Biol Blood Marrow Transplant 26 (2020) 380–391

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

# The Systemic Metabolic Profile Early after Allogeneic Stem Cell Transplantation: Effects of Adequate Energy Support Administered through Enteral Feeding Tube

Tor Henrik Anderson Tvedt[1,2,*], Kristin J. Skaarud[3,4], Geir Erland Tjønnfjord[3,5], Tobias Gedde-Dahl[4], Per Ole Iversen[3,6,7], Øystein Bruserud[1,2]

[1] *Section for Hematology, Department of Medicine, Haukeland University Hospital, Bergen, Norway*
[2] *Section for Hematology, Institute of Clinical Science, University of Bergen, Bergen, Norway*
[3] *Department of Hematology, University of Oslo, Oslo*
[4] *Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway*
[5] *Institute of Clinical Medicine, University of Oslo, Oslo, Norway*
[6] *Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway*
[7] *Division of Human Nutrition, Stellenbosch University, Tygerberg, South Africa*

*Article history:*
Received 23 June 2019
Accepted 3 October 2019

*Keywords:*
Allogeneic stem cell transplantation
Nutritional
Metabolome
Enteral nutrition
Graft-versus-host disease

A B S T R A C T

Patients undergoing allogeneic stem cell transplantation usually require nutritional support. There is no consensus on whether enteral support through tube feeding should be preferred. A recent randomized study could not detect any difference between enteral and parenteral feeding with regard to post-transplant outcomes, whereas 2 retrospective studies described an association between enteral feeding and a favorable post-transplant outcome. We compared pre- and post-transplant plasma metabolomic profiles for 10 patients receiving mainly enteral nutritional support and 10 patients receiving mainly parenteral support. Samples were collected before conditioning and 3 weeks post-transplant; 824 metabolites were analyzed using mass spectrometry. The pretransplant metabolite profiles showed a significant overlap between the 2 groups. Post-transplant samples for both patient groups showed an increase of secondary bile acids and endocannabinoids, whereas reduced levels were seen for food preservatives, plasmalogens, and retinol metabolites. The main post-transplant differences between the groups were decreased levels of fatty acids and markers of mitochondrial activation in the control group, indicating that these patients had insufficient energy intake. A significant effect was also seen for heme/bilirubin metabolism for the parenteral support. To conclude, allotransplant recipients showed altered metabolic profiles early after transplantation; this was mainly due to the conditioning/transplantation/reconstitution, whereas the type of nutritional support had minor effects.



## INTRODUCTION

Gastrointestinal complications are common after allogeneic (hematopoietic) stem cell transplantation (ASCT) [1,2]. Most patients experience weight loss during the early post-transplant period, but this weight loss is transient for most patients and with no or little clinical impact [3]. However, persistent weight loss and later chronic graft-versus-host disease (GVHD) are highly correlated and associated with an increased risk of nonrelapse mortality due to the combined effect of immune dysregulation and malnutrition [1,4,5].

Due to the high incidence of post-transplant weight loss, both European and US guidelines recommend allotransplant recipients to undergo nutritional screening and to receive parenteral nutritional support during the early post-transplant period [6]. However, there is currently no agreement with regard to the optimal time point to start (pre-emptive or "on-demand") and whether there is a benefit of early nutritional intervention [7-9]. Furthermore, the current clinical and biochemical tools used to assess when to initiate nutritional support rely on traditional and nonspecific clinical or laboratory parameters [7]. The dosing of total parenteral nutrition is often individualized based on calculations of estimated energy need [7,10]. Finally, there is no general agreement on the best route of nutritional support in allotransplant recipients. The experience from certain other diseases (e.g., pancreatitis) suggests that enteral nutrition reduces risk of complications in critically

*Financial disclosure:* See Acknowledgments on page 390.
*Correspondence and reprint requests: Tor Henrik Anderson Tvedt, MD, Section for Hematology, Department of Medicine, Haukeland University Hospital, Bergen, Jonas Lies vei 65. 5021, Bergen, Norway.
*E-mail address:* thetve@helse-bergen.no (T.H.A. Tvedt).

https://doi.org/10.1016/j.bbmt.2019.10.005
1083-8791/

ill patients [11-14]. Although 2 retrospective observational studies suggested that enteral feeding is associated with a reduction of post-transplant complications after ASCT [15,16], there is no consensus on whether tube feeding during the early post-transplant is beneficial [7,17,18]. In a recent randomized study, we investigated the effects of individualized and preferably enteral feeding early after allotransplantation [18,19]. In this study, enteral and individualized nutritional support did not have a significant effect on post-transplant nutritional status measured by the scored Patient-Generated Subjective Global Assessment, frequency and severity of infections, frequency of acute GVHD, or death rate at 3 months post-transplant. There was no significant effect on self-reported quality of life measured by the European Organisation for Research and Treatment of Cancer **Quality of Life Questionnaire Core 30** questionnaire either.

Further studies are definitely needed to clarify the optimal nutritional support for allotransplant recipients during the first weeks after transplantation. It has previously been shown that the pretransplant systemic metabolic profile is associated with clinical outcome after ASCT [20,21]. In this study, we therefore used data from our randomized trial and compared the early (i.e., day +21) post-transplant systemic metabolic profiles of allotransplant recipients receiving individualized and preferably enteral support to ensure adequate energy and protein intake compared with controls receiving standard and mainly parenteral nutritional support.

## METHODS

### *Study Design and Patient Selection*

All our patients were included in the NASQ clinical study (Individualized Nutrition for Adult Recipients of Allogeneic Stem Cell Transplants, clinical trial identifier NCT01181076); this study included collection of blood samples and was approved by the Regional Committee for Medical and Health Research Ethics South East Norway (#S-09136c 2009/2115; approved in 2009). A detailed description of the NASQ study including study design and clinical outcome has been described previously [18,19]. Briefly, in this study, allotransplant recipients were randomized to receive individualized nutritional support (referred to as the intervention group) or standard of care (referred to as the control group). In the intervention group, the intention was to secure adequate oral/enteral protein and energy intake based on resting energy requirement (30 to 40 kcal/kg/d, minimum of 1.5 g protein/kg/d). The intake was monitored daily and patients able to eat received oral supplements. A nasojejunal tube was inserted 3 to 5 days post-transplant to facilitate enteral nutrition during the post-transplant period. Thus, the major difference between the groups was the nutritional support from days 3 to 5 post-transplant when the tube was inserted until days 17 to 23 post-transplant when the second sample was collected. Patients in the intervention group not able to secure adequate enteral energy intake were given supplementary parenteral nutrition. The patients in the control group were treated according to institutional guidelines and total parenteral nutrition was provided by the attending physician. According to generally accepted guidelines, the daily nutrition should then be an energy intake corresponding to 25 to 30 kcal/kg/d, and this parenteral nutrition was usually started after 1 to 3 days with an oral intake less than 50% of the estimated energy requirements [7]. The nutritional support for the control patients was mainly based on parenteral nutrition, and placement of a nasogastric/jejunal feeding tube was not used as a routine. Even though the dietary intake of the control group was not monitored to avoid cross-contamination between the 2 study arms, the control group required more extensive parenteral nutritional (i.e., same number of days but a larger fraction of the energy demand) based on the daily clinical evaluation, including estimation of the oral intake. Parenteral nutrition always included supplementation with trace elements (Tracel, Fresenius Kab) together with water and fat-soluble vitamins (Solu-vit, Fresenius Kabi and Vitalipid, Fresenius Kabi), but glutamine supplementation was not provided. For a detailed description of the different products used for nutritional support, refer to Supplementary Table S1.

We investigated 2 contrasting patient groups, and each group included 10 patients. Our enteral nutrition or intervention group corresponded to those patients who received the lowest fraction of parenteral support and thus being able to receive a major part of their post-transplant nutrition by the oral or enteral route. The parenteral nutrition of our control group included those patients from the clinical study control group who received less oral/enteral nutrition than the others and thus received mainly parenteral nutrition. Both groups were allowed to eat by mouth.

The present project was approved by the Regional Ethics Committee (REK Vest 2013/634, March 19, 2013; REK Vest 2015/1410, June 19, 2015). In our present study, we investigated 2 groups of contrasting patients, each group including 10 patients (Table 1). The 10 selected patients from the intervention group included those patients in the study who tolerated enteral feeding best throughout the whole post-transplant period and thereby received the least parenteral nutrition (i.e., enteral feeding was the major part of their nutritional support and the use of parenteral nutrition was therefore significantly lower for these patients than for the 10 control patients who received the least enteral nutrition in the study and mainly received parenteral nutritional support). Only patients treated with a myeloablative conditioning regime were included in the NASQ trial. To minimize differences between the 2 groups with regard to transplant-related factors, we ensured that (1) all 20 patients were treated with either busulfan plus cyclophosphamide or total body irradiation plus cyclophosphamide as pretransplant conditional therapy, (2) all patients received GVHD prophylaxis based on cyclosporine and methotrexate, and (3) only patients being alive without morphologic relapse at day +100 were included in the present study.

### *Classification of Clinical Outcomes*

All patient data were collected and validated by 2 independent researchers. Acute GVHD (aGVHD) and veno-occlusive disease (VOD) were diagnosed according to established criteria [22-24]. All patients with aGVHD were assessed using the modified Glucksberg score [25]. All patients who required treatment for severe GVHD received 2 mg/kg/d intravenous methylprednisolone. Mucositis was evaluated using the World Health Organization oral toxicity score [26].

### *Sample Preparation and Analysis*

Pretransplant samples were collected from all patients on day −8 or −7; post-transplant samples were collected 17 to 24 days post-transplant (Table 1). Venous blood samples were collected into plastic tubes (BD Vacutainer SST ACD Plasma Separation Tubes; Becton-Dickenson, Franklin Lakes, NJ) and allowed to sediment for 120 minutes at room temperature before centrifugation (300 × *g* for 10 minutes) and plasma collection. The samples were immediately frozen and stored at −80°C until analyzed. The metabolic profile was analyzed (Metabolon, Durham, NC) as described previously [20,21,27]. Briefly, samples were prepared by the automated MicroLab Star system, Hamilton Company, Reno, NV, and before analysis, the samples were treated with methanol and rigorously shaken to remove proteins and to increase recovery of metabolites. After centrifugation, the resulting extract was analyzed by either (1) 2 separate reverse-phase ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS) methods with positive ion mode electrospray ionization (ESI), (2) reverse-phase UPLC-MS/MS with negative ion mode ESI, or (3) hydrophilic interaction liquid chromatography/UPLC-MS/MS with negative ion mode ESI. The routines for quality control of the analyses are summarized in Supplementary Table S2.

### *Statistical and Bioinformatical Analyses*

Statistical analyses of clinical variables were performed using Stata Version 14 (StataCorp 2009; Stata Statistical Software, College Station, TX). The Mann-Whitney *U* test was used to compare continuous variables, and the chi-square tests and Fisher's exact test were used to compare categorized data. The log-rank test was used to compare frequencies of aGVHD. Differences were regarded as statistically significant when *P* values were $<.05$.

The metabolomics data were analyzed by analysis of variance, principal component analysis (PCA), and random forest analysis. An alteration of a metabolite concentration was regarded as significant when the *P* value was $<.05$ and the corresponding *q* value was $<.10$. The random forest analysis has been described in detail previously [28]. Briefly, this analysis creates a list of metabolites that would best characterize the 2 different groups and the predictive accuracy is then calculated. Random chance alone would result in a predictive accuracy of 50%, whereas a perfect test would result in an observed predictive accuracy of 100%.

## RESULTS

### *Description and Comparison of the 2 Patient Groups*

A detailed description of the 2 groups of patients is given in Supplementary Table S3, and a summary is given in Table 1. There were no significant differences between the 2 study groups with respect to age, sex, incidence of severe mucositis, pretransplant body weight, or the weight 3 weeks post-transplant. One patient had type 2 diabetes at the time of transplantation, and 1 patient was treated with total body irradiation (both in the control group). The other 19 patients received busulfan + cyclophosphamide as their conditioning therapy. The GVHD prophylaxis was ciclosporin A plus methotrexate. All

**Table 1**
Clinical Characteristics of Patients Included in the Present Study: A Comparison of the 10 Patients Mainly Receiving Parenteral (Control Group) and the 10 Patients Mainly Receiving Enteral (Intervention Group) Nutritional Support

| Patient Characteristics | Parenteral Nutrition (Control Group, n = 10) | Enteral Nutrition (Intervention Group, n = 10) | *P*Value |
|---|---|---|---|
| Age, median (range), yr | 34 (18-60) | 53 (21-62) | .21 |
| Sex, male/female, No. | 6/4 | 5/ 5 | 1.00 |
| Donor type related/unrelated, No. | 2/8 | 5/5 | .35 |
| Hematologic diagnosis, No. | | | |
| Acute myeloid leukemia | 7 | 7 | |
| Acute lymphoblastic leukemia | 0 | 2 | |
| Chronic myelogenous leukemia | 1 | 1 | |
| Atypical CML | 1 | 0 | |
| Myelodysplastic syndrome | 1 | 0 | |
| Diabetes type 2, No. | 1 | 0 | 1.00 |
| Pretransplant sampling | Day −7 or day −8 | Day −7 or day −8 | NA |
| Post-transplant sampling (range) | Day +21 (17-24) | Day +21 (20-23) | NA |
| Pretransplant weight (range), kg | 77.5 (66.8-109.8) | 64.3 (50.0-105.9) | .12 |
| Post-transplant weight (range), kg | 79.2 (62.5-107.3) | 66.6 (49.9-102.0) | .10 |
| Pretransplant energy expenditure (range), kcal/d | 1937 (1335-2720) | 1858(1348-2937) | .97 |
| Post-transplant energy expenditure (range), kcal/d | 1674 (1161-2702) | 1943 (1474-2248) | .41 |
| Days with parenteral nutrition (range) | 14 (1-19) | 13.5 (8-22) | .79 |
| Total energy (kcal) given by parenteral nutrition (range) | 25,275 (13,750-35,400) | 13,000 (424-21,920) | **<.01** |
| Energy (kcal/d) given per day of parenteral nutrition (range) | 1805 (424-1565) | 1024 (424-1565) | **<.01** |
| Mucositis, No. | | | |
| Grade 1 | 1 | 1 | .47* |
| Grade 2 | 2 | 4 | |
| Grade 3 | 1 | 3 | |
| Grade 4 | 6 | 2 | |
| Patients with grade III-IV aGVHD, No. | 4 | 6 | .32 |

Summaries of continuous variables are presented as median and range. Significant differences (i.e., $P < .05$) are shown in bold.
CML indicates chronic myelogenous leukemia.
* Comparing patients with mucositis grades 1 to 3 versus grade 4.

patients in the intervention group managed to secure an adequate energy and protein intake. The number of days with parenteral nutritional support did not differ between the 2 groups (median 14 days versus 13.5 days, $P = .79$), but the control group received both a significantly higher average per day and total amount of parenteral nutrition (see Table 1). The post-transplant decrease in body weight did not differ significantly.

None of the patients were treated with palifermin, and the incidence of grade 4 mucositis (i.e., patient not able to tolerate liquid or solid food by mouth) did not differ ($P = .65$). The incidence of aGVHD did not differ between the 2 groups. The pretransplant energy expenditure did not differ from the energy expenditure 3 weeks post-transplant for any of the groups (repeated-measures analysis of variance, $P = .31$). None of the patients developed VOD or received VOD prophylaxis with ursodeoxycholate or defibrotide.

### *Nutritional Characterization of the Enteral and Parenteral Groups*

The 2 groups differed with regard to how they received their nutritional support (mainly oral/enteral versus mainly parenteral), but in addition, the ensured daily energy intake was also different with at least 30 kcal/kg/d (recommended 30 to 40 kcal/kg/d) for the enteral group and a recommended intake corresponding to 25 to 30 kcal/kg/d for the parenteral control group. As will be described in detail below, the parenteral group showed a more pronounced increase in acylcarnitines, consistent with increased mitochondrial fatty acid oxidation, and decreased N-formylmethionine, consistent with increased mitochondrial activity. These differences are consistent with a more fasting-like post-transpant metabolic profile for the parenteral group. Thus, our 2 groups differ not only with regard to the route of nutritional administration, but there seems to be an additional (and expected) difference in the amount of energy supplementation.

### *Patients Mainly Receiving Enteral or Parenteral Nutrition Show Similarities in Their Pretransplant Systemic Metabolic Profiles but Less Similarity in Post-Transplant Profiles*

The complete list of analyzed metabolites is given in the supplementary file. We first did a PCA to compare the systemic metabolic profiles for both patient groups and at both time points tested (Figure 1). There was a considerable overlap between the distributions of the pretransplant samples (i.e., samples derived before the conditioning treatment) from the 2 groups; they both showed an overlapping distribution to the lower left in Figure 1. This observation indicates that the 2 groups had similarities in their pretransplant systemic metabolic profiles, which are also consistent with the observed similarities in pretransplant clinical characteristics between the groups (Supplementary Table S3).

The post-transplant samples from the 2 groups showed different localizations in the PCA plot (Figure 1) and less degree of overlap compared with the pretransplant samples. Thus, the conditioning and transplantation procedures altered the systemic metabolic profile for both patient groups, and our PCA analyses suggest that these alterations are partly overlapping

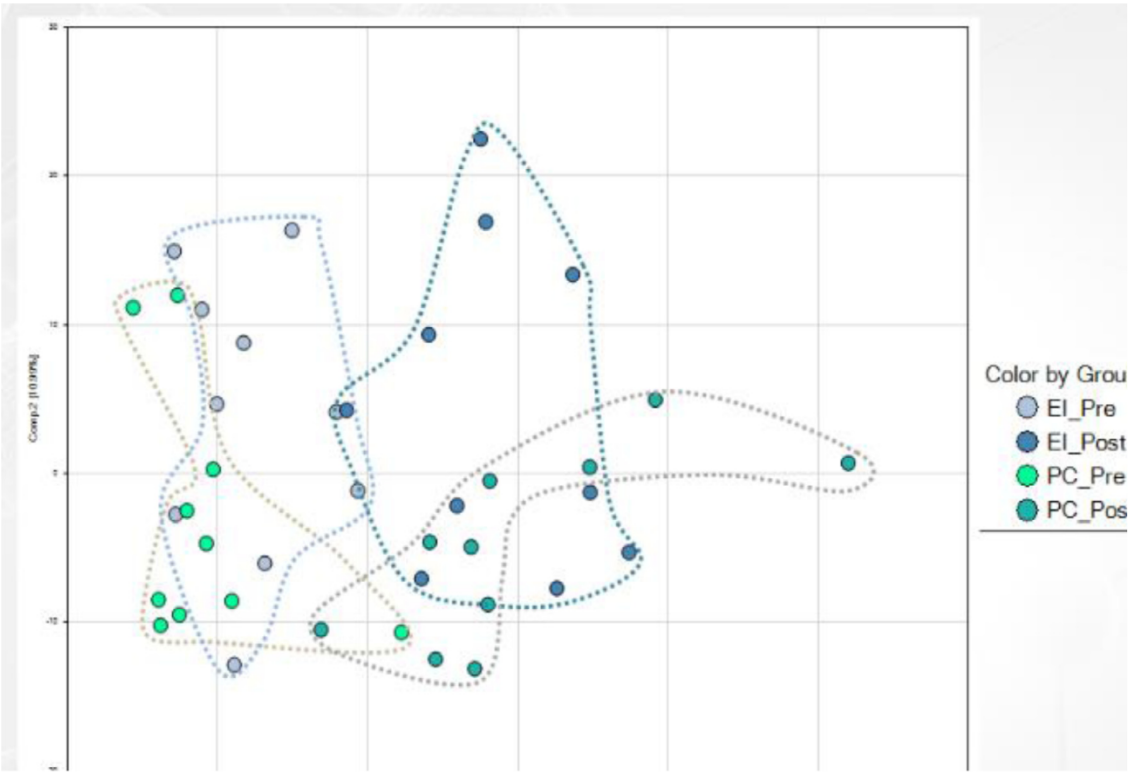



**Figure 1.** PCA of systemic metabolic profiles for patients mainly receiving enteral and parenteral support. The figure shows a comparison between the 4 different sample groups: pre- and posttransplant samples for patients mainly receiving enteral support (the enteral intervention group [EI]) and patients mainly receiving parenteral support (the patient control group [PC]) after the transplantation. Both groups included 10 patients. Thus, each symbol represents 1 sample at a specific time point, and each of the 4 different sample groups is encircled. The outer limits of each group of samples are indicated (EI pre- and EI post-transplant with light gray and gray, respectively; PC pre- and post-transplant with light blue and blue, respectively).

for patients mainly receiving enteral or parenteral nutrition during the study period.

### *The Post-Transplant Metabolic Profile Is Mainly Determined by the Transplantation Procedure and Not by the Nutritional Support*

We determined the numbers of significantly altered metabolites when comparing the corresponding pre- and post-transplant samples for each of the 2 patient groups, and we also determined the numbers of significantly altered metabolites when comparing the pretransplant samples and the post-transplant samples for the 2 groups (Table 2). There was a concordance between the 2 groups (i.e., mainly enteral or parenteral nutritional support) with regard to the post-transplant changes in systemic metabolic profiles relative to the matched pretransplant samples. We investigated 824 metabolites; 311 metabolites underwent significant changes after parenteral nutrition and 198 of them showed trending ($.05 < P < .10$) or significant ($P < .05$) changes with the same directionality as for the group receiving mainly enteral nutritional support. By comparison, 237 metabolites showed a significant post-transplant alteration in patients receiving mainly enteral nutritional support, and 179 of them showed trending or significant changes with the same directionality as for parenteral nutrition.

We did random forest analyses, and the predictive accuracy when comparing pre- and post-transplant samples from patients mainly receiving enteral support was 100% (Table 2). A similar comparison for patients mainly receiving parenteral nutritional support showed a predictive accuracy of 90%. These observations suggest that there is a considerable difference in the systemic metabolic profile for each of the patient cohorts when comparing pre- and post-transplant samples. In contrast, the predictive accuracy was lower when comparing the pretransplant (65%) and the post-transplant (70%) samples for the 2 cohorts.

These observations suggest that the differences between pre- and post-transplant samples for the 2 groups are larger than the differences observed when comparing the 2 groups at the same time point. Thus, our results are consistent with the hypothesis that the systemic post-transplant metabolic profile at day +21 is mainly determined by the conditioning treatment/stem cell transplantation/reconstitution and not by the kind of nutritional support during the early post-transplant period.

To summarize and conclude based on the overall data presented in this section, the PCA showed that there was a larger degree of overlap between pretransplant samples for the 2 patient groups than for the post-transplant samples (Figure 1). Furthermore, the comparison of pre- versus post-transplant samples identified a larger number of significantly different metabolites than the statistical comparisons of the 2 sets of post-transplant samples (Table 2). Finally, there was a considerable overlap between the top 30 ranked metabolites/metabolite classes in the random forest analyses that compared pre- versus post-transplant samples for the 2 groups (Figure 2). The predictive accuracy was also higher for pre- versus post-transplant samples than it was for the comparison of pre- versus pretransplant and post- versus post-transplant samples. Taken together, these observations suggest that the post-transplant metabolomics profiles show a relatively large degree of overlap between the enteral and parenteral groups, and the observations thereby are consistent with our hypothesis that the conditioning/transplantation/

**Table 2**
Metabolomic Comparison of Allotransplant Recipients Receiving Mainly Enteral and Parenteral Nutritional Support: An Overview of the Statistical Comparisons of Metabolite Levels and the Random Forest Analyses of Pre- versus Post-Transplant Systemic Profiles

| Statistical Summary of Transplantation and Nutrition Effects: Two-Way ANOVA with Repeated Measures (Presented as Number of Metabolites) | | | | |
|---|---|---|---|---|
| Number of Altered Metabolites | *P* Value | Nutrition-Dependent Differences: Comparison of Post-Transplant Profiles for the Enteral and Parenteral Group (Number of Altered Metabolites) | Transplantation-Dependent Differences: Comparison of Pre- versus Post-Transplant Metabolic Profiles (Number of Altered Metabolites) | Combined Effect: Number of Metabolites Differing in Both Comparisons |
| Significant difference | ≤.05 | 66 | 378 | 52 |
| Trend | .05-.10 | 53 | 55 | 45 |

| Statistical Comparison: ANOVA Contrasts (Presented as Number of Metabolites) | | | | |
|---|---|---|---|---|
| | Enteral Relative to Parenteral | | Post-transplant Relative to Pretransplant | |
| Total Metabolites | Pretransplant | Post-Transplant | Parenteral | Enteral |
| $P \leq .05$ | | | | |
| Upregulated | 8 | 19 | 138 | 91 |
| Downregulated | 37 | 55 | 173 | 146 |
| $.10 > P > .05$ | | | | |
| Upregulated | 10 | 7 | 26 | 35 |
| Downregulated | 41 | 44 | 46 | 41 |

| Random Forest Analysis: Prediction of Groups Based on Their Systemic Metabolic Profile | |
|---|---|
| Comparison | Predictive Accuracy, % |
| Pretransplant enteral versus pretransplant parenteral | 65 |
| Post-transplant enteral versus post-transplant parenteral | 70 |

ANOVA indicates analysis of variance.

reconstitution is more important for the post-transplant metabolomics profiles than the nutritional differences between the 2 patient groups.

### *Post-Transplant Metabolic Profiles Reflects the Nutritional Status as Well as Altered Bile Acid, Vitamin A, Plasmalogen, and Endocannabinoid Metabolism*

The top 30 ranked metabolites for the 2 comparisons of pretransplant versus post-transplant samples are shown in Figure 2. There were 8 overlapping top-ranked metabolites when comparing pre- and post-transplant samples for each of the 2 patient groups. These overlapping metabolites included 3 lipid metabolites (the amino fatty acid 2-aminooctanoate, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate, the secondary bile acid taurocholate) and the 5 xenobiotics that all are related to food intake (homostachydrine, 1-methylxanthine, theobromine, 4-alylphenol sulfate, pyrraline; see Supplementary Table S4).

A major part of the top-ranked metabolites for both patient groups were classified as xenobiotics: 14 metabolites for enteral nutrition and 16 metabolites for parenteral nutrition (5 being overlapping, see above). These metabolites included both the preservative benzoate, xanthine (caffeine) metabolites, and several food or plant components (Supplementary Table S5). These alterations are consistent with an altered enteral intake in both patient groups.

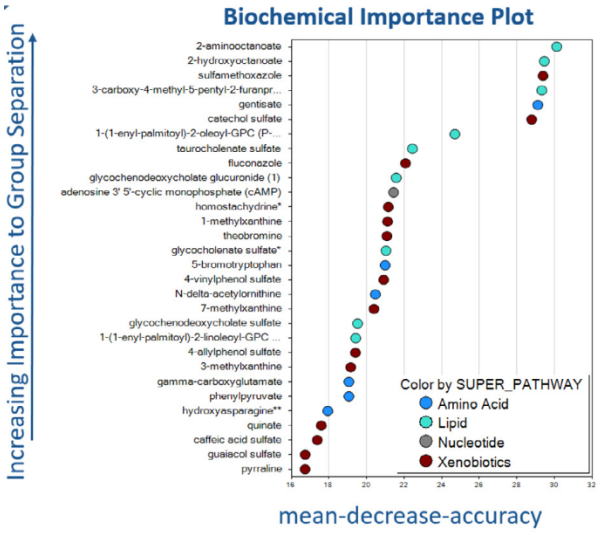
Primary bile acids are released by the liver, and gut bacterial metabolism then produces the secondary bile acids via dihydroxylation and deconjugation. Several bile acids were altered after transplantation for patients receiving mainly enteral and parenteral nutrition. The results for 6 of these bile acids are presented in Figure 3.

There was a significant increase in the systemic levels of the primary bile acids taurocholate and taurochenodeoxycholate. The derivatives glycochenodeoxycholate glucuronide and glycochenodeoxycholate sulfate were also significantly increased post-transplant for both patient groups, and the same was true for the secondary bile acids glycocholenate sulfate and taurocholenate sulfate. The variation between individual patients was generally wider post-transplant for both groups. We could in addition observe significantly decreased plasma levels of isoursodeoxycholate and ursodeoxycholate (0.24- and 0.20-fold decrease, respectively) for parenteral nutrition but not for the mainly enteral group.

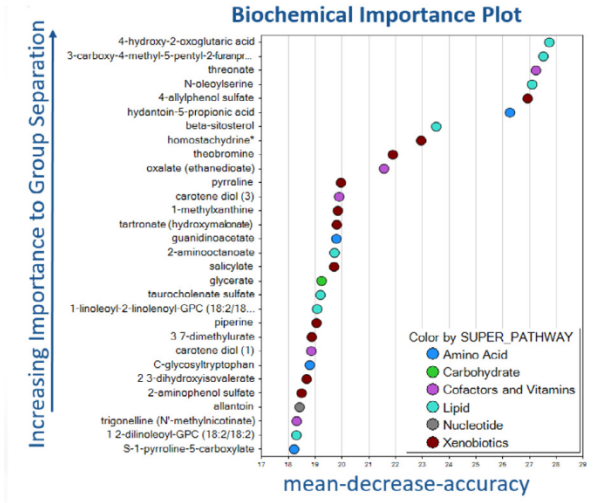
The liver is important in vitamin A metabolism; vitamin A absorption is facilitated by biliary acid metabolism, and a negative feedback mechanism links vitamin A absorption with hepatic bile acid synthesis [29]. Together with the altered bile acid levels, we also observed decreased post-transplant levels of retinol (vitamin A), 3 forms of the vitamin A metabolite carotene diol, and the pro-vitamin A metabolite beta-cryptoxanthin (Figure 4).

Plasmalogens are glycerophospholipid derivatives, and numerous plasmalogens and lysoplasmalogen lipids decreased after transplantation for patients receiving enteral or parenteral nutritional support (Supplementary Table S6).

The liver and the bile acids can also regulate the synthesis of endocannabinoids, and several endocannabinoids (especially oleoylethanolamide, N-olelyltaurine, and N-oleoylserine) showed altered post-transplant plasma levels (Supplementary Tables S4, S5, and S7). For patients receiving mainly enteral nutrition, the levels were significantly increased for only 2 of the 6 analyzed endocannabinoids, whereas 5 of the 6 metabolites were significantly increased for patients mainly receiving parenteral nutrition.




(a) Enteral




(b) Parenteral

**Figure 2.** Random forest analyses showing the top 30 ranked metabolites when comparing pre- versus post-transplant samples derived from allotransplant recipients mainly receiving enteral (a, 10 patients) or parenteral (b, 10 patients) nutritional support. The classification of individual metabolites is indicated in each figure. The predictive accuracy for the random forest plot was 100% for the enteral group and 90% for the parenteral group.

### *Post-Transplant Differences between Patients Receiving Enteral and Parenteral Nutritional Support Include Altered Tyrosine/Dopamine and Oxidative Metabolism*

We compared the post-transplant metabolic profiles for patients receiving enteral or parenteral nutritional support. Altered lipid metabolism was the most striking difference, and lipid metabolites constituted 16 of the 30 top-ranked metabolites from this comparison. The lipid metabolism differed especially with regard to fatty acid metabolism; several acylcarnitines were also increased (Supplementary Tables S4 and S5, supplementary file) and the 18 top-ranked lipid metabolites included 8 acylcarnitines (Figure 5). These alterations may reflect an altered oxidative metabolism, and this is further supported by the increased levels of N-formylmethionine that is linked to mitochondria-specific protein synthesis [30]. This may be due to a more fasting-like metabolic signature; this is supported by the increased levels of oleoylethanolamide that induces lipolysis and fatty acid oxidation [31].

Additional minor differences were observed when comparing the post-transplant plasma metabolic profiles for patients receiving parenteral and enteral nutritional support. First, tyrosine metabolites (dopamine 4-sulfate, dopamine 3-O-sulfate) were significantly increased in patients receiving enteral support, whereas tyrosine and its metabolites 3-(4-hydroxyphenyl)-lactate-phenol-sulfate were significantly decreased (Supplementary Tables S4 and S5, supplementary file). With the exception of beta-citrylglutamate, no effects on glutamate and aspartate metabolism were observed. Second, differences in bilirubin metabolism/clearance were observed with a significant increase in heme and porphyrin metabolites (bilirubin, biliverdin, and i-urobilinogen) in patients receiving parenteral nutrition. The 2 groups did not show any significant differences in pre- and post-transplant levels of bilirubin, hemoglobin, or rate of erythrocyte transfusions (Table 1, Supplementary Table S3).

Corticosterone levels were increased in patients receiving enteral support, and a similar nonsignificant trend was observed for cortisol. Additional differences in steroid metabolism were also detected, especially in pregnenolone/progestin and androgenic steroid metabolism. These additional differences were mainly seen when comparing pre- and post-transplant levels, and increased levels were observed especially for the enteral group (Figure 6).

## DISCUSSION

Previous studies suggest that the pretransplant systemic metabolic profile (i.e., the serum or plasma levels of metabolites derived from cellular metabolism) reflects the risk of post-transplant complications following ASCT [20,21]. The preconditioning profile is associated with risk of GVHD as well as the development of endothelial dysfunction and fluid retention [32-34]. In our opinion, such associations may also reflect altered immune regulation due to the altered levels of immunomodulatory metabolites as well as altered endothelial cell function, metabolite-induced alteration of the renal function, or treatment-induced organ toxicity leading to altered organ functions and thereby secondary metabolic effects. A recent study has demonstrated that the systemic metabolic profile can also be altered by therapeutic interventions (i.e., Granulocyte-colony stimulating factor treatment) [27]. In this context, we have investigated how the plasma metabolic profile is altered by conditioning therapy/stem cell transplantation/hematopoietic reconstitution and nutritional support.

The gut microbiome and the gastrointestinal barrier are important for immune regulation early after allotransplantation [35,36]. Regulation of metabolic pathways has been shown to be important for adequate T and B development and function [37,38], and targeting specific metabolic pathways has been proposed as a possible strategy to modulate post-transplant immune reconstitution [39,40]. Even though treatment with antibiotics, growth factors, probiotics, or specific nutrients has been proposed as possible approaches to reduce transplant-related morbidity, the overall impact of these strategies on the patient's metabolic regulation has so far been thought to be minimal [7,41,42]. Although clinical interventions through these mechanisms until now have failed to demonstrate an effect of clinical relevance, such an effect may be masked by patient heterogeneity, including differences with regard to post-transplant nutritional support, or be clinically relevant only for subsets of patients. Thus, to better understand the possible importance of metabolic regulation and nutritional support in allotransplant recipients, an aim of our study was to

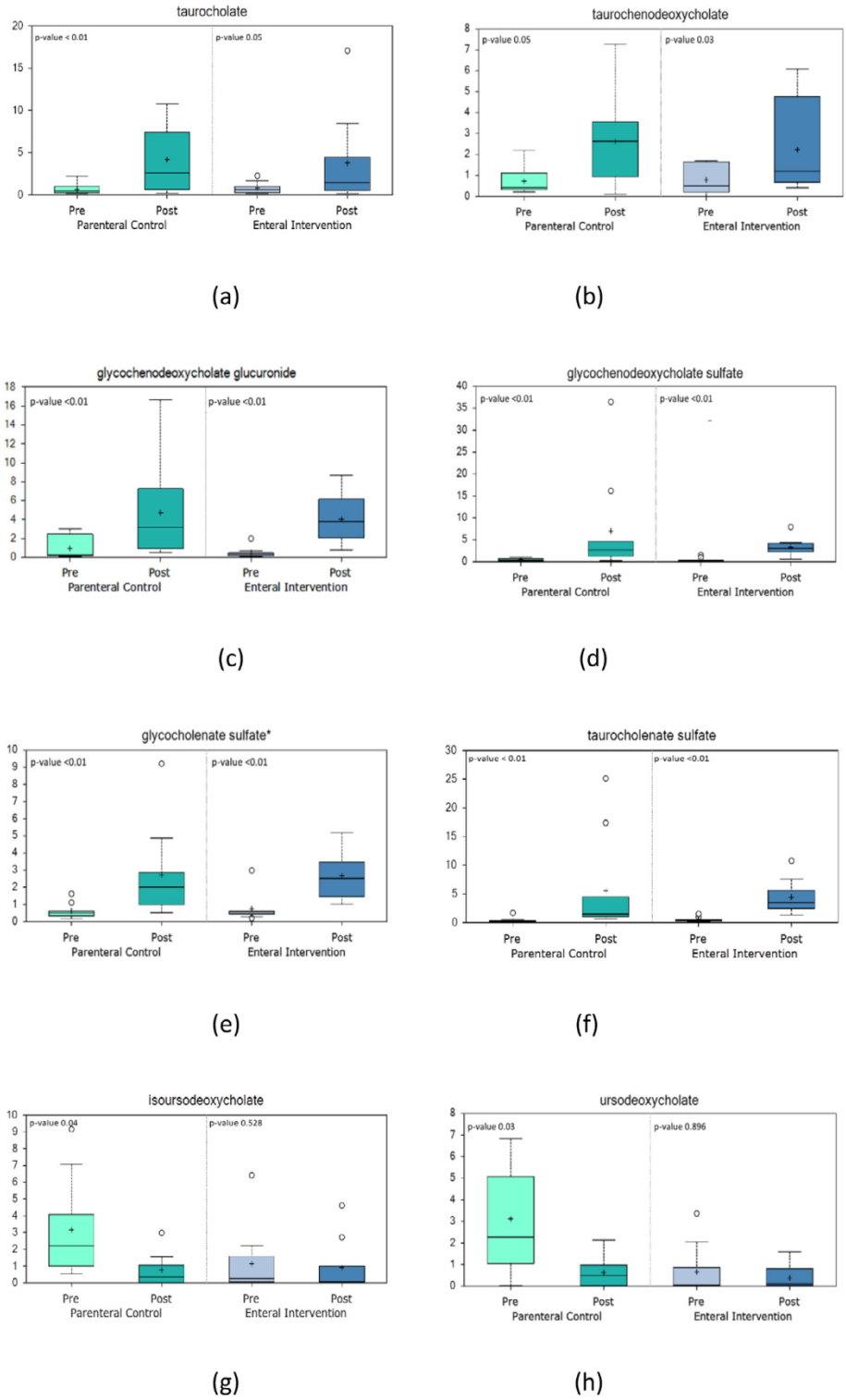



**Figure 3.** The effects of ASCT on bile acid metabolism. The figure presents the levels before the conditioning (pre) and after hematopoietic reconstitution (post). For each of the 8 metabolites, the effects of ASCT are compared for patients receiving mainly parenteral (left part of each individual figures) or enteral (right part of each figure) nutritional support. We show the results for the primary bile acids taurocholate (a), taurochenodeoxycholate (b), glycochenodeoxycholate glucuronide (c), and glycochenodeoxycholate sulfate (d). The lower part of the figure shows the results for the secondary bile acids glycocholenate sulfate (e), taurocholenate sulfate (f), ursodeoxycholate (g), and isoursodeoxycholate (h). The corresponding *P* values are given in each figure. All results are presented as box plots with the median, 25th to 75th percentiles, and range. Outliers are indicated by open symbols (o).

compare the metabolic effects of allotransplantation in patients receiving mainly enteral or parenteral nutritional support.

As described previously, our 2 contrasting patient groups included only patients receiving myeloablative conditioning, and the patients were selected based on the strategy for nutritional support alone. We did not apply any additional selection criteria, and for this reason, there are some differences between the groups; for example, 1 patient had diabetes, 2

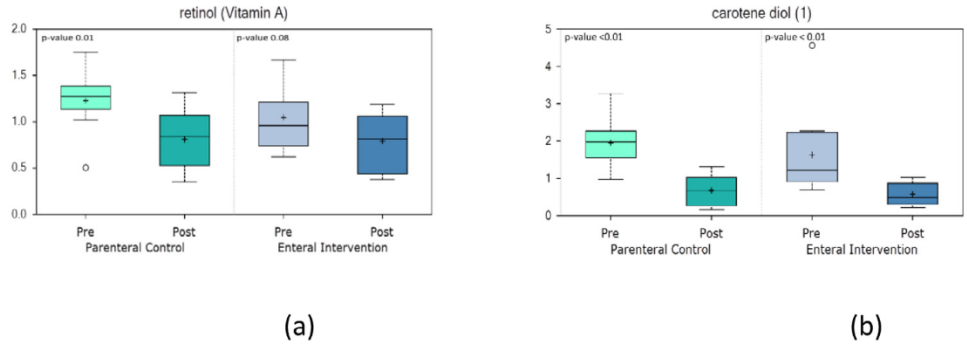
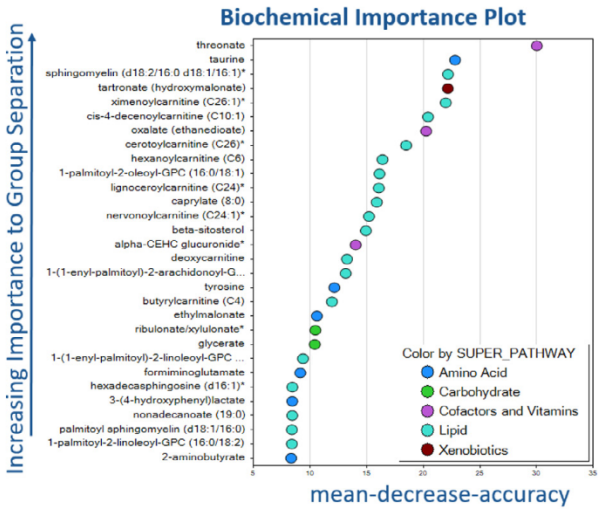



**Figure 4.** Effects of ASCT on vitamin A metabolism. The figure presents the levels for retinol/vitamin A and carotene diol when patients were tested before conditional therapy (pre) and after hematopoietic reconstitution (post). For each metabolite, the effects of ASCT for patients receiving mainly parenteral (left part of each figure) or enteral (right part of figures) nutritional support are compared. The corresponding *P*values are given in each figure. All results are presented as the median level, 25th to 75th percentiles (boxes), and variation range. Outliers are indicated by open symbols (o).

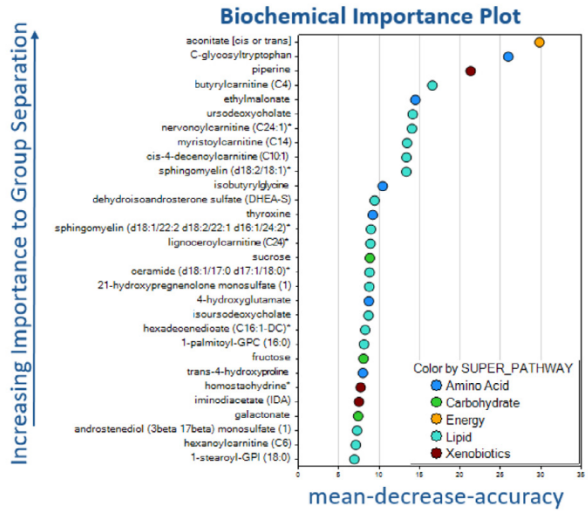
patients received total body irradiation, and 2 patients with acute lymphoblastic leukemia had previously received metabolism-modulating steroid therapy. We regard this as a minor heterogeneity, and despite these differences, we were able to demonstrate significant metabolomic differences between the 2 groups. These metabolic differences were mainly associated with the conditional therapy. Further studies have to clarify whether similar effects are present in other patients receiving intensive chemotherapy with severe gastrointestinal and hematologic/immunologic toxicity (e.g., acute myelogenous leukemia induction and consolidation chemotherapy, reduced-intensity conditioning, autologous stem cell transplantation).

Our 2 relatively small but contrasting patient groups differed with regard to the route of administration of their nutritional support, and the intervention (i.e., enteral) group in addition had very close nutritional monitoring [18,19]. We determined the levels of 824 metabolites, and a *P*value of <.05 was regarded as a significant difference. Thus, we would expect up to 42 metabolites to fulfill this criterion in each comparison by random chance, and in such cases, we would expect an equal distribution between increased or decreased metabolite levels. The low number of only 45 significantly differing metabolites when comparing pretransplant metabolite levels for the 2 groups is thus consistent with only minor differences as expected in a randomized study. However, the higher number of different metabolites when comparing the post-transplant samples (71 differing metabolites, different numbers of increased/decreased metabolites) and the high number of differing metabolites when comparing pre- versus post-transplant samples (237 for the enteral and 311 for the parenteral group) cannot be explained by random chance. By comparing small but well-characterized and contrasting groups, we were thus able to detect metabolic differences between the 2 groups as well as between pre- and post-transplant samples. The metabolic differences may even be more extensive than observed in our present study because additional but quantitatively less important differences may not reach statistical significance in this study.

We observed that the transplantation procedures (conditioning therapy and stem cell transplantation followed by hematologic reconstitution) altered the systemic metabolic profiles, and a major part of these effects was seen for patients receiving mainly enteral or parenteral nutritional support. First, ASCT significantly alters bile acid metabolism. Primary bile acids are synthetized in the liver by oxidation of cholesterol before they are secreted into the gastrointestinal tract and converted into secondary bile acids by the gut




(a) Pretransplant




(b) Posttransplant

**Figure 5.** Random forest analyses showing the top 30 ranked metabolites when comparing the 2 patient groups that received either mainly enteral or mainly parenteral nutritional support. (a) Results from the comparison of the 2 groups when tested before the transplantation. (b) Comparison of the post-transplant profiles. The classification of individual metabolites is indicated in each figure. The predictive accuracy for the random forest plot was 65% for the pretransplant samples and 70% for the posttransplant group.

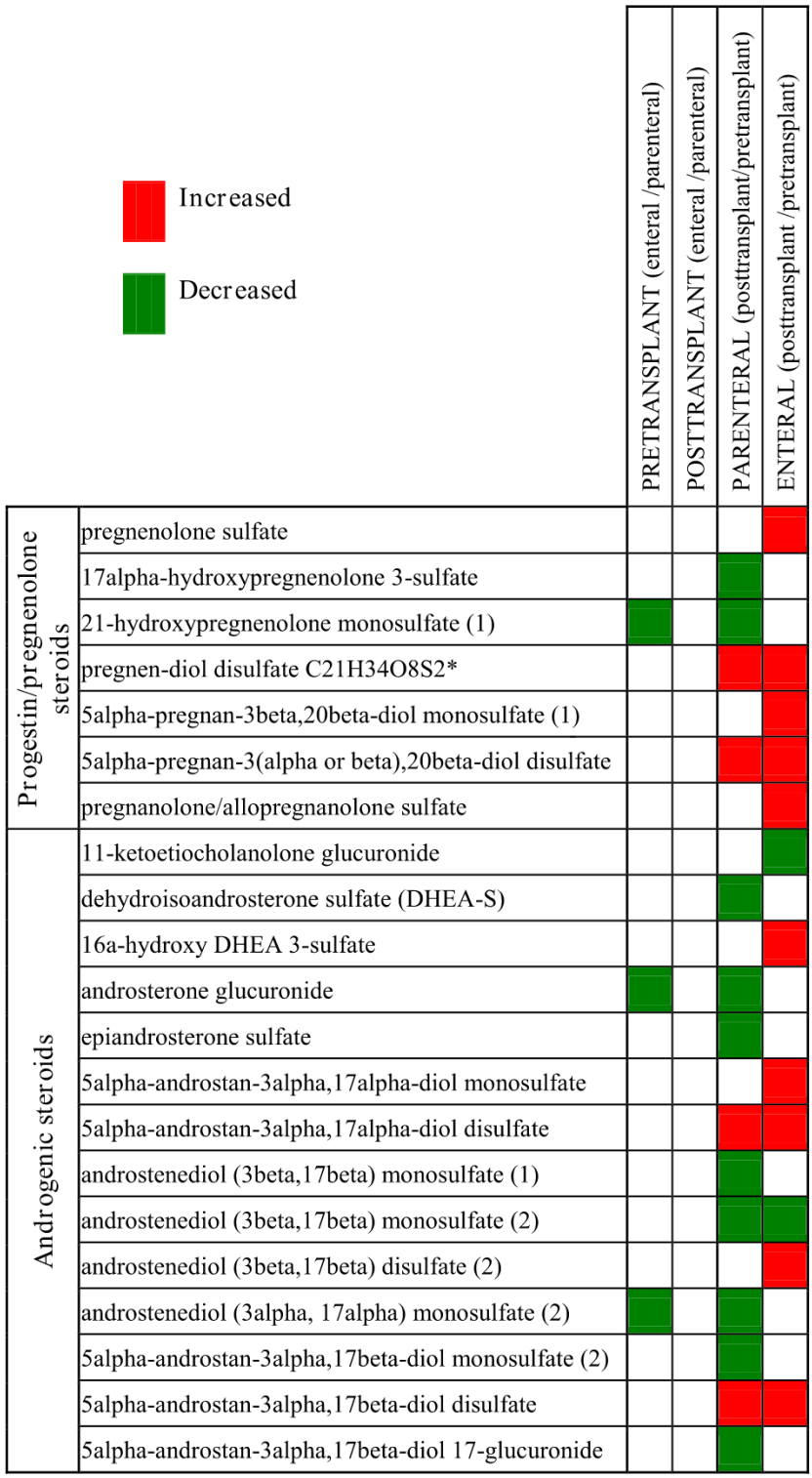



**Figure 6.** Comparison of progestin/pregnenolone and androgenic metabolites in allotransplant recipients. The figure presents the significant differences for the 4 statistical comparisons indicated at the top of the figure (i.e., comparison of the enteral versus the parenteral group with regard to pre- and post-transplant levels; separate comparison of post- versus pretransplant levels for the enteral and the parenteral group). A total of 14 progestin/pregnenolone metabolites were analyzed, and 7 showed significant differences. A total of 22 androgenic steroids were analyzed, and 14 showed statistically significant differences.

microbiota; these secondary bile acids are absorbed in the terminal ileum and enter the enterohepatic circulation [43]. We observed significant reductions of systemic levels of secondary bile acids. Possible explanations for this could be altered primary bile acid synthesis, reduced formation or intestinal reabsorption of secondary bile acids, or altered hepatic reuptake. In our opinion, the most likely explanation is reduced formation and/or reabsorption due to gastrointestinal toxicity with mucositis and altered gut microbiota. This hypothesis is supported by the observation that enteral

nutrition seems to counteract somewhat the reduction in the secondary bile acids isoursodeoxycholate and ursodeoxycholate, 2 bile acids that are produced by specific microbes in the gastrointestinal tract [44,45]. The reduction of ursodeoxycholate levels may have clinical relevance because it has antiapoptotic effects on liver cells, and substitution with ursodeoxycholate has been tried as a therapeutic strategy to improve overall survival by reducing drug-induced liver toxicity or liver involvement in aGVHD [46].

We also observed decreased levels of several metabolites derived from preservatives, caffeine, and food/plant components in both patient groups; this is probably due to gastrointestinal toxicity and thereby an altered dietary intake compared with pretreatment samples. The post-transplant levels of vitamin A metabolites and plasmalogens probably also reflect altered liver function and/or gastrointestinal absorption that are consistent with the changes in plasma bile acid levels (see above).

The increased post-transplant levels of endocannabinoids are surprising because these metabolites are thought to be generated in the small intestine during food intake; this could be secondary to the altered bile acid metabolism because certain bile acids bind to the enzyme N-acylphosphatidylethanolamide-hydrolyzing phospholipase D and thereby promote formation of oleoylethanolamide [47]. Endocannabinoids are arachnoidate-based lipids that are synthesized from membrane glycerophospholipids, and through their binding to specific G-protein-coupled receptors, they are involved in a large variety of biological processes, including immune regulation and carcinogenesis [48-51]. The possible clinical importance of this observation needs further studies to be clarified.

The most prominent differences between the 2 patient groups were increased levels of sulfated dopamine metabolites in patients receiving mainly enteral nutritional support. A major part of circulating dopamine sulfate is thought to be derived from the upper gastrointestinal tract where the dopamine sulfating enzyme is expressed [52]. This difference may thus simply reflect the larger oral intake by patients receiving enteral nutrition, but an alternative explanation could be reduced mucosal toxicity and persistence of a higher enzymatic activity for patients in the enteral group. The other major difference was increased medium- and long-chain carnitines; although this increase was seen for both groups, it was more pronounced in patients receiving parenteral nutrition, and it indicates an insufficient calorie intake in this group [53]. We also observed a significant difference in porphyrin metabolite and corticosterone levels between the 2 groups post-transplant, but there were no differences in pre/post-transplant hemoglobin or bilirubin levels, transfusion rate, or use of systemic steroids (Supplementary Table S3). The clinical impact of these differences is elusive.

Prolonged starvation leads to increased degradation of amino acids and an altered function of various cells (e.g., gut epithelium and lymphocytes) that are not capable of sufficient synthesis of certain nonessential amino acids [54-56]. Although our metabolomic data suggest that patients receiving parenteral nutrition exhibited a metabolic profile more similar to fasting-like metabolism, we could not detect any major differences in amino acid metabolism except for the dopamine metabolism. However, as discussed above, the increased dopamine levels in the enteral group may simply be caused by increased intake or preserved enzymatic activity (i.e., dopamine-specific mechanisms) and may not be caused (or at least not only caused) by a general starvation-like metabolic response.

For several reasons, our results should be interpreted with care. First, there may be treatment spillover between the 2 arms in the clinical study because all patients were treated in the same clinical unit; to minimize this risk, we selected patients included during the first 15 months of the trial period. Second, we cannot exclude significant effects of the previous diseases or their pretransplant treatment on the metabolic profiles, although the random forest and PCAs together with the patient characteristics summarized in Table 1 suggest that such factors do not have a major impact. Third, the timing of blood sampling and parenteral infusions may influence the post-transplant samples, even though strict routines were followed and blood samples were collected at least 4 hours after the end of parenteral infusions. Finally, due to the complexity of the transplant procedure and several competing risks factors, there is a risk of missing significant effects of the nutritional interventions. Despite this, we would emphasize that only a few previous studies have examined the metabolic profile in patients before ASCT [20,21], and to our knowledge, the present study is the first to make paired comparisons of pre- and post-transplant metabolic profiles.

The hypothalamic-pituitary-adrenal axis shows a close interaction with the gut microbiota, and this seems to be a bidirectional communication where altered microbiota is able to alter the hypothalamic-pituitary-adrenal axis [57]. Furthermore, the 2 main glucocorticoids in humans are cortisol and corticosterone; the functions of these 2 glucocorticoids are only partly overlapping, and under certain conditions, corticosterone has additional biochemical functions [58]. Corticosterone as well as its metabolites, but not cortisol, can be secreted into the bile, and the gastrointestinal bacteria (especially anaerobic bacteria) can thereby transform corticosterone into various metabolites that are absorbed into the portal circulation, later bypass the liver, and finally may have systemic effects [59]. These metabolites can then modulate the steroid metabolism through inhibition of certain steroid-modulating enzymes [58,59]. Corticosterone/cortisol levels may also be influenced by extra-adrenal production and/or activation; these enzymes are present in several organs, including liver and colon [60]. Furthermore, recent animal studies suggest that corticosterone has a protective effect against gastric ulcerations [61]; it is not known whether this is true also for the gut mucosa. Thus, differences in systemic levels of cortisol, corticosterone and their metabolites, and the larger difference for corticosterone (i.e., significant effect) than cortisol (a trend) may be explained by different effects on the gut microbiota by the 2 different nutritional regimens and possibly also by differences in mucosa-protecting effects.

We observed significantly altered post-transplant levels of several other steroid metabolites; this was especially seen for progestin/progranolone and androgenic metabolites. In our opinion, the most likely explanation for these differences is an altered microbiome. The gut bacteria are able to modulate steroids and form pregnene/pregnan/androstane metabolites [59], and our 2 groups differed in the serum levels of several such metabolites. Studies in animal models suggest that even certain antibiotics can alter steroid metabolism [57], but this explanation seems less likely because the clinical study did not detect any differences between the control and intervention groups with regard to time until neutrophil engraftment, episodes of bacteremia, fungal or viral infections, empirical use of antibiotics or antifungal, or days with fever [18,19].

Our study shows that the systemic metabolic profile is significantly altered early after ASCT, and these effects are more pronounced than the effects caused by differences in nutritional support (predominantly enteral versus parenteral). However, altered immune regulation early after

allotransplantation is important for outcome, and our study suggests that the possible clinical importance of differences in early nutritional support/metabolic regulation in allotransplant recipients should be further investigated with a focus on patient heterogeneity and standardization of the nutritional support.

## ACKNOWLEDGMENTS

*Financial disclosure:* The study received financial support from The Blix Family Foundation, Eivind Møllbach Pedersens Foundation, Helse-Vest, Norwegian Cancer Society, Oslo University Hospital, University of Bergen, and Throne Holst Foundation. The enteral feeding was supported by Nutricia.

*Conflict of interest statement:* There are no conflicts of interest to report.

*Authorship statement:* T.H.A.T. and Ø.B. conceived the idea, designed the study, interpreted data, and wrote the manuscript. P.O.I., K.J.S., G.E.T., and T.G.D, designed and conducted the initial clinical trial from which the samples were collected for the current study. K.J.S. was responsible for collection of samples and clinical data. K.J.S., together with T.H.A.T. and Ø.B., selected patients from the clinical study for metabolomic analysis. All participants read and accepted the final manuscript.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.10.005.

## REFERENCES

1. Lenssen P, Sherry ME, Cheney CL, et al. Prevalence of nutrition-related problems among long-term survivors of allogeneic marrow transplantation. *J Am Diet Assoc*. 1990;90:835–842.
2. Schulenburg A, Turetschek K, Wrba F, et al. Early and late gastrointestinal complications after myeloablative and nonmyeloablative allogeneic stem cell transplantation. *Ann Hematol*. 2004;83:101–106.
3. Rieger CT, Wischumerski I, Rust C, Fiegl M. Weight loss and decrease of body mass index during allogeneic stem cell transplantation are common events with limited clinical impact. *PLoS One*. 2015;10: e0145445.
4. Fuji S, Mori T, Khattry N, et al. Severe weight loss in 3 months after allogeneic hematopoietic SCT was associated with an increased risk of subsequent non-relapse mortality. *Bone Marrow Transplant*. 2015;50:100–105.
5. Jacobsohn DA, Margolis J, Doherty J, Anders V, Vogelsang GB. Weight loss and malnutrition in patients with chronic graft-versus-host disease. *Bone Marrow Transplant*. 2002;29:231–236.
6. Arends J, Bachmann P, Baracos V, et al. ESPEN guidelines on nutrition in cancer patients. *Clin Nutr*. 2017;36:11–48.
7. Tvedt TH, Reikvam H, Bruserud O. Nutrition in allogeneic stem cell transplantion—clinical guidelines and immunobiological aspects. *Curr Pharm Biotechnol*. 2016;17:92–104.
8. Aoyama T, Imataki O, Mori K, et al. Nutritional risk in allogeneic stem cell transplantation: rationale for a tailored nutritional pathway. *Ann Hematol*. 2017;96:617–625.
9. Sommacal HM, Gazal CH, Jochims AM, et al. Clinical impact of systematic nutritional care in adults submitted to allogeneic hematopoietic stem cell transplantation. *Rev Bras Hematol Hemoter*. 2012;34:334–338.
10. Guttormsen AB, Pichard C. Determining energy requirements in the ICU. *Curr Opin Clin Nutr Metab Care*. 2014;17:171–176.
11. Al-Omran M, Albalawi ZH, Tashkandi MF, Al-Ansary LA. Enteral versus parenteral nutrition for acute pancreatitis. *Cochrane Database Syst Rev*. 2010(1) CD002837.
12. Petrov MS, Pylypchuk RD, Emelyanov NV. Systematic review: nutritional support in acute pancreatitis. *Aliment Pharmacol Ther*. 2008;28:704–712.
13. Braunschweig CA, Sheean PM, Peterson SJ, et al. Intensive nutrition in acute lung injury: a clinical trial (INTACT). *JPEN J Parenter Enteral Nutr*. 2015;39:13–20.
14. Marik PE, Zaloga GP. Early enteral nutrition in acutely ill patients: a systematic review. *Crit Care Med*. 2001;29:2264–2270.
15. Mattsson J, Westin S, Edlund S, Remberger M. Poor oral nutrition after allogeneic stem cell transplantation correlates significantly with severe graft-versus-host disease. *Bone Marrow Transplant*. 2006;38:629–633.
16. Beckerson J, Szydlo RM, Hickson M, et al. Impact of route and adequacy of nutritional intake on outcomes of allogeneic haematopoietic cell transplantation for haematologic malignancies. *Clin Nutr*. 2019;38:738–744.
17. Thompson JL, Duffy J. Nutrition support challenges in hematopoietic stem cell transplant patients. *Nutr Clin Pract*. 2008;23:533–546.
18. Skaarud KJ, Hjermstad MJ, Bye A, et al. Effects of individualized nutrition after allogeneic hematopoietic stem cell transplantation following myeloablative conditioning; a randomized controlled trial. *Clin Nutr ESPEN*. 2018;28:59–66.
19. Skaarud KJ, Veierød MB, Lergenmuller S, Bye S, Iversen PO, Tjønnfjord GE. Body weight, body composition and survival after 1 year: follow-up of a nutritional intervention trial in allo-HSCT recipients. *Bone Marrow Transplantation*. 2019;54(12):2102–2109.
20. Reikvam H, Hatfield K, Bruserud O. The pretransplant systemic metabolic profile reflects a risk of acute graft versus host disease after allogeneic stem cell transplantation. *Metabolomics*. 2016;12:12.
21. Reikvam H, Gronningsaeter IS, Ahmed AB, Hatfield K, Bruserud O. Metabolic serum profiles for patients receiving allogeneic stem cell transplantation: the pretransplant profile differs for patients with and without posttransplant capillary leak syndrome. *Dis Markers*. 2015;2015: 943430.
22. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945–956.
23. McDonald GB, Sharma P, Matthews DE, Shulman HM, Thomas ED. Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. *Hepatology*. 1984;4:116–122.
24. Jones RJ, Lee KS, Beschorner WE, et al. Venoocclusive disease of the liver following bone marrow transplantation. *Transplantation*. 1987;44:778–783.
25. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295–304.
26. World Health Organization. *WHO Handbook for Reporting the Results of Cancer Treatment*. Geneva: Offset Publications; 1979.
27. Hatfield KJ, Melve GK, Bruserud O. Granulocyte colony-stimulating factor alters the systemic metabolomic profile in healthy donors. *Metabolomics*. 2017;13:2.
28. Breiman L. Random forests. *Machine Learn*. 2011;45:5–32.
29. Schmidt DR, Holmstrom SR, Fon Tacer K, Bookout AL, Kliewer SA, Mangelsdorf DJ. Regulation of bile acid synthesis by fat-soluble vitamins A and D. *J Biol Chem*. 2010;285:14486–14494.
30. Bianchetti R, Lucchini G, Crosti P, Tortora P. Dependence of mitochondrial protein synthesis initiation on formylation of the initiator methionyl-tRNAf. *J Biol Chem*. 1977;252:2519–2523.
31. Guzman M, Lo Verme J, Fu J, Oveisi F, Blazquez C, Piomelli D. Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). *J Biol Chem*. 2004;279:27849–27854.
32. Tvedt TH, Lie SA, Reikvam H, et al. Pretransplant levels of CRP and interleukin-6 family cytokines: effects on outcome after allogeneic stem cell transplantation. *Int J Mol Sci*. 2016;17(11):1823.
33. Lindas R, Tvedt TH, Hatfield KJ, Reikvam H, Bruserud O. Preconditioning serum levels of endothelial cell-derived molecules and the risk of post-transplant complications in patients treated with allogeneic stem cell transplantation. *J Transplant*. 2014;2014: 404096.
34. Dietrich S, Falk CS, Benner A, et al. Endothelial vulnerability and endothelial damage are associated with risk of graft-versus-host disease and response to steroid treatment. *Biol Blood Marrow Transplant*. 2013;19:22–27.
35. Holler E, Butzhammer P, Schmid K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant*. 2014;20:640–645.
36. Staffas A, Burgos da Silva M, van den Brink MR. The intestinal microbiota in allogeneic hematopoietic cell transplant and graft-versus-host disease. *Blood*. 2017;129:927–933.
37. Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. *Semin Immunol*. 2016;28:514–524.
38. Caro-Maldonado A, Wang R, Nichols AG, et al. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. *J Immunol*. 2014;192:3626–3636.
39. Glick GD, Rossignol R, Lyssiotis CA, et al. Anaplerotic metabolism of alloreactive T cells provides a metabolic approach to treat graft-versus-host disease. *J Pharmacol Exp Ther*. 2014;351:298–307.
40. Chang CH, Pearce EL. Emerging concepts of T cell metabolism as a target of immunotherapy. *Nat Immunol*. 2016;17:364–368.
41. Jagasia MH, Abonour R, Long GD, et al. Palifermin for the reduction of acute GVHD: a randomized, double-blind, placebo-controlled trial. *Bone Marrow Transplant*. 2012;47:1350–1355.
42. Beelen DW, Elmaagacli A, Muller KD, Hirche H, Schaefer UW. Influence of intestinal bacterial decontamination using metronidazole and ciprofloxacin or ciprofloxacin alone on the development of acute graft-versus-host disease after marrow transplantation in patients with hematologic malignancies: final results and long-term follow-up of an open-label prospective randomized trial. *Blood*. 1999;93:3267–3275.
43. Chiang JY. Bile acid metabolism and signaling. *Compr Physiol*. 2013;3: 1191–1212.

44. Fedorowski T, Salen G, Tint GS, Mosbach E. Transformation of chenodeoxycholic acid and ursodeoxycholic acid by human intestinal bacteria. *Gastroenterology*. 1979;77:1068–1073.
45. Beuers U, Fischer S, Spengler U, Paumgartner G. Formation of iso-ursodeoxycholic acid during administration of ursodeoxycholic acid in man. *J Hepatol*. 1991;13:97–103.
46. Ruutu T, Eriksson B, Remes K, et al. Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. *Blood*. 2002;100:1977–1983.
47. Magotti P, Bauer I, Igarashi M, et al. Structure of human N-acylphosphatidylethanolamine-hydrolyzing phospholipase D: regulation of fatty acid ethanolamide biosynthesis by bile acids. *Structure*. 2015;23:598–604.
48. Cabral GA, Ferreira GA, Jamerson MJ. Endocannabinoids and the immune system in health and disease. *Handb Exp Pharmacol*. 2015;231:185–211.
49. Tegeder I. Endocannabinoids as guardians of metastasis. *Int J Mol Sci*. 2016;17:230.
50. Chiurchiu V. Endocannabinoids and immunity. *Cannabis Cannabinoid Res*. 2016;1:59–66.
51. Lee Y, Jo J, Chung HY, Pothoulakis C, Im E. Endocannabinoids in the gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol*. 2016;311:G655–G666.
52. Goldstein DS, Swoboda KJ, Miles JM, et al. Sources and physiological significance of plasma dopamine sulfate. *J Clin Endocrinol Metab*. 1999;84:2523–2531.
53. Costa CC, de Almeida IT, Jakobs C, Poll-The BT, Duran M. Dynamic changes of plasma acylcarnitine levels induced by fasting and sunflower oil challenge test in children. *Pediatr Res*. 1999;46:440–444.
54. Wang B, Wu G, Zhou Z, et al. Glutamine and intestinal barrier function. *Amino Acids*. 2015;47:2143–2154.
55. van der Hulst RR, von Meyenfeldt MF, Soeters PB. Glutamine: an essential amino acid for the gut. *Nutrition*. 1996;12:S78–S81.
56. Ohnuma T, Holland JF, Arkin H, Minowada J. L-asparagine requirements of human T-lymphocytes and B-lymphocytes in culture. *J Natl Cancer Inst*. 1977;59:1061–1063.
57. Farzi A, Frohlich EE, Holzer P. Gut microbiota and the neuroendocrine system. *Neurotherapeutics*. 2018;15:5–22.
58. Morris DJ. Why do humans have two glucocorticoids: a question of intestinal fortitude. *Steroids*. 2015;102:32–38.
59. Morris DJ, Ridlon JM. Glucocorticoids and gut bacteria: "the GALF hypothesis" in the metagenomic era. *Steroids*. 2017;125:1–13.
60. Terao M, Katayama I. Local cortisol/corticosterone activation in skin physiology and pathology. *J Dermatol Sci*. 2016;84:11–16.
61. Filaretova L. The hypothalamic-pituitary-adrenocortical system: hormonal brain-gut interaction and gastroprotection. *Auton Neurosci*. 2006;125:86–93.