Increased protein-energy intake promotes anabolism in critically ill infants with viral bronchiolitis: a double-blind randomised controlled trial

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ABSTRACT

Objective The preservation of nutritional status and growth is an important aim in critically ill infants, but difficult to achieve due to the metabolic stress response and inadequate nutritional intake, leading to negative protein balance. This study investigated whether increasing protein and energy intakes can promote anabolism. The primary outcome was whole body protein balance, and the secondary outcome was first pass splanchnic phenylalanine extraction (SPE_{Phe}).

Design This was a double-blind randomised controlled trial. Infants (n=18) admitted to the paediatric intensive care unit with respiratory failure due to viral bronchiolitis were randomised to continuous enteral feeding with protein and energy enriched formula (PE-formula) (n=8; 3.1±0.3 g protein/kg/24 h, 119±25 kcal/kg/24 h) or standard formula (S-formula) (n=10; 1.7±0.2 g protein/kg/24 h, 84±15 kcal/kg/24 h; equivalent to recommended intakes for healthy infants <6 months). A combined intravenous-enteral phenylalanine stable isotope protocol was used on day 5 after admission to determine whole body protein metabolism and SPE_{Phe}.

Results Protein balance was significantly higher with PE-formula than with S-formula (PE-formula: 0.73±0.5 vs S-formula: 0.02±0.6 g/kg/24 h) resulting from significantly increased protein synthesis (PE-formula: 9.6±4.4, S-formula: 5.2±2.3 g/kg/24 h), despite significantly increased protein breakdown (PE-formula: 8.9±4.3, S-formula: 5.2±2.6 g/kg/24 h). SPE_{Phe} was not statistically different between the two groups (PE-formula: 39.8±18.3%, S-formula: 52.4±13.6%).

Conclusions Increasing protein and energy intakes promotes protein anabolism in critically ill infants in the first days after admission. Since this is an important target of nutritional support, increased protein and energy intakes should be preferred above standard intakes in these infants.

Dutch Trial Register number: NTR 515.

What is already known on this topic

- Critical illness in children is associated with increased protein breakdown, negative protein balance and adverse clinical outcome.
- Inadequate nutritional support further leads to protein-energy malnutrition during admission to the paediatric intensive care unit.

What this study adds

- Protein anabolism in critically ill infants can be achieved in the first days after admission by increasing protein and energy intakes above reference levels.
- The higher protein balance resulted from stimulated protein synthesis exceeding the rate of concomitant stimulated protein breakdown.
- Increased protein and energy intakes are recommended in critically ill infants with viral bronchiolitis.

INTRODUCTION

The preservation of nutritional status and growth is a specific aim in critically ill children, but difficult to achieve. This is due to a metabolic stress response with profound changes in protein metabolism leading to a negative protein balance and loss of lean body mass. Inadequate nutritional intake in the paediatric intensive care unit (PICU), often due to fluid restriction, further leads to protein and energy deficits, especially early after admission.[1] Other factors that hinder adequate nutrition are impaired intracellular insulin signalling,[2] impaired glucose uptake[3] and reduced mitochondrial capacity during critical illness.[4] These factors are probably the reason why protein-energy malnutrition is observed in 16–24% of critically ill children[5 6] and is associated with adverse clinical outcome.[7–9]

A common but threatening disease in infants is viral bronchiolitis, which in severe cases leads to respiratory failure with need for ventilatory support and PICU admission. Adequate nutritional support in these critically ill infants is important, with protein anabolism as goal. However, up to now common practice has been to use standard infant formulas to provide approximately 1.5 g protein/kg/day and 100 kcal/kg/day.

Increased protein intake with adequate energy provision promotes anabolism in preterm infants,[10–12] in neonates undergoing surgery[13] and in children with burns[14] and cystic fibrosis.[15] In relation to these observations, it is important to note that protein synthesis is a high-energy consuming process[16] and energy deficiency worsens nitrogen balance.[17 18] Hence, to induce net protein anabolism, it is essential to provide an adequate energy intake. We therefore hypothesised that increasing protein *and* energy

intakes would induce net protein anabolism in critically ill infants.

Stable isotope amino acid methods are used to determine net protein balance.[19] During feeding, amino acids appearing in the circulation originate from protein breakdown and from the fraction of meal-derived amino acids that are not retained in the splanchnic area. Protein synthesis during feeding can be calculated from the disappearance of essential amino acids (EAAs) such as phenylalanine from the circulation, corrected for non-protein synthesis related disposal (eg, oxidation, hydroxylation). Therefore, all these factors need to be considered if whole body net protein anabolism during feeding is to be calculated.[20 21] Splanchnic extraction (SPE) of meal-derived amino acids has not been reported before in critically ill children.

The present study was part of a larger study on the nutritional and metabolic effects of increased protein and energy intakes using a protein and energy enriched formula (PE-formula) compared with a standard infant formula (S-formula).[22] In the present study we studied the efficacy of increased protein and energy intakes to promote protein anabolism and the underlying mechanisms by using intravenous-enteral phenylalanine/tyrosine stable isotope method protocol. The primary outcome measure was whole body protein balance (WbPBal) at day 5 after admission. SPE of phenylalanine was a secondary outcome measure. The 24 h nitrogen balance was used as alternative method to assess protein balance. To gain more insight into the role of separate amino acids in protein kinetics, correlations between plasma amino acid concentrations and protein metabolism were assessed.

DESIGN

Setting and patients

Infants admitted to the PICU of Maastricht University Medical Center (MUMC) or ErasmusMC-Sophia Children's Hospital (ErasmusMC) meeting the following inclusion criteria were enrolled: (1) respiratory failure due to viral bronchiolitis; (2) age 4 weeks to 12 months; (3) >40 weeks postmenstrual age; (4) ability to start enteral feeding <24 h after admission; (5) expected length of stay >96 h; and (6) venous and arterial catheters present. Exclusion criteria were as follows: (1) gastrointestinal, metabolic or chromosomal disorder; (2) parenteral nutrition other than intravenous dextrose; and (3) breast feeding. The inclusion and exclusion criteria were chosen to create a homogenous population of infants. Inclusion criteria 4, 5 and 6 were necessary for performance of the study protocol.

The Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands) and local ethics committees approved this study. Written informed consent was obtained from parents or caregivers.

Anthropometric characteristics and severity of illness (Paediatric Risk of Mortality II)[23] were assessed at inclusion. Duration of mechanical ventilation and length of PICU stay were noted. To determine the metabolic state of the patients, plasma amino acid concentrations were determined in arterial blood collected in the fed state at the start of the stable isotope protocol on day 5 using fully automated high-performance liquid chromatography as described before.[24] The roles of specific amino acids were identified through correlation with whole body protein metabolism (WbPM).

Interventions

Patients were randomised (randomisation and blinding as described before[22]) within 24 h after admission to receive continuous enteral feeding with PE-formula (Infatrini: 2.6 g protein/100 ml, 100 kcal/100 ml) or with S-formula (Nutrilon 1: 1.4 g protein/100 ml, 67 kcal/100 ml) both from Nutricia Advanced Medical Nutrition, Zoetermeer, The Netherlands. Compositions are summarised in appendix 1. Formulas were administered as previously described, starting 25.3±5.6 versus 23.4±5.4 h after PICU-admission in the PE-group and S-group, respectively.[22] The ranges of protein and energy intakes on day 5 in the S-group (1.7±0.2 g protein/kg/24 h, 84±15 kcal/kg/24 h) covered recommended intakes for healthy infants <6 months (1.14–1.77 g protein/kg/24 h, 81–113 kcal/kg/24 h, depending on age in months).[16 25] The ranges were significantly higher in the PE-group (3.1±0.3 g protein/kg/24 h, p<0.001; 119±25 kcal/kg/24 h, p<0.001) and were 175–272% and 105–147% of recommended intakes for protein and energy, respectively. Intake by volume was not significantly different between groups; 120.6±13.4 ml/kg/24 h in the PE-group versus 118.5±13.4 ml/kg/24 h in the S-group. As the target volume was 130 ml/kg/day, this was the maximum achievable intake for both groups for medical reasons (eg, fluid restriction) as decided by the treating physician. Details of nutritional intake are summarised in appendix 2.

Main outcome measures

WbPM and splanchnic phenylalanine extraction

On day 5 WbPM and splanchnic phenylalanine extraction (SPE_{Phe}) were assessed by using a stable isotope protocol in the fed state. Several methods can be used to determine protein metabolism. We used the phenylalanine/tyrosine method because of the advantage that only blood samples are needed instead of both blood and breath samples as for methods based on leucine isotopes.[26] In order to attain steady state, the infusion rate of enteral nutrition was not changed in the 6 h before the start of or during the stable isotope protocol. The stable isotope protocol was conducted by a research physician or research nurse. Intravenous amino acid tracers were administered continuously for 2 h with calibrated syringe pumps after a priming dose, using the following tracers, priming doses (μmol/kg) and infusion rates (μmol/kg/h), respectively: L-[ring-$^2H_5$]phenylalanine, 4.4 μmol/kg, 4.5 μmol/kg/h; L-[ring-$^2H_2$]tyrosine, 1.9 μmol/kg, 1.5 μmol/kg/h; L-[ring-$^2H_4$]tyrosine, 0.63 μmol/kg. For assessment of SPE_{Phe}, L-[1-^{13}C]-phenylalanine was administered as a primed-continuous enteral infusion (4.4 μmol/kg, 9.0 μmol/kg/h, respectively). Stable isotope tracers (>98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, Massachusetts, USA). Infusates were prepared by the centres' clinical pharmacists. Arterial blood was sampled (500 μl) before isotope infusion to determine background enrichment and at 60, 90 and 120 min of infusion to determine isotopic enrichment. Samples were put on ice and centrifuged (3500×g) for 10 min at 4°C. Plasma was deproteinised with 5% sulfosalicylic acid, frozen in liquid nitrogen and stored at −80°C until analysis. Tracer-to-tracee ratios (TTRs) were analysed using a liquid chromatography–mass spectrometry system as described before.[27] TTRs were corrected for background enrichment and contribution to the measured TTRs of isotopomers with lower masses as described before.[28] Isotopic enrichment reached a steady state after 1 h infusion, as shown by the lack of a statistically significant slope of calculated TTRs at 60, 90 and 120 min (data not shown). The mean enrichment was used for further calculations as described before.[19] These calculations are explained in detail in appendix 3.

Nitrogen balance

The 24 h nitrogen balance on day 5 was assessed as described before, with urinary urea being converted to total urinary nitrogen (TUN) excretion.[22]

Statistical analysis

Power analysis was based on protein metabolism parameters in infants in earlier reports.[29] To detect a 20% difference in protein balance between groups with 0.05 two-sided significance and 0.80 sensitivity, eight patients per group were required. Data were analysed on an intention-to-treat basis with the SPSS statistical software package (v 12.0; SPSS, Chicago, Illinois, USA). Differences between groups were assessed with Mann–Whitney U analysis. Correlations among parameters were tested with Spearman correlation coefficients. Statistical significance was defined as two-tailed $p<0.05$. Data are presented as mean±SD.

RESULTS

Patients

Twenty infants with respiratory failure due to viral bronchiolitis were enrolled (MUMC: n=10; Erasmus MC: n=10; December 2003 to February 2006). Ten patients were randomised and allocated to receive PE-formula and 10 to receive S-formula. All patients received the allocated formula. Two patients in the PE-group were lost to follow-up because vascular catheters were removed after extubation before day 5, and hence WbPM could not be measured. Patient characteristics are shown in table 1. Gestational age was significantly lower in PE-infants, but other parameters did not differ significantly. There were no significant differences in characteristics between patients enrolled in MUMC and in Erasmus MC (data not shown).

Main outcome measures

WbPM and SPE_{Phe}

The rates of phenylalanine kinetics on day 5 are shown in table 2. These values are directly derived from the phenylalanine and tyrosine stable isotope tracer results and subsequently used to calculate whole body protein kinetics as shown in figure 1. Whole body phenylalanine kinetics were significantly higher in the PE-group than in the S-group, apart from phenylalanine hydroxylation, which was higher in the PE-group but did not reach significance. Although SPE_{Phe} (%) tended to be higher in the S-group than in the PE-group (p=0.08), absolute SPE was highest in the PE-group, but did not reach significance in either group.

Figure 1 depicts the rates of whole body protein synthesis (WbPS), whole body protein balance breakdown (WbPBal) and WbPBal in g/kg/24 h. It shows that WbPBal on day 5 was positive in the PE-group, while in the S-group it did not differ significantly from zero (0.73±0.5 vs 0.02±0.6 g/kg/24 h, p=0.026). The higher WbPBal was achieved through higher WbPS in the PE-group (9.6±4.4 vs 5.2±2.3 g/kg/24 h, p=0.019), despite concomitant higher WbPB (8.9±4.3 vs 5.2±2.6 g/kg/24 h, p=0.046). Negative WbPBal, reflecting catabolism, was found

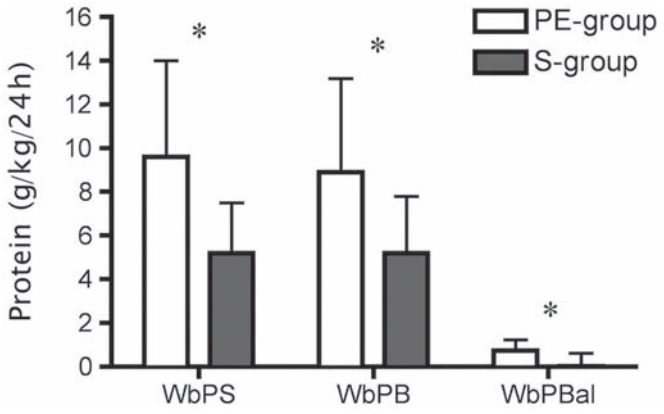

Figure 1 Rates of protein kinetics (g/kg/24 h) in both study groups on day 5. Data are presented as mean±SD. *p<0.05. PE-group, protein and energy enriched formula fed group; S-group, standard formula fed group; WbPB, whole body protein breakdown; WbPBal, whole body protein balance; WbPS, whole body protein synthesis. WbPS and WbPB were significantly higher in the PE-group than in the S-group. Consequently, a positive WbPBal was achieved in the PE-group, which was significantly higher than in the S-group.

Table 1 Patient characteristics of the study population

	PE-group (n=8)	S-group (n=10)	p Value
Medical centre (MUMC/ Erasmus MC)	4/4	4/6	
Gender (M/F)	2/6	3/7	
Age (months)	2.7±1.4	2.9±1.8	NS
Weight at inclusion (g)	3967±944	4791±1114	NS
Birth weight (g)	2299±903	2841±192	NS
Gestational age (weeks)	35.0±3.3	37.3±1.0	<0.05
Postmenstrual age (weeks)	46.8±7.6	49.9±8.2	NS
Crown–heel length (cm)	56.3±5.9	56.6±3.6	NS
PRISM score	20.3±4.3	18.6±4.5	NS
CRP on admission (mg/l)	75±65	75±51	NS
Mechanical ventilation (days)	7.1±6.2	5.0±2.2	NS
Length of PICU stay (days)	9.0±7.6	6.7±2.2	NS

Data are presented as number of subjects or mean±SD.
CRP, C-reactive protein; Erasmus MC, Erasmus Medical Center; MUMC, Maastricht University Medical Center; PE-group, protein and energy enriched formula fed group; PICU, paediatric intensive care unit; PRISM, Paediatric Risk of Mortality; S-group, standard infant formula fed group.

Table 2 Whole body and splanchnic phenylalanine kinetics on day 5

	PE-group (n=8)	S-group (n=10)	p Value
Whole body Phe kinetics			
$WbRa_{Phe}$	124.5±50.0	67.9±29.9	<0.05
$WbRa_{Tyr}$	115.4±56.3	57.6±9.0	<0.05
WbPhe utilised for PS	112.5±50.7	60.4±27.2	<0.05
$WbOH_{Phe\rightarrow Tyr}$	13.5±9.0	7.7±4.4	NS
WbPhe from PB	103.9±49.8	60.1±30.8	<0.05
WbPhe balance	8.5±6.5	0.3±5.7	<0.05
Splanchnic Phe kinetics			
Dietary Phe intake	34.0±3.8	16.4±2.1	<0.01
SPE_{Phe} (%)	39.8±18.3	52.4±13.6	NS
$ASPE_{Phe}$	13.4±6.6	8.7±2.8	NS
$PheI_{SPE}$	20.6±7.3	7.7±2.1	<0.01

All data are in μmol/kg/h unless otherwise specified and are presented as mean±SD.
$ASPE_{Phe}$, absolute splanchnic phenylalanine extraction; PE-group, protein and energy enriched formula fed group; Phe, phenylalanine; $PheI_{SPE}$, phenylalanine intake, corrected for SPE_{Phe}, thus available for peripheral protein synthesis and oxidation; S-group, standard formula fed group; SPE_{Phe}, splanchnic phenylalanine extraction; Tyr, tyrosine; $WbOH_{Phe\rightarrow Tyr}$, whole body hydroxylation of phenylalanine to tyrosine; WbPhe balance, whole body phenylalanine balance; WbPhe from PB, whole body phenylalanine originating from protein breakdown; WbPhe utilised for PS, whole body phenylalanine used for protein synthesis; WbRa, whole body rate of appearance.

in one subject (13%) in the PE-group, but in four infants in the S-group (40%).

Whole body protein turnover in the PE-group was higher than in the S-group (10.7±4.3 vs 5.8±2.6 g/kg/24 h, p=0.012). Whole body protein oxidation, calculated from hydroxylation of phenylalanine to tyrosine, was higher with the PE-formula than with the S-formula, but not significantly so (1.2±0.8 vs 0.7±0.4 g/kg/24 h, p=0.25).

Plasma amino acid concentrations on day 5 are shown in appendix 4. The concentrations of five EAAs (methionine, histidine, phenylalanine, lysine and valine) and ornithine were significantly higher in the PE-group. The sums of branched chain amino acids (BCAAs) and EAAs were also significantly higher. WbPS was positively correlated with concentrations of the EAAs histidine (r=0.46, p<0.05), methionine (r=0.64, p<0.01), tryptophan (r=0.51, p<0.05), leucine (r=0.56, p<0.05) and isoleucine (r=0.47, p<0.05) and with sums of BCAAs (r=0.51, p<0.05) and EAAs (r=0.51, p<0.05). WbPBal was positively correlated with isoleucine (r=0.52, p<0.05), valine (r=0.46, p<0.05) and the sum of BCAA (r=0.53, p<0.05).

Nitrogen balance

The 24 h nitrogen balance on day 5 was significantly higher in PE-infants (274±127 vs 137±53 mg/kg/24 h, p<0.05). Multiplication of the results by 6.25 (the average amount of nitrogen in protein) resulted in protein balances of 1.71 vs 0.85 g/kg/24 h for the PE-group and S-group, respectively. TUN excretion on day 5, as a measure of amino acid oxidation, was higher in PE-infants, but not significantly so (171±81 vs 103±54 mg/kg/24 h, respectively, p=0.37).

CONCLUSIONS

The present study is the first to show that protein anabolism, an important target of nutritional support in critically ill infants, can be achieved within the first days after admission to the PICU by increasing enteral protein and energy intakes above dietary reference levels using a protein-energy enriched formula. This target was not achieved with a standard infant formula. The higher protein balance resulted from stimulated protein synthesis exceeding the rate of concomitant stimulated protein breakdown. Nitrogen balance data confirmed our phenylalanine results.

Our findings of increased protein synthesis and protein balance are in agreement with several studies in premature and term neonates evaluating the effects of amino acid supplementation.[10–13 29–33] This is also true for protein breakdown which was either increased[33] or not affected by amino acid supplementation.[11 13 29 31] Although Poindexter[30] has also reported suppression of proteolysis, this was in healthy instead of critically ill infants, receiving short term supplementation. Our finding of both increased protein synthesis and protein breakdown with higher protein and energy intakes is probably due to overall stimulation of protein turnover, as shown by the increased whole body protein turnover rate in the PE-group.[34]

Increased protein intake promotes protein anabolism, but may lead to increased amino acid oxidation with urea formation as seen in neonates with increasing amino acid supplementation,[11 13 31] when exceeding needs. However, in the present study, neither phenylalanine hydroxylation nor TUN excretion (both reflecting amino acid oxidation), nor plasma urea concentrations (as described in our previous report)[22] differed significantly between groups, suggesting that protein intake up to, and probably above, 3.1 g/kg/day does not exceed these infants' needs.

We are aware that using a PE-formula makes it difficult to discern the influences of separate macronutrients on protein metabolism. However, studies in adults and children have shown that protein is the major dietary determinant of WbPM as long as energy intake is sufficient.[35] Additionally, supporting this hypothesis, the finding of a positive relationship between plasma EAAs and protein synthesis and balance suggests that EAA availability plays a crucial role in increasing protein synthesis and protein balance. It also agrees with previous observations in healthy adults indicating that (essential) amino acids are the primary stimulus for (muscle) protein synthesis.[36]

In these critically ill infants, receiving large amounts of intravenous fluids and medications, 120 ml/kg/day was the maximum achievable nutritional volume intake. Despite these fluid restrictions, an anabolic state was obtained within 5 days after admission using a protein-energy enriched formula, thereby limiting delay of growth and neurodevelopment during critical illness as much as possible. We have previously reported that the PE-formula is safe, well tolerated and improves nitrogen and energy balance at days 1–5 after admission.[22] This type of formula is thus preferable to standard formulas to achieve adequate nutrition in comparable clinical settings. Since the subjects were a typical sample of infants with respiratory insufficiency due to viral bronchiolitis, we suggest that the results apply to the general population of these critically ill infants.

Our study is also the first to report values of first pass SPE_{Phe} in continuously enterally fed critically ill infants. In this population, first pass SPE_{Phe} did not differ between groups with an average of 46.8%. Comparable values have been described in healthy adults after a meal[21] and in enterally fed piglets.[37] There is discussion about correcting protein intake for SPE in calculations of WbPBal, since these retained amino acids are used for constitutive or secreted (glyco-)proteins in the gut,[38 39] which is then considered part of WbPS. We have therefore also calculated the data without correction for SPE (not shown) and found that protein breakdown was 15–19% lower and protein balance 2.7–3.9 times higher. Only the absolute values are affected by this calculation, and the main conclusion of the study is not affected.

There are several limitations to this study. Despite using a randomised design, gestational age was significantly lower in the group receiving protein-energy enriched formula. This might have biased our results of protein metabolism as protein turnover decreases with increased (post-)conceptional age.[40]

Furthermore, the proportion of female subjects was relatively high. Protein deposition has been shown to be similar for healthy male and female children prior to adolescence and it is recommended that estimates of protein requirements for healthy children are calculated for both sexes combined.[25] However, in children with burns (8 years of age on average), females had a less negative net muscle protein balance compared to males, and females gained lean body mass whereas males lost lean body mass. These differences were possibly due to the observed attenuated hypermetabolic response in females.[41] Assuming that the same differences are true for critically ill infants, this would mean that the achievement of protein anabolism in the first days after admission in our study population could have been biased by the high proportion of females. However, gender differences in protein kinetics have not been described for critically ill infants. Moreover, our study population of infants with a viral infection is distinctly different from children with burns, who are subject to an extended

hypermetabolic stress response with high inflammation.[41] Also, when comparing the female with the male subjects within the PE- and S-groups of our study, the only notable difference was a non-significant trend towards higher protein turnover, synthesis and breakdown in the females compared to the males within the PE-group, but resulting in similar protein balances in both sexes. Therefore it seems unlikely that our results were affected by gender differences, despite the high proportion of females. Since the female subjects were equally distributed among both groups in our study, neither did it influence the comparison of groups.

Another limitation is that protein synthesis and protein breakdown were derived by extrapolating phenylalanine metabolism, which in fact only reflects the effects on the kinetics of this particular EAA. Other amino acid tracers may have shown different patterns, although the phenylalanine/tyrosine and leucine methods are considered to be reference methods to obtain reliable estimates of whole-body protein metabolism in most physiological conditions.[26] The present study was not designed to establish exact protein and energy needs in critically ill infants. Neither was it adequately powered to detect clinical effects. Dose–response studies and studies into the clinical effects of improved protein balance in larger groups of critically ill infants are therefore necessary.

In conclusion, protein anabolism in critically ill infants with viral bronchiolitis can be achieved in the first days after admission by increasing protein and energy intakes above reference levels. Since protein anabolism is an important goal of nutritional support in these infants, increased protein and energy intakes should be preferred over standard intakes.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands) and the local ethics committees of Maastricht University Medical Center, Maastricht, The Netherlands and Erasmus Medical Center–Sophia Children's Hospital, Rotterdam, The Netherlands.

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