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Dietary glycocalyx mimetic reduces vascular risk in Type 2 diabetes: evidence from urinary peptidomic classifiers in a South–Asian Surinamese Cohort

Sajjad Biglari [a,b], Lushun Yuan [c,d], Harald Mischak [a], Justyna Siwy [a], Agnieszka Latosinska [a], Miroslaw Banasik [b,1], Bernard M. van den Berg [d,*,1]

[a] Department of Biomarker Research, Mosaiques Diagnostics GmbH, 30659 Hannover, Germany
[b] Department of Nephrology, Transplantation Medicine and Internal Diseases, Institute of Internal Diseases, Wroclaw Medical University, 50-551 Wroclaw, Poland
[c] Department of Vascular Surgery, Intervention Center, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China
[d] Department of Internal Medicine (Nephrology) & Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands

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ABSTRACT

Aims: Following up on a prior placebo-controlled trial (NCT03889236), we examined the effects of an oral glycocalyx-mimetic supplement and a fasting-mimicking diet (FMD) on three urinary peptidomic-based classifiers, which indicate future heart failure (HF2), coronary artery disease (CAD160), and chronic kidney disease (CKD273) risk in South-Asian Surinamese adults with type 2 diabetes mellitus.

Methods: Forty-four participants were randomly allocated to one of three 12-week interventions: daily glycocalyx-mimetic capsules (n = 18), placebo (n = 14), or a five-day FMD repeated every four weeks (n = 12). Baseline and week-12 urine were profiled via capillary electrophoresis–mass spectrometry (CE-MS). The pre-validated support vector machine (SVM) classifiers (HF2, CAD160, CKD273) produced risk scores that were evaluated through paired t-tests for each group. Peptide-level changes were analyzed using paired Wilcoxon signed-rank tests, and all p-values were Benjamini–Hochberg corrected ($\alpha = 0.05$).

Results: Glycocalyx-mimetic supplementation significantly reduced HF2 scores (mean $\Delta = -0.58$, 95 % CI −0.83 to −0.33, adjusted $p < 0.001$) and altered the abundance of 17 peptides, primarily decreasing collagen-derived fragments, suggesting improved extracellular-matrix turnover. The risk scores for CAD160 and CKD273 remained unchanged. FMD and placebo did not produce any meaningful changes in classifier scores.

Conclusions: In this cohort, glycocalyx-mimetic supplementation improved the urinary peptidomic signature associated with heart-failure risk, whereas an FMD did not. Urinary peptidomics offers a sensitive molecular method for monitoring the effects of (dietary) interventions.

1. Introduction

Type 2 diabetes mellitus (T2DM) confers a markedly elevated risk of vascular complications, including chronic kidney disease (CKD), coronary artery disease (CAD), and heart failure (HF) [1,2]. This concern is especially relevant in South Asian populations, who have a higher prevalence of T2DM and experience cardiovascular disease at younger ages, and a 50 % higher age-adjusted mortality rate from coronary heart disease [3]. In particular, this increased vascular vulnerability results in a higher prevalence of micro- and macrovascular complications in diabetes [4–6]. These higher rates of complications are already present at the time of diagnosis [7–9], and progression is also much faster compared to other ethnic groups, translating into a 40 times higher risk for end-stage renal disease [10].

In our recent three-arm, parallel-group randomized controlled trial, it was observed that glycocalyx supplementation for 3 months

* Corresponding author at: Department of Internal Medicine C7-Q-36, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.
E-mail addresses: biglari@mosaiques-diagnostics.com (S. Biglari), yuanlushun@whu.edu.cn (L. Yuan), mischak@mosaiques.de (H. Mischak), siwy@mosaiques-diagnostics.com (J. Siwy), latosinska@mosaiques-diagnostics.com (A. Latosinska), miroslaw.banasik@umw.edu.pl (M. Banasik), bmvandenberg@lumc.nl (B.M. van den Berg).
[1] The authors contributed equally.

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significantly reduced urinary Monocyte Chemoattractant Protein-1 (MCP-1) and improved microvascular health in South-Asian Surinamese people with T2DM, which persisted at follow-up [11]. The glycocalyx, a glycosaminoglycan-rich layer coating the luminal surface of blood vessels, acts as a mechanosensor for shear stress and is essential for preserving the integrity of the microvascular barrier [12]. In T2DM, persistent hyperglycaemia and low-grade inflammation hasten the breakdown of the endothelial glycocalyx, leading to endothelial dysfunction and increased vascular permeability [11].

Both CKD and CAD are initiated at a molecular level, long before symptoms develop. These early stages of these diseases are asymptomatic and have non-specific symptoms, making early detection challenging and highlighting the need for sensitive early biomarkers. Conventional biomarkers (e.g., albuminuria), while helpful, appear late in the disease development and lack specificity for predicting which patients will progress to end-organ damage [13]. As a result, there is a large unmet need for biomarkers enabling early detection of CKD and CAD. In this context, urinary peptidomic-based classifiers have emerged as novel multimarker tools for risk stratification of diabetes complications.

Urine is a rich source of endogenous peptides that reflect ongoing physiological and pathological processes [14]. Large-scale mass spectrometry-based profiling has detected thousands of human urinary peptides, enabling the development of disease-specific proteomic classifiers [14]. One extensively studied panel is CKD273, a classifier comprising of 273 urinary peptides [15] that has been extensively validated across patient cohorts for prognosticating diabetic nephropathy and CKD progression [16].

In patients with T2DM, a high CKD273 score predicts incident microalbuminuria and faster glomerular filtration rate decline, outperforming albuminuria in the early stages of disease [17,18]. This disease-associated peptidomic pattern involves an imbalance of specific collagen fragments, mainly declining in progressive CKD, indicating reduced collagen degradation and increased collagen tissue deposition (fibrosis) [19].

Notably, CKD273 is not only prognostic for kidney outcomes but also correlates with cardiovascular risk [20]. In patients with CKD stages G1-G3b without overt cardiovascular disease, those in the highest tertile of CKD273 score had a >10-fold increased risk of fatal or non-fatal cardiovascular events compared to those in the lowest tertile [20]. Moreover, it has been demonstrated that health management strategies incorporating CKD273 yield QALY gains at acceptable incremental cost-effectiveness ratios and can even be cost-saving in higher-risk subgroups [21,22].

Similarly, for CAD, a panel of 160 urinary peptides (CAD160), including peptides derived from proteins involved in collagen turnover, lipid metabolism, and inflammation, has been developed [23]. CAD160 demonstrated an area-under-curve of 0.82 for 8-year CAD prediction, significantly improving risk reclassification beyond traditional risk scores (Framingham or SCORE2) [23].

Further, HF2, a panel of 671 peptides primarily derived from up- or down-regulated collagen fragments, allowed for the early detection of diastolic left ventricular dysfunction in the general population [24]. In a retrospective study, a hazard ratio of 3.84 for an HF event was reported when comparing patients in the fifth and first HF2 score quintiles [25].

An important question is whether such peptidomic risk signatures can also depict the beneficial impact of interventions on kidney and cardiovascular health; in essence, does improving a patient's metabolic or vascular health favourably change their urinary proteome?

In a previous study, it was shown that stratification based on the CKD273 signature has the potential to identify patients who may benefit from linagliptin treatment by helping to reduce kidney function loss [26]. Furthermore, a Sodium-Glucose Cotransporter-2 (SGLT2) inhibitor (dapagliflozin) trial reported a significant lowering of the CKD273 risk score after 12 weeks in patients with T2DM and albuminuria [27]. Along these lines, 6 weeks of olive oil consumption improved urinary CAD classifier score compared to baseline [28], while the use of sunflower and rapeseed oil showed no beneficial impact on CAD and CKD urinary peptidomics biomarkers [29]. To increase sensitivity in monitoring dietary interventions, urinary peptidomic classifiers (such as those described) could serve as sensitive surrogate endpoints to detect early intervention benefits and so support personalized complication risk management in T2DM [30].

In the present study, we evaluate the effects of two dietary interventions (glycocalyx-mimetics and FMD) on three urinary peptidomic risk classifiers in patients with T2DM. The primary objective was to test whether these interventions positively influence the classifiers, while the secondary objective was to identify individual urinary peptides significantly altered by each intervention.

This approach leverages cutting-edge biomarkers to gain insight into how lifestyle-based therapy might alter molecular determinants of cardiorenal risk in T2DM and aims to bridge the gap between the mechanistic benefits of diet and tangible prognostic biomarkers, ultimately contributing to a more personalized and pathophysiology-driven management of high-risk T2DM populations.

2. Materials and Methods

2.1. Study design

As previously described [11], the study included a three-arm, parallel-group randomized controlled trial in South-Asian Surinamese adults with T2DM in The Hague region, the Netherlands, between May 2018 and September 2020 (Fig. 1A).

2.2. Participants

Eligible participants were 18–75 years old, self-identified as being of South-Asian Surinamese descent, were on stable hypoglycaemic therapy, with albuminuria (albumin-to-creatinine ratio (ACR) 0.3–30 mg/mmol in the past 12 months) and an estimated glomerular filtration rate (eGFR) $\geq$ 45 mL/min/1.73 m^2 (Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI formula)). The protocol was approved by the Leiden University Medical Center (LUMC) Ethics Committee, registered at ClinicalTrials.gov (NCT03889236), and conducted per the Declaration of Helsinki [11].

2.3. Trial procedures

After informed consent, participants were randomized into one of three groups [11]:

1. Fasting-Mimicking Diet (FMD): Monthly 5-day cycles of Prolon® boxes (L-Nutra Inc., Los Angeles, CA, USA), containing energy bars, vegetable soups, kale chips, olives, energy drinks, supplements, and teas. Day 1 delivered 1090 kcal (34 % carbohydrate, 56 % fat, 10 % protein); days 2–5 delivered 725 kcal/day (47 % carbohydrate, 44 % fat, 9 % protein). Three consecutive monthly cycles were completed based on prior efficacy studies.
2. Glycocalyx-mimetics Supplementation (Endocalyx™): Four capsules daily for 3 months of a fucoidan-based blend (106.25 mg fucoidan, 375 mg glucosamine sulfate, 17.5 mg hyaluronic acid, 120 mg antioxidant enzymes/polyphenols per capsule) provided by MicroVascular Health Solutions LLC (Alpine, UT, USA).
3. Placebo: Four microcrystalline-cellulose capsules daily for 3 months, identical in appearance to Endocalyx capsules.

Triple-blinding was present in the glycocalyx-mimetic and placebo arms, but not in the FMD arm, due to the intervention being a diet.

First-morning midstream urine was collected at baseline and after 3 months, kept on ice immediately, and frozen at −20 °C within six hours. Urine samples used in the present study were from a total of 44

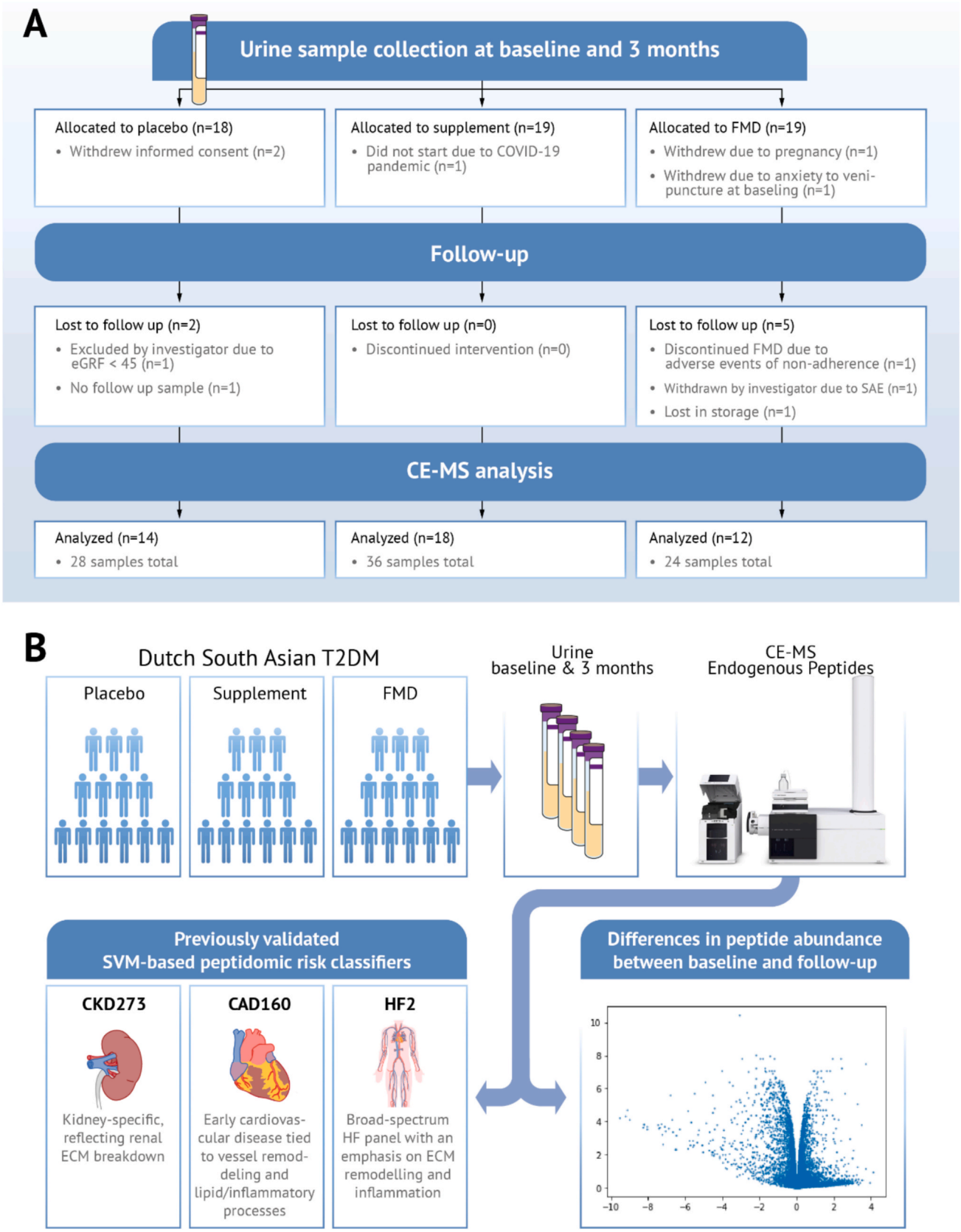

Fig. 1. Urine sample collection flow-chart and study design outline. (A) Short representative flow chart of study protocol as performed previously [11] of a subset of urine samples used in the present study and (B) Study design of endogenous peptidome research of a subset of urine samples.

individuals at baseline and after 3 months of intervention, divided over placebo (n = 14), glycocalyx-mimetic (n = 18), and FMD (n = 12) (Fig. 1A). Baseline demographics, medical history, medication use, and classifier risk score analysis results are available in Table 1.

2.4. Peptidomic profiling procedure

Urine sample preparation and capillary electrophoresis–mass spectrometry (CE-MS) analysis were carried out following previously established protocols (Fig. 1B) [31]. Samples were thawed and diluted 1:1 (v/v) with a denaturing buffer (2 M urea, 10 mM NH_4OH, 0.02 % SDS) to inhibit proteolysis and prevent peptide aggregation. High-molecular-weight proteins were removed by ultrafiltration through a Centrisart 20 kDa cut-off centrifugal filter device (Sartorius, Göttingen, Germany). Filtrates were desalted against 2 mM NH_4OH using PD-10 columns (GE Healthcare Bio Sciences, Uppsala, Sweden), lyophilized, and reconstituted in 10 μL HPLC-grade H2O before analysis.

CE separation employed a 90 cm × 50 μm ID bare fused-silica capillary with 20 % acetonitrile and 1 % formic acid (pH ≈2.0) as background electrolyte, applying 25 kV at 35 °C and hydrodynamic sample injection (2 psi, 99 s). The capillary was coupled via a sheath-flow interface (200 nL/min sheath liquid: 50 % isopropanol/0.1 % formic acid) to an electrospray-ionisation time-of-flight (ESI–TOF) mass spectrometer (Bruker micrOTOF II) operated in positive-ion mode, acquiring spectra from m/z 350–3000 every 3 s.

Raw spectra were processed via MosaiquesFinder software, which detects ion signals, deconvolutes charge states, and calibrates mass and migration time against internal peptide standards using linear and local regression algorithms. Normalized signal intensities served as relative abundance measures [32].

Normalized peptide abundances were input into pre-defined and previously validated and locked support vector machine (SVM) models to generate continuous scores for CKD273 (273-peptide panel for CKD risk) [15], CAD160 (160-peptide panel for subclinical CAD) [23], and HF2 (671-peptide panel for HF risk) [24], in which a higher resulting score indicates a greater risk of the respective pathology.

Sequencing was based on the previously collected MS/MS spectra, which were processed with Proteome Discoverer 2.4 (Thermo Fisher Scientific) using the SEQUEST search engine [33]. Searches were performed against the UniProt Swiss-Prot human database (canonical sequences only) without enzyme specificity, with a precursor mass tolerance of 5 ppm and a fragment mass tolerance of 0.05 Da. Search parameters included variable oxidation of methionine and proline, with no fixed modifications specified. Only peptide sequences with high confidence assignments were retained (spectra for all seventeen significant peptides are provided in the Supplementary Materials).

2.5. Statistical analysis

Normality of the distribution of within-subject changes (follow-up − baseline) for each classifier score [34] was assessed with the Shapiro–Wilk test and by visual inspection of Q–Q plots. Shapiro–Wilk p-values > 0.05 were interpreted as compatible with an approximately normal distribution; in this study, all classifier scores within-subject changes returned Shapiro–Wilk p > 0.20 and were therefore considered approximately normal for inference. Thus, paired two-sided t-tests were used to assess within-arm mean changes in SVM classifier scores (HF2, CAD160, CKD273).

Peptide-level distributions deviated from normality; therefore, non-parametric paired two-sided Wilcoxon signed-rank tests were applied to test within-subject changes at the peptide level. Peptides were retained for analysis if non-zero in ≥30 % of samples at either baseline or follow-up, to keep sufficient coverage while minimizing the influence of sparsely detected features. Missing values were not present in the datasets, and fold-change was calculated as a ratio of mean(follow-up) to mean(baseline).

Table 1
Baseline demographics* and classifier score analysis.

	Placebo (n = 16)	Supplement (n = 19)	FMD (n = 18)	Total (n = 53)
Demographics				
Age, years (SD)	63 (±7)	56 (±7)	61 (±6)	60 (±7)
Women, n (%)	7 (44)	12 (63)	12 (67)	31 (59)
Current tobacco smoking, n (%)	5 (31)	6 (32)	4 (22)	15 (28)
Medical history				
Duration diabetes mellitus, years (SD)	7 (±5)	9 (±5)	11 (±4)	9 (±5)
Retinopathy, n (%)	4 (25)	5 (26)	10 (56)	19 (36)
Neuropathy, n (%)	1 (6)	4 (21)	2 (11)	7 (13)
Coronary artery disease, n (%)	4 (25)	2 (11)	3 (16)	9 (17)
Angina pectoris, n (%)	4 (25)	1 (5)	2 (11)	7 (13)
CVA/TIA, n (%)	3 (19)	4 (21)	0	7 (13)
Medication use				
Metformin, n (%)	16 (100)	19 (100)	17 (94)	52 (98)
DPP4 inhibitor/GLP-1-RA/SGLT2 antagonist, n (%)	2 (13)	3 (16)	3 (17)	8 (15)
Sulfonylurea derivatives, n (%)	3 (19)	9 (47)	9 (50)	21 (40)
Insulin, n (%)	2 (13)	3 (16)	3 (17)	8 (15)
Anti-hypertensive medication, n (%)	11 (61)	12 (63)	12 (67)	35 (66)
RAAS inhibitors (n)	10 of 11	10 of 12	10 of 12	30 of 35
Statins, n (%)	12 (75)	16 (84)	16 (89)	44 (83)
Classifier risk score analysis	**Placebo (n = 14)**	**Supplement (n = 18)**	**FMD (n = 12)**	**Total (n = 44)**
CKD273				
Composite classifier score, baseline (SD)	−0.30 (±0.41)	−0.30 (±0.37)	−0.61 (±0.32)	
Composite classifier score, follow up (SD)	−0.39 (±0.48)	−0.32 (±0.27)	−0.58 (±0.31)	
Mean classifier score Δ (95 % CI)	−0.094 (−0.375, 0.188)	−0.02 (−0.188, 0.147)	0.031 (−0.214, 0.276)	
t-statistic (paired *t*-test)	0.7188	0.2582	−0.2764	
Paired effect size (Cohen's dz)	0.1921	0.0609	−0.0798	
Adjusted *p*-value	0.4850	0.7994	0.7874	
CAD160				
Composite classifier score, baseline (SD)	0.69 (±0.35)	0.64 (±0.45)	0.94 (±0.43)	
Composite classifier score, follow up (SD)	0.95 (±0.32)	0.85 (±0.35)	0.67 (±0.42)	
Mean classifier score Δ [FU − BL] (95 % CI)	0.26 (−0.009, 0.528)	0.208 (0.004, 0.412)	−0.267 (−0.498, −0.036)	
t-statistic (paired *t*-test)	−2.0918	−2.1489	2.5446	
Paired effect size (Cohen's dz)	−0.5591	−0.5065	0.7346	
Adjusted *p*-value	0.0850	0.0695	0.0818	
HF2				
Composite classifier score, baseline (SD)	−0.55 (±0.64)	−0.30 (±0.58)	−0.84 (±0.70)	
Composite classifier score, follow up (SD)	−0.94 (±0.85)	−0.88 (±0.63)	−1.13 (±0.40)	

(*continued on next page*)

Table 1 (*continued*)

	Placebo (n = 16)	Supplement (n = 19)	FMD (n = 18)	Total (n = 53)
Mean classifier score Δ [FU − BL] (95 % CI)	−0.39 (−0.706, −0.074)	−0.582 (−0.833, −0.332)	−0.286 (−0.74, 0.169)	
t-statistic (paired *t*-test)	2.6669	4.9063	1.3844	
Paired effect size (Cohen's dz)	0.7128	1.1564	0.3996	
Adjusted *p*-value	0.0581	0.0004	0.2905	

Data is presented as mean (SD), median (25–75 percentile) or number with percentage (partly from [11]).
Abbreviations: *CI* confidence interval, *CVA/TIA* cerebrovascular event/transient ischemic attack, *DPP4* dipeptidyl peptidase-4, *FMD* fasting mimicking diet. *GLP-1-RA* glucagon-like peptide-1 receptor agonist, *SGLT2* sodium-glucose cotransporter 2, *RAAS* renin-angiotensin-aldosterone system. Means within-arm changes from baseline to follow-up were compared in a paired-sample *t*-test followed by Benjamini-Hochberg correction.
*Clinical characteristics of participants included in the urinary peptidomic analysis are reported in Supplementary Table S1. No statistically significant differences were observed between any variables of this subset of patients and the original cohort (all $p \geq 0.141$).

Multiple testing correction was performed using the Benjamini–Hochberg [35] procedure to control the false discovery rate (FDR). The Benjamini–Hochberg method was chosen because it balances discovery and Type I error control in high-dimensional omics settings and is widely used for peptide/proteomic data. FDR correction was applied independently within each intervention arm for (i) the three classifier tests and (ii) the set of peptide-level tests; statistical significance was defined as FDR ≤ 0.05.

Analyses were performed in Python (3.11) using numpy [36], pandas [37], scipy [38] (Shapiro, ttest_rel, wilcoxon), and statsmodels [39] (multipletests for Benjamini–Hochberg). The Python code is available in the Supplementary Materials.

3. Results

3.1. Study population

In the original study [11], a total of 56 patients were included and randomized, 19 in the FMD group, 19 in the glycocalyx-mimetic group, and 18 patients in the placebo group (short representative flow chart, Fig. 1A). In the FMD group, one patient withdrew due to pregnancy at the start of the study, and one patient withdrew during the baseline visit due to venipuncture anxiety. Three patients discontinued due to adverse events or non-adherence during or after the first diet cycle, and one patient was withdrawn by the investigator due to a severe adverse effect after the first diet cycle. A total of thirteen patients completed the three diet cycles and the follow-up study visit at month six.

In the glycocalyx-mimetic group, nineteen patients were randomized and completed the baseline measurement. One patient did not start due to the COVID-19 pandemic, which resulted in eighteen patients completing the 3-month intervention and follow-up. In the placebo group, two patients withdrew informed consent before the baseline visit, and one patient was withdrawn by the investigator after the baseline measurements due to an eGFR below the inclusion criteria threshold. A total of fifteen patients completed the 3-month placebo intervention. Due to the COVID-19 pandemic, recruitment was halted, preventing the completion of the estimated number of patients per group.

3.2. Classifier score change between baseline and follow-up

Classification scores for the three biomarker panels CKD273, HF2, and CAD160 were calculated for all baseline and follow-up urine samples. Within each intervention group, changes in these scores following the intervention were analyzed. Only the peptidomic classifier HF2 in the glycocalyx-mimetic arm surpassed the significance threshold after correction, indicating a marked reduction in the urinary peptidomic HF risk signature (mean change −0.58; 95 % CI −0.83 to −0.33; adjusted $p < 0.001$).

No other classifiers significantly changed when comparing baseline and follow-up scores. Mean urinary peptidomic classifier scores at baseline and 3 months, along with *t*-test results, are summarized in Fig. 2.

3.3. Paired peptide-level comparisons between baseline and follow-up

Within each intervention group, we compared peptide abundances between baseline and follow-up. No peptides showed significant changes in the placebo or FMD groups after adjustment for multiple testing. In contrast, seventeen peptides were significantly altered between baseline and follow-up in the glycocalyx-mimetic group (adjusted $p \leq 0.05$); Fig. 3A, Table 2).

Of these, thirteen (76 %) decreased in abundance from baseline to follow-up, whereas four (24 %) increased (Fig. 3A). The down-regulated peptides showed fold-changes ranging from 0.01 to 0.41, whereas the up-regulated peptides exhibited fold-changes from 1.38 up to 75.00, indicating substantial induction in a subset of peptides (Table 2).

3.4. Protein-level patterns

Thirteen of the seventeen significant peptides (76 %) mapped to collagen family proteins, emphasizing the role of extracellular-matrix (ECM) remodelling in the glycocalyx-mimetic arm. COL6A1 contributed five peptides (29 % of all hits), four of which were down-regulated while one was up-regulated. COL5A1 accounted for three peptides, all of which were down–regulated. Single peptides originated from COL1A1 (upregulated), COL1A2 (downregulated), COL2A1 (upregulated), COL11A1 (downregulated), and COL17A1 (downregulated) (Fig. 3B and Table 2). Of the non-collagen peptides derived from MUC17, hornerin, chloride intracellular channel protein 6 (CLIC6), and neurosecretory protein VGF; three were reduced in abundance, whereas the CLIC6 fragment was increased.

4. Discussion

In this study, we successfully demonstrated that glycocalyx-mimetic supplementation can lower the urinary HF2 peptidomic risk score in South-Asian patients with T2DM, translating into a reduced HF risk, whereas the FMD had no such effect. The absence of significant shifts in CKD273 and CAD160 suggests glycocalyx-mimetics act preferentially on microvascular biology rather than on broader cardiorenal axes, consistent with its proposed mechanism. However, this lack of effect could also be due to a modest sample size. Moreover, we identified specific urinary peptides that were significantly altered by these interventions, thereby meeting both our primary and secondary study objectives.

Most of the seventeen glycocalyx-mimetic responsive peptides originated from collagen types I, II, V, and VI, indicating ECM remodelling. In large-scale datasets of patients with cardiorenal syndrome, collagens constitute almost 72 % of the quantified peptidome, and most differentially expressed peptides map to COL1A1, COL1A2, and COL3A1, reflecting fibrosis and changes in ECM [40]. Similarly, almost 77 % of HF-associated urinary peptides are collagen-derived [41].

The concomitant decline in urinary mucin-derived peptides (established surrogates of endothelial glycocalyx attrition [42]) and in hornerin fragments, a thrombomodulin interacting partner, of which knockdown limited leukocyte adhesion and tube formation capacity in vitro [43], may serve as novel biomarkers of microvascular vulnerability. Collectively, the data argue that preserving the endothelial surface layer may be central to the supplement's benefit.

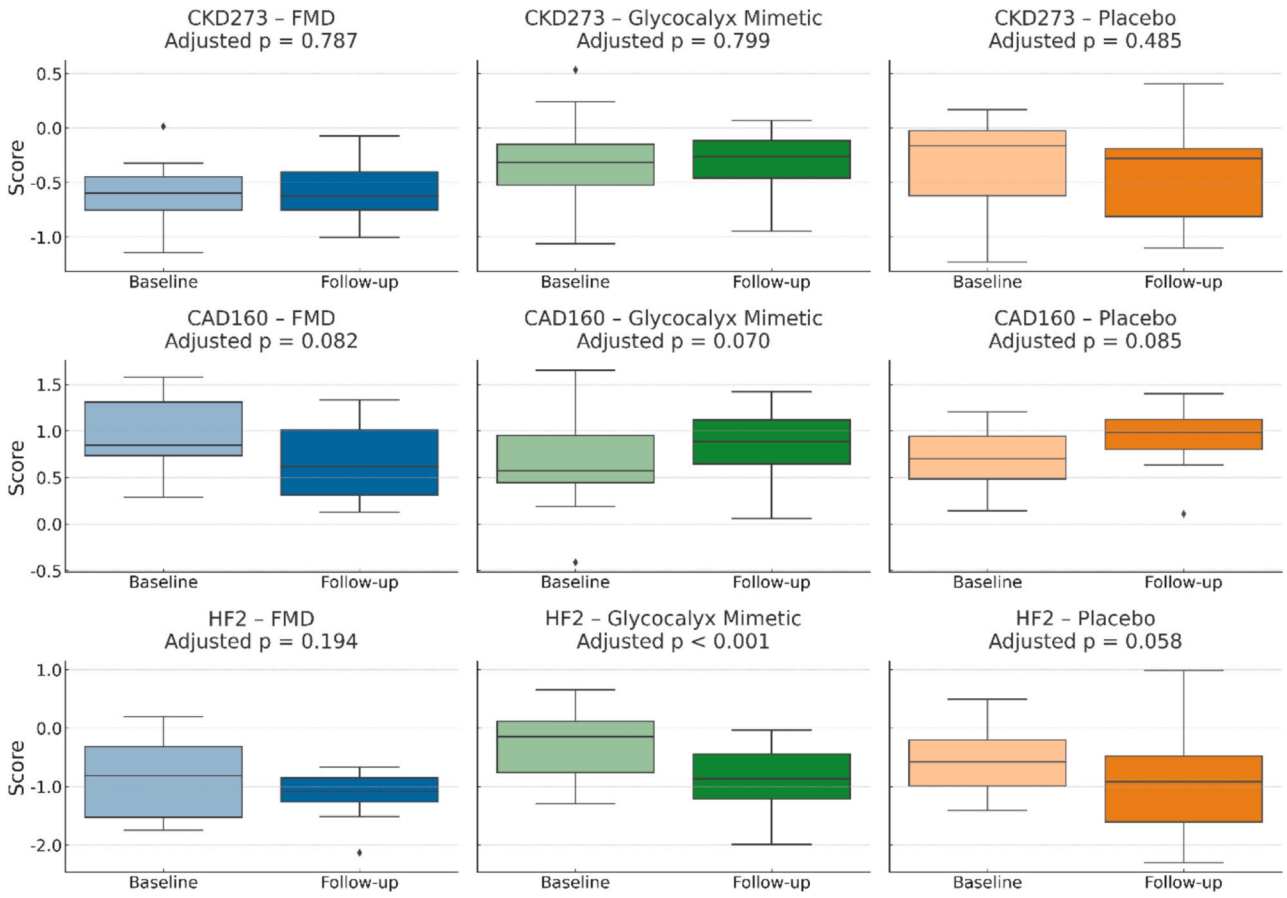

Fig. 2. Effect of 12–week interventions on urinary peptide classifiers Box–and–whisker plots compare baseline and follow–up scores for the three CE–MS–derived classifiers, CKD273 (kidney–disease risk), CAD160 (coronary–artery–disease risk) and HF2 (heart–failure risk), in participants assigned to (i) a monthly 5–day fasting–mimicking diet (FMD), (ii) daily glycocalyx–mimetic supplementation, or (iii) placebo. A downward score indicates lower predicted disease risk. Boxes denote the median and inter–quartile range; whiskers extend to 1.5 × IQR; overlaid dots are individual subjects. The Benjamini–Hochberg–adjusted paired *t*-test p–values are included.

Endothelial dysfunction plays an important role in the development and progression of HF [44], and peripheral endothelial function is a predictor of adverse outcomes in the early stages of HF [45]. Interventions that improve endothelial health have been associated with changes in urinary collagen peptides: for example, GLP-1 receptor agonist therapy led to concordant reductions in collagen fragments in urine [46]. The predominant downregulation of COL5/6 fragments (associated with poor prognosis in Heart Failure with Preserved Ejection Fraction (HFpEF) [47]) alongside upregulation of specific COL1/3 fragments implicated in physiological turnover, suggests a shift from maladaptive fibrotic signaling towards restorative matrix remodeling.

Glycocalyx-mimetics are formulated with key glycocalyx precursors and protectants, including a fucoidan-rich seaweed extract (a heparan sulfate mimetic), high-molecular-weight hyaluronan, and glucosamine sulfate. Fucoidan binds and inhibits heparanase [48], the enzyme that degrades the endothelial glycocalyx, thereby preventing glycocalyx shedding.

Preserving this surface layer stabilises the endothelium, reduces shear stress–induced injury, and limits inflammatory cell adhesion [49,50]. This endothelial protection may interrupt downstream pathways that lead to ECM remodelling [49,51,52]. Furthermore, glycocalyx-mimetics improved microvascular geometry in our original trial data, producing a lower dynamic perfused boundary region (PBR) and a + 0.7-point gain in the Microvascular Health Score [11].

Recent work shows that glycocalyx-mimetics can rebuild the glycocalyx via extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and phosphoinositide-3-kinase (PI3K) signalling and protect against uremic- or diabetic-induced endothelial damage [53]. By mitigating chronic inflammation and oxidative stress (known triggers of matrix metalloproteinases (MMP)), a healthier glycocalyx can interrupt downstream pathways of ECM remodelling [49]. Moreover, a previous study showed that pharmacological MMP inhibition prevents glycocalyx shedding, thus preserving endothelial integrity [54].

In conclusion, the observed patterns are biologically coherent with early endothelial surface-layer (glycocalyx) stabilization: (i) modulation of the thrombomodulin–hornerin axis suggests tighter anticoagulant/anti-inflammatory signaling at the endothelial interface; (ii) reductions in mucin-derived fragments are consistent with decreased surface-layer shedding; and (iii) fewer collagen-derived fragments are compatible with lower extracellular-matrix proteolysis. Alternative explanations (e.g., greater ECM cross-linking limiting peptide liberation) cannot be excluded without orthogonal assays. Together, these shifts align with an intervention effect on vascular surface integrity and perivascular remodeling, providing a mechanistic rationale for the HF2 risk-score reduction.

In contrast to this glycocalyx-stabilization signature, the FMD arm showed no meaningful changes in HF2, CAD160, or CKD273 despite exhibiting short-term metabolic improvements (lower HbA1c and body weight [11]). However, even with the limited statistical power available, a non-significant trend toward reduced CAD160 scores at 3 months was observed (adjusted p = 0.082). Given that microvascular and ECM remodeling typically evolve over longer timeframes, the FMD exposure and intensity may have been insufficient for peptide-level shifts to

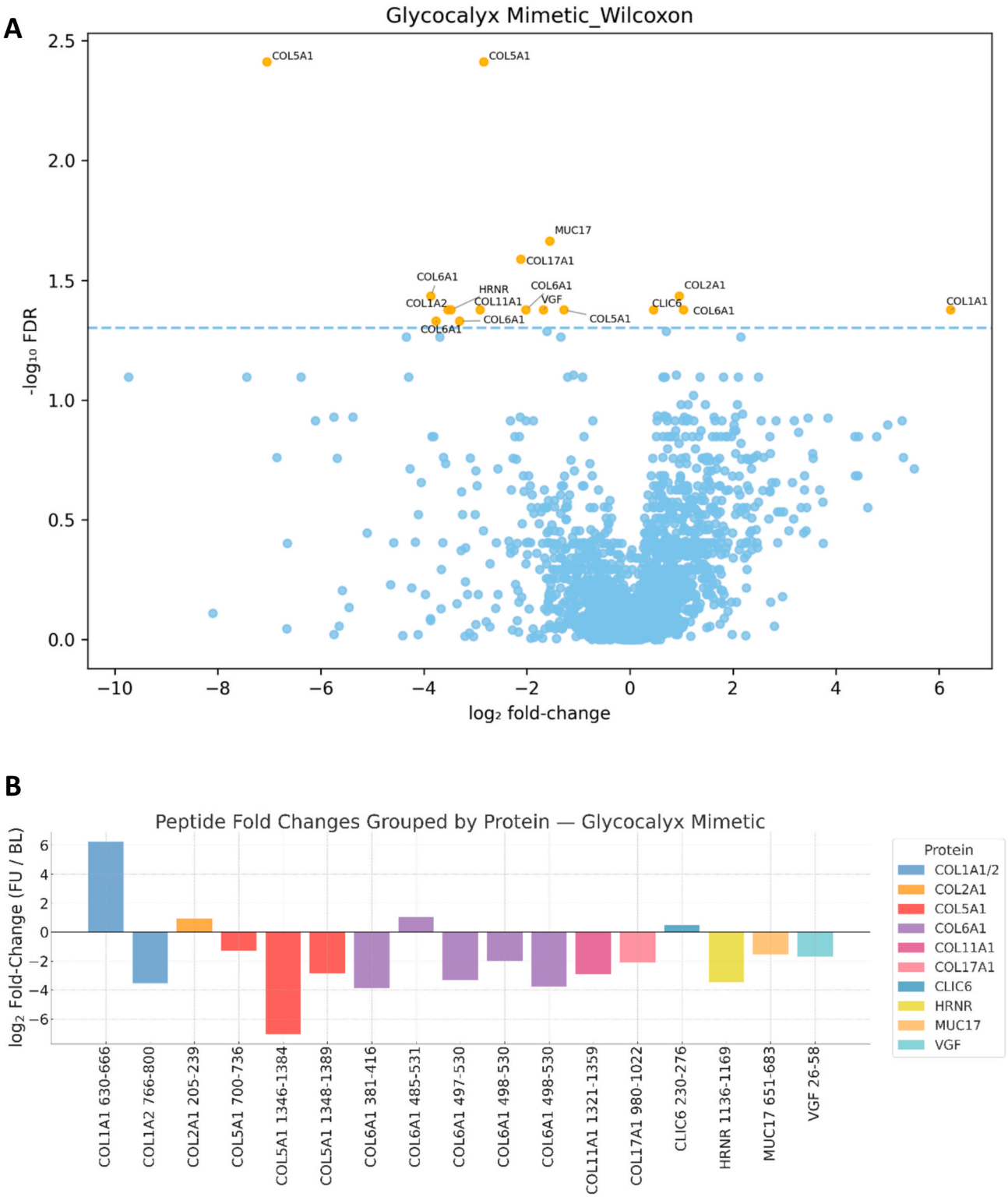

Fig. 3. Significance versus magnitude of peptide-level responses in the glycocalyx-mimetic group. **(A)** Volcano plot of all quantified peptides (points) showing median $\log_2$ fold-changes (x-axis) against $-\log_{10}$ FDR-adjusted *p*-values (y-axis). The horizontal dashed line indicates the 5 % FDR threshold ($-\log_{10} \approx 1.3$). Peptides that passed the 5 % FDR threshold (n = 17) are highlighted in orange and annotated with their parent protein symbols. The distributions mostly highlight a predominant downregulation of collagen-derived peptides, especially types V and VI, as shown in **(B)** in which 13 peptides (76 %) were decreased in abundance (negative bars) and 4 (24 %) increased (positive bars) after paired Wilcoxon signed-rank testing and Benjamini–Hochberg FDR correction (adjusted $p \leq 0.05$) in the glycocalyx-mimetic arm, highlighting a broad remodelling of the ECM after supplementation. Peptides are ordered by gene symbol and residue amino acid range for clarity. Bars are coloured by their parent protein (legend, upper-right). Abbr. COL1A1/2, collagen 1 α 1 and 2; COL2A1, collagen 2 α 1; COL5A1, collagen 5 α 1; COL6A1, collagen 6 α 1; COL11A1, collagen 11 α 1; COL17A1, collagen 17 α 1; CLIC6, chloride intracellular channel 6; HRNR, hornerin; MUC17, mucin 17; VGF, neurosecretory protein VGF.

Table 2
Significantly changed peptides in the glycocalyx-mimetic arm.

Number	Protein	Start AA	Stop AA	Mean Baseline intensity	Mean Follow up intensity	Fold Change	Adjusted p-value
1	Collagen alpha-1(V) chain	1346	1384	705.16	5.34	0.01	0.0038
2	Collagen alpha-1(V) chain	1348	1389	405.59	56.87	0.14	0.0038
3	Mucin-17	651	683	873.78	298.11	0.34	0.0217
4	Collagen alpha-1(XVII) chain	980	1022	1523.34	351.66	0.23	0.0258
5	Collagen alpha-1(II) chain	205	239	204.32	397.74	1.95	0.0368
6	Collagen alpha-1(VI) chain	381	416	304.88	20.96	0.07	0.0368
7	Chloride intracellular channel protein 6	230	276	183.91	15.91	0.09	0.0420
8	Collagen alpha-1(I) chain	630	666	3.66	274.7	75.00	0.0420
9	Collagen alpha-1(V) chain	700	736	5762.36	7939.01	1.38	0.0420
10	Collagen alpha-1(VI) chain	498	530	2150.24	887.04	0.41	0.0420
11	Collagen alpha-1(VI) chain	485	531	2956.23	266.49	0.09	0.0420
12	Collagen alpha-1(XI) chain	1321	1359	597.22	147.57	0.25	0.0420
13	Collagen alpha-2(I) chain	766	800	558.43	1151.24	2.06	0.0420
14	Hornerin*	1136	1169	992.84	132.49	0.13	0.0420
		1606	1639				
		2546	2579				
15	Neurosecretory protein VGF	26	58	339.46	106.27	0.31	0.0420
16	Collagen alpha-1(VI) chain	498	530	1081.75	79.78	0.07	0.0468
17	Collagen alpha-1(VI) chain	497	530	623.47	63.13	0.10	0.0468

*This peptide contains three distinct regions with repeated sequence motifs, resulting in three separate sets of start and stop amino acid positions. Consequently, it is not possible to unambiguously determine the specific locus of origin for this peptide.
Abbreviations: *AA* amino acid.

manifest.

It is also possible that the diet itself may impose physiological stress. FMD increased the dynamic PBR by 0.32 μm and left glycocalyx-shedding markers such as heparanase-1 (HPSE-1), angiopoietin-2 (ANG-2), and soluble thrombomodulin (sTM) unchanged [11]. This suggests that caloric restriction alone fails to stabilise the endothelial surface layer. Additionally, parallel work in an experimental model revealed that although FMD preserved certain glomerular structural parameters, it paradoxically heightened renal oxidative-stress markers [55], hinting at an unfavourable renal redox balance that could negate vascular gains.

Finally, adherence to FMD can be difficult, and patients may find the regimen demanding. After the FMD cycle, measurements of capillary or urinary ketone levels (reflecting ketosis) were only elevated in a few patients, suggesting that other patients may not have been compliant with the dietary regimen. However, another explanation could be that the switch from carbohydrate to lipid oxidation in response to fasting is impaired in South-Asians with T2DM (as compared to Europeans), reflecting metabolic inflexibility in South-Asian individuals [56]. As ketones are most likely involved in the health effects of fasting [57], this may indicate that fasting or fasting mimicking diets are less effective in individuals of South-Asian descent than in individuals of European descent.

The present study offers several strengths. It represents the first randomised comparison of a glycocalyx-targeted supplement and an FMD on urinary peptidomic profiles. By focusing on South-Asian Surinamese patients, a high-risk yet under-studied group, it addresses an important research gap in cardiometabolic medicine. Moreover, the use of multi-peptide classifiers such as HF2, CAD160, and CKD273, which integrate hundreds of pathophysiology-relevant peptides, allows a holistic assessment of risk beyond conventional single biomarkers.

Nevertheless, the study has some limitations. Unfortunately, participant recruitment was limited by a low response rate among eligible patients, and due to the COVID-19 epidemic, participant inclusion had to be discontinued and therefore, the low sample size may have restricted statistical power to show other effects in the intervention groups, even though clear primary endpoints were still observed in the original study [11]. In general, conducting lifestyle intervention studies in the South-Asian population has been proven to be extremely difficult due to low response rates, high drop-out rates, and lack of effect on lifestyle [58,59]. We experienced a dropout rate of 30 % in the diet group, comparable to other FMD studies [60].

Furthermore, blinding was impossible for the dietary arm, introducing potential performance bias. Finally, potential mechanistic inferences were indirect, based on urinary peptides and prior microvascular imaging rather than on simultaneous measures such as flow-mediated dilation.

Altogether, our findings have broader implications for deploying urinary peptidomic classifiers in clinical trials and lifestyle-intervention research. When an intervention lowers a patient's classifier score, it provides molecular evidence that pathogenic pathways are being favourably modulated, well before clinical end-points are observable.

As continuous measures with established prognostic validity, these classifiers enable standardized, cross-intervention comparisons and serve as plausible surrogate indicators of therapeutic impact, supporting their use as early readouts to prioritise which lifestyle strategies merit longer, outcome-powered evaluation.

5. Conclusion

Targeting the endothelial glycocalyx with an oral glycocalyx-mimetic supplement favourably shifted the urinary HF2 risk classifier (a predictor of HF-related vascular pathology) in high-risk South–Asian Surinamese T2DM patients. At the individual-peptide level, the decline in excreted collagen fragments at follow-up aligns with early extracellular-matrix remodeling. The two other urinary peptidomic classifiers (CKD273 and CAD160) were not significantly altered in the supplement group, and a fasting mimicking diet did not yield any significant changes in any classifier scores, although this could be due to the study's modest sample size. The data also show that urinary peptidomic panels are not only predictive but also sensitive to intervention-induced changes, highlighting their potential as early surrogate biomarkers in progression-modifying trials. Longer exposure, wider settings, and combined modalities are needed to build a large-scale picture of how peptide-level changes convert into long-term clinical outcomes.

6. Data Availability

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

CRediT authorship contribution statement

Sajjad Biglari: Writing – review & editing, Writing – original draft,

Visualization, Methodology, Investigation, Formal analysis, Data curation. **Lushun Yuan:** Writing – review & editing, Methodology, Investigation. **Harald Mischak:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Justyna Siwy:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Agnieszka Latosinska:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Miroslaw Banasik:** Writing – review & editing, Methodology, Investigation. **Bernard M. van den Berg:** Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

H.M. is the founder and co-owner of Mosaiques Diagnostics (Hannover, Germany). S.B., A.L., and J.S. are employed by Mosaiques Diagnostics. Other authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2025.112931.

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