Association between antioxidant metabolites and N-terminal fragment brain natriuretic peptides in insulin-resistant individuals

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Objectives **Oxidative stress plays a pivotal role in the development of metabolic syndrome, including heart failure and insulin resistance. The N-terminal fragment of brain natriuretic peptide (NT-proBNP) has been associated with heightened oxidative stress in heart failure patients. Yet, its correlation with insulin resistance remains poorly understood. Our objective is to investigate the association between oxidative stress markers and NT-proBNP levels in insulin-resistant individuals.**

Methods **In this cross-sectional study involving 393 participants from the Qatar Biobank, clinical and metabolic data were collected, and the association between NT-proBNP and 72 oxidative stress metabolites was compared between insulin-sensitive and insulin-resistant individuals.**

Results **Our results showed significantly lower NT-proBNP levels in insulin-resistant individuals (median = 17 pg/ml; interquartile range = 10.3–29) when compared to their insulin-sensitive counterparts (median = 31 pg/ml; interquartile range = 19–57). Moreover, we revealed notable associations between NT-proBNP levels and antioxidant metabolic pathways, particularly those related to glutathione metabolism, in insulin-resistant, but not insulin-sensitive individuals.**

Conclusion **The significant decrease in NT-proBNP observed in individuals with insulin resistance may be attributed to a direct or indirect enhancement in glutathione production, which is regarded as a compensatory mechanism against oxidative stress. This study could advance our understanding of the interplay between oxidative stress during insulin resistance and cardiovascular risk, which could lead to novel therapeutic approaches for managing cardiovascular diseases. Further investigations are needed to assess the practical utility of these potential metabolites and understand the causal nature of their association with NT-proBNP in the etiology of insulin resistance.** ***Cardiovasc Endocrinol Metab*** **13: 1–9**

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Introduction

Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize these harmful substances. It plays a pivotal role in the development of metabolic syndrome, including heart failure and insulin resistance. In heart failure, an excess production of ROS relative to antioxidant defense can lead to cardiac dysfunction, contributing to the pathophysiology of the condition [1]. Insulin resistance, a key component of metabolic syndrome, has also been linked to oxidative stress. Increased oxidative stress can lead to insulin resistance, dyslipidemia, β-cell dysfunction, and impaired glucose tolerance, ultimately leading to type 2 diabetes (T2D) [2]. Insulin resistance in the myocardium can generate damage through altered signal transduction, impaired regulation of substrate metabolism, and altered delivery of substrates to the myocardium. These alterations can contribute to cardiomyopathy and heart failure [3].

Natriuretic peptides are family of neurohormones that are genetically discrete and mainly synthesized by the heart and the brain [4]. N-terminal pro B-type natriuretic peptide (NT-proBNP) is one of the main types of natriuretic peptides released by cardiomyocytes as a counteraction to elevated stress on cardiac walls and vasoconstriction [5]. NT-proBNP is a prominent diagnostic marker of heart failure severity and cardiac dysfunction [6].

Studies have shown that assessing levels of NT-proBNP and oxidative stress markers may improve the detection of early stages of heart failure [7]. Indeed, reports have shown that lowered serum NT-proBNP was associated

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with high levels of carotenoids [8], superoxide dismutase, total antioxidant capacity, catalase [9], and ubiquinone [10]. The relationship between NT-proBNP and antioxidant metabolites is complex and multifaceted, with implications for the diagnosis, management, and prognosis of cardiovascular diseases [11]. Furthermore, the association between NT-pro-BNP and insulin resistance is inconsistent across the literature. While some recent studies suggest an inverse relationship between NT-proBNP and insulin resistance [12], others do not find this association [13]. Nevertheless, the interplay between NT-proBNP and antioxidant metabolites in the context of metabolic syndrome, such as insulin resistance, could be a potential area of focus for improving diagnostic and prognostic models in cardiovascular health. Metabolomics has a significant role, from understanding the metabolic pathways underlying heart failure and insulin resistance, to identifying potential biomarkers and therapeutic targets [14]. The comprehensive understanding of the interplay between oxidative stress, insulin resistance, and cardiovascular risk could potentially lead to the development of novel therapeutic strategies for metabolic disorders and cardiovascular diseases.

Our objective is to evaluate the association between circulating metabolic markers of oxidative stress and NT-proBNP levels in healthy individuals, then investigate this association after dichotomization of our cohort into insulin-resistant and insulin-sensitive individuals. This research aims to contribute to the development of improved diagnostic and prognostic models in the field of cardiovascular health.

Methods

Data and study participants

The study was approved by the Institutional Review Boards of the Qatar Biobank (QF-QBB-RES-ACC-00125). All participants provided informed consent. Data for 3000 participants was retrieved from Qatar Biobank (QBB), along with questionnaire related to medications, previous history of cardiovascular events, and laboratory measurements of clinical chemistry and endocrinology tests. Insulin resistance was determined by homeostatic model assessment of insulin resistance (HOMA-IR) calculated from fasting blood glucose (mmol/l) and fasting insulin mIU/ml measurements using the following equation:

$$\text{HOMA-IR} = \text{Insulin} \times \text{Glucose (mmol/l)} / 22.4$$

Exclusions were based on questionnaire-derived information regarding prior medical diagnosis. Participants with any history of cardiovascular disease or event were excluded from the study. Participants with documented diagnosis of T2D, and those using renin–angiotensin system inhibitors and beta-blockers, or any medication for managing hypertension or cholesterol were systematically removed from the dataset. Data cleaning process included the removal of incomplete records pertaining to NT-proBNP and essential demographic factors such as age, gender, and BMI. Outliers identified within NT-proBNP and HOMA-IR variables were meticulously identified and removed. The 15th and 85th quantiles of HOMA-IR were selected to ensure a clear categorization of individuals as insulin-sensitive and insulin-resistant, respectively. This selection prevents any ambiguity or placement within the 'gray zone', which encompasses individuals near the cutoff range for insulin-sensitive and insulin-resistant classification. Three hundred and ninety-three participants were included in the final analysis.

Metabolomics

Untargeted metabolomics of serum samples from all subjects was performed according to established techniques [15]. Metabolite measurement was performed via Waters ACQUITY ultra-performance liquid chromatography UPLC (Waters Corporation, Milford, Massachusetts, USA) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35 000 mass resolution. Compounds were identified by matching with existing library entries of purified standards of over 3300 compounds before being assigned to various categories according to their sources. Quality controls and internal standards are previously described [16]. To track the consistency and replicability of the method over time, quality control samples were employed. To reduce variability and guarantee sample integrity, pre-analytical sample handling procedures, such as sample collection, storage, and preparation, followed a standardized methodology.

Statistical analysis

The metabolomics data were inverse rank normalized. Metabolites/pathways associated with antioxidant activity [glutathione metabolism, methionine, cysteine, S-adenosylmethionine (SAM) and taurine, gamma-glutamyl amino acids, and vitamins (A, C, and E)] were selected for analysis. Principal component analysis was performed to derive a global view of the data. R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform linear models for NT-proBNP (as the response variable) versus each metabolite (as the explanatory variables). The model also included the following confounders: age, gender, and BMI. Nominal *P*-values were penalized using multiple testing correction methods (false discovery rate, FDR). FDR < 0.05 was considered statistically significant. Spearman's correlation was performed between NT-proBNP and clinical measurements. Statistical significance was determined by *P*-value <0.01.

Table 1 General characteristics of participants

Test	Variable	Total ($N = 393$)	Insulin sensitive ($N = 187$)	Insulin resistant ($N = 206$)	Insulin sensitive vs insulin resistant *P*-value
Vital signs	Gender (M/F)	196/197	73/114	123/83	<0.001
	Age	33 (27–42)	34.09 (10.14)	35.82 (9.71)	0.090
	BMI (kg/m^2)	26.77 (23.16–30.68)	24.42 (4.7)	29.33 (4.76)	<0.001
	Systolic blood pressure (mmHg)	110 (100–119)	106.96 (13.74)	113.9.3 (13.33)	<0.001
	Diastolic blood pressure (mmHg)	70 (65–77)	68.96 (8.98)	72.41 (10.06)	<0.001
	Pulse rate	67 (61–75)	64.01 (9.07)	71.69 (10.41)	<0.001
Blood sugar	HOMA-IR	5.15 (0.75–7.96)	0.72 (0.6–0.84)	7.85 (5.94–12.13)	<0.001
	Fasting blood glucose (mmol/l)	5 (4.6–5.6)	4.6 (4.4–4.8)	5.6 (5.1–6)	<0.001
	HbA_{1C} (%)	5.3 (5.1–5.6)	5.3 (5–5.5)	5.4 (5.1–5.7)	<0.001
	Insulin (μU/ml)	21 (3.8–34.2)	3.5 (3–4)	32.6 (25.5–47.08)	<0.001
	C-peptide	3.15 (1.19–5.4)	1.15 (0.98–1.39)	5.11 (4.26–6.38)	<0.001
Lipid profile	Total cholesterol (mmol/l)	4.8 (4.24–5.27)	4.7 (0.84)	4.95 (0.84)	0.002
	HDL-cholesterol (mmol/l)	1.35 (1.1–1.63)	1.62 (0.4)	1.2 (0.33)	<0.001
	LDL-cholesterol (mmol/l)	2.88 (2.23–3.29)	2.69 (0.81)	2.99 (0.76)	<0.001
	Triglyceride (mmol/l)	1.1 (0.68–1.66)	0.84 (0.37)	1.73 (1.08)	<0.001
Kidney function	Creatinine (μmol/l)	66 (55–79)	63.55 (14.08)	70.06 (15.47)	<0.001
	Urea (mmol/l)	4.3 (3.6–5.1)	4.31 (1.21)	4.53 (1.31)	0.096
	Total protein (g/l)	72 (70–75)	72.5 (4.17)	72.67 (3.73)	0.639
	Uric acid (μmol/l)	289 (229–343)	270.48 (72.87)	315.44 (82.81)	<0.001
Liver function	Albumin (g/l)	45 (43–47)	45 (44–48)	45 (43–46)	<0.001
	ALT (U/l)	17 (12–27)	16.3 (8.89)	27.17 (21.56)	<0.001
	AST (U/l)	17 (15–22)	18.52 (7.58)	20.82 (10.55)	0.013
	GGT (U/l)	14 (10–21.5)	14.97 (11.78)	26.12 (18.8)	<0.001
Hormones	TSH (mIU/l)	1.33 (0.94–1.95)	2.09 (7.25)	1.66 (1.28)	0.567
	Free thyroxine (pmol/l)	13.2 (12.2–14.1)	13.56 (1.86)	13.05 (1.65)	0.006
	Free triiodothyronine (pmol/l)	4.4 (4.02–4.8)	4.31 (0.69)	4.52 (0.73)	0.006
Red cells indices	Hematocrit (%)	40.95 (37.27–43.82)	39.64 (4.51)	41.36 (4.71)	0.001
	Hemoglobin (g/dl)	13.55 (12.3–14.7)	13.17 (1.66)	13.75 (1.72)	0.001
	Red blood cell $\times 10^6$ μl	4.9 (4.5–5.2)	4.72 (0.57)	5.01 (0.6)	<0.001

Data are presented as mean ± SD or median I(QR) based on the results of the normality test (Shapiro Wilks'). Student's *t*-test and Mann–Whitney *U* test were performed accordingly. Nominal variables were compared using the Chi-square test.

ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IQR, interquartile range; LDL, low density lipoprotein; TSH, thyroid stimulating hormone.

Results

General characteristics of participants

Three hundred ninety-three healthy individuals were included in the study. One hundred and eighty-seven were insulin sensitive, and 206 were insulin resistant. Significant differences (Table 1) were shown in BMI, systolic blood pressure, diastolic blood pressure, pulse rate, HOMA-IR, fasting glucose, HbA_{1C}, insulin, C-peptide, total cholesterol, HDL-cholesterol,low density lipoprotein (LDL)-cholesterol, triglyceride, albumin, alanine transaminase, aspartate aminotransferase, gamma-glutamyl transferase (GGT), creatinine, uric acid, hematocrit, and hemoglobin.

NT-proBNP and clinical traits in the general cohort

Spearman correlation was used to assess the relationship between NT-proBNP and clinical traits in the cohort (Fig. 1). Positive correlation was found with HDL. Negative correlation includes, among other clinical parameters, hematocrit, hemoglobin, and GGT.

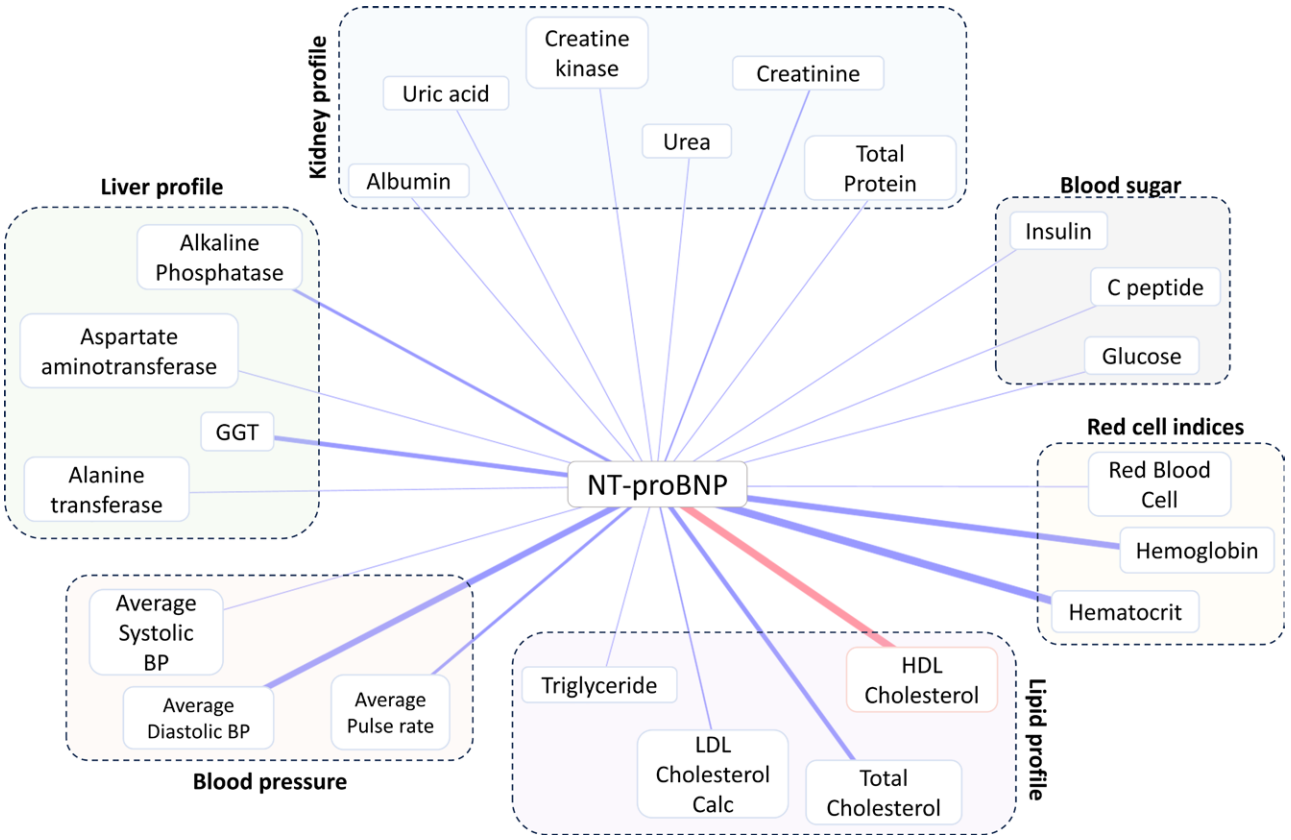
Effects of antioxidant metabolites on NT-proBNP in the general cohort

A linear model was used to assess the effect of antioxidant-associated metabolites on NT-proBNP level after adjusting for age, gender, and BMI (Table 2). Data suggested a possible decrease in NT-proBNP level with increase of glutamate, while it showed an increase in gamma-glutamylglutamine, S-methylcysteine, cysteine-glutathione disulfide, gamma-glutamylcitrulline, 5-oxoproline, S-methylcysteine sulfoxide methionine, and sulfone gamma-glutamylglycine. Significant correlations are also illustrated in supplementary (Supplementary Figure 1, Supplemental Digital Content 1, *http://links.lww.com/CAEN/A59*).

NT-proBNP and antioxidant metabolites in insulin-sensitive and insulin-resistant participants

In order to investigate whether of NT-proBNP levels are affected by insulin resistance status, its levels were compared between insulin sensitive and insulin resistant. Figure 2 shows significantly lower levels of NT-proBNP in insulin-resistant individuals when compared to their insulin-sensitive counterparts. In order to determine metabolites associated with of NT-proBNP in relation to insulin resistance, metabolites differentiating insulin sensitive and insulin resistant were determined. Figure 3 shows higher levels of 5-oxoproline, gamma-glutamylglutamine, gamma-glutamylcitrulline, cysteine-glutathione disulfide, methionine sulfone, S-methyl cysteine (SMC), and SMC sulfoxide in insulin sensitive, whereas methionine, cysteine, cystathionine, glutamate, S-adenosylhomocysteine, cysteinyl glycine, methionine sulfoxide, and many gamma glutamyl amino acids were higher in insulin resistant.

Fig. 1



Network of significant correlation between clinical traits and NT-proBNP in the study group. Red/blue reflects the positive/negative Spearman's ρ, respectively and the thickness of the line depicts the weight of association. (See Supplementary Table 1, Supplemental Digital Content 1, *http://links.lww.com/CAEN/A59* for spearman's ρ and *P*-values).

Table 2 Metabolites associated with NT-proBNP after adjusting for age, gender, and BMI. Estimate reflects the effect of the following metabolites on NT-proBNP

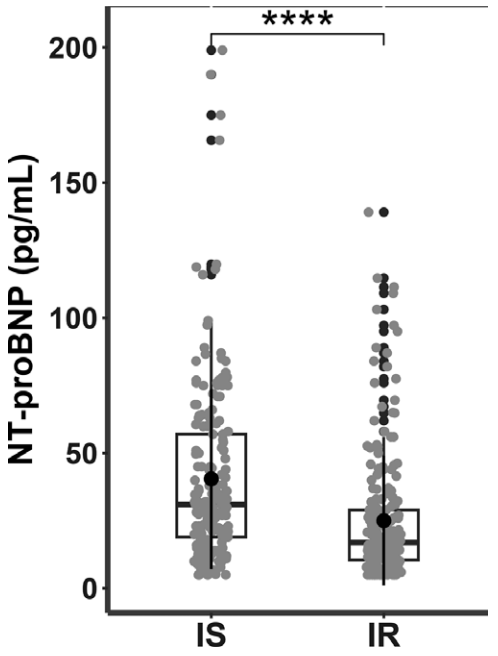
Metabolite	Super pathway	Estimate	SE	*P*-value	FDR
Gamma-glutamylglutamine	Gamma-glutamyl amino acid	0.170	0.039	1.78×10^{-05}	0.001
S-methylcysteine	Methionine, cysteine, SAM, and taurine metabolism	0.151	0.036	2.98×10^{-05}	0.001
Glutamate	Glutamate metabolism	−0.161	0.039	4.93×10^{-05}	0.001
Cysteine-glutathione disulfide	Glutathione metabolism	0.144	0.037	1.25×10^{-04}	0.002
Gamma-glutamylcitrulline*	Gamma-glutamyl amino acid	0.148	0.040	2.70×10^{-04}	0.004
Pyroglutamine*	Glutamate metabolism	0.171	0.050	7.39×10^{-04}	0.009
S-methylcysteine sulfoxide	Methionine, cysteine, SAM, and taurine metabolism	0.110	0.038	3.53×10^{-03}	0.035
Gamma-glutamylglycine	Gamma-glutamyl amino acid	0.111	0.038	3.90×10^{-03}	0.035
Methionine sulfone	Methionine, cysteine, SAM, and taurine metabolism	0.107	0.040	7.31×10^{-03}	0.058

*Indicates a compound that has not been officially confirmed based on a standard but that Metabolon is confident in its identity.
FDR, false discovery rate; SAM, S-adenosylmethionine.

Discussion

Cardiac dysfunction remains a serious global public health problem, and its diagnosis is extremely important. N-terminal fragment brain natriuretic peptides are frequently used in the diagnosis of congestive heart failure [17]. Heart failure is characterized by an exhaustion of the innate antioxidant defense mechanism, leading to an increase in oxidative stress [18]. Metabolomics is now considered an irreplaceable tool in understanding biological functions, by providing insights into the metabolic changes and biomarkers associated with a particular disease or condition. Here, we investigated the possible association between metabolites possessing antioxidant activity, and NT-proBNP in a cohort of healthy individuals; then, we dichotomized our cohort into insulin sensitive and insulin resistant, and compared the levels of

Fig. 2



Comparing the NT-proBNP level between insulin-sensitive and insulin-resistant individuals in the study group. [****P-value <0.0001].

these metabolites along with the levels of NT-proBNP between these two groups.

NT-proBNP and clinical traits

Our results showed a significant and inverse association between NT-proBNP with hemoglobin and hematocrit. This is in line with several studies that have found lower hemoglobin levels, often indicative of anemia, are associated with higher levels of NT-proBNP [19–21]. Given that our cohort includes only healthy participants, this suggests that anemia itself can influence NT-proBNP levels, independent of heart failure.

A direct positive association was shown between NT-proBNP and HDL cholesterol. This relation was inversed but less pronounced between NT-proBNP and LDL cholesterol, total cholesterol, and triglyceride. This suggests that an increase in blood NT-proBNP is associated with a beneficial lipide profile. While our findings contradict Zhu *et al*. [22], they are consistent with Masuch *et al*. [23], and Birukov *et al*. [24]. In fact, NT-proBNP has been found to be associated with lipid metabolism, and suggesting a positive role on circulating lipid levels [25]. More studies, however, are needed to extend our understanding of the metabolic properties of NT-proBNP.

NT-proBNP and antioxidant metabolites in the general cohort

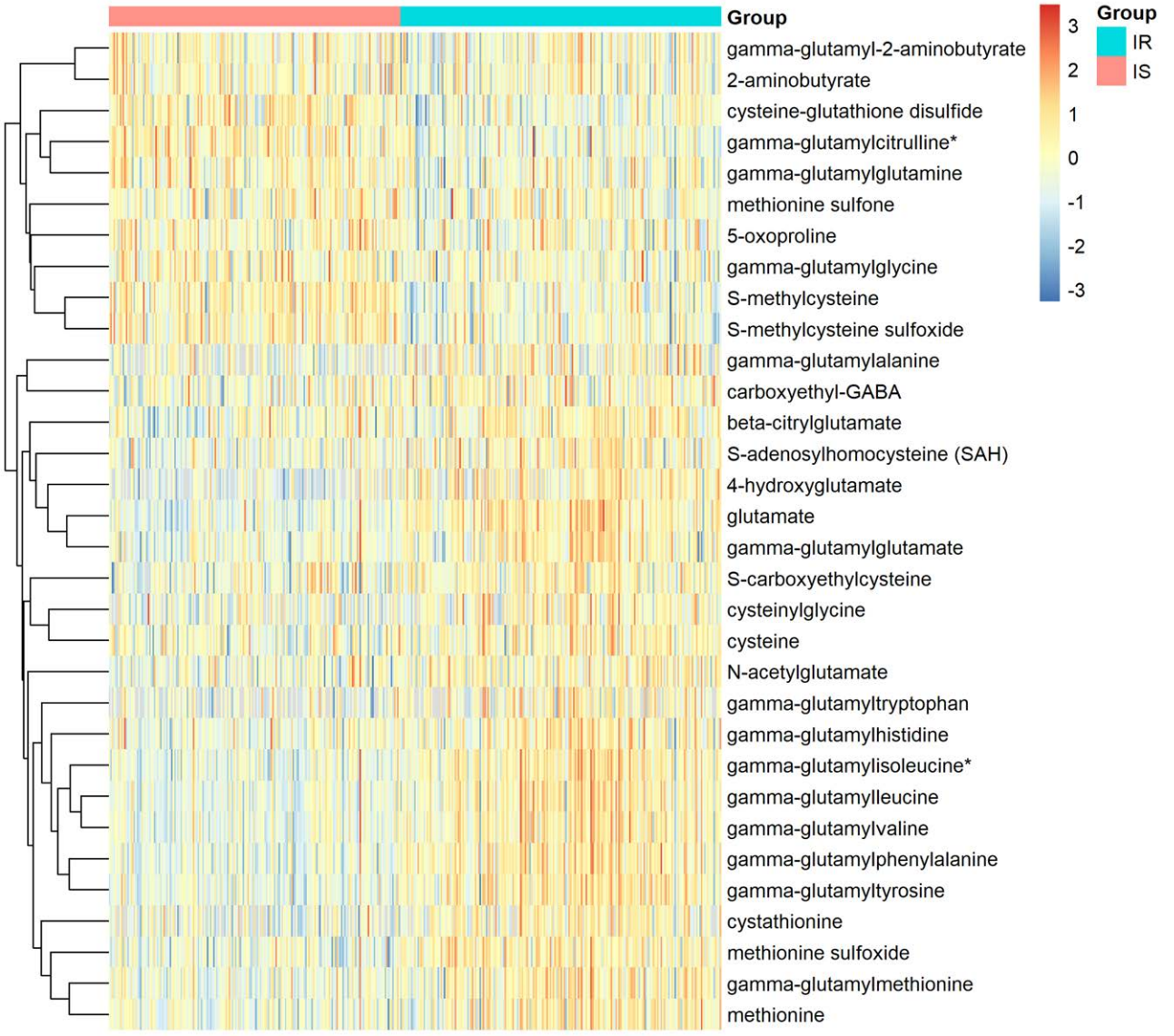
Oxidative stress is a critical hallmark in the pathophysiological cardiac dysfunction in heart failure [1,26]. Glutathione is a tripeptide that plays an important role as antioxidant in the cardiovascular system by counteracting the deleterious effects of ROS and maintaining redox homeostasis [27,28]. Our emerging data showed that increased levels of NT-proBNP was associated with an increase in 5-oxoproline and gamma-glutamylglutamine, and a decrease in glutamate, which may ultimately lead to a reduction in glutathione biosynthesis. Indeed, the enzyme GGT catalyzes the hydrolysis of gamma-glutamyl bonds to form 5-oxoproline, and the enzyme 5-oxoprolinase hydrolyzes 5-oxoproline into glutamate to reconstitute glutathione (Fig. 4). Interestingly, an accumulation in 5-oxoproline owing to downregulation of 5-oxoprolinase or an increased in GGT activity, was shown to be an important factor in heart failure [29,30]. Relatedly, Sourdon *et al*. showed that disturbing 5-oxoprolinase activity can trigger what they called a redox storm in the ischemic heart [31]. Moreover, many studies suggest participation of GGT in the pathophysiology of cardiovascular diseases [32,33].

Cumulative evidence attests that altered GSH cycling can trigger cardiac oxidative stress. In heart failure, a significant decrease in glutathione levels has been observed [30,34]. Kobayashi *et al*. showed that an increased vulnerability of mitochondria to oxidative damage caused by a reduction in GSH, was associated with a higher susceptibility of myocardial injury in mice [35].

Our findings were further corroborated by the direct significant association between NT-ProBNP levels with SMC and its metabolite SMC sulfoxide. The increase in the levels of these metabolites could indicate a divergence in cysteine metabolism away from glutathione synthesis due to the low availability of glutamate (Fig. 4). Relatedly, El-Magd *et al*. [36] demonstrated that high doses of SMC have been found to result in a significant increase in serum cardiac injury biomarkers. Further experiments, however, are required to check the exact involvement of SMC in cardiac function.

Our emerging data also showed a direct and significant association between NT-ProBNP levels with cysteine-glutathione disulfide levels. The accumulation of cysteine-glutathione disulfide can indicate an increased level of oxidative stress in the body [37], which leads to formation of glutathione mixed disulfides with protein thiol groups, leading to a process called S-glutathionylation [38]. Intriguingly, S-glutathionylation is emerging as a critical signaling mechanism in cardiovascular diseases [39,40]. Additionally, S-glutathionylation of cysteine is catalyzed by glutathione S-transferase P1 [41], an enzyme strongly associated with heart failure [42]. More research, however, is needed to understand the role of S-glutathionylation in cardiac disorders, as well as its potential use in therapies and as a biomarker.

Fig. 3



Comparing the metabolite difference between insulin-sensitive and insulin-resistant individuals, FDR < 0.05. (See Supplementary Table 2, Supplemental Digital Content 1, *http://links.lww.com/CAEN/A59* for estimate values, nominal, and FDR significance).

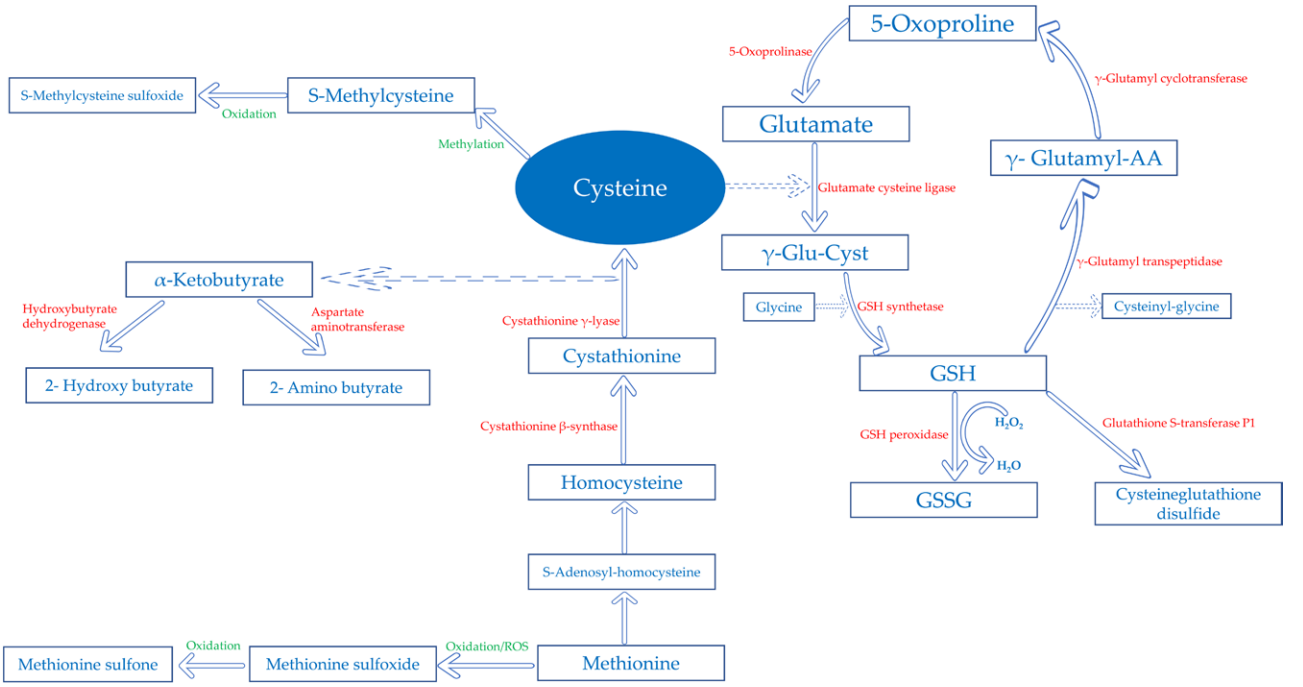
The increase of NT-ProBNP associated with the increase in methionine sulfone can also be explained by an increase in ROS overwhelming the cellular antioxidant defense system. Methionine is oxidized to methionine sulfoxide; this oxidation can be reversed by enzymes in the methionine sulfoxide reductase family which scavenge ROS; however, the further oxidation of methionine sulfoxide to form methionine sulfone is irreversible [43]. Strikingly, methionine sulfoxide reductase A was shown to protect the heart from ischemia-reperfusion injury [44], the latter being a significant factor in the development of heart failure [45].

NT-proBNP and antioxidant metabolites in insulin sensitivity and insulin resistance

Our results showed significantly lower levels of NT-ProBNP in insulin resistant participants when compared to insulin-sensitive counterparts. This is in line with many studies [12,46–48], which showed that insulin resistance, is generally associated with reduced NT-proBNP levels.

Interestingly, our results showed that all metabolites positively associated with NT-ProBNP in the general cohort, were lower in the insulin resistant group when compared to insulin-sensitive counterpart. This includes

Fig. 4



Metabolic antioxidant pathways significantly associated with NT-proBNP in this study.

5-oxoproline, gamma-glutamylglutamine, gamma-glutamylcitrulline, cysteine-glutathione disulfide, methionine sulfone, SMC, and SMC sulfoxide. Moreover, glutamate, negatively associated with NT-ProBNP in the general cohort, was higher in insulin resistant group when compared to insulin sensitive. This shows that the decrease of NT-ProBNP observed in insulin resistant group may be associated, at least in part, with the change of these metabolites.

Besides glutamate, the decrease of NT-ProBNP in the insulin resistant group, was also accompanied by an increase of cystathionine, cysteine, S-adenosyl-homocysteine, cysteinyl glycine, methionine, and many gamma-glutamyl amino acids. This may indicate a potential indirect enhancement in the glutathione cycle via the transsulfuration pathway. Indeed, cysteine is the immediate precursor of glutathione, and the trans-sulfuration pathway is the only route for biosynthesis of cysteine from homocysteine via cystathionine [49] (Fig. 4). Studies have shown that an activation of the transsulfuration pathway significantly contributes to an increase in glutathione biosynthesis [50]. The increase in glutathione synthesis in insulin resistant could be a compensatory mechanism to reduce oxidative stress and improve insulin signaling [51]. Moreover, recent research has shown that glutathione supplementation can improve insulin sensitivity [52]. Conversely, the insulin-sensitive group featuring higher NT-ProBNP exhibited higher levels of 2-amino butyrate and 2-hydroxy butyrate. A study on murine cardiomyopathy model showed that increased ROS are accompanied by 2-amino butyrate accumulation and compensatory maintenance of myocardial GSH levels [53]. Remarkably, α-hydroxybutyrate dehydrogenase (α-HBDH) is a marker of cell death reflecting myocardial injury [54]; the latter condition was shown to be associated with an increase in NT-proBNP [55].

Taken together, our results provide evidence that an increase in NT-proBNP levels could be ascribed, at least partially, to an imbalance in glutathione cycle and accumulation of ROS; and the reduction of NT-proBNP observed in insulin-resistant individuals, could be a result of a direct or indirect restoration of glutathione biosynthesis.

Conclusion

Our study provides the research community with a wealth of antioxidant metabolites associated with NT-proBNP in insulin resistance. These metabolites are directly or indirectly involved in the glutathione metabolism. We believe that the compensation mechanisms demonstrated in insulin resistance, which target the reduction of oxidative stress and enhancement of insulin signaling, may play a role in the observed decrease in NT-proBNP levels. Therefore, these metabolites can be of clinical relevance to provide valuable information about the heart's functional status. Additionally, the study could advance our understanding of the interplay between oxidative stress during insulin resistance

and cardiovascular risk, which could lead to novel therapeutic approaches for managing cardiovascular diseases. We acknowledge limitations in our study; while glutathione data is very useful to our interpretation, its measurement in nontargeted metabolomics is not typically performed due to the nature of the nontargeted approach and the specific challenges associated with accurately measuring glutathione levels. An additional limitation is the absence of echocardiographic data for participants. Another limitation is the measurement of the levels of NT-proBNP in healthy individuals; since reference ranges for NT-proBNP in healthy individuals are not well-established and can vary depending on the population studied and it can be affected by various extracardiac factors. Further studies are warranted to investigate the relevant usage of these metabolites in clinical practice, and to understand how these differences in oxidative stress markers translate into variations in NT-proBNP values.

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K.N. wrote the article, N.A. wrote the article and supported the statistical analysis, and M.A.E. supervised the work. All authors have read and agreed to the published version of the manuscript.

The study was approved by the Institutional Review Boards of the Qatar Biobank (QF-QBB-RES-ACC-00125).

Informed consent was obtained from all participants involved in the study.

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

There are no conflicts of interest.

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