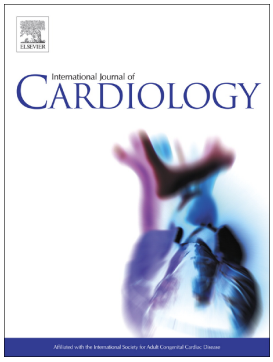
Associations of systemic oxidative stress with functional outcomes after ST-segment elevation myocardial infarction

de Koning, Marie-Sophie L. Y.; Al Ali, Lawien; Assa, Solmaz; Bourgonje, Arno R.; Pasch, Andreas; van Goor, Harry; Lipsic, Erik; van der Harst, Pim

Published in:
International Journal of Cardiology

DOI:
10.1016/j.ijcard.2023.131214

Publication date:
2023



Associations of systemic oxidative stress with functional outcomes after ST-segment elevation myocardial infarction

Marie-Sophie L.Y. de Koning, Lawien Al Ali, Arno R. Bourgonje, Solmaz Assa, Andreas Pasch, Harry van Goor, Erik Lipsic, Pim van der Harst

PII: S0167-5273(23)01057-4

DOI: https://doi.org/10.1016/j.ijcard.2023.131214

Reference: IJCA 131214

To appear in: *International Journal of Cardiology*

Received date: 22 March 2023

Revised date: 23 July 2023

Accepted date: 24 July 2023

Please cite this article as: M.-S.L.Y. de Koning, L. Al Ali, A.R. Bourgonje, et al., Associations of systemic oxidative stress with functional outcomes after ST-segment elevation myocardial infarction, *International Journal of Cardiology* (2023), https://doi.org/10.1016/j.ijcard.2023.131214

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

Marie-Sophie L.Y. de Koning[1] MD PhD, Lawien Al Ali[1] MD, Arno R. Bourgonje[2] MD PhD, Solmaz Assa[1] MD PhD, Andreas Pasch[3,4,5] MD PhD, Harry van Goor[6] PhD, Erik Lipsic[1] MD PhD, Pim van der Harst[1,7] MD PhD

[1] University of Groningen, University Medical Center Groningen, Department of Cardiology, Groningen, the Netherlands
[2] University of Groningen, University Medical Center Groningen, Department of Gastroenterology and Hepatology, Groningen, The Netherlands
[3] Institute for Physiology and Pathophysiology, Johannes Kepler University, Linz, 4040, Austria
[4] Lindenhofspital, Department of Nephrology, Bern, 3011, Switzerland
[5] Nierenpraxis Bern, Bern, 3011, Switzerland
[6] University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, the Netherlands
[7] Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, Utrecht, the Netherlands

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Corresponding Author: Dr. Marie-Sophie L.Y. de Koning, University of Groningen, University Medical Center Groningen, Department of Cardiology, Groningen, the Netherlands, Hanzeplein 1, PO Box 30.001, 9700 RB, Groningen, the Netherlands. Fax: (+31) 0503611347 | Tel: (+31) 0503612355 | E-mail: m.s.l.y.de.koning@umcg.nl

Acknowledgements: The authors would like to thank Marian L.C. Bulthuis and Janny E. Takens for their technical assistance with the free thiol measurements.

Funding: The GIPS-III trial was supported by grant 95103007 from the Netherlands Organization for Health Research and Development (ZonMw), The Hague, the Netherlands.

Conflict of interest: The authors have no conflict of interest to disclose.

Keywords: Acute coronary syndrome, free thiols, oxidative stress, major adverse cardiovascular events

Abstract

Background: Ischemia-reperfusion is accompanied by oxidative stress. Serum free thiols (FTs; sulfhydryl groups) reliably reflect systemic oxidative stress. This study evaluates longitudinal changes in FTs and their associations with outcomes after ST-segment elevation myocardial infarction (STEMI).

Methods: FTs were detected in archived serum samples from 378 participants of a neutral randomized trial on metformin therapy after STEMI. FT levels were determined at presentation with STEMI and at 24 hours, 2 weeks, 4 months and 1 year thereafter. Outcomes included infarct size and left ventricular ejection fraction (LVEF), both determined with cardiac magnetic resonance imaging after 4 months, and 5-year major adverse cardiovascular events (MACE).

Results: Serum FT concentrations at presentation and at 24 hours were 356 ± 91 and 353 ± 76 µmol/L, respectively. The change in FTs between presentation and 24 hours (ΔFTs) was associated with outcomes in age- and sex-adjusted analysis (per 100 µmol/L FT increase, β=-0.87 for infarct size, 95%-confidence interval (CI): -1.75 to -0.001, *P*=0.050; β=1.31, 95%-CI: 0.37 to 2.25 for LVEF, *P*=0.007). Associations between ΔFTs and LVEF were markedly stronger in patients 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 FTs 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. Our findings warrant validation in larger cohorts, and then may be used as rationale for development of 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. 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 S1**. 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 triglycerides (*P*<0.001)(**Table S2**).

Free thiols during follow-up

Longitudinal changes in serum free thiols are presented in **Figure S1**. 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. Female 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 1**).

Free thiols and functional outcomes

At 4 months 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 S3 and Table 2**). 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 S3**). 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 2, Table S4**). 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 2**), but lost statistical significance in multivariable analysis.

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 3, Table S5, 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 3, Table S5**). 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, **Table S6**).

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, more events during 5-year follow-up were observed, however this difference was non-significant (log rank *P*=0.09; **Figure S2**). For free thiols at other timepoints, no associations with clinical outcomes were observed.

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 results provide rationale for future mechanistic studies that investigate the role of free thiols early post-PCI, and for studies investigating free thiols as potential treatment target 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 central hubs in our redox system by controlling redox exchange reactions between organs and between extracellular and intracellular environments, and by mediating protein structure,[23] activity and functions.[7] Since free thiols play a central role in our redox system, a decline in circulating free thiols reliably reflects systemic or local oxidative stress.[24] A decrease in free thiol concentration has indeed been linked to a wide variety of oxidative stress-associated diseases,[6, 10, 13, 25] 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. 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. Targeting I/R injury in the acute phase in patients presenting with STEMI is complex and requires timely administration and adequate levels of a therapeutic compound during the very first moments after reperfusion.[34] We observed that an ongoing depletion of free thiols during the first 24 hours post-STEMI confer predictive value in relation to functional outcomes, especially LVEF. This may

suggest that there is a possible therapeutic window for redox-directed interventions that might target oxidative stress in the remote non-infarcted area, which may potentially improve LVEF.[35] 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.[36] Similarly, hydrogen sulfide (H_2S)-targeted compounds e.g. N-acetylcysteine,[37] sodium thiosulfate (STS),[38, 39] or taurine[40, 41] 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.[42] However, to date trials with N-acetylcysteine for cardioprotection have been inconclusive.[43] 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.[36] 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, this study was not designed to prove or suggest an additional prognostic role for free thiols, on top of existing predictors, e.g. TIMI flow and enzymatic infarct size. Third, 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. Especially for the TIMI flow subgroups our results should be considered as hypothesis generating. In addition, Cox proportional hazards regression analyses had to be omitted due to paucity of events. Therefore, our results warrant validation in larger cohorts of patients with atherosclerotic cardiovascular diseases.

Conclusions

Our hypothesis generating study shows that 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. If our findings can be validated in larger cohorts, it might be of interest to study whether free thiol modulation may hold therapeutic potential in patients with ischemic heart disease.

References

1. Ferrari R, Ceconi C, Curello S, et al. Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. Am J Med 1991; 91:95S-105S

2. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol 2015; 4:180–183

3. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol 2020; 21:363–383

4. Ferrari R, Guardigli G, Mele D, Percoco GF, Ceconi C, Curello S. Oxidative stress during myocardial ischaemia and heart failure. Curr Pharm Des 2004; 10:1699–1711

5. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. Free Radic Biol Med 2013; 65:244–253

6. Banne AF, Amiri A, Pero RW. Reduced level of serum thiols in patients with a diagnosis of active disease. J Anti Aging Med 2003; 6:327–34

7. Cortese-Krott MM, Koning A, Kuhnle GGC, et al. The Reactive Species Interactome: Evolutionary Emergence, Biological Significance, and Opportunities for Redox Metabolomics and Personalized Medicine. Antioxid Redox Signal 2017; 27:684–712

8. Schillern EEM, Pasch A, Feelisch M, et al. Serum free thiols in type 2 diabetes mellitus: A prospective study. J Clin Transl Endocrinol 2015; 16:100182

9. Gur M, Elbasan Z, Yildiray Sahin D, et al. DNA damage and oxidative status in newly diagnosed, untreated, dipper and non-dipper hypertensive patients. Hypertens Res 2013; 36:166–171

10. Bourgonje AR, Abdulle AE, Bourgonje MF, et al. Serum free sulfhydryl status associates with new-onset chronic kidney disease in the general population. Redox Biol 2021; 48:102211

11. Bourgonje AR, Gabriëls RY, de Borst MH, et al. Serum Free Thiols Are Superior to Fecal Calprotectin in Reflecting Endoscopic Disease Activity in Inflammatory Bowel Disease. Antioxidants 2019; 8:351

12. Ashfaq S, Abramson JL, Jones DP, et al. The relationship between plasma levels of oxidized and reduced thiols and early atherosclerosis in healthy adults. J Am Coll Cardiol 2006; 47:1005–1011

13. Abdulle AE, Bourgonje AR, Kieneker LM, et al. Serum free thiols predict cardiovascular events and all-cause mortality in the general population: a prospective cohort study. BMC Med 2020; 18:130

14. Giustarini D, Tazzari V, Bassanini I, Rossi R, Sparatore A. The new H2S-releasing compound ACS94 exerts protective effects through the modulation of thiol homoeostasis. J Enzyme Inhib Med Chem 2018; 33:1392–1404

15. Atkuri KR, Mantovani JJ, Herzenberg LA, Herzenberg LA. N-Acetylcysteine--a safe antidote for cysteine/glutathione deficiency. Curr Opin Pharmacol 2007; 7:355–359

16. Murphy MP. Mitochondrial thiols in antioxidant protection and redox signaling: distinct roles for glutathionylation and other thiol modifications. Antioxid Redox Signal 2012; 16:476–495

17. Lexis CPH, van der Horst ICC, Lipsic E, et al. Metformin in non-diabetic patients presenting

with ST elevation myocardial infarction: rationale and design of the glycometabolic intervention as adjunct to primary percutaneous intervention in ST elevation myocardial infarction (GIPS)-III trial. Cardiovasc drugs Ther 2012; 26:417–426

18. Lexis CPH, van der Horst ICC, Lipsic E, et al. Effect of metformin on left ventricular function after acute myocardial infarction in patients without diabetes: the GIPS-III randomized clinical trial. JAMA 2014; 311:1526–1535

19. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70–77

20. Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. J Lab Clin Med 1993; 121:257–262

21. Ferrari R, Ceconi C, Curello S, et al. Oxygen-mediated myocardial damage during ischaemia and reperfusion: role of the cellular defences against oxygen toxicity. J Mol Cell Cardiol 1985; 17:937–945

22. Chen QM. Nrf2 for protection against oxidant generation and mitochondrial damage in cardiac injury. Free Radic Biol Med 2022; 179:133–143

23. Santolini J, Wootton SA, Jackson AA, Feelisch M. The Redox architecture of physiological function. Curr Opin Physiol 2019; 9:34–47

24. Sutton TR, Minnion M, Barbarino F, et al. A robust and versatile mass spectrometry platform for comprehensive assessment of the thiol redox metabolome. Redox Biol 2018; 16:359–380

25. de Koning MLY, Emmens JE, Romero-Hernández E, et al. Systemic oxidative stress associates with disease severity and outcome in patients with new-onset or worsening heart failure. Clin Res Cardiol 2023; 1–11

26. Kundi H, Ates I, Kiziltunc E, et al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med 2015; 33:1567–1571

27. Kavakli HS, Sezer AA, Yilmaz H, et al. Thiol disulphide homeostasis in patients with acute myocardial infarction (AMI). J Pak Med Assoc 2018; 68:1631–1635

28. Barsotti A, Fabbi P, Federici M, et al. Role of advanced oxidation protein products and Thiol ratio in patients with acute coronary syndromes. Clin Biochem 2011; 44:605–611

29. Kundi H, Erel O, Balun A, et al. Association of thiol/disulfide ratio with syntax score in patients with NSTEMI. Scand Cardiovasc J 2015; 49:95–100

30. Weaver JC, Ullah I, Qi M, et al. Free Thiol β2-GPI (β-2-Glycoprotein-I) Provides a Link Between Inflammation and Oxidative Stress in Atherosclerotic Coronary Artery Disease. Arterioscler Thromb Vasc Biol 2020; 40:2794–2804

31. Rajic D, Jeremic I, Stankovic S, et al. Oxidative stress markers predict early left ventricular systolic dysfunction after acute myocardial infarction treated with primary percutaneous coronary intervention. Adv Clin Exp Med 2018; 27:185–191

32. Xuan Y, Bobak M, Anusruti A, et al. Association of serum markers of oxidative stress with myocardial infarction and stroke: pooled results from four large European cohort studies. Eur J Epidemiol 2019; 34:471–481

33. Akkus O, Topuz M, Koca H, et al. The relationship between low thiol levels and major adverse cardiovascular events after primary percutaneous coronary intervention in patients with

STEMI. Turk Kardiyol Dern Ars 2018; 46:248–259

34. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007; 357:1121–1135

35. Oskarsson HJ, Coppey L, Weiss RM, Li WG. Antioxidants attenuate myocyte apoptosis in the remote non-infarcted myocardium following large myocardial infarction. Cardiovasc Res 2000; 45:679–687

36. Andreadou I, Efentakis P, Frenis K, Daiber A, Schulz R. Thiol-based redox-active proteins as cardioprotective therapeutic agents in cardiovascular diseases. Basic Res Cardiol 2021; 116:44

37. Bourgonje AR, Offringa AK, van Eijk LE, et al. N-Acetylcysteine and Hydrogen Sulfide in Coronavirus Disease 2019. Antioxid Redox Signal 2021; 35:1207–1225

38. de Koning MLY, Assa S, Maagdenberg CG, et al. Safety and Tolerability of Sodium Thiosulfate in Patients with an Acute Coronary Syndrome Undergoing Coronary Angiography: A Dose-Escalation Safety Pilot Study (SAFE-ACS). J Interv Cardiol 2020; 2020:6014915

39. de Koning MLY, van Dorp P, Assa S, et al. Rationale and Design of the Groningen Intervention Study for the Preservation of Cardiac Function with Sodium Thiosulfate after ST-segment Elevation Myocardial Infarction (GIPS-IV) Trial. Am Heart J 2022; 243:167–176

40. Sun Q, Wang B, Li Y, et al. Taurine Supplementation Lowers Blood Pressure and Improves Vascular Function in Prehypertension: Randomized, Double-Blind, Placebo-Controlled Study. Hypertension 2016; 67:541–549

41. DiNicolantonio JJ, OKeefe JH, McCarty MF. Boosting endogenous production of vasoprotective hydrogen sulfide via supplementation with taurine and N-acetylcysteine: a novel way to promote cardiovascular health. Open Heart 2017; 4:e000600

42. Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 2012; 92:791–896

43. Khan SA, Campbell AM, Lu Y, An L, Alpert JS, Chen QM. N-Acetylcysteine for Cardiac Protection During Coronary Artery Reperfusion: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Front. Cardiovasc. Med. 8:752939

Tables and figures

Table 1 | Age- and sex-adjusted and multivariable associations of baseline parameters with delta free thiols during the first 24 hours

	Age- and sex-adjusted			*Multivariable*		
Variable	**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 0.00 01	**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*	-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 baseline, µmol/L	-0.85	-0.92 to -0.78	**<0.001**	-0.80	-0.87 to -0.783	**<0.001**

P-values ≤0.05 are bold printed.

* 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 that model was lower.

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

Table 2 | Associations of free thiols with LVEF at 4 months follow-up

	Univariable			*Age- and sex-adjusted*			*Multivariable* [#]		
Variable	**β**	**95% CI**	***P*-value**	**β**	**95% CI**	***P*-value**	**β**	**95% CI**	***P*-value**
Free thiols before PCI*	-1.02	-2.20 to 0.15	0.088			> 0.10			
Δ Free thiols, first 24 hours*	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 at 24 hours*	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 at 2 weeks*	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 at 4 months*			> 0.10			> 0.10			

β: unstandardized regression coefficient

P-values ≤0.05 in bold print.

* Free thiols at each time point were modelled separately, β values are given for every 100 µmol/L increase in free thiols.

[#] 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.

[^] The full regression model is depicted in Table S4.

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

Table 3 | Associations of free thiols with LVEF in subgroups with and without reperfusion at presentation

	Univariable			*Age- and sex-adjusted*			*Multivariable **		
Variable	**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

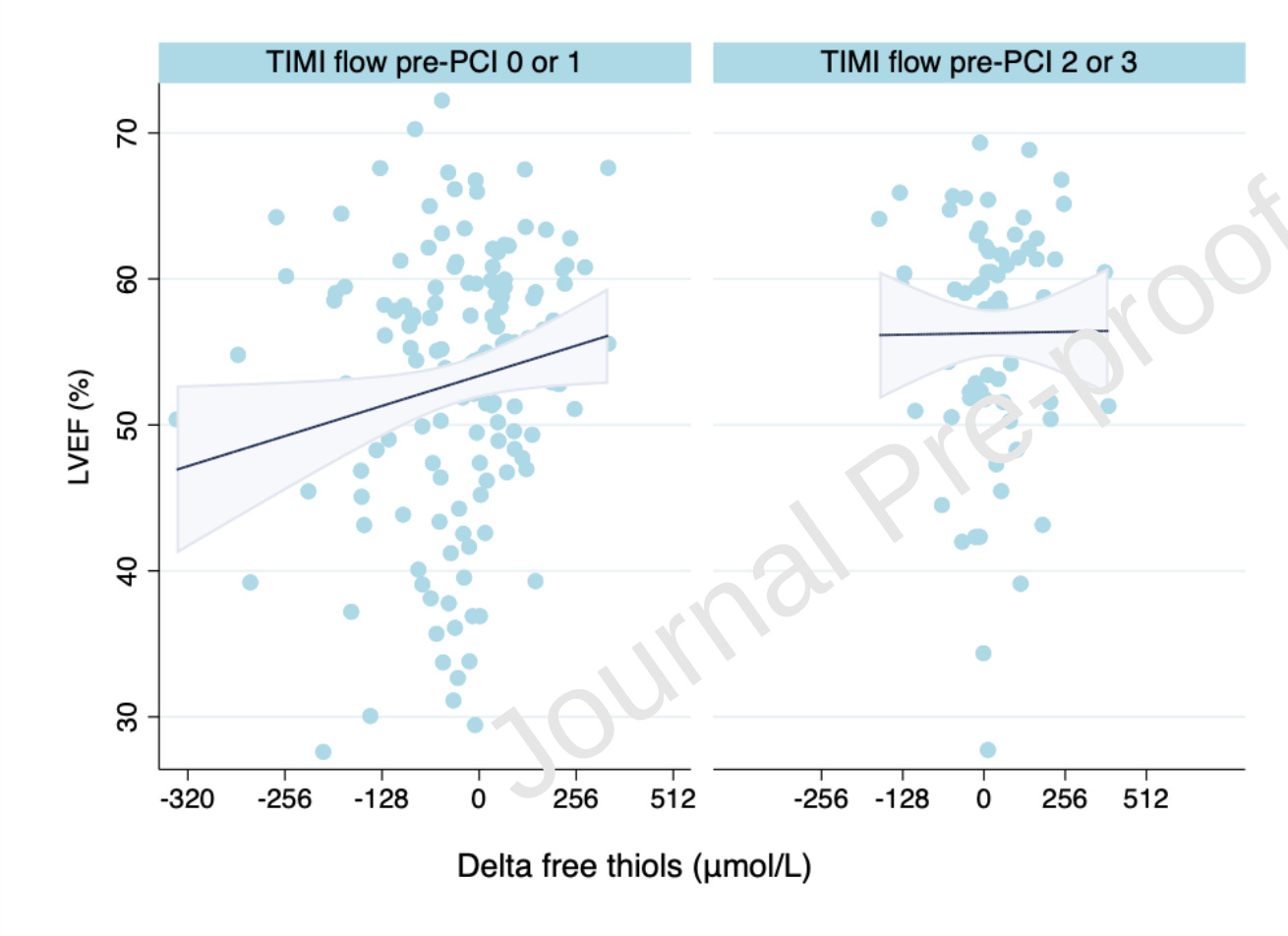
β: unstandardized regression coefficient. *P*-values ≤0.05 in bold print.

* Next to age, sex and metformin treatment, the following variables were entered into the multivariable 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.

[^] The full regression model is depicted in Table S5.

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

Figure 1 | Associations between delta free thiols and LVEF by TIMI flow pre-PCI subgroups



Plot showing unadjusted regression lines with 95% confidence interval and LVEFs (n=237) for each individual data point of delta free thiols during the first 24 hours after admission on a log 2 scale. Plots were depicted separately for patients with TIMI flow pre-PCI of 0/1 at presentation with STEMI (left panel) and for patients with TIMI flow pre-PCI of 2/3 (right panel). Abbreviations: LVEF, left ventricular ejection fraction; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Highlights

- Free thiols (FT) are antioxidants and their serum levels reflect oxidative stress
- FT depletion post-PCI is associated with larger infarct size and lower LVEF
- Associations of FT with outcomes were diluted in multivariable analysis
- Associations of FT with outcomes were stronger in patients presenting without reperfusion
- FT may have a therapeutic potential in acute myocardial infarction