receiving a mean natural protein intake below 100% WHO safe values during the observation period were considered to have a protein restricted diet. To monitor BCAA levels during the patients’ individual observation periods, mean plasma levels for L-valine, L-leucine, L-isoleucine, and L-arginine (all in µmol/l) were calculated, where available. Moreover, use of nitrogen scavengers was documented within the individuals’ observation periods. Only individuals until the age of 18 years were included into this study.

Inclusion and exclusion criteria. Overall, 307 individuals with UCDs were eligible for data analysis (mOTC-D: n = 82, 26.7%; fOTC-D: n = 76, 24.8%; ASS1-D: n = 78, 25.4%; ASL-D: n = 71, 23.1%). Individuals belonging to specific UCD subgroups were included in subsequent analysis, defined by distinct inclusion and exclusion criteria. Descriptive characteristics of those subgroups are presented in the corresponding results section and in Supplementary Table S1. Analysis 1 (“Anthropometrical parameters at birth are within normal range”) investigated if individuals with UCD suffered from intrauterine retardation of growth, weight, and head circumference (inclusion criteria: birth weight, length and head circumference measured within the first 2 weeks.
of life). Analysis 2 ("Asymptomatic individuals have a normal postnatal growth and weight development") evaluated whether asymptomatic and untreated individuals had a normal or impaired postnatal anthropometrical development (inclusion criteria: asymptomatic individuals with UCDs; study inclusion within 12 months after age at diagnosis; no scavengers and no protein restricted diet within the individuals' observation periods). Analysis 3 ("Symptomatic individuals have a risk of postnatal growth retardation") investigated whether symptomatic individuals with UCDs receiving conservative management were at risk of growth and weight retardation (inclusion criteria: symptomatic individuals with UCDs, study inclusion within 12 months after age at diagnosis; exclusion criteria: asymptomatic individuals with UCDs, individuals receiving a LTx). Analysis 4 ("Postnatal growth retardation in symptomatic individuals with UCDs is not associated with a protein restricted diet") studied whether symptomatic individuals with a protein restricted diet are at risk of developing impaired growth and weight compared to individuals without a protein restricted diet (inclusion criteria: see analysis 3, additionally only individuals with sufficient information on biochemical and therapy-related longitudinal data allowing the calculation of mean values were included; exclusion criteria: see analysis 3). Analysis 5 ("Liver transplantation rescues postnatal growth retardation in symptomatic UCDs") investigated whether individuals receiving LTx suffered from pre- and post-transplant growth and weight retardation [inclusion criteria: individuals receiving a LTx with three distinct time points of growth and weight (first time point: first observation; second time point: last observation prior to LTx; third time point: last observation after LTx), time lag between 2nd and 3rd time point at least 12 months; exclusion criteria: individuals with conservative management].

Data availability. The datasets generated and analyzed during the current study are not publicly available due to existing data protection laws. Furthermore, data ownership is retained by the members of the UCDC and E-IMD consortia making anonymized data available for specific research purposes. Data availability is subject to the consent of both consortia upon request.

Statistical analysis. All statistical analyses were performed using R, a language for statistical computing and graphics (https://www.r-project.org). Multiple regression analyses were used to model a numeric dependent outcome variable with respect to several independent predictor variables. Longitudinal data was modelled with a linear mixed effect model (LME) with individuals as random factor. For multiple regression and LME, results were displayed with analysis of variance (ANOVA) tables with type two F-tests and post-hoc comparisons (contrasts) were carried out with t-tests based on estimated marginal means. Two groups were compared with Welch two sample t-test, paired data with a paired t-test.

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**Author contributions**

The STROBE statement was used when preparing this manuscript. RP and MZ contributed to conception and design of the study. RP, SFG, FG, ALG, PDL, GFH, AGC, SCSN, MRB, AS, DD, MY, SK, MZ, and all individual contributors from the UCDC and E-IMD consortia study group (Supplementary Table S2) contributed to acquisition and analysis of data. RP, SFG, FG, SK, and MZ contributed to drafting the text and preparing the figures.

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Additional information

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