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# Mannose as a biomarker of coronary artery disease: Angiographic evidence and clinical significance

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ABSTRACT

*Background:* High mannose has previously associated with insulin resistance and cardiovascular disease (CVD). Our objective is to establish whether mannose is associated with anatomical evidence of coronary artery disease (CAD).

*Methods:* Plasma mannose concentrations were measured by liquid chromatography/tandem mass spectrometry in a discovery cohort ($n = 513$) and a validation cohort ($n = 221$) of carefully phenotyped individuals. In both cohorts CAD was quantitated using state-of-the-art imaging techniques (coronary computed coronary tomography angiography (CCTA), invasive coronary angiography and optical coherence tomography). Information on subsequent CVD events/death was collected. Associations of mannose with angiographic variables and biomarkers were tested using univariate and multivariate regression models. Survival analysis was performed using the Kaplan-Meier estimator.

*Results:* Mannose was related to indices of CAD and features of plaque vulnerability. In the discovery cohort, mannose was a marker of quantity and quality of CCTA-proven CAD and subjects with a mannose level in the top quartile had a significantly higher risk of CVD events/death ($p = 3.6e\text{-}5$). In the validation cohort, mannose was significantly associated with fibrous cap thickness $< 65$ μm (odds ratio $= 1.32$ per each 10 μmol/L mannose change [95% confidence interval, 1.05–1.65]) and was an independent predictor of death (hazard ratio for mannose$\geq$*vs* $< 84.6$ μmol/L: 4.0(95%CI, 1.4–11.3), $p = 0.006$).

*Conclusion:* The current data add novel evidence that high mannose is a signature of CAD with a vulnerable plaque phenotype, consistently across measures of severity of vessel involvement and independent of the traditional correlates of CVD, and that it is an independent predictor of incident adverse outcomes.

*Abbreviations:* AUC, area under the curve; CAD, coronary artery disease; CCTA, computed tomography angiography; CVD, cardiovascular disease; FFR, fractional flow reserve; GDP-mannose, guanosine-diphospho-mannose; GCK, glucokinase; GMPPA/B, GDP-mannose pyrophosphorylases; HDL, high density lipoprotein; HK, hexokinase; HPLC-MS-MS, liquid chromatography and tandem mass spectrometry; hsCRP, High-sensitivity C-reactive protein; hs-TnT, High-sensitivity cardiac troponin T; IQR, interquartile range; MPI, Mannose-6 phosphate isomerase; MinFCT, Minimal fibrous cap thickness; OCT, optical coherence tomography; OR, odds ratio; PFK, phosphofructokinase; PMM 1, phosphomannose mutase 1; ROC, receiver operating characteristics; SD, standard deviation; T2D, type 2 diabetes.

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## 1. Introduction

Previous work – using cell-specific analysis of genome-scale metabolic models, transcriptional regulatory networks, and protein-protein interaction networks – identified elevated circulating mannose concentrations as a novel marker of insulin resistance [1]. More recently, studies using the euglycaemic hyperinsulinaemic clamp technique to measure whole-body insulin sensitivity provided direct evidence that plasma mannose – quantitated by liquid chromatography and tandem mass spectrometry (HPLC-MS-MS) – is insulin regulatable and closely tracks *in vivo* insulin resistance [2]. Moreover, in epidemiological cohorts higher plasma mannose concentrations have been associated not only with insulin resistance but also with risk of developing diabetes, diabetic kidney disease, and cardiovascular disease (CVD) [3]. The mechanisms underlying these associations have not been explored. In particular, it is not clear whether mannose is related to CVD only because it marks insulin resistance or because of some intrinsic biological property. Preliminary to this inquiry is to establish whether circulating mannose concentrations are indeed associated with anatomical evidence of extent and kind of atherosclerotic lesion characteristics of the coronary vasculature.

To address this question, the present study measured plasma mannose concentrations in two large datasets, a discovery cohort and an external validation cohort, in whom coronary atherosclerosis was quantitated using state-of-the-art imaging techniques in carefully phenotyped groups of individuals.

## 2. Materials and methods

### 2.1. Subjects and protocols

#### 2.1.1. Discovery cohort ($n = 513$)

CAPIRE (ClinicalTrials.gov Identifier: NCT02157662) is a multicentre, prospective, observational study aimed at identifying new mechanisms promoting or protecting against coronary atherothrombosis; in its longitudinal phase, subjects have been followed for five years. Details of the study have been reported [4]. Briefly, the study enrolled subjects 45 to 75 years of age (64 with type 2 diabetes (T2D)), without previous clinical manifestations of coronary artery disease (CAD) including acute myocardial infarction, unstable angina, chronic stable angina, previous percutaneous or surgical coronary revascularisation and heart failure, who underwent a 64-slice coronary computed tomography angiography (CCTA) because of suspected CAD. The main indications for CCTA were (a) uninterpretable, equivocal, or contraindicated functional stress test (44% of patients), (b) new-onset chest pain syndrome at low-intermediate pre-test likelihood of CAD (25% of patients), and (*c*) other heart disorders, documented previously or identified at CCTA, such as dilated or obstructive cardiomyopathy, atrial fibrillation, myocarditis, and inflammatory vascular disease; (*d*) documented peripheral vascular disease (stroke, transient ischaemic attack, claudication, revascularisation); and (*e*) active inflammatory or neoplastic disease (31% of patients in the last three categories altogether).

Patients with T2D were further selected to be eligible for the CCTA procedure, *i.e.,* if they were in good clinical condition and stable metabolic control. Exclusion criteria were: (a) CCTA not meeting the quality control criteria; (b) previous cardiovascular events (myocardial infarction, stable or unstable angina, percutaneous or surgical coronary revascularisation, heart failure), both clinically evident and confirmed by clinical and conventional diagnostics; (c) other previous heart disorders documented or identified at CCTA, such as dilated cardiomyopathy (regardless of aetiology), obstructive hypertrophic cardiomyopathy, atrial fibrillation, myocarditis, and inflammatory vascular disease; (d) previous documented acute or chronic peripheral vascular disease (stroke, transient ischaemic attack, previous revascularisation); (e) claudication at rest or at low-grade effort); and (f) active inflammatory or neoplastic disease.

At follow-up, the occurrence of cardiac death, acute coronary syndrome (defined as both ST-segment elevation myocardial infarction and unstable angina or non–ST-segment elevation myocardial infarction) and non-urgent revascularisation was recorded.

#### 2.1.2. Validation Cohort ($n = 221$)

The Aachen OCT-cohort includes patients (130 with T2D) referred to the Cardiology Department of the University Hospital of the RWTH Aachen for planned coronary angiography due to suspected chronic coronary syndrome. Chronic coronary syndrome was defined as stable symptoms in the last 6 weeks; in case of multivessel disease, identification of the target lesion(s) relied on stress imaging and/or fractional flow reserve (FFR). Further inclusion criteria were age $> 30$ years and written informed consent to the study protocol. Exclusion criteria included haemodynamic or rhythmic instability, acute or chronic renal insufficiency (serum creatinine level $> 1.5$ mg/dL), systemic acute or chronic infections, pregnancy, and anti-inflammatory medications such as steroids. Extensive medical history recording concomitant cardiovascular risk factors and relevant pharmacotherapy was available for all patients [5]. Of the 221 patients undergoing coronary angiography, 134 (61%) underwent pre-interventional optical coherence tomography (OCT) in order to assess stenosis and plaque morphology [6]. The reason for not performing OCT was absence of occlusive coronary artery disease in 59 cases, multivessel disease with indication for coronary artery bypass graft in 12 cases, totally occluded and/or severely tortuous and/or calcified lesions impeding the safe passage of the OCT catheter in eight cases. In further eight cases, reason for not performing OCT was represented by other exclusion criteria (among them, left main coronary artery stenosis, graft stenosis and in-stent restenosis). OCT images were acquired using a Frequency-Domain-OCT C7XR system and the DragonFly catheter (St. Jude Medical Systems; Lightlab Imaging, Inc., Westford, Massachusetts, USA) [5]. Complete blood removal was obtained through the injection of 14 mL iodixanol at a flow rate of 4 mL/s in the guiding catheter. The image was acquired with an automated pullback at a rate of 20 mm/s. Two independent and blinded observers with expertise in OCT performed the plaque analysis. The analysis was carried out frame by frame in 0.4 mm intervals using St. Jude's proprietary software, throughout the entire lesion. The intraclass correlation coefficients for intraobserver and interobserver agreements were 0.979 and 0.893 for calcium arc, 0.989 and 0.902 for calcium area and 0.949 and 0.927 for percent area stenosis. According to consensus [6], fibrous cap thickness could only be assessed in lipid plaques.

A standardised follow-up was performed reporting cause of death, myocardial infarction and/or emergent revascularisation.

### 2.2. Analytical methods

In all subjects, a peripheral venous blood sample was obtained under fasting conditions (*i.e.,* in the morning after an overnight, 10–12-h fast) between 7 a.m. and 8 a.m. on the day of coronary angiography. EDTA-plasma samples were immediately put on ice, processed according to standard operating procedures and subsequently frozen at −80 °C. All biomarkers were measured in a central laboratory, in a single batch, by personnel blinded to the patient characteristics. Serum creatinine, $HbA_{1c}$ and lipids were measured by standard, automated laboratory methods. High-sensitivity C-reactive protein (hsCRP) was measured with an automatic immunoturbidimetric method (Beckman-Coulter, Galway, Ireland). High-sensitivity cardiac troponin T (hs-TnT) was measured on an automated platform (ECLIA Cobas e411; Roche Diagnostics, Rotkreutz, Switzerland).

Plasma mannose concentrations were measured by an HPLC-MS-MS method developed and validated in the Pisa Metabolism laboratory [7].

### 2.3. Ethics

Both studies comply with the Declaration of Helsinki on ethical principles for medical research involving human subjects and were approved by research ethics boards at each site. All participants provided written informed consent.

### 2.4. Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD), variables with a skewed distribution (by the Shapiro-Wilk test) are given as median and interquartile range [IQR]. Continuous variables with a normal distribution were compared using ANCOVA, variables with a skewed distribution were compared by Kruskal-Wallis tests for independent samples and logarithmically transformed for use in ANOVA. Proportions were compared using a $\chi^2$ or Fisher exact test, as appropriate. The population of the validation cohort was subdivided into tertiles of plasma mannose concentrations. Univariate associations between mannose levels and clinical characteristics and biomarkers were performed. Associations of plasma mannose concentrations with CCTA and angiographic variables were tested using univariate and multivariate regression models and logistic regressions. Adjustments included those covariates that resulted significantly associated with plasma mannose concentrations, by Spearman correlation coefficient in the discovery cohort and by ANOVA according to tertiles of plasma mannose concentrations in the validation cohort.

Survival analysis was carried out using the Kaplan-Meier estimator. To determine the diagnostic efficiency and the optimal cut-off value of plasma mannose in predicting mortality in the discovery cohort, we performed time-dependent receiver operating characteristics (ROC)-analysis using the R-package "timeROC" [8]; for this analysis we used a Kaplan-Meier estimator of the censoring distribution. Diagnostic efficiency was classified as previously described [9]. We then subdivided the study population based on the optimal cut-off value derived from the ROC analysis and performed Kaplan-Meier survival analysis. Cox regression was used to analyse the impact of plasma mannose tertiles on survival.

Statistical analyses were performed with SPSS (v 26.0, IBM Corp., Armonk, NY, USA) and R (v 4.0.0, The R Project) software. Statistical significance was awarded for $p < 0.05$.

## 3. Results

### 3.1. Discovery cohort

Based on CCTA, the CAPIRE subjects were grouped into $CAD^-$ (clean coronaries, $n = 340$) and $CAD^+$ (diffuse coronary atherosclerosis with or without coronary stenosis, $n = 173$). Participants in the $CAD^+$ category were more often men, older and heavier than subjects in the $CAD^-$ category; most clinical and metabolic parameters were different between the two groups, as expected (Supplementary Table 1). In the entire cohort, plasma mannose concentrations showed a skewed distribution, with a median of 60 μmol/L and an IQR of 23 μmol/L; levels in $CAD^+$ subjects were significantly higher than in $CAD^-$ subjects (68 [29] *vs* 56 [20] μmol/L) (Fig. 1a). In univariate analysis, several anthropometric and metabolic factors were significantly associated with plasma mannose (Supplementary Table 2). Using these variables as covariates in a multiple regression model, plasma mannose concentrations were still significantly associated with the presence of CAD ($p = 0.0061$). In a multivariate logistic model of presence of CAD with the same covariates, the odds ratio (OR) for 1 SD plasma mannose concentrations (= 22 μmol/L) was 1.51 [95% CI: 1.07–2.15], with an area-under-ROC of 0.833. By including pharmacologic treatments (use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers, statin, aspirin, and diuretics), the OR for plasma mannose was 1.45 [95% CI, 1.01–2.11], with an area-under-ROC of 0.858.

#### 3.1.1. Association between mannose and CCTA features

Plasma mannose concentrations were directly related to several indices of coronary artery anatomy at CCTA (Table 1), including total plaque volume, the segment stenosis score (or overall plaque extent), the segment involvement score (or number of segments with at least one plaque irrespective of degree of stenosis), and total Leaman score (Fig. 1b).

#### 3.1.2. Association between mannose and outcomes

Over a follow up of 60 [26] months, there were 74 first hospital admissions for CVD and 8 deaths. Using this outcome, subjects with a plasma mannose level in the top 25% of the distribution did significantly worse than the remainder of the cohort (Fig. 1c).

### 3.2. Validation cohort

In the whole cohort, median plasma mannose concentrations were 92 [46] μmol/L. Mannose levels trended to be higher in patients with CAD *vs* those without CAD (100 ± 35 *vs* 87 ± 28 μmol/L, $p = 0.070$). The tertiles of plasma mannose concentrations were: tertile 1: <77 μmol/L; tertile 2: 77–100 μmol/L; tertile 3: ≥100 μmol/L. As reported in Supplementary Table 3, across increasing plasma mannose levels patients were older and heavier and had a higher prevalence of diabetes and use of ß-blockers, lower levels of high density lipoprotein (HDL)-cholesterol, and higher levels of hsCRP; angiographic evidence of CAD also was higher. By multivariate logistic analysis of the data in Supplementary Table 3, presence of CAD was predicted by male sex (OR = 3.00 [95% CI:1.39–6.44]), age (OR = 1.04 [95%CI:1.00–1.09 per year]) and serum HDL-cholesterol (OR = 0.68 [95%CI:0.52–0.90 for 10 mg/dL]), while plasma mannose levels fell just short of full statistical significance (OR = 1.13 [95%CI: 0.99–1.28 for 10 μmol/L], $p = 0.072$).

#### 3.2.1. Plaque vulnerability by OCT

The association of plasma mannose with features of plaque vulnerability was analysed by subdividing the 134 patients who received pre-interventional OCT into tertiles of plasma mannose concentrations (Table 2). Minimal fibrous cap thickness (MinFCT) in lipid plaques was significantly smaller across tertiles of plasma mannose; furthermore, patients in the highest tertile of plasma mannose presented more thin-capped fibroatheromas and trended to present more macrophage accumulations than patients in the lower tertiles. An example of plaque morphology analysis with fibrous cap thickness is shown in Supplementary Fig. 1. By univariate logistic analysis, the presence of a fibrous cap thickness < 65 μm was significantly associated with plasma mannose levels (OR = 1.32 [95%CI:1.05–1.65 for 10 μmol/L]), presence of diabetes being the only other univariate predictor (OR = 7.82 [95% CI:1.55–2.87]). The reciprocal curvilinear association between plasma mannose and MinFCT is shown in Supplementary Fig. 2.

#### 3.2.2. Association between mannose and outcomes

We next tested whether the association between plasma mannose level and OCT-based plaque vulnerability translated into a worse prognosis. Follow-up data were available for 198 (90%) of the included patients, median follow-up time was 56 months (range 47–65 months). First, we performed a Kaplan-Meier analysis to assess the association between plasma mannose tertiles and death from any cause (29 events). Higher plasma mannose was associated with a higher all-cause mortality (Fig. 2a). The hazard ratio for the comparison of tertile 3 with tertile 1 is 5.2 [95%CI:1.5–17.8, $p = 0.014$]. We then performed a time-dependent ROC analysis in order to gauge the diagnostic efficiency of plasma mannose level in predicting death at a follow-up time of 60 months. A satisfactory efficiency (area under the curve AUC = 0.748) was evident at an optimal cut-off value of 84.6 μmol/L (sensitivity: 88%; specificity: 56%) (Supplementary Fig. 3). By subdividing the cohort according to this cut-off, we obtained a clear separation of the Kaplan-Meier curves (Fig. 2b).

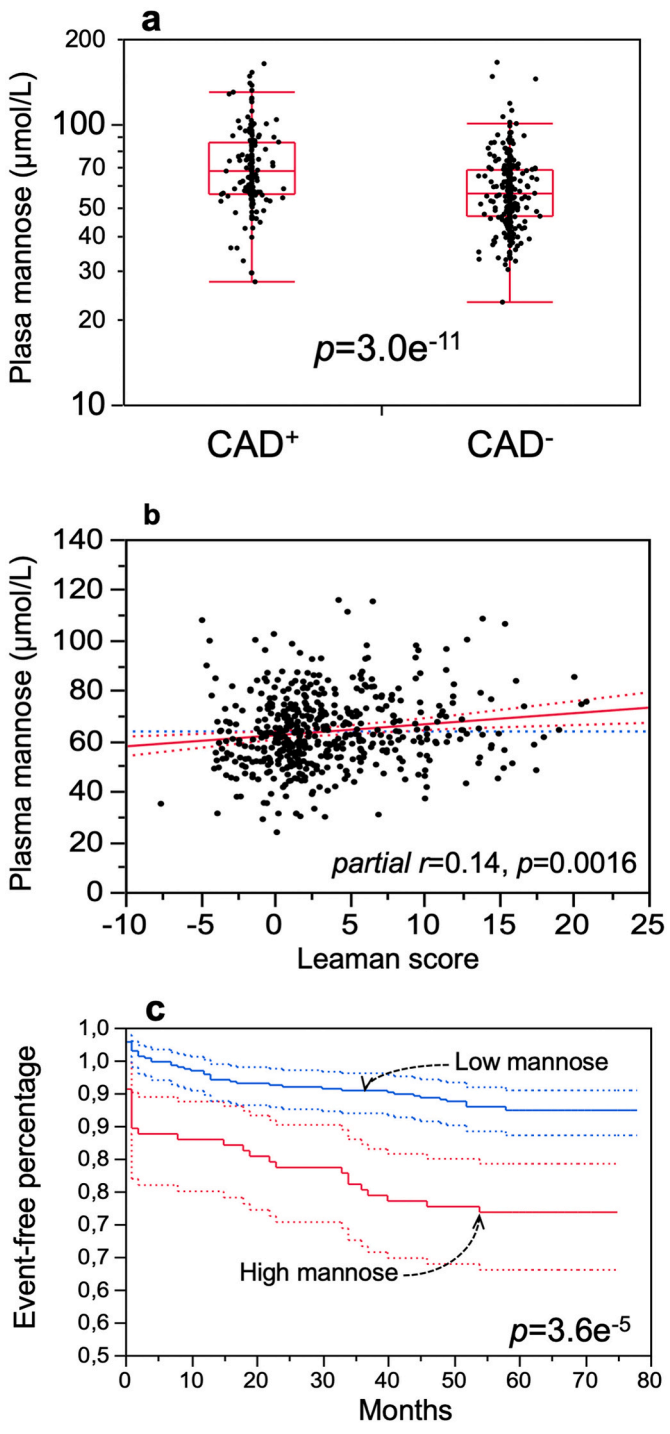


**Fig. 1.** Discovery cohort – (a) Box plot of plasma mannose concentrations in subjects with ($CAD^+$) or without ($CAD^-$) coronary artery disease. (b) Independent association of plasma mannose concentrations with Leaman score; the fit (*full line*) and its 95% confidence interval (*dotted lines*) are leverage after adjustment for sex, age, BMI, presence of diabetes, cigarettes/day, plasma glucose and serum $HbA_{1c}$ levels. (c) Kaplan-Meier plot of first hospital admission for cardiovascular causes comparing patient with "high mannose" (top 25% of the distribution) and "low mannose" (the other three quartiles); dotted lines are 95% confidence intervals of the fit; *p* value is by log rank.
Legend: CAD = coronary artery disease.

**Table 1**
Univariate (rho) and multivariate (partial *r*) associations of plasma mannose levels with high-risk CCTA features in the discovery cohort.

| | *rho*[*] | *p* | *partial r*[*] | *p*[*] |
|---|---|---|---|---|
| Segment Stenosis Score (1–16) | 0.31 | <0.0001 | 0.13 | 0.0036 |
| Segment Involvement Score (1–16) | 0.29 | <0.0001 | 0.13 | 0.0040 |
| Plaque total volume ($mm^3$) | 0.28 | <0.0001 | 0.10 | 0.0210 |
| Leaman score (units) | 0.28 | <0.0001 | 0.14 | 0.0016 |
| Remodelling Index, n (%) | 0.27 | <0.0001 | 0.16 | 0.0023 |

[*] Adjusted for sex, age, BMI, presence of diabetes, cigarettes/day, plasma glucose and serum $HbA_{1c}$ levels.

## 4. Discussion

The main findings of the present study are that plasma mannose concentrations are associated with the presence of CAD and with a more vulnerable plaque phenotype, and that mannose is an independent predictor of incident CV events and death.

In the discovery cohort, plasma mannose behaved not only as a strong surrogate for insulin resistance but also as a consistent marker of quantity and quality of CCTA-proven coronary atherosclerosis. Notably, in the latter capacity plasma mannose was superior to both plasma troponin-T and hsCRP levels. Moreover, baseline mannose concentrations were an independent predictor of incident CV deaths and CVD hospitalisations. The validation cohort included older and more obese participants with a chronic coronary syndrome; their plasma mannose levels were significantly higher than those of the discovery cohort, also on account of the strong association of mannose with age, body mass index, and cardiovascular risk factors.

Coronary lesions have the potential to rupture and cause acute coronary syndromes. Recently, pathology studies and investigations using intravascular imaging have identified several morphological features of coronary plaques that are prone to rupture. Among these features, a pivotal role is played by the thickness of the fibrous cap overlying the necrotic lipid core, which may be effectively assessed *in vivo* using OCT [5]. Specifically, a lower MinFCT is consistently associated with a higher incidence of acute coronary syndromes [10,11]. In the present study, we extend the current knowledge by showing that higher plasma mannose levels are associated with a lower MinFCT and trended to have more macrophage accumulation, suggesting a more vulnerable plaque

**Table 2**
Features of plaque vulnerability in OCT by tertiles of plasma mannose in the validation cohort.

| | Tertile 1 (*N* = 40) | Tertile 2 (*N* = 52) | Tertile 3 (*N* = 42) | *p*[*] |
|---|---|---|---|---|
| Minimal FCT (µm) | 105 ± 33 | 89 ± 25 | 76 ± 30 | 0.022 |
| Mean FCT (µm) | 143 [19] | 118 [36] | 116 [36] | Ns |
| Mean Lipid Arc (degrees) | 142 ± 47 | 142 ± 53 | 136 ± 51 | Ns |
| Lipid Volume Index (mm) | 505 ± 409 | 501 ± 394 | 592 ± 463 | Ns |
| Presence of TCFA (n, %) | 2 (5) | 6 (11) | 14 (33) | 0.019 |
| Presence of microcalcification (n, %) | 4 (10) | 13 (25) | 5 (12) | Ns |
| Calcium Volume Index (mm) | 524 [1270] | 662 [1413] | 652 [1338] | Ns |
| Presence of macrophages (n, %) | 12 (30) | 25 (48) | 20 (48) | Ns |
| Macrophage Volume Index ([†]mm) | 0 [36] | 2 [42] | 18 [56] | Ns |

Entries are mean ± standard deviation or median [interquartile range].
FCT = fibrous cap thickness; Ns: non-significant; OCT = optical coherence tomography; TCFA = thin-capped fibroatheroma.
[*] *p* by ANOVA for continuous variables with normal distribution; Kruskal-Wallis test for continuous variables with skewed distribution; Fisher's exact test for dichotomic variables.
[†] Entries are mean ± SD or median [interquartile range].

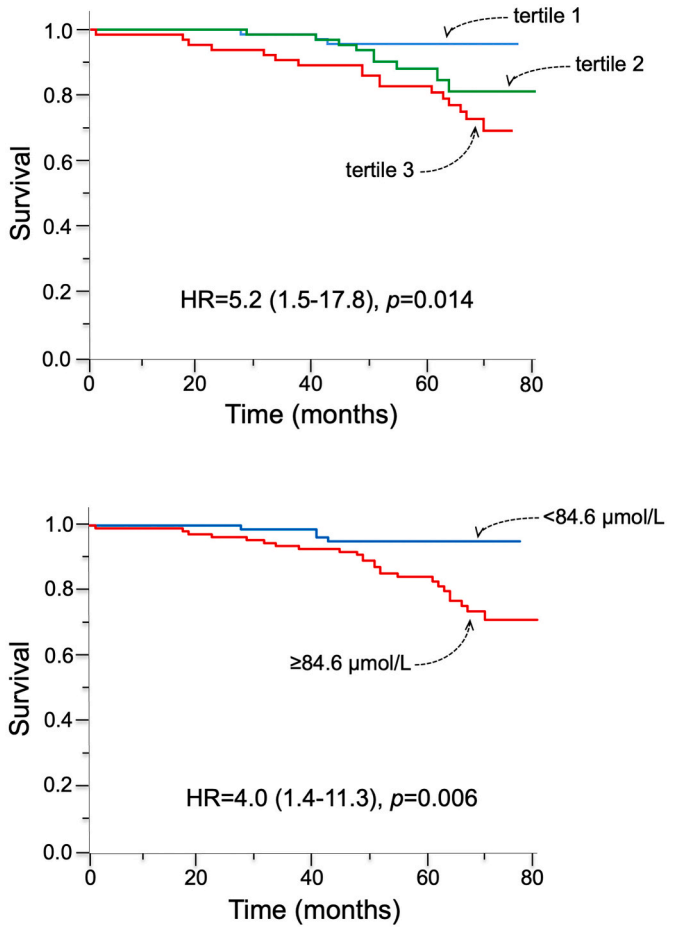


**Fig. 2.** Validation cohort - (*top*) Kaplan-Meier survival curves by tertiles of plasma mannose. (bottom) Kaplan-Meier survival curves by optimal cut-off of plasma mannose concentrations.
Legend: CI = confidence interval; HR = hazard ratio.

phenotype. Finally, in our cohort the association between plasma mannose levels and plaque vulnerability translated into a worse prognosis, despite the relatively small numbers. Altogether, our findings support that mannose may be a useful biomarker in detecting patients with higher risk for CAD, vulnerable plaques and cardiovascular events. Clinically, this may prompt toward a more aggressive management of patients with higher mannose levels, for instance with high-dose statins, which have been shown to have plaque-stabilising properties [12].

### 4.1. Biological plausibility

Importantly, the association of mannose with coronary atherosclerosis is of special significance because of its biological plausibility.

Mannose is a C2-epimer of glucose, which circulates at concentrations ~1/100th those of glucose and enters many cell types by facilitated diffusion [13]. Once taken up into organs, phosphorylated (by hexokinase 1/2, HK) and isomerised (by mannose-6 phosphate isomerase MPI) to fructose-6-phosphate, mannose is channelled through glycolysis or gluconeogenesis just like incoming glucose. Cytosolic mannose-6-phosphate can also be funnelled toward glycan (especially N-glycan) synthesis following conversion to mannose-1-phosphate – a reaction catalysed by phosphomannose mutase 1 (PMM1) – and conversion of mannose-1-phosphate into guanosine-diphospho-mannose (GDP-mannose) by GDP-mannose pyrophosphorylases (GMPPA/B). As mannose has a much higher glycosylation affinity than glucose, GDP-mannose-derived glycans are avidly attached to proteins in a non-enzymatic, covalent manner. Thus, the relative partitioning of cytosolic mannose-6-phosphate through glycolysis *vs* N-glycan synthesis depends on the ratio of MPI to PMM1 activity. Physiologically, MPI-catalysed mannose utilisation greatly exceeds N-glycan synthesis [14]; however, glycans also derive from the lysosomal catabolism of misfolded proteins, mainly in the liver [15]. Mannose generated through this pathway escapes the hexokinase step and is exported to the bloodstream as free mannose [16]. Thus, higher circulating mannose concentrations may result from (a) reduced uptake, (b) increased conversion of glucose, or (c) enhanced degradation of glycoproteins (or combinations thereof).

In obese, insulin resistant individuals [1] (Supplementary Fig. 4), hepatic glucose utilisation is increased due to higher activities of glucokinase (GCK) and phosphofructokinase (PFK), whereby glycolysis, gluconeogenesis, and glycogen and lipid synthesis are enhanced. In contrast, mannose phosphorylation is reduced, so that mannose builds up in the cytoplasm and regurgitates into the plasma. This likely accounts for the close association of plasma mannose with plasma glucose concentrations ($r = 0.51$, $p < 0.0001$ in our discovery cohort) and with clamp-based insulin resistance [2]. Both PMM1 and GMPP activities are increased in the liver of obese, insulin resistant subjects; as a consequence, traffic through the N-glycan path is augmented, by increased glycoprotein synthesis, glycoprotein degradation or both.

N-glycosylation controls protein folding and, through this process, the physicochemical properties of many membrane and secreted proteins, including lipoproteins, cadherins, and plexins. Moreover, high mannose-type oligosaccharides linked to *N*-acetylglucosamine – a molecule that has been associated with insulin resistance and diabetic complications [17–20] – serve as the recognition marker that mediates enzyme uptake by various cell types and targeting to lysosomes [21]. Protein misfolding is crucially involved in immune modulation and atherogenesis [14,15]. Thus, high plasma mannose not only marks metabolic insulin resistance but may also track abnormal post-translational protein modification in the endoplasmic reticulum. Increased glycosylation is likely to be a previously unrecognised mechanism through which insulin resistance promotes atherosclerosis with or without hyperglycaemia [22–24].

### 4.2. Limitations

Some limitations should be taken into account. First of all, plaque features and burden were assessed by different techniques in the discovery and in the validation cohort. Although mannose concentrations resulted associated with well-established features of vulnerable plaque phenotypes in both, if anything further supporting our findings, this important difference between the two cohorts should be kept in mind. Likewise, the validation cohort included patients that can be considered at a somewhat higher risk of CAD compared with the discovery cohort, and although this serves the scope of the present investigation, residual confounding due to the diversity of patients' characteristics in the two cohorts cannot be ruled out. Finally, the present findings support the described biological plausibility of mannose being a biomarker of high-risk coronary atherosclerosis, but further evidence is needed to confirm its role in clinical prediction models. In particular, in individuals with diabetes the exact impact of different glucose-lowering medications on circulating plasma mannose levels is unknown.

## 5. Conclusion

The current data add novel evidence that high circulating mannose is a signature of coronary atherosclerosis that is consistent across measures of severity of vessel involvement and independent of the canonical correlates of CVD. In addition, despite the small number of cardiovascular outcomes, baseline mannose also had some predictive power for incident clinical events, confirming the observations of larger epidemiological surveys obtained with the use of untargeted metabolomics [3]. In the perspective of personalized medicine, plasma mannose

concentrations could be used for screening and risk prediction of patients at high cardiovascular risk, providing further information when added to established risk markers.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.11.038.

## Author contributions

E.F. has the main role in work conception, design, analysis and interpretation of data and drafted the first manuscript; D.A. and M.M. participated in design, analysis and interpretation of data for the CAPIRE study and critically revised the manuscript; B.C. and A.S. participated in analysis and interpretation of data and contributed in manuscript drafting; M.G. is responsible for design, analysis and interpretation of data of the CAPIRE study and critically revised the manuscript; G.F. contributed to data analysis and interpretation and drafting of manuscript; A.M., M.B. and N.M. participated in design, analysis and interpretation of data for the Aachen study and drafted the manuscript; A.M. and A.P.M. participated in design, analysis and interpretation of data for the CAPIRE study and critically revised the manuscript. All authors approve the final version of the manuscript.

Ele Ferrannini is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of their analysis.

## Disclosures

*Conflicts of Interest statement:* Dr. Andreini, Dr. Campi, Dr. Saba, Dr. Gorini, Dr. Milzi, Dr. Magnoni, Prof. Maseri, and Prof. Burgmaier have no conflicts of interest to report; Prof. Ferrannini E. reports receiving consultancy/speaker fees, outside the present work, from Boehringer Ingelheim, Lilly&Co., AstraZeneca, and Sanofi. Prof. Marx is supported by the Deutsche Forschungsgemeinschaft (German Research Foundation; TRR 219; Project-ID 322900939 [M03, M05]) and has received support for clinical trial leadership from Boehringer Ingelheim, Novo Nordisk, served as a consultant to Boehringer Ingelheim, Merck, Novo Nordisk, AstraZeneca, BMS, received grant support from Boehringer Ingelheim, Merck, Novo Nordisk, and served as a speaker for Boehringer Ingelheim, Merck, Novo Nordisk, Lilly, BMS, and Astra Zeneca. Dr. Ferrannini G. received speaker fees from the European Society of Cardiology and grant support from the Erling-Persson foundation, outside the submitted work; Prof Maggioni reports receiving fees, outside the present work, from Bayer, Fresenius, Novartis for participation in study committees;

The data underlying this article will be shared on reasonable request to the corresponding author.

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