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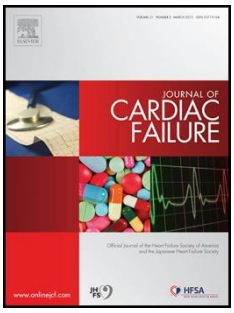

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Increased Secondary/Primary Bile Acid-ratio in Chronic Heart Failure

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Highlights

- In this cross sectional study, we find decreased serum levels of primary bile acids and increased levels of specific secondary bile acids, resulting in increased ratio of secondary to primary bile acids in patients with chronic heart failure.
- The ratio of secondary to primary bile acids was associated with reduced overall survival in univariate analysis, but not in multivariate analyses.
- Although the production of secondary bile acids depends on the gut microbiota, several factors affecting the production, regulation and elimination of bile acids could contribute to these findings.
- Future studies should assess the potential role of bile acid composition in patients with heart failure, and investigate whether manipulation of factors influencing bile acid metabolism, including the intestinal microbial community, could alter clinical outcome.

Abstract

Aims: Bile acids (BAs) are now recognized as signaling molecules and emerging evidence suggests that BAs affect cardiovascular function. The gut microbiota has recently been linked to the severity of heart failure (HF), and microbial metabolism has a major impact on BA homeostasis. We aimed to investigate the pattern of BAs, and in particular *microbiota-transformed* (secondary) BAs, in patients with chronic HF.

Methods and Results: This was a prospective, observational, single-center study including 142 patients with chronic HF and 20 age- and sex-matched healthy control subjects. We measured plasma levels of primary, secondary and total BAs, and explored their associations with clinical characteristics and survival. Plasma levels of primary BAs were lower ($p<0.01$) and the ratio of secondary to primary BAs was higher ($p<0.001$) in patients with HF compared to controls. Approximately 40% of patients in the upper tertile of the ratio of secondary to primary BAs died during 5.6 years of follow-up (unadjusted Cox-regression:

HR 1.93, 95% Confidence Interval 1.01-3.68 compared to the lower tertiles). However, this association was attenuated and no longer significant in multivariate analyses.

Conclusions: Levels of primary BAs were reduced and specific secondary BAs increased in patients with chronic HF. This pattern was associated with reduced overall survival in univariate analysis, but not in multivariate analyses. Future studies should assess the regulation and potential role of BA metabolism in HF.

Key words: Chronic Heart Failure; Gut Microbiota; Bile Acids Profile; Clinical Outcome

List of Abbreviations and Definitions of Terms

Abbreviation or special term	Explanation
HF	Heart failure
BAs	Bile acids
NYHA	New York Heart Association
CAD	Coronary artery disease
DCM	Dilated cardiomyopathy
LVEF	Left ventricular ejection fraction
CDC	Chenodeoxycholic acid
GCDC	Glycine conjugate of chenodeoxycholic acid
TCDC	Taurine conjugate of chenodeoxycholic acid

CA	Cholic acid
GCA	Glycine conjugate of cholic acid
DC	Deoxycholic acid
LC	Lithocholic acid
GLC	Glycine conjugate of lithocolic acid
TLC	Taurine conjugate of lithocolic acid
UDC	Ursodeoxycholic acid
GUDC	Glycine conjugate of ursodeoxycholic acid
TMAO	Trimethylamine-N-oxide
LPS	Lipopolysaccharide

Introduction

Heart failure (HF) is a major public health issue with increasing prevalence due to an aging population and improved management of the disease [1]. However, the mortality and morbidity of HF are still high, at least partly reflecting the possibility that important pathogenic mechanisms are not targeted by the current therapeutic options [2]. Metabolic and inflammatory disturbances have been suggested to play a role in the development and progression of chronic HF [3], but these issues remain far from clear.

Research over the last decades has expanded the traditional view of bile acids (BAs) beyond their role in cholesterol elimination and emulsification of dietary fat. BAs are now recognized as signaling molecules that interact with both plasma membrane and nuclear receptors, exerting regulatory effects on glucose and lipid metabolism [4], energy homeostasis [5] and other physiological processes [6]. We have recently reported decreased ratio of 12α-hydroxylated/non 12α-hydroxylated BA six months after bariatric surgery [7], associated with an improvement in insulin sensitivity and downregulation of inflammatory responses [8]. Emerging evidence suggests that BAs may also play a role in regulating cardiovascular function. In the heart and systemic circulation, BAs interact with plasma membrane G-protein coupled receptors, e.g. TGR5, and nuclear receptors, e.g. the farnesoid (FXR) and pregnane/steroid and xenobiotic receptors (PXR/SXR) (9). It has been suggested that BAs reduce heart rate by regulating channel conductance and calcium dynamics in sino-atrial and ventricular cardiomyocytes, and regulate vascular tone via both endothelium-dependent and -independent mechanisms [10]. Hence, a potential role of BAs in cardiovascular disease has been proposed.

The gastrointestinal tract contains a dynamic microbial community, denoted the gut microbiota. Altered gut microbiota has been linked to diseases associated with chronic

systemic inflammation and metabolic disturbances such as obesity, type 2 diabetes mellitus [11] and cardiovascular disease [12]. Of note, BA metabolism is tightly connected with the gut microbiota. *Primary* BAs are synthesized in the liver from cholesterol through the classic and alternative pathway, involving a variety of different enzymes [13]. Before excretion into bile, primary BAs are conjugated with either glycine or taurine, rendering the BAs water soluble and permitting high concentrations to persist in bile and intestinal content [14]. In the gut, primary BAs undergo metabolism to *secondary* BAs by the gut microbiota [15] before reabsorption as a part of the enterohepatic cycle. Thus, secondary BAs may also be considered *microbiota-transformed* BAs.

At present, data on BAs in HF patients are lacking. Given the proposed involvement of intestinal dysfunction, microbiota alterations and BAs in cardiovascular diseases[16], we aimed to investigate the regulation of BAs in patients with chronic HF and their association with disease severity including mortality.

Material and Methods

Patients

The study population has previously been described in details [17]. In brief, a total of 142 patients with HF for >6 months, stabilized on state-of-art treatment for at least 3 months, were consecutively included in a prospective cohort study at the Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway (Table 1). These patients had reduced systolic function and were in New York Heart Association (NYHA) functional class II–IV. Patients with acute coronary syndromes during the last six months and patients with significant concomitant disease such as infection, malignancy or autoimmune disease were excluded. The patients were evaluated by echocardiography. The underlying cause of HF was classified as coronary artery disease (CAD, n=66), dilated cardiomyopathy (DCM, n=70) or other (n=6) on the basis of patient history, echocardiography and coronary angiography. We also included 20 sex- and age–matched healthy individuals. The study was approved by the by the Regional Committee for Medical and Health Research Ethics (REK Sør-Øst) and conducted according to the Declaration of Helsinki. Informed consent was obtained from all individuals.

Blood sampling and biochemistry

For measurement of primary BAs, secondary BAs and their conjugated (glycine and taurine) fractions, we collected peripheral venous blood in pyrogen-free tubes with EDTA as anticoagulant. The samples were non-fasting, drawn after a light, standard Norwegian breakfast. The tubes were immediately immersed in melting ice and centrifuged within 30 minutes at 2,000*g* for 20 minutes to obtain platelet-poor plasma. All samples were stored at -80ºC until analysis and thawed <three times. BAs analyses were performed using liquid chromatography tandem mass spectrometry (LC-MS/MS) by an established in-house assay [18].

C-Reactive protein (CRP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were analyzed with the use of the Modular platform (Roche Diagnostics, Basel, Switzerland). Estimated glomerular filtration rate (eGFR) was calculated based on the Cockcroft–Gault formula. Plasma levels of LPS were measured with the limulus amoebocyte lysate assay (Lonza, Walkersville, MD, USA).

Right-sided heart catheterization

Right-sided heart catheterization was performed using a Swan–Ganz pulmonary artery thermodilution catheter (Baxter Health Care Corp, Santa Ana, CA, USA). Right heart haemodynamics were recorded, and cardiac output was measured using the thermodilution technique.

Echocardiography

Echocardiography was performed using Vivid 7 or E9 ultrasound scanners (GE Vingmed Ultrasound, Horten, Norway), with phased array transducers. Cine loops were digitally stored and analyzed offline using ECHO-PAC software (GE Vingmed Ultrasound). Two-dimensional parameters and conventional Doppler measurements were recorded according to current recommendations. Left ventricular ejection fraction (LVEF) was measured using Simpson's biplane method.

Statistical analysis

Differences between groups were analyzed with the use of Student t tests, Mann-Whitney U tests, or chi-square tests as appropriate. When comparing ≥3 groups, the Kruskal-Wallis test or 1-way analysis of variance (ANOVA) was used for continuous data, depending on distribution (Kolmogorov-Smirnov test). Correlations were analyzed with Pearson`s r for BA-ratio, whereas individual BAs and sum of primary and secondary BAs were analyzed by

Spearman`s Rho due to their skewed distribution. Kaplan-Meier analysis with log rank test was used to analyze all-cause/anticipated mortality stratified by tertiles of the secondary/primary BA ratio. A linear regression model was used to adjust differences in secondary/primary BA ratio between patients and controls. Cox proportional hazard analysis was performed to estimate hazard ratios (HRs) adjusting for age, renal function and NT-pro B-Type Natriuretic Peptide (NT-proBNP). *P* values are 2-sided and were considered significant when <.05. In this exploratory study, adjustments for multiple comparisons were not performed.

Results

Clinical characteristics

Clinical characteristics are provided in Table 1. The healthy control subjects were matched for age and gender, and had no previous MI, hypertension or diabetes, or concurrent medication. There were significantly higher eGFR measurements and fewer smokers among the controls. Furthermore, there was a tendency to higher BMI in HF patients, although it should be noted that BMI was available from only half the control subjects.

Altered bile acid composition in HF patients

As shown in Figure 1A and Supplementary Table, levels of primary BAs were significantly lower in patients with HF than in controls. This difference was mainly driven by lower levels of chenodeoxycholic acid (CDC) and its glycine (GCDC) and taurine (TCDC) conjugates, as well as the glycine conjugate of cholic acid (GCA) in patients with HF.

In the gut, glycine-conjugated and taurine-conjugated BAs are deconjugated by bacterial bile salt hydrolases. Primary BAs are subsequently dehydroxylated to secondary BAs by bacterial 7-alpha-dehydroxylases (i.e., CA is converted to deoxycholic acid (DC) and CDC to lithocholic acid [LC]). Reabsorbed BAs are further conjugated to glycine and taurine in the liver. Notably, in parallel with the observed reduction in GCDC (and numerically also CDC and TCDC), there was an increase in LC (p=0.001) and its conjugates GLC (p<0.001) and TLC (p<0.001) in patients with chronic HF. Finally, the glycine conjugate of ursodeoxycholic acid (GUDC) was also increased (p=0.001) in these patients (Figure 1A).

In contrast, the ratio of 12α-hydroxylated BAs to non-12α-hydroxylated BAs (i.e. the 12α-hydroxylated BAs [CA, deoxycholic acid (DC) and their conjugated forms]/the non-12α-hydroxylated BA [chenodeoxycholic acid (CDC), lithocholic acid (LC), UDC and their

conjugated forms) was similar in HF patients (ratio 0.69 [0.31, 1.06]) and controls (ratio 0.68 [0.42, 1.18] p=0.74).

Increased secondary/primary BA-ratio in chronic heart failure patients

Our findings so far suggested that in HF, there were decreased levels of primary BAs and increased levels of specific secondary BAs. Indeed, the ratio of secondary to primary BAs was significantly higher in patients with HF than in healthy control subjects (Figure 1B). Adjusting for eGFR and current smoking in a linear regression model, the difference in BA-ratio between HF patients and controls was still significant ($p<0.001$).

There was a higher proportion of patients with hypertension and use of β-blockers with higher tertiles of the secondary/primary BA-ratio, but this ratio was not associated with other baseline characteristics outlined in Table 1 such as age, renal function, New York Heart Association (NYHA) class, LVEF, NT-proBNP or CRP.

Association between the individual BAs and NT-proBNP as well as potential determinants including renal function, liver function, glycemic control and albumin are presented in Table 2. NT-proBNP correlated positively with the primary BAs TCDC and TCA and negatively with the secondary BA DC, but not with total primary or secondary BAs. The secondary BA LC was negatively correlated with eGFR. The primary BAs GCA and TCA, as well as total primary BAs and the secondary BA GUDC were correlated with ALT. None of the BAs were associated with HbA1c. Finally, the taurine-conjugated primary BAs TCDC and TCA were negatively correlated with albumin.

Association with pulmonary artery pressure and plasma lipopolysaccharide

Intracardiac pressures obtained from right heart catheterization had a moderate negative correlation with the secondary/primary BA-ratio (right atrium pressure, *r= -0.27, p= 0.01*; pulmonary artery mean pressure, *r= -0.25, p=0.024;* pulmonary capillary wedge pressure, *r= -0.27, p=0.012*), whereas no correlations were found with cardiac output (*r=0.13, p=0.228)* or pulmonary vascular resistance (*r= -0.12, p= 0.288)*. Plasma levels of lipopolysaccharide had a moderate association with both secondary BAs (*r=0.18, p= 0.033*) and secondary/primary BA-ratio (*r= 0.18, p= 0.031*).

Association with clinical outcome

During a median follow-up of 5.6 years (range 0.1 – 7.6 years), 37 patients died. None of the primary or secondary BAs *per se* were associated with mortality in our HF patients (data not shown). However, when we compared patients across tertiles of secondary/primary BAs, we found a trend (*p*=0.10) for decreased survival with increasing ratio.

As depicted in Figure 2, approximately 40% of patients in the upper tertile of secondary/primary BA-ratio died during follow- up (unadjusted Cox-regression: Hazard ratio (HR) 1.93 [95% Confidence Interval 1.01-3.68], p=0.041). However, this association was attenuated and no longer significant after adjustment for age, renal function, hypertension, use of beta-blockers and NT-pro-BNP (adjusted HR 1.83 [95% CI 0.91-3.66], *p*=0.089).

Discussion

The main findings in this study can be summarized as follows: i) Levels of primary BAs were lower and levels of specific secondary BAs were higher in patients with HF than in healthy control subjects, resulting in an increased secondary to primary BA ratio in HF, ii) an increased ratio of secondary to primary BAs tended to be associated with reduced overall survival on univariate analysis, but this association was not significant after adjustment for clinical characteristics and NT-proBNP.

BAs have been shown to have effects on the cardiovascular system including interaction with endothelial function, heart rate and various metabolic pathways at least partly involving signaling through nuclear and plasma membrane receptors [10]. Recently, the effect of ursodeoxycholic acid (UDC), a BA used in patients with cholestatic liver diseases, was investigated in 16 patients with HF, showing improved markers of liver function as well as improved peripheral blood flow [19]. However, data on the composition and homeostasis of BAs in HF patients are scarce.

The present study is, to the best of our knowledge, the first to assess a wide range of BAs in HF patients, observing reduced levels of multiple primary BAs in HF patients compared with healthy controls, while several of the secondary BAs, were higher. Although the formation of secondary BAs is dependent on transformation of primary BAs by intestinal bacteria[15], several factors could influence the secondary to primary BA ratio. In this context, one could argue that our observations are possibly just an epiphenomenon of cardiac congestion that affects patients with HF. However, data obtained from right heart catheterization of these patients showed a modest negative correlation with pulmonary artery pressures, refuting this hypothesis. Furthermore, there was a modest, but positive correlation between plasma levels

of secondary BAs and lipopolysaccharide in the patients, suggesting that an increased microbial activity could contribute to some of the observations in this study.

Although an increased ratio of secondary to primary BAs tended to be associated with reduced overall survival in univariate analysis, this association was no longer significant after adjustment for NT-proBNP. The negative association between the BA-ratio and pulmonary artery pressures further weakens the argument that this ratio has an independent prognostic value in HF. However, it should be noted that the current study was an exploratory rather than a prognostic study, and larger, independent samples are needed to evaluate secondary to primary BAs in relation to clinical end points.

Individual BAs have highly variable agonist activity and affinity to the proposed target receptors in the heart [20] and a reasonable hypothesis is therefore that specific BAs could be more distinctly associated with outcome. Notably, none of the primary or secondary BAs alone were associated with reduced survival, suggesting that the metabolism of BAs, rather than BAs *per se* may play a role in chronic HF. The levels of circulating BAs, however, are low and subject to large inter-individual variation [21], and given the moderate sample size of our study, the low number of clinical events and the single time-point blood draw, there is therefore a risk of underestimating the potential effect of individual BAs. In particular, the potent effect of the secondary bile acid LC (elevated in HF) on the bile acid receptor TGR5, expressed in the heart, could have physiological consequences not detected in the present study [22]. On the other hand, it was recently reported from a mouse model that an elevated level of total serum BA could contribute to liver-associated cardiac dysfunction, possibly due to impaired cardiac fatty acid oxidation, suggesting the involvement of the total BA pool, rather than individual BAs in HF[23].

In addition to gut microbiota dependent conversion from primary to secondary BAs, other factors influencing the production, metabolism and clearance of individual BAs could have affected the secondary/primary BA ratio. First, it should be noted that this ratio was driven by higher levels of some secondary BAs, and lower levels of some primary BAs, and does not necessarily reflect an increased global transformation from primary to secondary BAs in HF. Although dietary habits of the study population were not controlled for, we observed that the taurin conjugated primary BAs TCDC and TCA were negatively correlated with albumin. Furthermore, the primary BAs GCA and TCA, as well as total primary BAs were correlated with ALT. Hence, protein malnutrition and liver congestion could both could be associated with higher levels of primary BAs in some patients, which could have reduced rather than amplified the secondary/primary BA ratio in HF patients. Furthermore, although BAs may interact with glucose metabolism and energy homeostasis [4,5], none of the individual BAs were associated with glycemic control as measured by HbA1c. Finally, with the exception of a negative correlation between the secondary BA LC and eGFR, levels of BAs did not seem to be influenced by renal clearance, and the difference in BA ratio between HF patients and controls remained significant after adjustment for eGFR and current smoking.

Factors influencing the gut microbiota could also have affected our results. Although none of the patients used antibiotics at the time of blood collection, we do not have data on the use of antibiotics in the period before hospitalization, and these medications can perturb the gut microbiota, resulting in failure of the BA dehydroxylation necessary for the formation of secondary BAs. Another limitation is lack of BMIs in half of the control subjects, although the BA ratio was not associated with BMI in the HF patients. Moreover, relatively few patients were included and the number of fatal events during follow-up was also low. Finally,

this is a purely descriptive study and at present no conclusion can be drawn on causes and consequences of the disturbed BA metabolism in HF.

In conclusion, levels of primary BAs were reduced and specific secondary BAs increased in patients with chronic HF. Although this pattern was associated with reduced overall survival in univariate analysis, this association was lost in multivariate analyses. Future studies should thoroughly assess the potential role of the BA composition in patients with HF, and investigate whether manipulation of factors influencing BA metabolism, including the intestinal microbial community, could alter clinical outcome.

Funding

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Conflicts of interest

None declared.

References

1. Bui, Anh L, Horwish, Tamara B, Fonarow, Gregg C. Epidemiology and risk profile of heart failure. *Natl Health Stat Report* 2008;**8**:1–20.
2. Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJ. More 'malignant' than cancer? 5-year survival following a first admission with heart failure. *Eur J Hear Fail* 2001;**3**:315–322.
3. Aukrust P, Yndestad A, Ueland T, Damås JK. Anti-Inflammatory Trials in Chronic Heart Failure. *Heart Fail Monit* 2006;**5**:2–9.
4. Staels B, Fonseca VA. Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. *Diabetes Care* 2009;**32 Suppl 2**.
5. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC, Auwerx J. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006;**439**:484–489.
6. Vallim TQ de A, Edwards PA. Bile Acids Have the Gall to Function as Hormones. *Cell Metab* Elsevier Inc.; 2009;**10**:162–164.
7. Belgaumkar AP, Vincent RP, Carswell KA, Hughes RD, Alaghband-Zadeh J, Mitry RR, Roux CW Le, Patel AG. Changes in Bile Acid Profile After Laparoscopic Sleeve Gastrectomy are Associated with Improvements in Metabolic Profile and Fatty Liver Disease. *Obes Surg* 2015;1195–1202.
8. Haeusler RA, Astiarraga B, Camastra S, Accili D, Ferrannini E. Human insulin resistance is associated with increased plasma levels of 12alpha-hydroxylated bile acids. *Diabetes* 2013;**62**:4184–4191.
9. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* 1999;**3**:543–553.

10. Khurana S, Raufman JP, Pallone TL. Bile acids regulate cardiovascular function. *Clin Transl Sci* 2011;**4**:210–218.

11. Burcelin R, Serino M, Chabo C, Blasco-Baque V, Amar J. Gut microbiota and diabetes: From pathogenesis to therapeutic perspective. *Acta Diabetol* 2011;**48**:257–273.

12. Bäckhed F. Meat-metabolizing bacteria in atherosclerosis. *Nat Med* Nature Publishing Group; 2013;**19**:533–534.

13. Russell DW. The Enzymes, Regulation and Genetics of Bile Acid Synthesis. *Annu Rev Biochem* 2003;**72**:137–174.

14. Hofmann A. The Continuing Importance of Bile Acids in Liver and Intestinal Disease. *Arch Intern Med* 1999;**159**:2647–2658.

15. Chiang JYL. Bile acids: regulation of synthesis. *J Lipid Res* 2009;**50**:1955–1966.

16. Sandek A, Anker S, Haehling S. The Gut and Intestinal Bacteria in Chronic Heart Failure. *Curr Drug Metab* 2009;**10**:22–28.

17. Yndestad A, Finsen AV, Ueland T, Husberg C, Dahl CP, Øie E, Vinge LE, Sjaastad I, Sandanger Ø, Ranheim T, Dickstein K, Kjekshus J, Damås JK, Fiane AE, Hilfiker-Kleiner D, Lipp M, Gullestad L, Christensen G, Aukrust P. The homeostatic chemokine CCL21 predicts mortality and may play a pathogenic role in heart failure. *PLoS One* 2012;**7**.

18. Taylor DR, Alaghband-Zadeh J, Cross GF, Omar S, Roux CW Le, Vincent RP. Urine bile acids relate to glucose control in patients with type 2 diabetes mellitus and a body mass index below 30 kg/m2. *PLoS One* 2014;**9**:1–10.

19. Haehling S Von, Schefold JC, Jankowska EA, Springer J, Vazir A, Kalra PR, Sandek A, Fauler G, Stojakovic T, Trauner M, Ponikowski P, Volk HD, Doehner W, Coats AJS, Poole-Wilson PA, Anker SD. Ursodeoxycholic acid in patients with chronic heart

failure: A double-blind, randomized, placebo-controlled, crossover trial. *J Am Coll Cardiol* Elsevier Inc.; 2012;**59**:585–592.

20. Schaap FG, Trauner M, Jansen PLM. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* Nature Publishing Group; 2013;**11**:1–13.
21. Steiner C, Othman A, Saely CH, Rein P, Drexel H, Eckardstein A von, Rentsch KM. Bile acid metabolites in serum: Intraindividual variation and associations with coronary heart disease, metabolic syndrome and diabetes mellitus. *PLoS One* 2011;**6**.
22. Sato H, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, Saladin R, Schoonjans K, Pellicciari R, Auwerx J. Novel potent and selective bile acid derivatives as TGR5 agonists: Biological screening, structure-activity relationships, and molecular modeling studies. *J Med Chem* 2008;**51**:1831–1841.
23. Desai MS, Mathur B, Eblimit Z, Vasquez H, Taegtmeyer H, Karpen SJ, Penny DJ, Moore DD, Anakk S. Bile acid excess induces cardiomyopathy and metabolic dysfunctions in the heart. *Hepatology* 2017;**65**:189-201.

Fig. 1. Primary and secondary bile acids in chronic heart failure.
A) Primary (green) and secondary (orange) bile acids (BAs) in chronic heart failure patients, given as relative median difference compared to controls. *p<0.05, **p<0.01 vs. controls. BAs that are either zero or divided by zero are not included in the figure. Complete data for all individual BAs are provided in the Supplementary table. B) Ratio of Secondary/Primary bile acids in chronic heart failure (CHF) patients compared to controls (CTR), presented as median (25th/75th percentile).

Chenodeoxycholic acid (CDC), Cholic acid (CA), Deoxycholic acid (DC), Glycine conjugate of chenodeoxycholic acid (GCDC), Glycine conjugate of cholic acid (GCA), Glycine conjugate of deoxycholic acid (GDC), Glycine conjugate of lithocholic acid (GLC), Glycine conjugate of ursodeoxycholic acid (GUDC), Lithocholic acid (LC), Taurine conjugate of chenodeoxycholic acid (TCDC), Taurine conjugate of deoxycholic acid (TDC), Taurine conjugate of lithocholic acid (TLC).

Fig. 2. Ratio of Secondary/Primary bile acids and overall survival in chronic heart failure. Data are given as tertiles (T), with the upper tertile (T3) compared to the two lower ones (T1/T2). Unadjusted Cox-regression: HR 1.93 [95% Confidence Interval 1.01-3.68], p=0.041).

Table 1. Characteristics of the patients with heart failure stratified by tertiles of the ratio of secondary to primary bile acids. Comparison with control subjects.

Characteristic	Heart failure (n=142)	Tertile 1 (n=48)	Tertile 2 (n=46)	Tertile 3 (n=48)	P for trend	Controls (n=20)	P-value heart failure vs. controls
Age, y	57±11	56±11	55±12	58±10	0.382	56±7	0.798
Female sex (%)	17	13	17	21	0.522	15	0.831
BMI kg/m^2	27±6	26±5	28±7	27±6	0.597	24±6*	0.069
NYHA class (%) II/III/IV	28/47/25	24/50/26	35/39/26	25/52/23	0.686	-	
LVEF	29±11	28±11	28±12	30±9	0.578	-	
Current smoker (%)	49	43	48	57	0.306	25	0.043
Medical history (%)							
Previous MI	38	38	39	36	0.955	0	<0.001
Hypertension	17	**6**	**20**	**26**	**0.042**	0	<0.001
Diabetes mellitus	14	6	20	17	0.155	0	<0.001
Medication (%)							
ACE inhibitors	74	81	74	67	0.291	0	<0.001

Betablockers	85	**74**	**87**	**94**	**0.028**	0	<0.001
Diuretics	74	64	83	75	0.117	0	<0.001
Laboratory measurements							
eGFR, mL/min	78±27	79±29	79±25	76±27	0.831	90±11	0.014
NT-proBNP, pM	229 (91, 427)	309 (131, 553)	211 (76, 418)	190 (80, 312)	0.088	4.85 (2.60, 6.55)	<0.001
CRP, mg/L	3.5 (1.8, 8.7)	4.7 (2.1, 16)	3.0 (1.4, 6.8)	3.2 (1.8, 5.7)	0.110	1.2 (1.0, 1,8)	<0.001

For demographics in the whole sub-population, categorical data are reported as percentages and continuous data as mean±SD, or for CRP and NT-proBNP as median and 25th and 75th percentile. Conversion factor for NT-proBNP: 1 pmol/L=8.457 pg/mL. BMI indicates body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; NT-proBNP, amino-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association. * BMI was available for half of the control subjects only.

Table 2. Individual bile acids and association with NT-pro-BNP, renal function, liver function, glycemic control and albumin in heart failure patients (n=142).

Bile acids	NT-ProBNP	eGFR	ALT	HbA1c	Albumin
CDC	-0.02	0.01	0.11	-0.09	0.06
GCDC	0.07	0.05	0.17	-0.01	-0.10
TCDC	**0.18***	0.05	0.13	0.02	**-0.23***
CA	-0.03	-0.01	0.10	-0.13	0.05
GCA	0.09	0.06	**0.28***	-0.01	-0.11
TCA	**0.20***	-0.05	**0.22***	0.02	**-0.24***
Sum primary	0.06	0.06	**0.20***	-0.02	-0.12
DC	**-0.28****	0.10	0.08	-0.06	0.13
GDC	-0.09	0.04	0.11	-0.09	-0.06
TDC	-0.01	-0.06	0.09	-0.08	-0.14
UDC	0.00	-0.05	0.10	-0.05	0.16
GUDC	-0.01	0.00	**0.20***	-0.11	0.09
TUDC	-0.01	0.02	0.14	-0.08	0.01
LC	0.07	**-0.23***	0.09	-0.12	0.05
GLC	-0.07	0.01	0.01	-0.12	0.03
TLC	0.02	-0.16	0.03	-0.14	0.01
Sum secondary	-0.11	0.09	0.14	-0.10	0.05
BA-ratio	-0.14	-0.01	-0.10	-0.02	-0.06

Spearman`s rho are given for individual BAs, sum primary and sum secondary BAs, whereas Pearson`s r is given for BA-ratio, significant correlations in bold. *p<0.05, **p<0.01. BA indicates bile acid; BA-ratio, Secondary/Primary BA-ratio; eGFR, estimated glomerular

filtration rate; NT-proBNP, amino-terminal pro-brain natriuretic peptide; ALT, Alanin Aminotransferase; HbA1c, Hemoglobin A1c.

Supplementary table. Individual bile acids in heart failure patients and controls.

Bile acids (μM)	Heart failure (n=142)	Control (n=20)	p-value
CDC	0.09 (0.05,0.24) [0.02, 4.59]	0.23 (0.03,0.825) [0.01, 4.72]	0.306
GCDC	**0.80 (0.36, 1.89) [0.05, 10.2]**	**1.65 (1.04, 2.63) [0.60, 7.45]**	**0.005**
TCDC	0.14 (0.05, 0.42) [0.00, 10.0]	0.20 (0.11, 0.64) [0.03, 1.81]	0.131
CA	0.01 (0.00, 0.05) [0.00, 2.47]	0.01 (0.00, 0.90) [0.00, 4.08]	0.438
GCA	**0.22 (0.09, 0.56) [0.00, 7.55]**	**0.42 (0.23, 0.70) [0.02, 2.97]**	**0.050**
TCA	0.01 (0.00, 0.12) [0.00, 5.84]	0.00 (0.00, 0.73) [0.00, 0.58]	0.100
Sum Primary	**1.70 (0.72, 3.74) [0.07, 32.9]**	**3.98 (1.98, 5.71) [0.83, 18.7]**	**0.003**
DC	0.21 (0.08, 0.43) [0.00, 3.01]	0.28 (0.04, 0.64) [0.00, 2.08]	0.429
GDC	0.25 (0.11, 0.65) [0.00, 3.44]	0.44 (0.08, 1.06) [0.00, 1.49]	0.497
TDC	0.05 (0.02, 0.17) [0.00, 3.56]	0.08 (0.00, 0.14) [0.00, 0.38]	0.516
UDC	0.00 (0.00, 0.02) [0.00, 0.80]	0.00 (0.00, 0.05) [0.00, 0.15]	0.303
GUDC	**0.07 (0.04, 0.14) [0.01, 4.22]**	**0.03 (0.00, 0.08) [0.00, 0.29]**	**0.001**
TUDC	0.00 (0.00, 0.00) [0.00, 0.26]	0.00 (0.00, 0.00) [0.00, 0.00]	0.248
LC	**0.05 (0.00, 0.09) [0.00, 0.48]**	**0.00 (0.00, 0.01) [0.00,0.01]**	**0.001**
GLC	**0.08 (0.07, 0.10) [0.06, 0.22]**	**0.05 (0.04, 0.07) [0.03, 0.13]**	**<0.001**
TLC	**0.04 (0.02, 0.05) [0.00, 0.12]**	**0.01 (0.01, 0.02) [0.01, 0.06]**	**<0.001**
Sum Secondary	1.06 (0.45, 1.75) [0.12, 7.49]	1.10 (0.25, 2.27) [0.040, 3.19]	0.984
BA-ratio	0.29 (0.07, 0.52) [0.04, 4.02]	0.63 (0.31, 1.21) [0.02, 2.38]	0.001

Data as median (25th/75th percentile) [total range]. BA-ratio indicates Secondary/Primary BA-ratio.