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Postprandial variability of novel heart failure biomarkers in Fontan patients compared to healthy volunteers

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ABSTRACT

Background: Blood-based biomarkers reflecting different components of cardiovascular pathophysiology are now used increasingly in patients with Fontan circulation due to univentricular congenital heart disease. Feeding alters haemodynamics significantly and, thus, may affect biomarker levels. As the haemodynamic responses to a meal differ between Fontan patients and normal subjects, we hypothesised that biomarker kinetics may also vary between these populations.

Methods: In 15 patients with Fontan physiology, and 15 matched healthy volunteers, 4 heart failure biomarkers were measured under fasting conditions, and 3 after additional timepoints over 2 h following ingestion of a standardised liquid meal. Changes in biomarker levels over time and the effect of Fontan physiology, sex and age were tested using repeated-measures mixed models.

Results: Under fasting conditions, high-sensitivity C-reactive protein (hsCRP), mid-regional pro-adrenomedullin (MR-proADM) and C-terminal pro-endothelin-1 (CT-proET-1) were raised significantly in Fontan patients compared to controls. Postprandially, mid-regional pro-atrial natriuretic peptide (MR-proANP) decreased significantly in patients (max. mean decrease ~7% after 120 min). Conversely, it increased in normal subjects (max. mean increase ~8% after 60 min). The remaining biomarkers did not change significantly.

Conclusions: The ingestion of food triggers contrary deflections of MR-proANP levels in patients with Fontan circulation compared to normal subjects. Therefore, this parameter should be assessed under fasting conditions in order to correct for postprandial variability.

1. Introduction

In the Fontan circulation, the addition in series of the pulmonary to the systemic vascular bed results in numerous pathophysiological alterations, such as abnormal cardiac loading, reduced organ perfusion, and consequently, neurohumoral activation [1–3]. These changes determine long-term outcome and clinical management critically. Blood-based biomarkers are now explored increasingly for the management of patients with Fontan circulation due to univentricular congenital heart disease [4–6]. These biomarkers typically reflect different aspects of cardiovascular pathophysiology, and are now standard in the management of other types of cardiovascular disease, including heart failure (HF) [7].

Recently, we demonstrated that the ingestion of a meal triggers a range of important haemodynamic responses in patients with Fontan physiology, including a decrease in cardiac afterload, an increase in cardiac output, as well as regional changes in vascular tone of the limbs and kidneys. Some of these responses contrasted those of normal subjects significantly [2]. We hypothesised that due to their sensitivity to haemodynamic alterations, levels of common HF biomarkers could also be affected by a meal, and that any postprandial kinetics may vary similarly between these populations. As this would confer important implications for their clinical use, we addressed this in the present study.

Abbreviations: ANP, atrial natriuretic peptide; BNP, ventricular / B-type natriuretic peptide; CT-proET-1, C-terminal pro-endothelin-1; HF, heart failure; HsCRP, high-sensitivity C-reactive protein; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-atrial natriuretic peptide.

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2. Material and methods

2.1. Subjects

Patients with Fontan circulation ≥16 years of age (n = 15) were recruited from a cardiac outpatient clinic between November 2016 and March 2018. Local hospital staff volunteered as age- and sex-matched control subjects (n = 15). Exclusion criteria were: Chronic diseases requiring hospital management (in control subjects); hepatic or renal comorbidity; infection; dairy allergy. The study complies with the Declaration of Helsinki and was approved by National Research Ethics Service London – Queen Square. Informed consent was obtained from all participants in writing prior to their participation.

2.2. Protocol

Subjects were instructed to fast overnight and consume nothing but water until after the experiment. Study visits took place at ~9:00 a.m. Height and weight were measured using calibrated devices, and body mass index (= weight [kg]/height [m]^2) was calculated. A cannula, sized ≥20 G, was sited and blood was collected using standard collection techniques following ~20 min of rest in supine position. Following baseline blood sampling, subjects consumed a standardised liquid meal consisting of 170 mL of double cream and 45 g of maltose syrup (energy 925 kcal, total fluid volume ~200 mL), as described previously [2]. Follow-up blood samples were collected at 30, 60, 90, and 120 min (±1 min) relative to the onset of meal ingestion.

2.3. Analytical methods

EDTA-blood samples were spun, and plasma was stored at −80 °C for later batch-analysis. Serum samples were processed immediately after collection. Investigators who were blinded to participant history measured mid-regional pro-adrenomedullin (MR-proADM), mid-regional pro-atrial natriuretic peptide (ANP; MR-proANP), and C-terminal pro-endothelin-1 (CT-proET-1) from EDTA-plasma using an automated immunofluorescent assay, as described previously (KRYPTOR® System, BRAHMS AG, Hennigsdorf/Berlin, Germany) [4]. High-sensitivity C-reactive protein (hsCRP) was measured from serum at baseline (Vitros 5600 Clinical Chemistry analyser, Ortho Clinical Diagnostics, Raritan, NJ, USA). The detection limits and the functional assay sensitivities (20% of inter-assay variation coefficient) were, for CT-proET-1: 2.94 pmol.L^{-1} (estimated) and 9.78 pmol.L^{-1}; for MR-proANP: 4.5 pmol.L^{-1} and <10 pmol.L^{-1}; and for MR-proADM: 0.23 nmol L^{-1} and 0.25 nmol L^{-1}, respectively. Additional information on the analytical performance of the assays used is presented in the supplemental material.

2.4. Statistics

Associations of baseline biomarker levels with Fontan status, sex and age were assessed by linear regression analysis and 95% CI determined. Changes in biomarker levels over time and the effect of Fontan physiology, sex and age were assessed using repeated-measures mixed models, with the exception of hsCRP (Stata SE v14.2 software; StataCorp, USA). Continuous data are represented as medians (IQR), or as mean ± SD, and compared between groups by Mann-Whitney-U-test or by Student *t*-test, as appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Baseline data

At baseline, patients had significantly higher levels of MR-proADM, hsCRP and CT-proET-1 than controls (Table 1). Fontan status (B = 31.8 [95% CI: 8.4, 55.2], $P = 0.01$), age (B = 1.5 [95% CI: 0.1, 2.9], $P = 0.037$) and female sex (B = 27.2 [95% CI: 3.8, 50.7], $P = 0.025$) were associated independently with greater MR-proANP. Fontan status was also associated with greater CT-proET-1 (B = 15.0 [95% CI: 3.6, 26.4] unadjusted, $P = 0.012$), even after adjustment for age and sex. It was further linked to greater MR-proADM, but this association was no longer statistically significant after adjustment for age and sex ($P = 0.068$). HsCRP showed no significant associations in the regression analysis.

Table 1
Baseline characteristics.

	Fontan (n = 15)	Control (n = 15)	*P*
Age (years)	27.6 (IQR: 21.8, 34.6)	32.1 (IQR: 29.1, 38.6)	0.059
Female	5 (33.3%)	7 (46.7%)	0.456
Body mass index	23.3 (SD: 3.6)	22.8 (SD: 2.9)	0.659
CT-proET-1 (pmol.L^{-1})	62.2 (SD: 16.0)	45.1 (SD: 9.6)	0.002
MR-proADM (nmol.L^{-1})*	0.526 (IQR: 0.465, 0.582)	0.409 (IQR: 0.384, 0.472)	0.009
MR-proANP (pmol.L^{-1})*	53.2 (IQR: 28.6, 86.9)	45.5 (IQR: 35.6, 55.6)	0.406
hsCRP (nmol.L^{-1})*	16.5 (IQR: 6.8, 22.7)	5.9 (IQR: 2.1, 11.0)	0.034

Data were compared between groups by Mann-Whitney-U-test or by *t*-test, as appropriate (except for female: X^2). *Variables with skewed distribution were log-transformed, and their geometric mean back-transformed for presentation in natural units.

3.2. Postprandial responses

Following ingestion of the study meal, MR-proANP was significantly lower than baseline at all time points in the Fontan group (max. Δ_{mean} −7% at 120 min; Fig. 1). By contrast, it increased significantly in the control group from 30 min onwards (max. Δ_{mean} + 8% at 60 min), and decreased again after 120 min. A trend was seen for lower CT-proET-1 at 120 min in the Fontan group but not in controls ($P = 0.08$). No statistically significant changes were observed for MR-proADM compared to baseline in either group.

4. Discussion

In this experiment, a standardised meal triggered opposite deflections of MR-proANP levels in patients with Fontan circulation compared to healthy volunteers. Our findings have potential implications for clinical routine as these changes exceed the commonly accepted margins of error. This is particularly important in the Fontan population, who may undergo frequent, serial biomarker measurements in clinical routine.

Blood-based biomarkers, in particular ventricular natriuretic peptide (BNP) and its derivatives, are crucial tools for the management of HF [8]. They are now also used increasingly in other cardiovascular disorders, including congenital heart disease [4,9]. In patients with Fontan physiology, however, data on their clinical utility have been inconsistent, highlighting the need to investigate new biomarkers [8].

In HF, several 'novel' biomarkers have been described since the discovery of the natriuretic peptides, reflecting different components of cardiovascular pathophysiology [4,7]. As biomarker profiles vary significantly by subpopulation and between individuals, their combined assessment has been shown to be advantageous [10–12]. For example, MR-proANP has been reported to provide additional prognostic power in certain subpopulations when combined with BNP or its derivatives [8, 11]. Consequently, novel biomarkers are now also explored in patients with Fontan circulation [4,5].

As contemporary biomarkers are highly sensitive to haemodynamic alterations, it is important to study possible confounders in a clinical setting. Feeding affects haemodynamics significantly and, thus, may affect biomarker levels [2]. Indeed, previous research in healthy volunteers suggests that MR-proANP may be prone to postprandial fluctuation [13], unlike other natriuretic peptides [14]. We have demonstrated that a meal triggers different haemodynamic responses in Fontan patients than

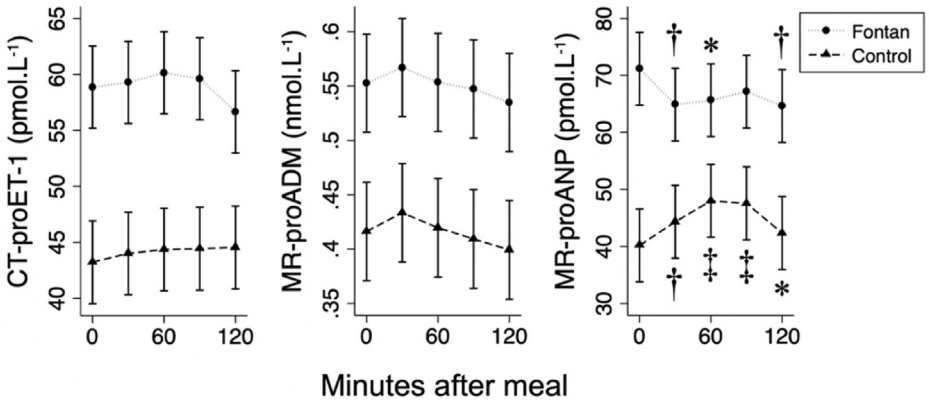

Fig. 1. Postprandial biomarker responses (mean ± SE). Repeated-measures mixed models were used to assess biomarker changes over time and the effect of Fontan status, adjusted for sex and age. $^{*}P < 0.05$, $^{\dagger}P < 0.01$ and $^{\ddagger}P \leq 0.001$ for biomarker changes with respect to baseline.

in normal subjects [2]. Thus, we inferred that any postprandial biomarker kinetics may vary between these populations, too. Indeed, we demonstrated that MR-proANP responses differed significantly. While we did not assess haemodynamics in this study, several mechanisms could explain this.

MR-proANP is released predominantly by the atria in response to wall stretch due to abnormal loading [8]. In Fontan physiology, afterload is raised by default due to the addition in series of the pulmonary to the systemic vascular bed [1]. This may explain the association between Fontan status and greater MR-proANP in this study. Recently, we demonstrated that the ingestion of food triggered a substantial drop in systemic vascular resistance due to mesenteric vasodilation in patients with Fontan circulation [2]. Though a change in afterload does not affect atrial stretch directly, it may have mediated this indirectly through lowering ventricular filling pressure.

Conversely, MR-proANP increased in volunteers postprandially. It is possible that the increased systemic venous return elevated atrial wall tension, stimulating ANP release. This may not have been the case in patients with Fontan circulation, where the venae cavae are attached directly to the pulmonary arteries and therefore, an increase in systemic venous return does not translate directly into an increase in preload [1]. Glucagon-like peptide-1 may also upregulate ANP postprandially [15], but its role in Fontan physiology has not yet been resolved. Future research could investigate its involvement in natriuretic peptide regulation.

Our findings align with one previous report showing a postprandial MR-proANP increase in healthy volunteers after an oral glucose challenge [13]. Other, similar experiments reached different conclusions [14, 16]. However, differences in sample size, analytical assays, and study meals limit comparability between these experiments. By contrast, several studies found that levels of BNP and its derivatives were largely unaffected by the ingestion of food [16–18]. Consequently, these markers were not investigated further in this study.

MR-proADM and CT-proET-1 are derivatives of 2 counteracting vasoactive peptides: adrenomedullin and endothelin-1. MR-proADM is an excellent prognostic marker in HF, especially when combined with CT-proET-1 [7,12]. Our findings concord with a previous study, where it was elevated and associated with poor haemodynamics in Fontan patients [5]. Previous data on the responsivity of adrenomedullin to other types of cardiovascular stress have been inconsistent [19]. Our findings suggest that the ingestion of food does not cause sufficient haemodynamic stress to alter MR-proADM levels significantly.

Endothelin-1 is central in the regulation of pulmonary vascular tone, one of the main determinants of pulmonary blood flow in the Fontan circulation. It is, therefore, an important therapeutic target and a potential diagnostic marker in this population [6]. While our study conflicts previous data showing postprandial variation [20], its marked elevation in Fontan patients at baseline supports that CT-proET-1 deserves further investigation as a diagnostic tool in this population.

The study meal we used has been validated previously, and used to investigate the effects of food ingestion on cardiovascular physiology [2, 21]. Importantly, we have shown previously that the haemodynamic changes such a meal triggered were not due to an increase in preload [22]. While similar biomarker studies have relied on oral carbohydrate challenges [13,14,18], the addition of dairy-based fat in our protocol resulted in a meal that was more similar to the high-sugar, high-fat foods commonly found in Western diets.

5. Limitations and future work

The prognostic significance of the biomarkers assessed has yet to be established in the Fontan population in longitudinal studies. While we only measured the inflammatory marker, hsCRP, for exploratory purposes at baseline, we could show that it was markedly elevated in clinically well Fontan patients. In a previous cross-sectional study, low-level inflammation was linked to protein-losing enteropathy, one of the most formidable long-term complications in this population [3]. Poor gut perfusion has been discussed as a possible cause. However, we have demonstrated that intestinal perfusion may, in fact, be normal in Fontan patients [2]. Future research could address whether hsCRP can predict protein-losing enteropathy before symptoms are reported, or abnormalities in gut perfusion are detectable.

6. Conclusions

MR-proANP should be determined under fasting conditions in order to mitigate the possible confounding effects of a meal. Additional, longitudinal studies are needed to better understand the prognostic significance of novel HF biomarkers in patients with Fontan physiology.

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Analytical performance

For CT-proET-1, the intra-assay coefficient of variation (CV) was <10% for concentrations between 10 and 44 pmol.L^{-1}, and <4% for concentrations >44 pmol.L^{-1}. The inter-assay CV was <10% for concentrations between 44 and 80 pmol.L^{-1}, and <6% for concentrations >44 pmol.L^{-1}.

For MR-proANP, the intra-assay CV was ≤5% for concentrations between 10 and 20 pmol.L^{-1}, and <3.5% for concentrations >20 pmol.L^{-1}.

The inter-assay CV was ≤6.5% for concentrations between 10 and 20 pmol.L^{-1}, and <6.5% for concentrations >20 pmol.L^{-1}. For MR-proADM, the intra-assay CV was ≤10.8% for concentrations >0.2 and ≤ 0.5 nmol L^{-1}, ≤3.1% for concentrations >0.5 and ≤ 2 nmol L^{-1}, ≤1.2% for concentrations >2 and ≤ 6 nmol L^{-1}, ≤3.0% for concentrations >6 and ≤ 10 nmol L^{-1}, and ≤6.3% for concentrations >10 nmol L^{-1}. The inter-assay CV was ≤17.5% for concentrations >0.2 and ≤ 0.5 nmol L^{-1}, ≤10.4% for concentrations >0.5 and ≤ 2 nmol L^{-1}, ≤7.3% for concentrations >2 and ≤ 6 nmol L^{-1}, ≤5.6% for concentrations >6 and ≤ 10 nmol L^{-1}, and ≤6.8% for concentrations >10 nmol L^{-1}.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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