

STANDARD ARTICLE

Plasma amino acid profiles in dogs with closed extrahepatic portosystemic shunts are only partially improved 3 months after successful gradual attenuation

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Abstract

Background: Dogs with portosystemic shunts have an altered blood amino acid profile, with an abnormal branched-chained amino acid (BCAA)-to-aromatic amino acid (AAA) ratio being the most common abnormality. Different liver diseases have distinctive amino acid profiles.

Objectives: Determine the changes in plasma amino acid profiles in dogs with extrahepatic portosystemic shunts (EHPSS) from diagnosis to complete closure.

Animals: Ten client-owned dogs with EHPSS closed after surgical attenuation.

Methods: Prospective cohort study. Medical treatment was instituted in dogs diagnosed with EHPSS. At least 4 weeks later, gradual surgical attenuation was performed. Three months postoperatively, EHPSS closure was confirmed by transsplenic portal scintigraphy. Clinical signs were scored and blood was taken before institution of medical treatment, at time of surgery, and 3 months postoperatively. At the end of the study, the plasma amino acid profiles were analyzed in batch.

Results: The median BCAA-to-AAA ratio was extremely low (0.6) at time of diagnosis and remained low (0.5) at time of surgery, despite the fact that median neurological score significantly improved from 22 to 2 after starting medical treatment ($P = .04$). Three months after surgical attenuation, a significantly higher BCAA-to-AAA ratio (1.5) was observed ($P < .001$).

Conclusions and Clinical Importance: Medical treatment does not improve the BCAA-to-AAA ratio in dogs with EHPSS, despite substantial clinical improvement. Although the ratio significantly increased after EHPSS closure, it was still indicative of moderate to severe hepatic dysfunction in all dogs.

KEYWORDS

canine, liver dysfunction, protein metabolism, vascular anomaly

Abbreviations: AAA, aromatic amino acids; BCAA, branched-chain amino acids; BCS, body condition score; EHPSS, extrahepatic portosystemic shunt; HE, hepatic encephalopathy.

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1 | INTRODUCTION

The liver is an important organ involved in many essential metabolic functions, including metabolism and synthesis of proteins, carbohydrates, and fats.¹ Portosystemic shunts (PSS) are vascular anomalies that connect the portal system to the systemic circulation, bypassing the liver parenchyma.² Clinical signs associated with PSS are growth retardation, hepatic encephalopathy (HE), gastrointestinal signs, and urinary signs secondary to the development of ammonium urate urolithiasis.³ Gastrointestinal signs can occur because of decreased bile production secondary to hepatic dysfunction, leading to maldigestion and malabsorption.³ Long-term malabsorption can cause deficiencies in proteins, fatty acids, and vitamins, resulting in growth retardation and skin and hair coat changes.^{3,4}

In dogs with hepatobiliary disease, an adequate amount of high quality protein should be provided in the diet to avoid negative nitrogen balance.^{5,6} It is, however, important to assure that dogs with HE do not receive an excessive amount of protein, which can exacerbate clinical signs.⁶ Blood ammonia concentrations often are linked to the presence of HE but ammonia concentrations can be normal despite the presence of HE.⁷ Other substances such as increased amounts of glutamine, glutamate, manganese, and systemic inflammation can contribute to HE.⁸⁻¹⁰ Also a decreased ratio of branched-chain amino acids (BCAA)-to-aromatic amino acids (AAA) can induce derangements in central neurotransmitter function that trigger HE.¹¹

Two experimental studies, in which a portocaval shunt was created in adult mongrel dogs clearly showed that amino acid profiles changed over time. The most important alterations were a significant increase of AAAs and a significant decrease of BCAAs, changes that were linked to the development of HE.^{11,12} Similarly, a recent study that assessed blood amino acid concentrations in dogs with congenital PSS found lower concentrations of BCAA and higher concentrations of AAA in dogs with PSS compared with dogs with chronic hepatitis and healthy dogs.¹³ Normal BCAA-to-AAA ratios in dogs are reported to be 3.0 to 4.0, whereas dogs with severe hepatic insufficiency have ratios <1.5.¹⁴ In an experimental study in dogs in which a portocaval shunt was created after partial hepatectomy, a BCAA-enriched diet did not have a beneficial effect on the severity of HE.¹⁵

No research has been performed to identify the effect of medical or surgical treatment on the amino acid profiles in dogs with congenital PSS receiving medical treatment and undergoing surgical attenuation. Yet, this information is important because surgical treatment is not always performed or successful, and consequently a number of dogs with congenital PSS need life-long medical treatment.

Our aim was to determine whether the plasma amino acid profile changes after starting medical treatment and if the plasma amino acid profile normalizes in dogs with extrahepatic PSS (EHPSS) after complete EHPSS closure.

2 | MATERIALS AND METHODS

2.1 | Animals

The study was approved by the local ethical (EC2017-49) and deontological committee (2017N06), and, before inclusion of their dog, all owners signed an informed consent. Client-owned dogs with congenital EHPSS were prospectively enrolled. Inclusion criteria were dogs that had not yet received medical treatment (including a liver-supportive diet) at the time of diagnosis and that would undergo surgical attenuation of the EHPSS.

After confirmation of the diagnosis of EHPSS, medical treatment was given for a minimum of 4 weeks. All dogs received a strict liver-supportive diet (Royal Canin hepatic, Royal Canin, Zaventem, Belgium) divided into several small meals per day. Additional medication consisted of lactulose (0.5 mL/kg q8h), metronidazole (7.5-10 mg/kg q12h) or both lactulose and metronidazole. All dogs underwent gradual attenuation of the EHPSS using either an ameroid constrictor or thin film banding, always placed as close as possible to the systemic circulation. Postoperatively, medical treatment was continued until the 1-month follow-up visit. In dogs that had clinical signs compatible with PSS or dogs with fasting ammonia concentration above the upper limit 1 month postoperatively, lactulose was continued; in all other dogs only the liver-supportive diet was continued until the 3-month follow-up visit. Three months postoperatively, transsplenic portal scintigraphy was performed. After ultrasound-guided injection of ^{99m}Tc pertechnetate into the spleen, the percentage of blood bypassing the liver (shunt fraction) was calculated. Dogs with a shunt fraction <4.3% were considered to have a closed EHPSS.¹⁶ Dogs that had persistent shunting were excluded from the study.

Standardized questionnaires were completed by all owners at diagnosis, surgery, and 1 and 3 months postoperatively, and body weight and body condition scores (BCS, on a scale of 9) were recorded at each visit. Gastrointestinal, urinary and neurological signs were scored, based on a published scoring system (Table 1).¹⁷

2.2 | Blood tests

At diagnosis, at the day of surgery and 1 and 3 months after surgery, a blood sample was taken from a jugular vein. Owners were asked to fast their dogs for 12 hours before blood sampling. On all occasions, 1 drop of fresh blood was used to immediately measure fasting ammonia concentration using a hand-held ammonia analyzer (PocketChem BA, A. Menarini Diagnostics; upper limit, 45 μ mol/L; detection range, 8-285 μ mol/L). At diagnosis, on the day of surgery and 3 months after surgery, blood also was collected in a 2-mL EDTA tube and centrifuged at 3500g for 5 minutes at 2°C. Obtained plasma was aliquoted at 250 μ L and stored at -80°C.

At the end of the study, frozen plasma samples were sent to an external laboratory for analysis of a targeted metabolomics amino acid panel, measuring 22 amino acids including the AAAs (phenylalanine,

TABLE 1 Clinical scoring system, based on a previously published scoring system¹⁷

	Frequency often - occasionally - never	Multiplication factor	Maximal score
Gastrointestinal signs			
Salivation, decreased appetite, weight loss, diarrhea, vomiting, melena, hematochezia	2 - 1 - 0 for each gastrointestinal sign	×1 for each gastrointestinal sign	14
Urinary signs			
Hematuria, stranguria, dysuria, pain while urinating	2 - 1 - 0 for each urinary sign	×1 for each urinary sign	8
Neurological signs			
Lethargic, unresponsive, disorientation, circling head pressing, ataxia, blindness	2 - 1 - 0 for each neurological sign	×2 for each neurological sign	40
Seizure activity, comatose		×3 for each neurological sign	

tryptophan and tyrosine) and the BCAAs (isoleucine, leucine and valine). All samples were spiked with stable labeled internal standards, extracted, and subjected to protein precipitation with an organic solvent. After centrifugation, an aliquot of the supernatant was diluted and injected onto an Agilent 1290/AB Sciex QTrap 5500 liquid chromatography-tandem mass spectrometry unit equipped with a C18 reversed phase ultra-high performance liquid chromatography column. The mass spectrometer was operated in positive mode using electrospray ionization. The peak areas of the individual analyte parent ions were measured against the peak areas of the parent ions of the corresponding internal standards in pseudo-multiple reaction monitoring mode. Quantification was performed using a weighted least squares regression analysis generated from fortified calibration standards prepared immediately before each run. Liquid chromatography-tandem mass spectrometry raw data were collected and processed using SCIEX OS-MQ software v1.7. Data reduction was performed using Microsoft Excel for Office 365 v.16.

Sample analyses were carried out in 96-well plates containing 2 calibration curves and 6 quality samples per plate to monitor assay performance. Accuracy was evaluated using high-, medium-, and low-quality controls based on historical values from TAM146 validation. Precision was evaluated using the quality control replicates in each sample run.

The ratio of BCAAs-to-AAAs was determined for each dog by dividing the sum of all BCAA plasma concentrations by the sum of all AAA plasma concentrations at all 3 sampling points.

2.3 | Statistical analyses

Statistical analyses were performed using SPSS Statistics 26 (IBM, Armonk). Friedman 2-way analyses were performed to assess changes in body weight and BCS over time within dogs (at diagnosis, surgery, 1 and 3 months postoperatively). Kruskal-Wallis tests were used to evaluate the median clinical scores and fasting ammonia concentrations (at diagnosis, surgery, 1 and 3 months postoperatively) and the median plasma concentrations of the different amino acid concentrations and the BCAA-to-AAA ratio at different sampling points (at diagnosis, surgery and 3 months postoperatively). Thereafter Friedman 2-way analyses were performed to assess changes in clinical

scores, fasting ammonia concentrations and in plasma amino acid concentrations and the BCAA-to-AAA ratio over time within dogs. If statistical differences in Friedman 2-way analyses and in Kruskal-Wallis tests were present, pairwise multiple comparison tests using Bonferroni correction were performed. Finally, Mann-Whitney *U* tests were performed to assess differences in the different plasma amino acid concentrations between dogs <1 year of age and dogs ≥1 year of age. Results were considered significant if $P < .05$.

3 | RESULTS

3.1 | Study sample

Initially, 15 dogs were enrolled. Three months postoperatively, 5 dogs were excluded because of persistent shunting. The remaining 10 dogs had a median age of 12.5 months (range, 3-105 months); 5 were <1 year of age. Eight dogs had a portocaval shunt, 1 had a portophrenic shunt and 1 dog had both a portophrenic and a portoazygos shunt. In the latter dog, both EHPSSs were attenuated using an ameroid constrictor during 2 separate surgeries. In that dog, only blood samples taken at diagnosis, at time of the first surgery and 3 months after the second surgery were analyzed. Surgical attenuation using an ameroid constrictor was performed in another 7 dogs and thin film banding was used in the 2 remaining dogs. Demographic data, clinical scores and fasting ammonia concentrations are depicted in Table 2. Both body weight and BCS significantly increased over time ($P = .001$ and $P = .01$, respectively). Pairwise comparisons, however, only identified a significant increase in body weight from diagnosis to 3 months postoperatively ($P = .001$) and from 1 to 3 months postoperatively ($P = .004$).

Within dogs, all clinical scores (gastrointestinal, urinary, and neurological scores) significantly decreased over time ($P < .001$, $P = .02$, and $P < .001$, respectively). Pairwise comparisons showed a significant decrease in gastrointestinal scores from diagnosis to 3 months postoperatively ($P < .001$), and a significant decrease in neurological scores from diagnosis to 1 month postoperatively ($P = .001$) and from diagnosis to 3 months postoperatively ($P < .001$).

Fasting ammonia concentrations significantly decreased over time within dogs ($P < .001$). Pairwise comparisons indicated that fasting

TABLE 2 Median (range) of demographic data, clinical scores, and fasting ammonia concentrations at diagnosis, surgery, 1 and 3 months postoperatively of dogs with closed congenital extrahepatic portosystemic shunts after surgical attenuation

	Diagnosis	Surgery	1 month postoperatively ^a	3 months postoperatively ^a
Body weight (kg)	3.0 (1.4-8.0)	2.9 (1.8-9.8)	3.0 (1.6-10.0)	3.3 (2.0-11.4)
Body condition score (on scale of 9)	3 (2-6)	3.5 (3-7)	3.5 (2-7)	4 (4-9)
Weeks since start medical therapy	NA	6 (4-7)	11.5 (10-28)	20.5 (19-37)
Gastrointestinal score	5 (2-10)	2 (1-4)	2 ^b (0-5)	0 ^c (0-2)
Urinary score	0 (0-4)	0 (0-1)	0 (0-1)	0 (NA)
Neurological score	22 (2-40)	2 ^d (0-18)	0 ^b (0-10)	0 ^{c,e} (0-12)
Fasting ammonia (μmol/L)	150 (43-256)	76 (21-high)	14.5 ^{b,f} (low-32)	14 ^{c,e} (low-24)

Abbreviations: EHPSS, extrahepatic portosystemic shunts; NA, not applicable.

^aOnly data after the second surgery are reported of the dog with 2 single EHPSS that underwent 2 surgeries.

^bSignificant difference between median concentrations at diagnosis and 1 month postoperatively.

^cSignificant difference between median concentrations at diagnosis and 3 months postoperatively.

^dSignificant difference between median concentrations at diagnosis and time of surgery.

^eSignificant difference between median concentrations at time of surgery and 3 months postoperatively.

^fSignificant difference between median concentrations at time of surgery and 1 month postoperatively.

ammonia concentration significantly decreased from diagnosis to 1 and to 3 months postoperatively ($P = .002$ and $P < .001$, respectively) and from surgery to 1 and to 3 months postoperatively ($P = .02$ and $P = .01$, respectively).

3.2 | Amino acids

Median plasma concentrations of the different amino acids at different time points are presented in Table 3.

For the AAAs, significant decreases were found in the plasma concentrations of phenylalanine and tyrosine within dogs from diagnosis to 3 months postoperatively ($P = .01$ and $P = .003$, respectively) and from surgery to 3 months postoperatively ($P < .001$ and $P = .001$, respectively). No significant changes were found in plasma tryptophan concentrations within dogs ($P = .67$).

Regarding BCAAs, plasma valine concentrations were the only concentrations that changed significantly within dogs, with a significant increase from surgery to 3 months postoperatively ($P = .04$).

The median BCAA-to-AAA ratios at diagnosis and surgery were 0.6 (range, 0.43-0.95) and 0.5 (range, 0.32-1.07), which were both significantly lower than the median BCAA-to-AAA ratio 3 months postoperatively (1.5; range, 0.58-2.24; $P = .004$ and $P < .001$, respectively). Friedman 2-way analysis determined that the BCAA-to-AAA ratio within dogs was significantly higher 3 months postoperatively compared to the BCAA-to-AAA ratio at diagnosis as well as at time of surgery ($P = .004$ and $P = .002$, respectively).

Concerning the remaining amino acids, a significant increase in plasma concentrations of both methionine and serine were found within dogs from diagnosis to surgery ($P = .02$ and $P = .01$, respectively), these plasma concentrations, however, significantly decreased again from surgery to 3 months postoperatively ($P = .02$ and $P < .001$, respectively). Plasma concentrations of arginine, citrulline, and trans-hydroxyproline significantly increased within dogs from diagnosis to

3 months postoperatively ($P = .02$, $P = .01$, and $P = .02$, respectively). Finally, plasma concentrations of glutamine, histidine and proline significantly decreased within dogs from surgery to 3 months postoperatively ($P = .01$, $P = .01$, and $P = .04$, respectively).

Aspartic acid was significantly higher at diagnosis in dogs <1 year of age compared to older dogs. No other statistical differences between dogs <1 year of age and older dogs were identified.

4 | DISCUSSION

Our study determined that the plasma concentrations of individual amino acids changed differently over time. The AAAs phenylalanine and tyrosine, but not tryptophan, were significantly higher at the time of diagnosis and surgery compared to 3 months postoperatively. The plasma concentration of valine, a BCAA, was significantly higher 3 months postoperatively compared to diagnosis and surgery. The BCAA-to-AAA ratio was extremely low at the time of diagnosis (0.6) and did not improve after a minimum of 4 weeks of medical treatment. Although the BCAA-to-AAA ratio significantly increased 3 months after gradual surgical attenuation, it was, at 1.5, still indicative of the presence of moderate to severe liver dysfunction.¹⁴ The plasma concentrations of arginine, citrulline and trans-hydroxyproline were significantly lower at time of diagnosis compared to 3 months postoperatively, whereas the plasma concentrations of glutamine, histidine, and proline were significantly lower 3 months postoperatively compared to the time of surgery.

Both BCAAs and AAAs are considered to be key factors in the pathogenesis of HE.²⁰ In contrast to other amino acids, BCAAs are primarily metabolized in muscle tissue,²¹ where they serve as precursors of other amino acids and as an energy substrate.²² In the presence of hyperammonemia, BCAA metabolism in muscle tissue changes and helps to detoxify ammonia by forming glutamine.^{20,23} Also in brain tissue, BCAAs are used as an alternative pathway to detoxify

TABLE 3 Median plasma concentrations of different serum amino acids at different sampling points (diagnosis, surgery, 3 months postoperatively) in dogs with closed congenital extrahepatic portosystemic shunts after surgical attenuation. Essential amino acids are depicted in italic. Ranges of amino acids in healthy dogs, published previously, are added

	Diagnosis nmol/mL (range)	Surgery ^a nmol/mL (range)	3 months postoperatively ^a nmol/mL (range)	Ranges in healthy dogs ^{18,19}
Aromatic amino acids				
<i>Phenylalanine</i>	115.3 (85.1-175.8)	137.2 (83.3-181.0)	49.5 ^{b,c} (41.2-70.5)	51.1-75.2
<i>Tryptophan</i>	57.1 (41.7-109.2)	50.5 (39.6-128.2)	48.0 (33.4-159.5)	35.7-94.8
Tyrosine	83.1 (49.9-127.3)	87.8 (60.5-129.8)	29.0 ^{b,c} (21.8-44.8)	32.9-81.5
Branched-chain amino acids				
<i>Isoleucine</i>	37.1 (22.2-79.2)	35.7 (26.2-59.2)	47.0 (32.3-74.4)	37.1-77.1
<i>Leucine</i>	74.7 (45.3-163.4)	71.9 (53.6-124.4)	98.3 (67.8-150.7)	61.4-187.5
<i>Valine</i>	101.0 (97.0-107.0)	87.7 (60.2-169.1)	133.9 ^c (83.2-202.2)	97.8-230.0
Other amino acids				
Alanine	271.3 (169.1-505.2)	363.3 (261.1-542.6)	371.0 (278.7-612.4)	353.6-726.5
Arginine	86.8 (47.1-99.9)	100.8 ^d (64.9-180.3)	119.7 (80.3-182.1)	69.9-158.9
Asparagine	55.3 (38.7-108.5)	60.0 (32.8-89.4)	50.0 (31.1-68.3)	37.2-78.3
Aspartic acid	2.7 (2.1-4.8)	3.1 (2.0-6.6)	3.2 (2.2-4.4)	NA
Citrulline	34.1 (10.7-100.8)	67.2 (19.1-156.0)	69.2 (33.9-126.2)	22.2-127.3
Glutamic acid	26.2 (13.5-44.2)	25.9 (16.3-48.6)	28.5 (11.9-44.4)	14.1-52.2
Glutamine	912.2 (565.0-1171.0)	888.9 (556.6-1489.7)	629.6 ^{b,c} (535.4-936.5)	557-1003.4
Glycine	190.4 (132.2-292.4)	226.9 (132.2-392.2)	215.7 (128.4-279.2)	145-335.3
<i>Histidine</i>	80.1 (53.3-105.3)	101.7 (65.5-128.4)	68.6 ^c (60.4-77.2)	67.7-95.3
<i>Lysine</i>	128.8 (47.0-243.0)	143.5 (44.6-250.7)	176.6 (82.9-244.3)	87.6-254.2
<i>Methionine</i>	42.8 (32.9-92.2)	62.5 ^d (55.4-89.0)	44.7 ^c (35.0-71.0)	51.0-83.6
Ornithine	15.0 (6.7-29.3)	18.8 (10.3-26.8)	14.8 (9.8-22.6)	7.3-31.2
Proline	124.6 (55.8-169.6)	162.7 (94.5-263.9)	121.0 (76.4-139.6)	70.5-376.7
Serine	151.2 (111.8-204.8)	226.2 ^d (144.3-278.0)	116.6 ^c (73.8-195.6)	59.1-222.9

(Continues)

TABLE 3 (Continued)

	Diagnosis nmol/mL (range)	Surgery ^a nmol/mL (range)	3 months postoperatively ^a nmol/mL (range)	Ranges in healthy dogs ^{18,19}
Threonine	76.4 (55.8-164.5)	103.0 (40.1-223.9)	138.1 (64.3-189.8)	147.8-444.8
Trans-hydroxyproline	13.6 (2.8-27.6)	16.4 (3.9-33.3)	22.2 (4.8-28.0)	NA

Abbreviations: EHPSS, extrahepatic portosystemic shunts; NA, not applicable.

^aOnly data after the second surgery are reported of the dog with 2 single EHPSS that underwent 2 surgeries.

^bSignificant difference between median plasma concentrations at diagnosis and 3 months postoperatively.

^cSignificant difference between median plasma concentrations at time of surgery and 3 months postoperatively.

^dSignificant difference between median plasma concentrations at diagnosis and time of surgery.

ammonia,²⁴ which becomes the main route of detoxification in case of malnutrition.²⁵ The glutamine that is formed, however, causes astrocyte swelling, which in turn can aggravate HE.²⁶ Previous experimental studies, in which portocaval shunts were created in adult mongrel dogs that subsequently developed HE, indeed found a decrease in plasma BCAA concentrations, which might be the consequence of increased BCAA metabolism secondary to hyperammonemia.^{11,12} Nevertheless, BCAA concentrations in cerebrospinal fluid remained stable.¹¹ Meanwhile, it was found that plasma AAA concentrations increased, and simultaneously, AAA concentrations in cerebrospinal fluid increased substantially.^{11,12} Increased concentrations of AAAs in cerebrospinal fluid can exacerbate HE by increasing the availability of precursors for neurotransmitters in the brain and by disturbing brain neurotransmission by promoting the synthesis of cerebral catecholamines and false neurotransmitters, such as phenylethanolamine and octopamine.²⁷ In a recent study in which amino acid profiles of dogs with congenital PSS, dogs with chronic hepatitis, and healthy dogs were analyzed, serum concentrations of BCAAs were significantly lower whereas serum concentrations of tyrosine and phenylalanine (both AAAs) were significantly higher, leading to a lower BCAA-to-AAA ratio in dogs with congenital PSS compared to healthy dogs and dogs with chronic hepatitis.¹³ Although both tyrosine and phenylalanine concentrations were very high at diagnosis in our study, all BCAAs were at the lower end of the reference range reported in dogs,^{18,19,28-30} which is consistent with previous studies.¹¹⁻¹³ Although valine significantly increased after surgical attenuation, it remained within normal limits. This observation suggests that changes in AAAs are more important than changes in BCAAs, both before and after surgery.

After starting medical treatment, clinical scores improved significantly, but, in contrast, fasting ammonia concentrations and the BCAA-to-AAA ratio did not. Medical treatment mainly aims at decreasing absorption of ammonia from the intestine on the one hand and by providing a highly digestible protein source on the other hand. This improvement was not entirely unexpected because clinical signs are influenced by several other substances and situations such as manganese and systemic inflammation.^{9,10} A previous experimental study in dogs concluded that an overall improved nutritional state is more important in improving clinical

signs than the proportions of amino acids that are present within the food.¹¹ In contrast to BCAAs, AAAs typically have a very high extraction ratio and are rapidly metabolized by the liver,³¹ which can explain why AAAs significantly decreased after surgical attenuation of the EHPSS. In our study, gradual attenuation devices were used to obtain EHPSS closure. Although ameroid constrictors are used for gradual attenuation, complete EHPSS closure can occur as early as 10 days postoperatively.³² Thin film banding typically is reported to cause slower EHPSS closure, which can take up to 8 weeks if the internal diameter of the vessel is decreased to <2.5 mm.³³ Although liver perfusion is normalized once the EHPSS is completely closed, it might take longer for liver function to completely normalize and to restore amino acid balance. Further research is needed to verify if the BCAA-to-AAA ratio will completely normalize in the long-term.

In our cohort of dogs, the installed medical treatment device did not improve the overall amino acid profile. This is an important finding, because some dogs with PSS require life-long treatment in case of persistent shunting after surgical attenuation or if owners prefer not to have the EHPSS treated surgically. Because the liver plays a critical role in the metabolism of most amino acids,³¹ it is important to identify which amino acid imbalances are present, in order to try and adjust the diet to compensate for these imbalances. Liver-supportive diets are diets specifically manufactured for dogs with liver disease. Liver diseases, however, are very diverse, and amino acid profiles found in dogs with different types of liver diseases vary markedly.^{18,28} Dogs with focal liver masses have higher plasma glutamic acid concentrations compared to age-matched healthy dogs regardless of whether the lesions were benign or hepatocellular carcinomas, and plasma glutamic acid concentrations were not significantly different 3 to 6 months after surgical resection of hepatocellular carcinomas.¹⁸ Dogs with superficial necrolytic dermatitis (also called hepatocutaneous syndrome) have mean plasma amino concentrations that are significantly lower than those of dogs with acute and chronic hepatitis, most likely because of increased hepatic catabolism.²⁸ In dogs with acute and chronic hepatitis, many plasma amino acid concentrations however are increased because of compromised hepatic metabolism.²⁸ The liver-supportive diet used in our study contains 16% protein in kibble, with the main ingredients being rice, corn, animal fat, soy protein and hydrolyzed animal

protein. In the canned food, 6.5% protein is present; the main ingredients are corn, rice, and poultry liver. In the dry food, methionine and lysine are supplemented; no amino acid supplements are added to the canned food.

After at least 4 weeks of medical treatment using a liver-supportive diet, only methionine and serine concentrations increased significantly. Surprisingly, both amino acid concentrations decreased again to the concentration observed initially at diagnosis, 3 months after surgical attenuation, despite any change in diet. Both methionine and serine have a high liver extraction ratio.³¹ The most likely explanation for this finding is the relatively high intake of both amino acids in the liver-supportive diet that could only be metabolized efficiently after surgical attenuation of the EHPSS. Methionine is an essential amino acid that is converted into S-adenosylmethionine in the liver, and has important physiological roles such as decreasing tissue oxidative stress.³⁴ In human medicine, controversy exist as to whether methionine supplementation is useful in patients with chronic liver disease.³⁵ Our findings suggest that the liver-supportive diet used might not be ideally balanced in terms of amino acids for dogs with portosystemic shunting. A double-blind cross-over study found that clinical signs of HE significantly improved in dogs with PSS that received a diet based on soy instead of meat protein.³⁵ Yet, the BCAA-to-AAA ratio of the soy-based diet was only 2.05 compared to 2.30 for the meat-based diet. Despite a lower BCAA-to-AAA ratio in the food, improved HE scores in that study were attributed to the fact that vegetable proteins are highly digestible leading to a higher absorption of amino acids in the small intestine and subsequently less nitrogen reaching the colon.³⁶ Blood amino acid concentrations were not examined in that study. The digestibility of amino acids in food is dependent not only on the protein source, but also on the way the food is processed and on the presence of other components in the food. It remains to be investigated how the composition of diets can influence blood amino acid profiles in dogs with impaired hepatic function.³⁷⁻³⁹

Our study had some limitations. Only a small number of dogs was included; different small dog breeds were represented and half of the dogs were young at the time of inclusion. In dogs, it has been shown that blood amino acid concentrations differ between small and large breeds.⁴⁰ Because only small breed dogs were included in the study, no influences are expected based on the breed. In people, it is well known that blood amino acid concentrations are age-specific, with histidine, arginine, cysteine, tyrosine and taurine being semi-essential amino acids in children because these are important in normal development and growth.^{41,42} Similar age-specific blood amino acid concentrations would be expected in dogs. Nevertheless, no age-specific differences were identified in our study between dogs with EHPSS <1 year of age and those that were older.

An age- and size-matched healthy control group could have been included to strengthen our findings. Blood amino acid concentrations in healthy dogs however vary among studies, with all studies describing relatively wide ranges.^{18,19,30} In addition, differences in reported normal values can be explained by the fact that most studies only included a small number of apparently healthy dogs that received

different types of food, and also by the fact that amino acids were analyzed using different laboratory techniques.

The fact that dogs were fed different foods at inclusion makes interpretation of concentrations obtained at diagnosis difficult. Nevertheless, our study clearly showed that even if dogs were subsequently placed on a standardized liver-supportive diet, no differences in the amino acid profile occurred, apart from the increases in methionine and serine concentrations. Therefore, the influence of diets the dogs were receiving before treatment did not seem to have much impact.

In accordance with previous studies, dogs with EHPSS had a marked decreased BCAA-to-AAA ratio. Medical treatment did not influence this ratio, notwithstanding the fact that clinical signs attributable to HE improved substantially. Despite the fact that the BCAA-to-AAA ratio increased significantly after surgery, it still indicated moderate to severe liver dysfunction in all dogs with closed EHPSS, suggesting that functional recovery requires more time than does clinical recovery.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Ethical approval: EC 2017/49; Deontological committee: 2017 N06.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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