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Authors: Heather R. Hoffmann, Justin R. Perrault, Carsten Bandt, Leonel Londoño, Charles A. Manire, et. al.

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PLASMA COLLOID OSMOTIC PRESSURE IN CHRONICALLY DEBILITATED LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*)

Heather R. Hoffmann, D.V.M., Justin R. Perrault, Ph.D., Carsten Bandt, D.V.M., Dipl. A.C.V.E.E.C., Leonel Londoño, D.V.M., Dipl. A.C.V.E.E.C., Charles A. Manire, D.V.M., Natalie H. Hall, D.V.M., Dipl. A.C.Z.M., Nicole Montgomery, C.V.T., and Nicole I. Stacy, D.V.M., Dr. Med.Vet., Dipl. A.C.V.P.

Abstract: Colloid osmotic pressure (COP) is an important component of the forces that manage capillary filtration and is determined by circulating plasma proteins. Patients affected by conditions resulting in hypoproteinemia often suffer severe hemodynamic derangements, including decreased COP. Because chronically debilitated sea turtles (CDT) present with severe hypoproteinemia, the objectives of this study were to 1) determine differences in plasma COP and blood analyte data (packed cell volume [PCV], sodium, chloride, plasma protein fractions) in CDT at admission compared with data from apparently healthy rehabilitated turtles at time of release (HRT) admitted from various stranding causes, and 2) to investigate correlations of COP with these selected blood analytes. COP, PCV, and most plasma protein fractions (excluding pre-albumin and γ-globulins) were significantly lower in CDT upon admission as compared with HRT. Sodium and chloride did not significantly differ between CDT and HRT. A significant increase was observed with PCV and all plasma protein fractions as COP increased. Of all protein fractions tested, albumin contributed the most toward COP ($r^2 = 0.88$, $P < 0.001$). The results of this study suggest that COP is significantly lower in CDT as compared with HRT, providing insight into the complexity of this critical clinical condition and a small step in advancing the understanding of associated hemodynamic imbalances. Although COP analysis is not readily available as a diagnostic test, this preliminary baseline data suggests that additional research studies are warranted, given the potential for optimization of fluid therapy during rehabilitation of CDT.

Key words: albumin, chronic debilitation, colloid osmotic pressure, loggerhead sea turtle, rehabilitation.

INTRODUCTION

Chronic debilitation is a frequent stranding cause in loggerhead sea turtles (*Caretta caretta*) worldwide. The pathophysiology of this condition is only partially understood and is presumably multifactorial or from any condition preventing food intake and/or digestion, leading to end-stage starvation.[9] Stranded chronic debilitated sea turtles (CDT) present with lethargy, emaciation, panhypoproteinemia, anemia, hypoglycemia, and heavy epibota coverage (e.g., barnacles, leeches) on the shell and skin. Traditionally, this condition is treated with various combinations of fluid therapy, antimicrobials, iron supplementation, blood transfusions, erythropoietin, and nutritional support.[9]

One essential goal in the treatment of CDT is the stabilization of hemodynamic imbalances resulting from anemia and hypoproteinemia. Colloid osmotic pressure (COP; i.e., oncotic pressure) is an important component of the forces that manage capillary filtration, as described in Starling's law.[16,17] Plasma COP is mainly determined by circulating plasma proteins and varies greatly among species, with limited availability of baseline data for many veterinary species in health.[6] The osmotic effects of proteins are measured with a colloid osmometer.[17] Various formulas have been used to estimate or calculate COP in humans; however, these methods are not recommended in veterinary medicine due to interspecies variability in plasma protein concentrations.[17] Albumin has been identified as the major contributor to COP in many taxa, including reptiles.[10,17] In humans, up to 75–80% of oncotic pressure is influenced by the albumin fraction, with the remaining 25% resulting from globulins.[5,6] The balance between hydraulic and COP gradients helps maintain blood volume in healthy animals, but alterations in these forces during disease can cause fluid to move into the interstitial space, resulting in edema.[17] A more recently revised Starling equation considers the contributions of the endothelial glycocalyx layer, the

From the Department of Comparative, Diagnostic, and Population Medicine (Hoffmann, Stacy), Department of Small Animal Clinical Sciences (Londoño), College of Veterinary Medicine, University of Florida, 2015 Southwest 16th Avenue, Gainesville, FL 32608, USA; Loggerhead Marinelife Center, 14200 US Highway 1, Juno Beach, FL 33408, USA (Perrault, Manire, Montgomery); Canada West Veterinary Specialists, 1988 Kootenay Street, Vancouver, BC V5M 4Y3, Canada (Bandt); and Disney's Animals, Science, and Environment Team, Disney's Animal Kingdom, 1200 North Savannah Circle East, Bay Lake, FL 32830, USA (Hall). Correspondence should be directed to Dr. Stacy (stacyn@ufl.edu).

endothelial basement membrane, and the extracellular matrix.[19] This research demonstrates that the COP difference across the endothelial glycocalyx layer opposes the filtration rate, but does not reverse it as presumed prior to this elementary discovery.[19] This concept is important for understanding the limitations of attempting to prevent or treat edema by administration of colloids.[19]

There is very little known about COP in reptiles. The first report on this topic from 1968 characterized plasma albumin of reptiles and confirmed that this protein is the main contributor of COP.[10] In humans, COP has been used to help form a prognosis in various critical conditions such as sepsis and shock, predict pulmonary edema and mortality in critically ill or septic patients, and indicate injury severity in trauma patients.[1,8,12,13] In other mammals, correlations between COP and albumin, total protein, and globulins have been documented.[7,11,18] There are substantial knowledge gaps regarding COP in reptiles, including species-specific variations, reference ranges in health, and changes in disease. However, understanding COP in health and disease in any species is of clinical importance for diagnosis, patient monitoring, guidance for treatment, and prognosis. Because the majority of CDT present with severe hypoproteinemia,[9] understanding the mechanisms of COP may contribute to understanding the pathophysiology of this condition.

The objectives of this study were to 1) determine differences in plasma COP and blood analyte data (packed cell volume [PCV], sodium, chloride, plasma protein fractions) in CDT at admission compared with data from healthy rehabilitated turtles at time of release (HRT) admitted from various stranding causes, and 2) to investigate correlations of COP with these selected blood analytes. We predicted that plasma COP and the measured blood analytes in CDT would be significantly lower compared with HRT and that correlations would exist between COP and other blood analytes, including PCV, sodium, chloride, and plasma protein fractions.

MATERIALS AND METHODS

Study animals

The study described herein is retrospective. To identify appropriate samples for this study, medical records from loggerhead sea turtles spanning a period of 3 yr were reviewed from Loggerhead Marinelife Center, a private nonprofit rehabilitation, research, education, and conservation facility (Juno Beach, FL 33408, USA). Sea turtle condition was determined by physical examination findings and supporting results of hematological and biochemical analyses. CDT were defined as turtles with emaciation, lethargy, heavy skin and shell epibiota coverage, and supportive clinical pathology findings of panhypoproteinemia, anemia, hypoglycemia, and/or hypocalcemia.[9] Blood samples from CDT were previously collected at admission and prior to administration of any medical treatment. In addition, HRT were initially admitted from various stranding causes (e.g., chronic debilitation, motorcraft injury, cold stunning, hook-line ingestion, or injury-trauma), successfully rehabilitated, and were identified to use as comparison with CDT. The HRT samples were collected at time of turtle release when any health issues due to stranding or admission were apparently resolved, turtles were off medications, and hematology and plasma chemistry data were within normal limits for a minimum of 4 wk for each turtle.

Sample collection and analysis

Blood samples were previously obtained from the external jugular vein (i.e., dorsal cervical sinus) by routine sampling and processing methods. Heparinized plasma was banked in an ultralow freezer at −80°C (American BioTech Supply, Salem, NH 03079, USA). PCV was determined at time of sampling using centrifugation at 12,000 *g* for 5 min (Mobilespin, Vulcan Technologies, Grandview, MO 64030, USA), as previously described.[6] Archived plasma was analyzed for protein electrophoresis at the University of Miami Avian and Wildlife Laboratory (Miami, FL 33101, USA) using SPEP-II agarose gels and the Beckman paragon electrophoresis system (Beckman-Coulter Corporation, Brea, CA 92821, USA). Total protein was quantified by biuret method (Kodak 750 X R, Ortho Clinical Diagnostics, Rochester, NY 14626, USA) and protein electrophoresis gels were run as described previously.[4] The percentage of protein fractions was quantified by laser densitometry and then each fraction value was calculated by multiplying the percentage of the fraction by the total protein concentration. Sodium and chloride were also determined using the Kodak 750 X R. COP data of archived plasma samples were obtained using a benchtop commercial colloid osmometer (Osmomat 050; Gonotec, 10553 Berlin, Germany) according to manufacturer's instructions.

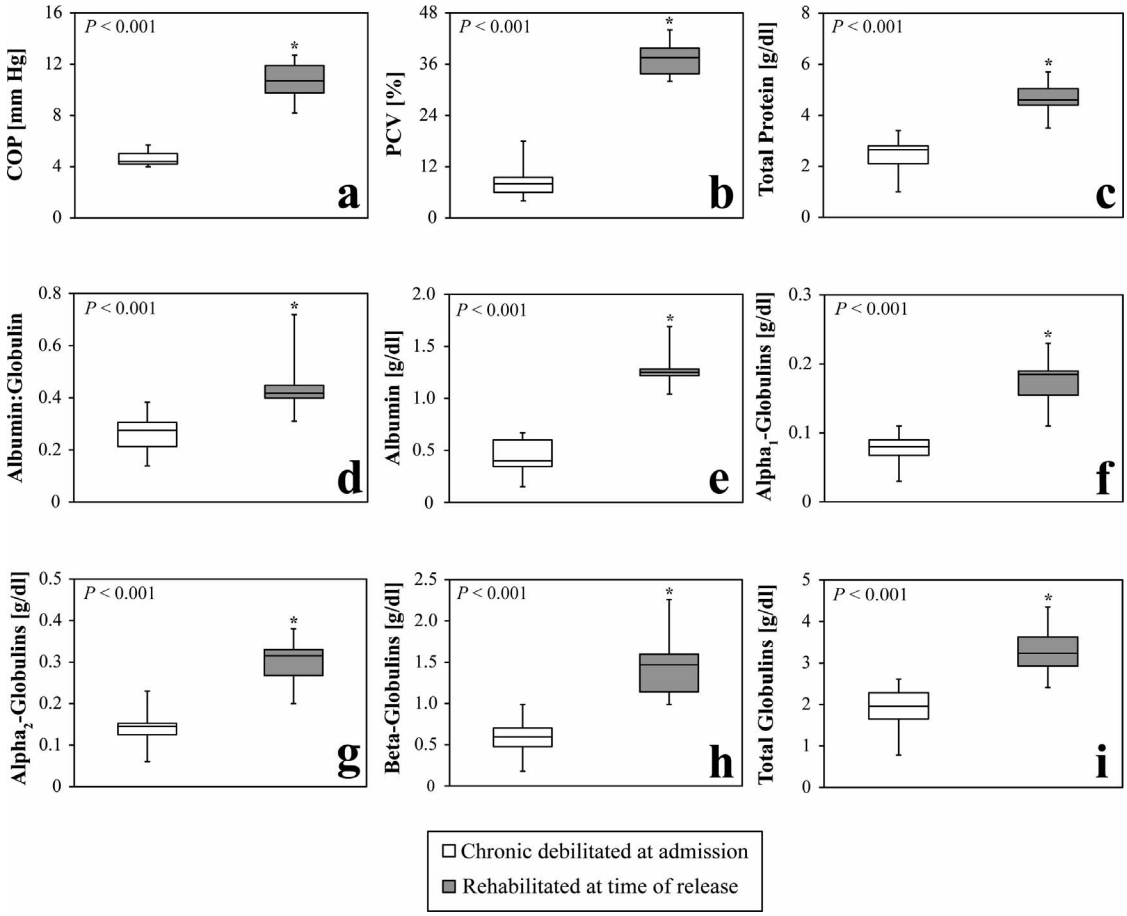

Figure 1. Results of significant Mann–Whitney *U* tests comparing colloid osmotic pressure (COP; **a**), packed cell volume (PCV; **b**), and protein fractions (total protein; **c**), albumin : globulin (**d**), albumin (**e**), α_1-globulins (**f**), α_2-globulins (**g**), β-globulins (**h**), total globulins (**i**) determined by protein electrophoresis between chronically debilitated turtles upon admission (white) and healthy rehabilitated turtles at release (gray). The stars above the box-plots indicate that the values were significantly different at $P < 0.05$. *P* values are shown. The middle lines in the box of the box and whisker plots represent the median, the outer lines of the boxes represent the first and third quartiles, and the whiskers represent values that fall outside of the first and third quartiles, with minimum and maximum values at the end of each whisker.

Statistical analysis

Statistical analyses were performed using a commercial software package (IBM SPSS Statistics 24 software, SPSS, Inc, Chicago, IL 60606, USA). Mean, standard deviation, standard error, median, and range were reported for COP and measured blood analytes for CDT upon admission and for HRT. Non-parametric Mann–Whitney *U* tests were selected to compare COP and blood analytes between CDT and HRT as the majority of the data did not meet the assumptions of normality using the Shapiro–Wilk statistic. All *P* values were adjusted using the Bonferroni correction ($P = 0.05/13$ analytes $= 0.0039$). Additionally, regression analyses were appropriately fitted to the trends (polynomial or exponential) and used to determine if COP was significantly related to the measured blood analytes (PCV, sodium, chloride, plasma protein fractions). Data were transformed when necessary to meet the assumptions of regression analyses. For those analytes where the residuals of the regression could not be normalized, nonparametric Spearman correlations were used to determine relationships between COP and the measured analytes.

Table 1. Colloid osmotic pressure (COP), packed cell volume (PCV), and protein fractions by plasma protein electrophoresis in plasma from chronically debilitated loggerhead sea turtles upon admission (CDT; $n = 12$; white rows) and from healthy rehabilitated turtles after recovery from various stranding causes at time of release (HRT; $n = 10$; grey rows).

Analyte	Time	Mean	SE	SD	Median	Min	Max
COP (mm Hg)	CDT	4.7	0.2	0.6	4.4[A]	4.0	5.7
	HRT	10.7	0.5	1.5	10.7[A]	8.2	12.7
PCV (%)	CDT	9	1	4	8[A]	4	18
	HRT	37	1	4	38[A]	32	44
Sodium (mM/L)	CDT	149	1	4	150	143	157
	HRT	148	1	2	148	144	150
Chloride (mM/L)	CDT	112	1	5	111	107	120
	HRT	115	1	4	116	109	120
Total protein (g/dl)	CDT	2.5	0.2	0.6	2.7[A]	1.0	3.4
	HRT	4.7	0.2	0.6	4.6[A]	3.5	5.7
Albumin : globulin ratio	CDT	0.26	0.02	0.07	0.28[A]	0.14	0.38
	HRT	0.44	0.03	0.11	0.42[A]	0.31	0.72
Pre-albumin (g/dl)	CDT	0.07	0.01	0.02	0.06	0.05	0.11
	HRT	0.12	0.02	0.05	0.12	0.05	0.19
Albumin (g/dl)	CDT	0.44	0.05	0.16	0.40[A]	0.15	0.67
	HRT	1.27	0.05	0.17	1.25[A]	1.04	1.69
α-1 globulins (g/dl)	CDT	0.08	0.01	0.02	0.08[A]	0.03	0.11
	HRT	0.18	0.01	0.03	0.19[A]	0.11	0.23
α-2 globulins (g/dl)	CDT	0.14	0.01	0.04	0.15[A]	0.06	0.23
	HRT	0.30	0.02	0.05	0.32[A]	0.20	0.38
β-globulins (g/dl)	CDT	0.60	0.06	0.20	0.60[A]	0.18	0.99
	HRT	1.46	0.12	0.40	1.47[A]	0.99	2.26
γ-globulins (g/dl)	CDT	1.11	0.08	0.29	1.02	0.51	1.58
	HRT	1.33	0.13	0.42	1.21	0.92	2.07
Total globulins (g/dl)	CDT	1.93	0.14	0.48	1.96[A]	0.78	2.61
	HRT	3.27	0.18	0.58	3.24[A]	2.41	4.35

[A] Indicates significant difference between both groups ($P < 0.0039$).

RESULTS

Nonhemolytic, clear plasma samples from 21 loggerhead sea turtles were included in the study, of which 12 samples were from CDT and 10 samples were from HRT.

COP ($U = 120$; $P < 0.001$), PCV ($U = 120$; $P < 0.001$), total protein ($U = 120$; $P < 0.001$), A : G ratio ($U = 116$; $P < 0.001$), albumin ($U = 120$; $P < 0.001$), α_1-globulins ($U = 119$; $P < 0.001$), α_2-globulins ($U = 118.5$; $P < 0.001$), β-globulins ($U = 119.5$; $P < 0.001$), and total globulins ($U = 118$; $P < 0.001$) were all significantly lower in CDT at time of admission compared with RHT at the time of release (Fig. 1; Table 1). Sodium, chloride, pre-albumin, and γ-globulins did not significantly differ between admission and release time points ($P > 0.0039$). COP significantly increased with increasing PCV (polynomial regression; $r^2 = 0.87$; $P < 0.001$), total protein (polynomial regression; $r^2 = 0.91$; $P < 0.001$), A : G ratio (polynomial regression; $r^2 = 0.44$; $P = 0.004$), pre-albumin (polynomial regression; $r^2 = 0.39$; $P = 0.009$), albumin (polynomial regression; $r^2 = 0.88$; $P < 0.001$), α_1-globulins (exponential regression; $r^2 = 0.76$; $P < 0.001$), α_2-globulins (exponential regression; $r^2 = 0.75$; $P < 0.001$), β-globulins (polynomial regression; $r^2 = 0.69$; $P < 0.001$), γ-globulins (polynomial regression; $r^2 = 0.29$; $P = 0.038$), and total globulins (polynomial regression; $r^2 = 0.76$; $P < 0.001$) (Fig. 2). Albumin contributed the most toward COP, as indicated by the highest coefficient of variation ($r^2 = 0.88$).

DISCUSSION

This study provides valuable baseline information for plasma COP in sea turtles and includes an investigation of COP in loggerhead sea turtles affected by chronic debilitation in comparison with apparently healthy, successfully rehabilitated loggerhead sea turtles. Similar to humans, other mammals, and some reptile species, we identified albumin as the major macromolecule contributing to COP in sea turtles. In contrast to humans (mean ± SD COP: 21.6 ± 2.6 mm Hg) and mammals (example Camelidae, median: 24.6 mm

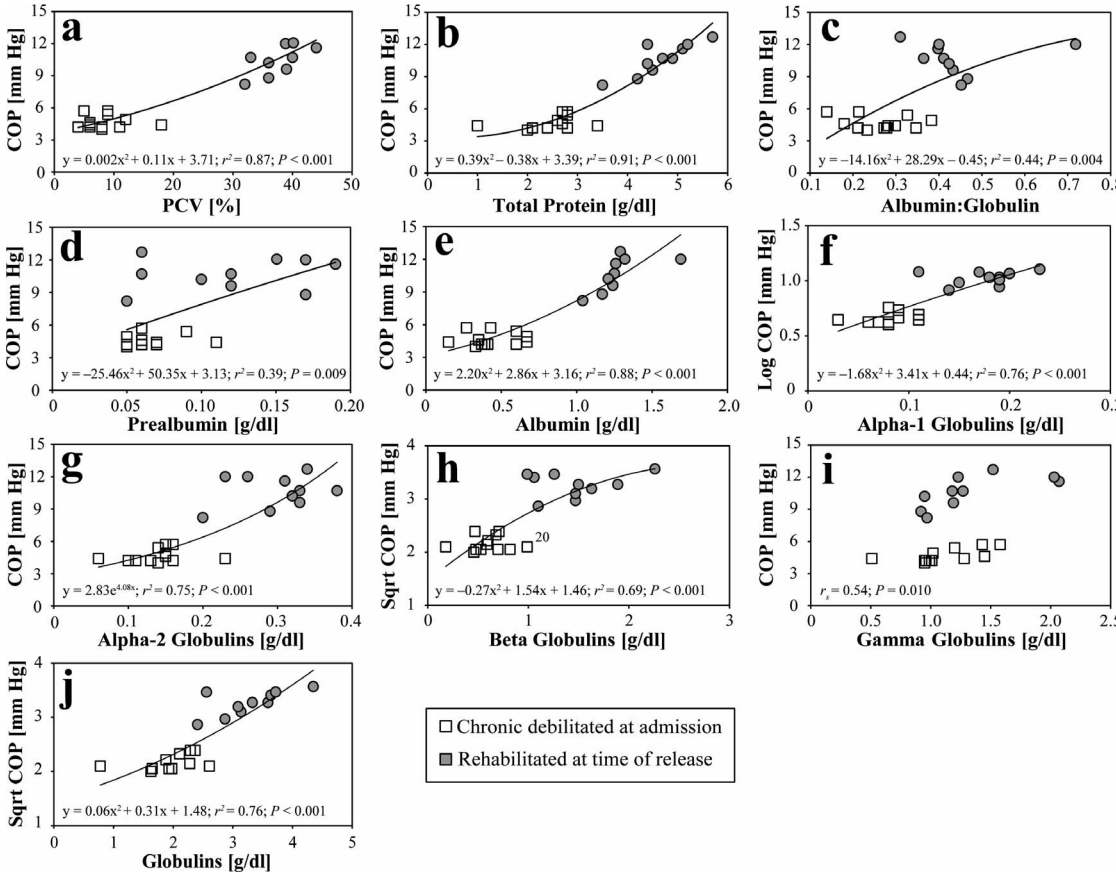

Figure 2. Results of significant linear regressions (or Spearman correlations) between colloid osmotic pressure (COP), packed cell volume (PCV, **a**), and protein fractions (total protein, **b**; albumin : globulin, **c**; prealbumin, **d**; albumin, **e**; α_1-globulins, **f**; α_2-globulins, **g**; β-globulins, **h**; γ-globulins, **i**; globulins, **j**) determined by protein electrophoresis. Chronically debilitated turtles upon admission are represented by white squares, whereas gray circles represent healthy rehabilitated turtles at release. The equations of the lines-of-best-fit, r^2 values, and P values are shown. For γ-globulins, the residuals could not be normalized, and the r_s and P values are shown. A number of values had to be transformed to meet the assumptions of normality of a regression test; those are indicated on the axes when necessary.

Hg; range: 19.3–28.1 mm Hg), COP in rehabilitated loggerhead sea turtles is comparatively lower (median: 10.7 mm Hg; range: 8.2–12.7 mm Hg).[1,3,8,11,13] Previous studies in reptiles have postulated that the inherent properties of albumin and its comparatively lower plasma concentrations in reptiles may explain the presence of coelomic fluid that often occurs in healthy freshwater turtles. This phenomenon may be a natural adaptation to diving in freshwater turtles, with the high amount of bicarbonate in the fluid acting as a buffer against anaerobic processes during lengthy dives.[10] There may be a similar protective mechanism of coelomic fluid in sea turtles as in freshwater turtles, but there is no information available on the chemical composition and amount of coelomic fluid in healthy sea turtles to date. However, small changes in COP appear to be of clinical relevance, as described in a study of human trauma patients (mean COP: 17.7 mm Hg) compared with healthy controls (mean COP: 120.7 mmHg).[13] In this study, COP of CDT was less than half (mean: 4.7 mm Hg) of HRT (mean: 10.7 mm Hg). This difference demonstrates the presence of hemodynamic imbalances (e.g., hypovolemia) in sea turtles affected by chronic debilitation and explains the observation of transudative effusions with this condition as described by Manire et al.[9]

The importance of albumin as the main contributor to the maintenance of COP within blood vessels in loggerhead sea turtles is emphasized by

the results of this study. Although it is well documented that panhypoproteinemia, which includes hypoalbuminemia, is a frequent observation in CDT, the clinical relevance and consequences for treatment are not fully understood to date. Chronic debilitation represents an end-stage condition of starvation, with the initial causes including any reason that prevents a sea turtle from eating or absorbing nutrients.[9] The observed severe panhypoproteinemia in these patients is considered the result from a combination of reduced protein absorption, reduced production, and/or increased protein loss.[15] In addition, increased vascular permeability from systemic inflammation also may contribute to extravasation of fluid and plasma proteins into the interstitial space. The lack of a difference between γ-globulins in CDT and HRT may suggest a similar mechanism in turtles as described in mammals in that an increased synthesis of immunoglobulins is attempted to counteract a decreased COP.[17] In addition, a systemic immune response with antibody production may result from antigens entering the portal blood and consequently gaining access to the systemic circulation due to reduced removal by the liver during hepatic insufficiency.[17]

Although plasma sodium and chloride are closely tied with COP in order to achieve electroneutrality across membranes through the Gibbs–Donnan equilibrium, there was no significant difference in debilitated and rehabilitated turtles for both ions. This may be associated with changes in plasma electrolytes and osmolality in CDT from a variety of potential causes, because major electrolyte-regulating organs such as intestines, kidneys, and/or salt glands may be variably affected. Therefore, changes in electrolytes may be complex in CDT and measurement of osmolality may further the understanding of underlying mechanisms considering that electrolytes contribute a notable portion to COP through the Gibbs–Donnan equilibrium, i.e., close to less than half of plasma proteins in mammals.[17]

The limited number of study turtles presents a major limitation of this study with regard to relevance of conclusions. Therefore, this study should be considered preliminary baseline information on the topic of COP in sea turtles. Despite the low number of study animals and thus the limitation of interpretation of results, our results demonstrate the clinical relevance of COP and suggest future use of COP as a research tool, for instance for studies on patient monitoring and guidance for treatment. The recent paradigm shift in small animal emergency critical care medicine has resulted in decreasing use of colloids in favor of a combination of crystalloid and enteral nutritional therapy to support endothelial health and to restore hemodynamic stability.[2,14] Further studies are required to investigate the applicability of this approach in reptile critical care medicine and whether fluid treatment modalities may need to be reconsidered.

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