Thiol-based cardioprotection

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CHAPTER 3

Associations of Systemic Oxidative Stress with Functional Outcomes after ST-Segment Elevation Myocardial Infarction

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Submitted

ABSTRACT

Background

Myocardial ischemia and subsequent reperfusion are accompanied by oxidative stress. Serum free thiols (R-SH, sulfhydryl groups) reliably reflect systemic oxidative stress. This study evaluates longitudinal changes in free thiols and their associations with outcomes after ST-segment elevation myocardial infarction (STEMI).

Methods

Free thiols were detected in archived serum samples from 378 participants of a randomized trial on early metformin therapy after STEMI. Free thiol levels were determined at presentation with STEMI and at 24 hours, 2 weeks, 4 months and 1 year after reperfusion. Outcomes included infarct size and left ventricular ejection fraction (LVEF) as functional outcomes, determined with cardiac magnetic resonance imaging at 4 months follow-up, and 5-year major adverse cardiovascular events (MACE).

Results

Serum free thiol concentrations at presentation and at 24 hours were 356 ± 91 and 353 ± 76 μmol/L, respectively. The change in free thiols between presentation and 24 hours (Δ free thiols) was associated with functional outcomes in age- and sex-adjusted analysis (per 100 μmol/L increase free thiols, β=-0.87 for infarct size, 95% CI: -1.75 to -0.001, *P*=0.050 and β=1.31, 95% CI: 0.37 to 2.25 for LVEF, *P*=0.007). Associations with Δ free thiols and LVEF were markedly stronger in patients presenting with Thrombolysis in Myocardial Infarction flow of 0 or 1 before percutaneous coronary intervention (PCI) (β=2.73, 95% CI: 0.68 to 4.77, *P*=0.009). Declining free thiols during the first 24 hours might be associated with higher incidence of 5-year MACE (*P*=0.09).

Conclusions

Changes in oxidative stress early post-PCI may predict functional outcomes after STEMI. Since free thiols are amendable to therapeutic intervention these data may support the development of future thiol-targeted therapy in ischemic heart disease.

INTRODUCTION

Patients presenting with ST-segment elevation myocardial infarction (STEMI) suffer from ischemic and reperfusion injury, which are both characterized by oxidative stress.[1] Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of oxidants, leading to the disruption of redox signaling and control and/or molecular damage.[2] Although reactive oxygen species (ROS) fulfill pivotal physiological functions, overproduction of ROS during ischemia-reperfusion (I/R) may result in cellular and molecular damage and accompanying cell death.[3,4]

Thiols are organic antioxidant compounds containing a sulfhydryl (-SH) moiety. They exist both extracellularly (i.e. circulating or free thiols, of which albumin is the most relevant example), as well as intracellularly (predominantly low-molecular-weight thiols including glutathione and cysteine).[5] Free thiols play an important role in redox signaling, but also act as one of the most potent and versatile endogenous defense mechanisms against oxidative stress due to their ability to scavenge ROS and to serve as the main transducers of kinetically controlled redox exchange reactions.[5] Free (i.e. reduced) thiols are being oxidized in the presence of ROS to form disulphide bonds, which prevents ROS from inflicting oxidative modifications to lipids and proteins and subsequent myocardial structural damage.[5] Lower levels of free thiols are therefore a reflection of higher levels of oxidative stress.[6] Conversely, higher levels of free thiols are indicative of a more favorable systemic redox status.[6]

In previous studies, lower levels of free thiols have been linked to a variety of cardiovascular risk factors (e.g. smoking, hypertension and diabetes mellitus),[6–9] as well as to disease severity and outcomes in a number of oxidative stress-mediated human conditions.[10–13] To the best of our knowledge, serum free thiols have never been longitudinally evaluated after STEMI, nor linked to functional and clinical outcomes after STEMI. Especially, levels of free thiols in the (sub)acute phase after STEMI are of interest, because this could be helpful in establishing a therapeutic window, since free thiols are amendable by therapeutic modulation with N-acetylcysteine.[1,14–16]

This study addressed several objectives. First, we investigated longitudinal changes in serum free thiols. Second, we studied whether serum free thiols were associated with myocardial infarct size and left ventricular ejection fraction (LVEF) at 4 months follow-up, and major adverse cardiovascular events (MACE) during 5 years after STEMI.

METHODS

Study population

Serum free thiols were measured in archived serum samples of the GIPS-III (Metabolic modulation with metformin to reduce heart failure after acute myocardial infarction: Glycometabolic Intervention in Adjunct to Primary Percutaneous Coronary Intervention in STEMI; NCT01217307) randomized controlled trial. This trial was designed to evaluate the effect of 4 months metformin therapy on preservation of left ventricular function in patients without known diabetes that presented with a first STEMI. The design and outcomes of this trial were previously published.[17,18] In brief, all patients admitted to the University Medical Center Groningen with a STEMI between January 2011 and May 2013 were considered eligible for the trial. Inclusion criteria were age older than 18 years, the presence of STEMI, and primary percutaneous coronary intervention (PCI) with implantation of at least 1 stent with a diameter of at least 3 mm resulting in TIMI flow grade 2 or 3 post-PCI. Key exclusion criteria were previous myocardial infarction (MI), known diabetes, the need for coronary artery bypass graft surgery, severe renal dysfunction, and standard contraindications for cardiac magnetic resonance imaging (CMR). The study protocol of the GIPS-III trial was in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Groningen, the Netherlands) and national regulatory authorities. Informed consent was obtained before any study related procedures.

Characteristics during hospitalization

On admission, standard laboratory assessment and physical examination parameters were measured according to protocol. During hospitalization, blood was sampled at admission (before PCI) and at 3, 6, 9, 12 and 24 hours thereafter to monitor cardiac enzymes. Serum samples for biobanking were drawn at presentation with STEMI, at 24 hours, 2 weeks, 4 months and 1 year.

Measurement of serum free thiols

Serum samples were stored at -80 °C until free thiol measurement. Free thiol groups were detected as previously described, with minor modifications.[19,20] In short, after thawing, 75 µl serum samples were diluted 1:4 with a 0.1 M Tris buffer (pH 8.2) and then transferred to a microplate. The background absorption was measured, using a Sunrise microplate reader (Tecan Trading AG, Männedorf, Switzerland) at 412 nm, with a reference filter at 630 nm. Subsequently, 10 µl 3.8 mM 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB, CAS-number 69–78–3, Sigma Aldrich Corporation, Saint Louis, MO, USA) in a 0.1 M phosphate buffer (pH 7.0) was added to the samples. Following 20 min of incubation at room temperature, absorption was measured again. The concentration of free thiols in the samples was determined by parallel measurement of a L-cysteine (CAS-number 52–90–4, Fluka Biochemika, Buchs, Switzerland)

calibration standard in the concentration range of 15.6–1000 µM in 0.1 M Tris and 10 mM EDTA (pH 8.2). All measurements were performed in triplicate, where the mean value of three measurements was used as the serum free thiol concentration. The mean concentration was based on duplicate measurement in case that one out of three values was an obvious outlier based on visual inspection. Hemolytic samples or measurements with a coefficient of variation >15% were excluded from further analysis.

Outcome parameters

Infarct size and left ventricular ejection fraction (LVEF) were used as functional outcomes. Both were determined with cardiac magnetic resonance imaging (CMR) at 4 months follow-up. Details on imaging acquisition and analysis were reported elsewhere.[17,18] An independent core laboratory (Image Analysis Center, VU University Medical Center, Amsterdam, the Netherlands) evaluated the CMR scans, blinded for clinical patient data and treatment allocation. In addition, in GIPS-III, a clinical follow-up was performed by telephone during 5 years follow-up for the assessment of major adverse cardiovascular events (MACE). MACE was defined as the composite of cardiovascular death, reinfarction or unscheduled revascularization.

Statistical analysis

Normally distributed data were presented as mean ± standard deviation (SD). Skewed data were presented as median and interquartile range [IQR] and were normalized by logarithmic transformation for analyses. Discrete variables were presented as frequencies with percentages (%). Student's t tests were used to compare groups for normally distributed continuous variables, Mann–Whitney *U* tests for skewed continuous variables and Chi-square and Fisher's exact tests for categorical variables. Associations between clinical parameters and free thiols levels at presentation and delta free thiols during the first 24 hours after STEMI were assessed using uni- and multivariable linear regression analyses. Variables with a *P*-value <0.1 in age- and sex-adjusted analyses were included in stepwise multivariable regression. Unless otherwise stated, identical models were composed using forward and backward regression analyses. Assumptions of residual variance normality and absence of collinearity were fulfilled. Subsequently, associations between free thiols and functional outcomes (infarct size and LVEF) were investigated with regression analysis, adjusting for age, sex, treatment allocation and relevant baseline parameters. Associations with 5-year MACE were assessed using Kaplan-Meier survival analysis in which groups were compared with log rank tests. Cox proportional hazards regression analyses were omitted due to paucity of events. Statistical analysis was performed with STATA version 14.0 (Stata Corp, College Station, Texas, USA). Graphs were drawn in GraphPad Prism 8. A two-tailed P-value of ≤0.05 was considered statistically significant.

RESULTS

Characteristics at presentation

Serum free thiols were measured in 378 patients that presented with STEMI and participated in the GIPS-III trial.[18] Baseline characteristics of the study population are presented in **Table 1**. Mean age was 59 ± 12 years old and 25% were women. The mean (±SD) serum free thiol concentration at presentation was 356 ± 91 µmol/L. Relatively lower serum free thiols at presentation with STEMI, indicative of systemic oxidative stress, were associated with older age, female sex, and higher creatinine (all $P<0.01$). Other predictors of lower free thiols were non-anterior MI ($P<0.01$), TIMI flow before PCI of 2/3 ($P<0.05$), lower heart rate ($P<0.01$) and lower log triglycerides ($P<0.001$)(**Supplementary Table 1**).

Free thiols during follow-up

Longitudinal changes in serum free thiols are presented in **Supplementary Figure 1**. We observed that free thiol concentrations at 24 hours (353 ± 76 µmol/L) were on average comparable with free thiols at presentation ($P=0.74$), but a large distribution in change during the first 24 hours (delta free thiols; 2 ± 125 µmol/L) was observed. Females, patients with older age, higher free thiols at presentation, TIMI flow before PCI of 0/1 and higher heart rate at presentation were more likely to have a decline in free thiols during the first 24 hours (**Table 2**).

Table 1 | Baseline characteristics

Characteristic	n=378
Age, years, mean ± SD	58.8 ± 11.6
Female sex, n (%)	95 (25%)
Caucasian ethnicity, n (%)	364 (96%)
Medical history and risk factors, n (%)	
Hypertension	112 (30%)
Dyslipidemia	238 (63%)
Current smoking	209 (55%)
Cerebrovascular accident	3 (0.8%)
Previous PCI	4 (1%)
Clinical parameters, mean ± SD	
Body Mass Index, kg/m^2	27.0 ± 3.8
Systolic blood pressure, mmHg	134 ± 23
Diastolic blood pressure, mmHg	84 ± 15
Heart rate, bpm	76 ± 16

PCI parameters	
Total ischemic time, min, median (IQR)	161 [109-250]
Single vessel disease, n (%)	258 (68%)
Anterior myocardial infarction, n (%) [a]	146 (39%)
TIMI flow pre-PCI, n (%)	
0	208 (55%)
1	27 (7%)
2	66 (18%)
3	77 (20%)
TIMI flow post-PCI, n (%)	
2	34 (9%)
3	344 (91%)
Myocardial blush grade, n (%)	
0	10 (3%)
1	29 (8%)
2	74 (20%)
3	262 (70%)
Laboratory parameters, median (IQR)	
Hemoglobin, mmol/L	9.0 [8.4-9.4]
Creatinine, µmol/L	72 [62-82]
eGFR, ml/min x 1.73m^2	93 [80-106]
NT-proBNP, ng/L	81 [40-200]
CK, U/L	130 [83-210]
CK-MB, U/L	16 [13-25]
Total cholesterol, mmol/L	5.3 [4.7-6.0]
LDL cholesterol, mmol/L	3.8 [3.2-4.4]
HDL cholesterol, mmol/L	1.1 [0.9-1.3]
Triglycerids, mmol/L	0.95 [0.64-1.52]
Glucose, mmol/L	8.2 [7.0-9.6]
HbA_{1c}, %	5.8 [5.6-6.0]
Serum free thiols, µmol/L, mean (SD)	356 ± 91

[a] *defined as culprit in left anterior descending artery.*

Abbreviations: CK, creatine kinase; CK-MB, creatine kinase myocardial band; eGFR, estimated glomerular filtration rate; IQR, interquartile range; HbA_{1c}, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TIMI, Thrombolysis in Myocardial Infarction; PCI, percutaneous coronary intervention; SD, standard deviation.

Table 2 | Age- and sex-adjusted and multivariable associations of baseline parameters with delta thiols during the first 24 hours

	Age- and sex-adjusted			Multivariable		
	Std β	95% CI	P-value	Std β	95% CI	P-value
Age, years	0.01	-0.10 to 0.12	0.85	-0.16	-0.23 to -0.08	**<0.001**
Female sex	0.013	-0.10 to 0.12	0.82	-0.12	-0.19 to -0.05	**0.001**
Treatment allocation	0.012	-0.10 to 0.12	0.84	0.002	-0.07 to 0.07	0.96
ACE-inhibitor at admission	-0.12	-0.23 to -0.004	**0.04**			
Systolic BP, mmHg	-0.11	-0.22 to 0.0001	**0.05**			
Diastolic BP, mmHg	-0.14	-0.25 to -0.03	**0.01**			
Heart rate, bpm	-0.21	-0.32 to -0.10	**<0.001**	-0.08	-0.15 to -0.007	**0.031**
TIMI flow pre-PCI 0/1 vs 2/3 (ref)	-0.14	-0.25 to -0.03	**0.01**	-0.08	-0.15 to -0.010	**0.026**
Anterior myocardial infarction [a]	-0.18	-0.29 to -0.07	**<0.001**			
log ASAT at presentation, U/L	-0.15	-0.26 to -0.03	**0.01**			
ALAT at presentation, U/L	-0.11	-0.23 to -0.003	**0.05**			
Alkaline phosphatase at presentation, U/L	-0.12	-0.24 to 0.002	0.06			
log Glucose at presentation, mmol/L	-0.11	-0.22 to 0.001	**0.05**			
log LDH at presentation, U/L	-0.11	-0.22 to 0.004	0.06			
Serum free thiols at presentation, µmol/L	-0.85	-0.92 to -0.78	**<0.001**	-0.80	-0.87 to -0.78	**<0.001**

P-values ≤0.05 are bold printed, [a] defined as culprit in left anterior descending coronary artery. Next to age, sex and treatment allocation, variables with P-values <0.1 in age- and sex-adjusted analyses were considered for multivariable regression analysis. In the forward regression model instead of heart rate and TIMI flow pre-PCI, LDH was a significant predictor, however the overall R^2 of the model was lower.

Abbreviations: ACE, angiotensin converting enzyme; ALAT, Alanine transaminase; ASAT, Aspartate transaminase; BP, blood pressure; bpm, beats per minute; CI, confidenc LDH, lactate dehydrogenase; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Free thiols and functional outcomes

At 4 month follow-up, mean infarct size and LVEF were 9.0% (±7.9) and 54% (±8.5), respectively. Free thiols at presentation with STEMI were not associated with infarct size or LVEF (**Table 3** and **Table 4**). Delta free thiol levels (defined as the change in free thiols between presentation and 24 hours), however, were associated with infarct size in univariate analysis (per 100 µmol/L increase in free thiols: β=-0.90, 95% CI: -1.77 to -0.03, *P*=0.044), and age- and sex-adjusted analysis (per 100 µmol/L increase free thiols: β=-0.87, 95% CI: -1.75 to -0.001, *P*=0.050; **Table 3**). Delta free thiols during the first 24 hours were also associated with LVEF in univariate and age- and sex-adjusted analysis (per 100 µmol/L increase: β=1.34, 95% CI: 0.40 to 2.28, *P*=0.005 and β=1.31, 95% CI: 0.37 to 2.25, *P*=0.007, respectively; **Table 4**). After adjustment for additional covariates in the model, however, the statistical significance of the associations with functional outcomes vanished. Free thiol levels at 24 hours were not associated with infarct size, only with LVEF (per 100 µmol/L increase: β=1.75, 95% CI: 0.27 to 3.24, *P*=0.021). This association with LVEF was still present after adjustment for age- and sex-adjusted (per 100 µmol/L increase: β=1.97, 95% CI: 0.43 to 3.51, *P*=0.012; **Table 4**), but lost statistical significance in multivariable analysis.

Table 3 | Associations of free thiols with infarct size at 4 months follow-up

	Univariable			Age- and sex-adjusted			Multivariable [a]		
	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
Free Thiols before PCI, per 100 µmol/L			>0.10			>0.10			
Δ Free Thiols, first 24 hours, per 100 µmol/L	-0.90	-1.77 to -0.03	**0.044**	-0.87	-1.75 to -0.001	**0.050**	-0.23	-1.09 to 0.62	0.59
Free Thiols, 24 hours, per 100 µmol/L	-1.30	-2.69 to 0.84	0.065	-1.39	-2.84 to 0.07	0.061	-0.61	-1.99 to 0.77	0.39
Free Thiols, 2 weeks, per 100 µmol/L			>0.10			>0.10			
Free Thiols, 4 months, per 100 µmol/L			>0.10			>0.10			

β: unstandardized regression coefficient. P-values ≤0.05 in bold print. [a] Free thiols at each time point were modelled separately. Next to age, sex and metformin treatment, the following variables were entered into the stepwise model: BMI, TIMI flow pre- and post-PCI, myocardial blush grade, anterior myocardial infarction (defined as culprit in left anterior descending coronary artery) and log NT-proBNP.

Abbreviations: BMI, body mass index; CI, confidence interval; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction

Table 4 | Associations of free thiols with LVEF at 4 months follow-up

	Univariable			Age- and sex-adjusted			Multivariable [a]		
	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
Free thiols, pre-PCI, per 100 µmol/L	-1.02	-2.20 to 0.15	0.088			> 0.10			
Δ Free thiols, first 24 hours, per 100 µmol/L	1.34	0.40 to 2.28	**0.005**	1.31	0.37 to 2.25	**0.007**	0.76	-0.19 to 1.71	0.12
Free thiols, 24 hours, per 100 µmol/L	1.75	0.27 to 3.24	**0.021**	1.97	0.43 to 3.51	**0.012**	1.11	-0.41 to 2.64	0.15
Free thiols, 2 weeks, per 100 µmol/L	1.87	-0.04 to 3.78	0.054	1.98	0.05 to 3.90	**0.044**	1.29	-0.59 to 3.18	0.18
Free thiols, 4 months, per 100 µmol/L			> 0.10			> 0.10			

β: unstandardized regression coefficient. P-values ≤0.05 in bold print. [a] Free thiols at each timepoint were modelled separately. Next to age, sex and metformin treatment, the following variables were entered into the stepwise model: TIMI flow pre- and post-PCI, myocardial blush grade, anterior myocardial infarction (defined as culprit in left anterior descending coronary artery), ischemic time and log NT-proBNP.

Abbreviations: CI, confidence interval; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Associations between free thiols at 24 hours and LVEF were modified by interaction of TIMI flow pre-PCI subgroups (*P* for interaction=0.039). In patients that presented with a TIMI flow pre-PCI of 0 or 1, a 100 µmol/L increase in free thiol levels over the first 24 hours was associated with higher LVEF (β=1.44, 95% CI: 0.22 to 2.66, *P*=0.021) in age- and sex-adjusted analysis, whereas in patients that presented with TIMI flow 2 or 3 at reperfusion no association was observed (**Table 5**, **Figure 1**). For free thiols at 24 hours similar results were observed (TIMI 0/1; per 100 µmol/L increase: β=2.73, 95% CI: 0.68 to 4.77, *P*=0.009 vs. TIMI 2/3; per 100 µmol/L increase: β=0.05, 95% CI: -2.14 to 2.24, *P*=0.96; **Table 5**). For the associations with infarct size, comparable differences were observed within the TIMI flow subgroups, although the associations between free thiols and infarct size in the TIMI flow 0/1 group did not reach statistical significance (*P*=0.05 to 0.1, **Figure 1, Supplementary Table 2**).

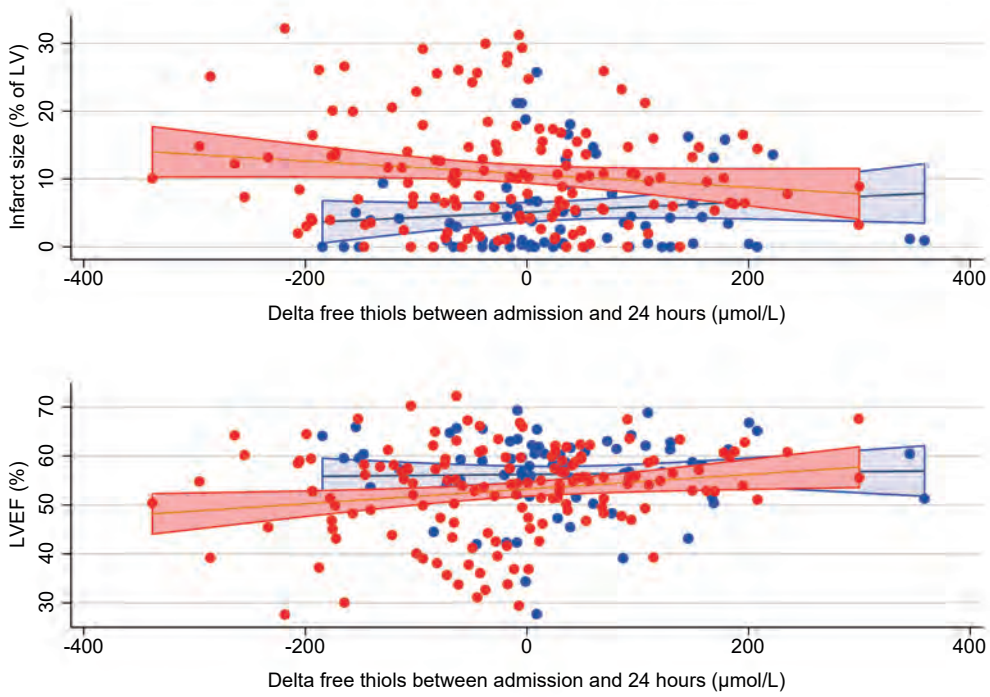

Figure 1 | Associations between delta free thiols and functional outcomes by TIMI flow pre-PCI

Scatterplot showing infarct sizes (top, n=224) and LVEF (bottom, n=237) for each individual data point of delta free thiols during the first 24 hours after admission. Associations with infarct size and LVEF are depicted by TIMI flow pre-PCI. The blue unadjusted regression line with 95% confidence interval depicts patients with TIMI flow pre-PCI of 2/3 at presentation with STEMI and the red unadjusted regression line with 95% confidence interval patients with TIMI flow pre-PCI of 0/1. Abbreviations: LV, left ventricle; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

Table 5 | Associations of free thiols with LVEF in subgroups with and without reperfusion at presentation

	Univariable			Age- and sex-adjusted			Multivariable [a]		
	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
Δ free thiols, first 24 hours, per 100 µmol/L									
No reperfusion, TIMI 0 or 1	1.50	0.27 to 2.72	**0.017**	1.44	0.22 to 2.66	**0.021**	1.11	-0.06 to 2.28	0.063
Reperfusion, TIMI 2 or 3	0.20	-1.32 to 1.71	0.80	0.24	-1.27 to 1.74	0.75			>0.10
Free thiols 24 hours, per 100 µmol/L									
No reperfusion, TIMI 0 or 1	2.83	0.85 to 4.82	**0.005**	2.73	0.68 to 4.77	**0.009**	1.79	-0.20 to 3.78	0.078
Reperfusion, TIMI 2 or 3	-0.56	-2.66 to 1.54	0.59	0.05	-2.14 to 2.24	0.96			>0.10

β: unstandardized regression coefficient. P-values ≤0.05 in bold print. [a] Each thiol was modelled separately. Next to age, sex and metformin treatment, the following variables were entered into the stepwise model: TIMI flow post-PCI, myocardial blush grade, anterior myocardial infarction (defined as culprit in left anterior descending coronary artery), ischemic time and log NT-proBNP.

Abbreviations: CI, confidence interval; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Free thiols and clinical outcomes

During 5 years of follow-up 63 patients (17.7%) underwent an ischemic driven intervention (n=48) or deceased (n=16, of which 1 patient died after ischemic driven re-intervention). In patients with a net decrease in serum free thiols during the first 24 hours, a trend towards more events during 5-year follow-up was observed, log-rank (*P*=0.09; **Figure 2**). For free thiols at other timepoints, however, no associations with clinical outcomes were observed.

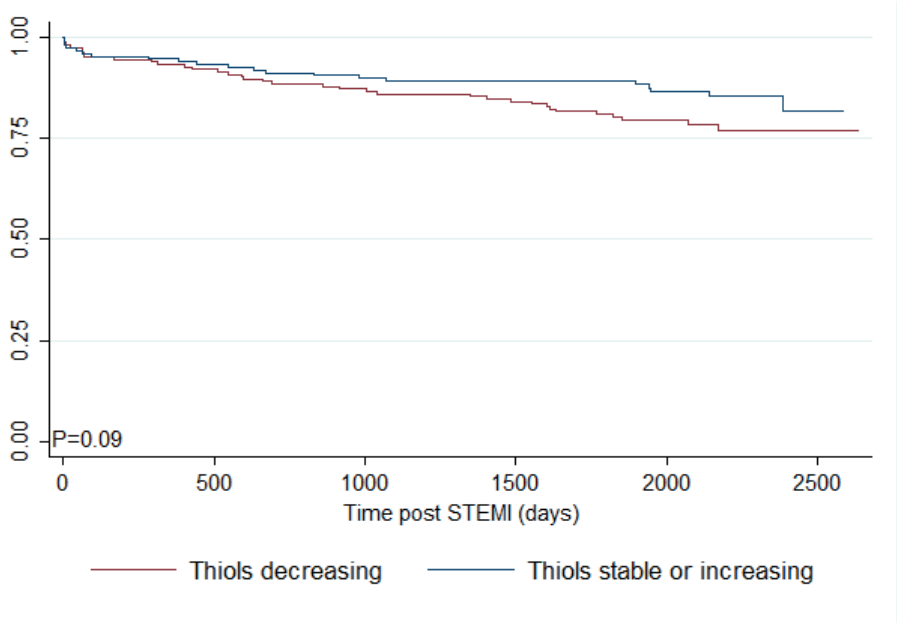

Figure 2 | Kaplan-Meier for delta free thiols on MACE up to 5 years follow-up

Incidences of major adverse cardiovascular events (MACE) at 5 years follow-up for patients with stable or increasing free thiols during the first 24 hours after admission (blue line) and for patients with decreasing free thiols during the first 24 hours (red line). MACE was defined as all-cause death, re-infarction or unscheduled re-intervention.

DISCUSSION

This study demonstrates that changes in serum free thiols, indicative of variations in oxidative stress levels, early after PCI, associate with functional outcomes after STEMI, especially in patients presenting with closed coronary arteries (TIMI flow pre-PCI 0/1). Our data support the hypothesis that extracellular free thiols might offer a novel monitoring tool for risk stratification and might be used as a therapeutic target to modify outcomes for patients presenting with STEMI and coronary artery occlusion.

Ischemia and the subsequent reperfusion are major triggers for oxidative stress. During ischemia, the antioxidant enzyme superoxide dismutase (SOD) becomes less active, together with a decline in reduced glutathione and a reduction in free thiols.[21] Subsequently, the reintroduction of oxygen to an ischemic area leads to a burst of ROS, triggering oxidative stress. Moreover, permanent mitochondrial damage and inflammatory responses drive a prolonged course of ROS generation.[22] Free thiols are potent antioxidants that directly scavenge ROS, thereby preventing ROS from inflicting cellular damage. Aside from functioning as potent oxidant scavengers, extracellular free thiols also act as multimodal redox relays by controlling redox exchange reactions between organs and between extracellular and intracellular environments, and by mediating protein structure,[23] activity and functions.[7] Plasma free thiols mainly encompass circulating cysteine-based proteins, of which albumin is the most relevant compound.[5,24] Moreover, a smaller part of circulating free thiols is represented by low-molecular-weight free thiols such as glutathione and cysteine. The aggregate of high- and low-molecular-weight free thiols is referred to as *total free thiols*. Since free thiols are central biomarkers in our redox system, a decline in circulating free thiols reliably reflect systemic or local oxidative stress.[25] A decrease in free thiol concentration has indeed been linked to a wide variety of oxidative stress-associated diseases,[6,10,13] but have not been longitudinally evaluated in the acute phase after STEMI.

A few cross-sectional studies investigated thiol-disulfide homeostasis in patients with acute MI, and although different measurement techniques were employed, they consistently showed lower levels of free thiols and higher levels of oxidation products (e.g. oxidized protein thiols: disulfides) in patients with acute MI.[26–28] In addition, several studies have demonstrated inverse associations between free thiols and coronary atherosclerosis severity,[29,30] complications early after STEMI such as left ventricular systolic dysfunction and acute heart failure,[31] and fatal MI outcomes.[32] Only one study reported on associations between free thiol levels and MACE 6 months after MI.[33] Until date, however, no studies have yet been performed that measure free thiols before and after PCI, with adequate long-term follow-up and pre-defined functional outcomes after STEMI such as infarct size and LVEF.

Although free thiols were longitudinally measured, this study focused on changes during the acute phase since these appeared to be most strongly predictive for long-term functional outcomes. Notably, we observed a significant interaction with TIMI flow before PCI for the association between the change in serum free thiols during the first 24 hours (delta free thiols) and the functional outcomes. While we observed that in patients with a TIMI flow pre-PCI of 0 or 1 (representing absence of flow/poor reperfusion) delta free thiols were significantly associated with LVEF, patients with TIMI flow 2 or 3 (representing partial and complete reperfusion) did not demonstrate these associations. A potential explanation for this phenomenon could be that profound changes in free thiols levels already occurred before presentation in patients with TIMI flow pre-PCI of 2/3, as a result of the reperfusion that already took place, limiting the predictive value of the delta free thiols between presentation and 24 hours. Another explanation may be a different health/redox status before the onset of MI, which might influence reperfusion and alters the associations with functional outcomes. In line with this hypothesis, previous studies also observed significant interactions (i.e. stronger associations between free thiols and cardiovascular outcomes) with albuminuria and the presence of chronic heart failure,[10,13] in which it was hypothesized that ongoing endothelial and/or structural damage to the cardiovascular system inflicts a higher level of systemic oxidative stress that could in turn augment the predictive capacity of free thiols. Unfortunately, due to the nature of the disease, we were not able to evaluate baseline redox status before the onset of ischemia.

Next to the prognostic value of serum free thiols, our results also shed light on the potential for future development of redox-targeted therapeutics in the context of ischemic heart disease. In particular, since we observed that free thiols during the first 24 hours post-STEMI confer predictive value in relation to functional outcomes, this may aid in establishing a therapeutic window for redox-directed interventions. For example, redox-active compounds capable of reversing oxidative thiol modifications such as thioredoxins, glutaredoxins and peroxiredoxins represent a complex network of antioxidants still requiring further study but holding potential to reveal relevant therapeutic targets for cardioprotection.[34] Similarly, hydrogen sulfide (H_2S)-targeted compounds e.g. N-acetylcysteine,[35] sodium thiosulfate (STS),[36,37] or taurine[38,39] may become relevant therapeutic candidates, because H_2S, is a gaseous signaling molecule, which has indirect antioxidant properties by activating antioxidant pathways and increase glutathione levels, but also has the capacity to scavenge ROS and reduce disulphide bonds, resulting in free thiol formation.[40] Notably, it remains important to cautiously analyze a patient's individual redox status before implementing therapeutic modulation, because it has been suggested that thiol-modulating strategies should be reserved for patients with an evidently disturbed redox system, since thiol supplementation could potentially disrupt physiological redox signaling.[34] Therefore, more mechanistic studies and studies with strict protocols, evaluating to which extent long-term functional outcomes after STEMI could be improved by exogenous redox system modifications, are warranted.

Limitations

Strengths of our study include the size, well-documented and longitudinal nature of our study, combined with an extensive follow-up and detailed pre-defined functional outcomes. Several limitations however also warrant recognition. For instance, the observational character of the study did not allow the establishment of potential causality between extracellular free thiol status and functional outcomes. Second, we lacked total protein or albumin levels, which precluded a more precise estimation of circulating free thiol levels since adjustment to one of both would be an appropriate but indirect way of accounting for total thiol content and fluid status.[7,10] Finally, due to a possible lack of statistical power we were unable to draw firm conclusions on the studied associations between free thiols and functional outcomes across TIMI flow subgroups. Therefore, our results warrant validation in larger cohorts of patients with atherosclerotic cardiovascular diseases.

CONCLUSIONS

Changes in levels of systemic oxidative stress early post-PCI may predict infarct size and LVEF in patients experiencing STEMI, especially those presenting without reperfusion. Since free thiols are amendable to therapeutic intervention our data may support the development of future thiol-targeted therapy in patients with ischemic heart disease.

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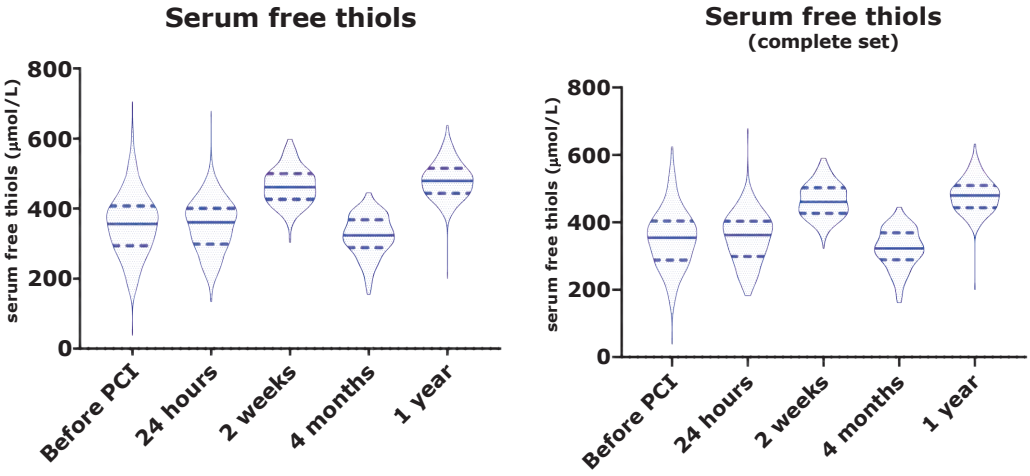
SUPPLEMENTARY MATERIAL



Supplementary Figure 1 | Serum free thiol concentrations after ST-segment elevation myocardial infarction

Violin plots of serum free thiol concentrations (µmol/L) at presentation with STEMI (n=358) and at 24 hours (n=330), 2 weeks (n=328), 4 months (n=313) and 1 year (n=271) after reperfusion on the left side. Likewise, the plot on the right depicts serum free thiol concentrations for patients in which serum free thiol measurements were obtained at all timepoints (n=216). Abbreviations: PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction.

Supplementary Table 1 | Age- and sex-adjusted and multivariable associations of baseline parameters with serum free thiols before PCI

	Age- and sex adjusted			Multivariable		
	Std β	**95% CI**	***P*-value**	**Std β**	**95% CI**	***P*-value**
Age, years	-0.21	-0.31 to -0.11	**<0.001**	-0.13	-0.23 to -0.03	**0.012**
Female sex	-0.15	-0.25 to -0.05	**<0.001**	-0.17	-0.27 to -0.06	**0.002**
Hypertension in medical history	0.10	-0.001 to 0.21	**0.05**			
Systolic BP, mmHg	0.11	0.02 to 0.21	**0.02**			
Diastolic BP, mmHg	0.13	0.03 to 0.23	**0.01**			
Heart rate, bpm	0.18	0.08 to 0.27	**<0.001**	0.16	0.06 to 0.26	**0.002**
TIMI flow pre-PCI, 0/1 vs 2/3 (ref)	0.10	0.001 to 0.20	**0.05**	0.12	0.02 to 0.21	**0.019**
Anterior myocardial infarction [a]	0.15	0.05 to 0.25	**<0.001**	0.13	0.04 to 0.23	**0.008**
Hemoglobin, mmol/L	0.11	-0.0004 to 0.22	**0.05**			
Creatinine, µmol/L	-0.11	-0.22 to 0.004	0.06	-0.15	-0.26 to -0.04	**0.007**
Log glucose, mmol/L	0.11	0.01 to 0.21	**0.04**			
Total cholesterol, mmol/L	0.09	-0.01 to 0.20	0.08			
Log Triglycerides, mmol/L	0.17	0.07 to 0.28	**<0.001**	0.19	0.09 to 0.30	**<0.001**

P-values ≤0.05 are bold printed, [a] defined as culprit in left anterior descending coronary artery

Next to age and sex, variables with P- values<0.1 in age- and sex-adjusted analyses were considered for multivariable regression analysis.

Abbreviations: BP, blood pressure; bpm, beats per minute; CI confidence interval; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Supplementary Table 2 | Associations of delta free thiols during the first 24 hours and free thiols at 24 hours after presentation with infarct size in subgroups with and without reperfusion at presentation

	Univariable			Age- and sex-adjusted			Multivariable [a]		
	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
Δ free thiols, first 24 hours, per 100 µmol/L									
No reperfusion, TIMI 0 or 1	-0.97	-2.07 to 0.13	0.083	-0.96	-2.06 to 0.14	0.087			>0.10
Reperfusion, TIMI 2 or 3	0.77	-0.51 to 2.05	0.23	0.80	-0.48 to 2.07	0.22			>0.10
Free thiols 24 hours, per 100 µmol/L									
No reperfusion, TIMI 0 or 1	-1.81	-3.62 to 0.02	0.053	-1.63	-3.53 to 0.27	0.092			>0.10
Reperfusion, TIMI 2 or 3	0.67	-1.13 to 2.46	0.46	0.26	-1.64 to 2.16	0.79			>0.10

β: unstandardized regression coefficient. P-values ≤0.05 in bold print. [a] *Free thiols at each timepoint were modelled separately. Next to age, sex and metformin treatment, the following variables were entered into the stepwise model: TIMI flow post-PCI, myocardial blush grade, anterior myocardial infarction (defined as culprit in left anterior descending coronary artery), ischemic time and log NT-proBNP.*

Abbreviations: CI, confidence interval; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.