

Temporal changes in soluble angiotensin-converting enzyme 2 associated with metabolic health, body composition, and proteome dynamics during a weight loss diet intervention: a randomized trial with implications for the COVID-19 pandemic

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ABSTRACT

Background: Angiotensin-converting enzyme 2 (ACE2) serves protective functions in metabolic, cardiovascular, renal, and pulmonary diseases and is linked to COVID-19 pathology. The correlates of temporal changes in soluble ACE2 (sACE2) remain understudied.

Objectives: We explored the associations of sACE2 with metabolic health and proteome dynamics during a weight loss diet intervention.

Methods: We analyzed 457 healthy individuals (mean \pm SD age: 39.8 \pm 6.6 y) with BMI 28–40 kg/m² in the DIETFITS (Diet Intervention Examining the Factors Interacting with Treatment Success) study. Biochemical markers of metabolic health and 236 proteins were measured by Olink CVDII, CVDIII, and Inflammation I arrays at baseline and at 6 mo during the dietary intervention. We determined clinical and routine biochemical correlates of the diet-induced change in sACE2 (Δ sACE2) using stepwise linear regression. We combined feature selection models and multivariable-adjusted linear regression to identify protein dynamics associated with Δ sACE2.

Results: sACE2 decreased on average at 6 mo during the diet intervention. Stronger decline in sACE2 during the diet intervention was independently associated with female sex, lower HOMA-IR and LDL cholesterol at baseline, and a stronger decline in HOMA-IR, triglycerides, HDL cholesterol, and fat mass. Participants with decreasing HOMA-IR (OR: 1.97; 95% CI: 1.28, 3.03) and triglycerides (OR: 2.71; 95% CI: 1.72, 4.26) had significantly higher odds for a decrease in sACE2 during the diet intervention than those without ($P \leq 0.0073$). Feature selection models linked Δ sACE2 to changes in α -1-microglobulin/bikunin precursor, E-selectin, hydroxyacid oxidase 1, kidney injury molecule 1, tyrosine-protein kinase Mer, placental growth factor, thrombomodulin, and TNF receptor superfamily member 10B. Δ sACE2 remained

associated with these protein changes in multivariable-adjusted linear regression.

Conclusions: Decrease in sACE2 during a weight loss diet intervention was associated with improvements in metabolic health, fat mass, and markers of angiotensin peptide metabolism, hepatic and vascular injury, renal function, chronic inflammation, and oxidative stress. Our findings may improve the risk stratification, prevention, and management of cardiometabolic complications. This trial was registered at clinicaltrials.gov as NCT01826591. *Am J Clin Nutr* 2021;114:1655–1665.

Keywords: angiotensin-converting enzyme 2, weight loss diet intervention, metabolic health, body composition, proteomics

Introduction

As part of the renin–angiotensin system (RAS), angiotensin-converting enzyme 2 (ACE2) is a key regulator of vascular tone and blood pressure (BP) and serves protective functions in metabolic, cardiovascular, renal, and pulmonary diseases (1, 2). Recently, research on the genetic, environmental, and metabolic determinants of ACE2 has increased exponentially, predominantly because of its strong link with COVID-19 pathology (3). Indeed, the SARS-CoV-2 virus binds to the ACE2 receptor for cell entry (4, 5). The measurement of ACE2 receptor tissue density is technically unfeasible on a large population level. Instead, sensitive assays now enable population-wide quantification of the soluble form of the catalytic ACE2 ectodomain [soluble ACE2 (sACE2)] in body fluids. sACE2 may be a relevant biomarker of COVID-19 susceptibility and prognosis (6).

Identifying factors modulating sACE2 may provide insights into susceptibility to COVID-19 infection. Previous observational studies have reported cross-sectional associations between higher sACE2 and clinical characteristics associated with higher likelihood of COVID-19 infection and worse prognosis of COVID-19-related complications (7–10), such as higher age, male sex, and metabolic disturbances including insulin resistance and hypertriglyceridemia (11–13). sACE2 is elevated in patients with cardiovascular (2, 14, 15) and metabolic disorders (16), most likely to counteract the adverse effects of increased local or systemic angiotensin II activity. Of note, higher plasma ACE2 was independently associated with increased cardiovascular mortality in a case–control analysis on 10,753 community-dwelling individuals (13).

Also, experimental studies have consistently linked ACE2 to the metabolic syndrome and associated aberrations in glucose and lipid metabolism (1, 17, 18). Indeed, animal studies suggest that RAS overactivation and improper compensation by ACE2 can contribute to organ-specific disorders associated with the metabolic syndrome, including pancreatic β -cell apoptosis, deficits in insulin secretion, and decreased insulin responsiveness in skeletal muscle and adipose tissue (1, 17, 18). In turn, metabolic disturbances can enhance the proinflammatory and profibrotic actions of angiotensin II, while hampering the compensatory actions of ACE2 (1).

However, the cross-sectional design of the aforementioned observational studies did not allow for inference of the causal relation between sACE2 and metabolic health profiles. Instead, interventional trials should evaluate the temporal and dynamic interplay between metabolic health and sACE2 in order to translate the experimental data on sACE2 dynamics to clinically relevant applications. However, to date, longitudinal evidence for linked dynamics in sACE2 concentrations and markers of

metabolic health has been lacking. Moreover, no longitudinal study has yet explored the molecular associations underlying sACE2 fluctuations, whereas this may identify novel protein targets for better detection, prevention, and management of cardiovascular and COVID-19-related complications. Recent advancements in high-throughput proteomic profiling (19) and analytics capable of processing large protein networks (20, 21) have facilitated the search for pathologically relevant proteins, including the search for metabolic and inflammatory proteins associated with temporal fluctuations in sACE2.

Here, we hypothesized that perturbations in sACE2 are paralleled by alterations in distinct metabolic and proteomic health profiles. We investigated in generally healthy but overweight and obese individuals the association between sACE2, metabolic health, and body composition before, during, and after a weight loss diet intervention. In addition, we combined high-throughput proteomic profiling and multidimensional network analyses to identify metabolic, cardiovascular, and inflammatory protein dynamics associated with sACE2 changes during the dietary intervention.

Methods

Study design

The DIETFITS (Diet Intervention Examining the Factors Interacting with Treatment Success) study is a single-site, parallel-group weight loss diet trial that randomly assigned healthy adults to a healthy low-fat diet or a healthy low-carbohydrate diet (NCT01826591) (22, 23). The primary outcome of the DIETFITS study was 12-mo weight change (23). Men and premenopausal women aged 18–50 y with a BMI of 28–40 kg/m² were recruited (22). The major criteria for exclusion were having uncontrolled hypertension or metabolic disease; diabetes; cancer; heart, renal, or liver disease; and being pregnant or lactating. Individuals were excluded if taking hypoglycemic, lipid-lowering, antihypertensive, psychiatric, or other medications known to affect body weight or energy expenditure. The Stanford University human subjects committee approved the study. All participants provided written informed consent.

Study protocol

Participants were recruited using media advertisements and email lists from previous recruitment for nutrition studies conducted by the Stanford Nutrition Studies Group. The screening, run-in, and randomization procedures as well as the dietary interventions have been described in detail previously (22). Briefly, 608 participants underwent a 1-mo run-in period during which they were instructed to maintain their habitual diet, physical activity level, and body weight. Random assignment to a healthy low-fat diet or a healthy low-carbohydrate diet was performed using an allocation sequence determined by random number generation (Blockrand in R version 3.4.0; R Foundation for Statistical Computing) by a statistician not involved in intervention delivery or data collection. The diet interventions consisted of intensive class-based education, a dietary strategy (a healthy low-fat diet or a healthy low-carbohydrate diet for 12 mo), promotion of physical activity, and behavioral modification (22).

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Supplemental Tables 1–7 and Supplemental Figures 1–3 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: ACE, angiotensin-converting enzyme; AMBP, α -1-microglobulin/bikunin precursor; Ang(1–7), angiotensin-(1–7); APN, aminopeptidase N; BP, blood pressure; DIETFITS, Diet Intervention Examining the Factors Interacting with Treatment Success; HAOX1, hydroxyacid oxidase 1; KIM-1, kidney injury molecule 1; MERTK, tyrosine-protein kinase Mer; NPX, Normalized Protein eXpression; PGF, placenta growth factor; PLS, partial least squares; PRSS8, prostasin; RAS, renin–angiotensin system; sACE2, soluble angiotensin-converting enzyme 2; TRAIL-R2, TNF receptor superfamily member 10B; VIP, variable importance for projection; XGBoost, eXtreme Gradient Boosting.

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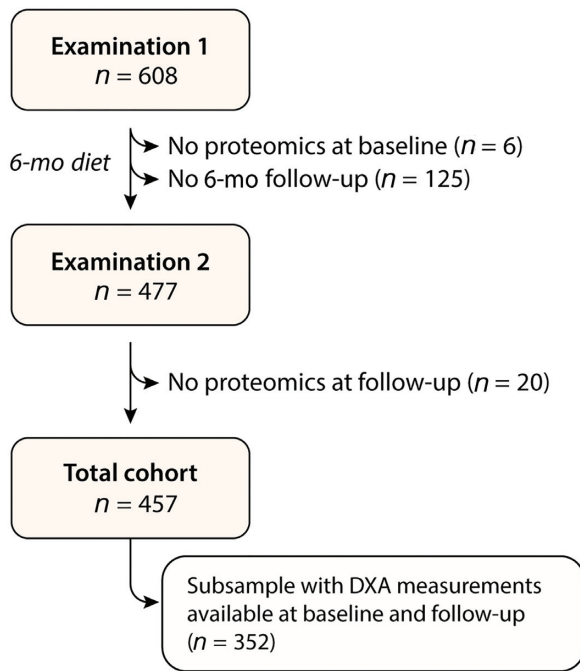


FIGURE 1 Flowchart of the DIETFITS (Diet Intervention Examining the Factors Interacting with Treatment Success) substudy.

Participants underwent a clinical examination at baseline and at months 3, 6, and 12. All clinic visits took place at the Stanford Clinical and Translational Research Unit starting between 07:00 and 09:30 with the participants fasting for ≥ 10 h. Of note, there was no significant difference in weight loss between the healthy low-fat and healthy low-carbohydrate diets at the end of the 12-mo trial (23). For the current substudy on sACE2, we derived the following clinical measurements taken at baseline and at month 6: body height, body weight, and BP levels; routine biochemistry and high-throughput proteomics; and—in all but cohort 1 from the 5 study cohorts—body composition based on DXA. In total, 457 individuals with proteomic measurements available at baseline and at month 6 were included in our analysis (from hereon referred to as the full DIETFITS cohort), of whom 352 had available DXA measurements at baseline and month 6 (from hereon referred to as the DIETFITS subsample with available DXA) (Figure 1). The data set analyzed in this study will be made available upon reasonable request.

Blood sampling and routine biochemistry

At all time points, fasting blood samples were taken via venipuncture, processed, and stored as plasma and serum samples in a -80°C freezer until biochemical analysis. Plasma triglycerides, total cholesterol, and HDL cholesterol were measured by enzymatic endpoint analysis on a clinical chemistry analyzer (Liasys 330) (22). LDL cholesterol was calculated using the Friedewald equation. Insulin concentrations were assessed by RIA by the Core Laboratory for Clinical Studies at Washington University School of Medicine, St. Louis, MO (22). Glucose concentrations were determined using a Beckman Glucose Analyzer II by electrochemical technique (22). We calculated the

HOMA-IR as the product of fasting glucose (mg/dL) and serum insulin ($\mu\text{IU/L}$) divided by 405.

Proteomic measurements

At both time points, we performed high-throughput proteomic measurements on plasma samples using Proseek® multiplex platforms (Olink Proteomics). The proteomic profiling in DIETFITS has been described in detail before (24). To be specific, the arrays CVDII (which includes sACE2), CVDIII, and Inflammation I were used to determine 276 proteins (92/panel) related to immune regulation, metabolic pathways, and cardiovascular disease. The platforms apply proximity extension assay technology (19), where each protein gets linked to a unique pair of oligonucleotide-labeled antibodies. Next, hybridization, amplification, and quantification of the complementary oligonucleotide strands linked to the paired antibodies enabled protein quantification by real-time qPCR using a Fluidigm BioMark HD platform. Quantitation data were quality controlled with the help of internal and external controls. Data were normalized, providing Normalized Protein eXpression (NPX). NPX is an arbitrary unit on a log₂ scale used to quantify relative changes in protein concentrations. Higher NPX corresponds to higher protein expression. The 3 panels used in this study have been thoroughly validated in terms of measurement range, specificity and precision, repeatability, reproducibility, and endogenous interference. Validation data documents and the list of biomarkers included in each panel are available at <https://www.olink.com/resources-support/document-download-center/>. In the DIETFITS study, the average intra- and interassay CVs were 8% and 15% (CVDII), 9% and 13% (CVDIII), and 6% and 13% (Inflammation I), respectively. For sACE2, the intra-assay CV was 9% and interassay CV was 17%. Of the 276 proteins, 40 were excluded owing to most measurements ($>90\%$) being below the detection limit ($n = 28$), overlap between the panels ($n = 11$), or an irregular distribution ($n = 1$), leaving 236 proteins for analysis. Of the 215,704 individual proteomic data points, 64 missing protein concentrations (0.03%) were imputed as the cohort's mean NPX.

DXA

In a subset of 352 individuals, DXA scans were performed to assess bone mineral content, lean body mass, and whole-body adiposity at baseline and at 6 mo. A Hologic QDR-4500 W fan-beam scanner was used in compliance with the manufacturer's guidelines. Quality control procedures were carried out regularly and the scanner was calibrated weekly following the manufacturer's recommendations. To minimize observer variability, 1 technician completed all scans for all participants at both time points.

Other measurements

Body weight was recorded without shoes to the nearest 0.1 kg using a calibrated Scale-Tronix scale. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca). After 5 min of rest, 3 BP readings were obtained on the right arm 1 min apart using a WelchAllyn, Spot Vital Signs LXi, with the

participant in a seated position. The mean of the second and third BP measurements was used.

Statistical analysis

For database management and analysis, we used SAS version 9.4 and JMP Genomics 9.0 (SAS Institute). Means and proportions were compared by a large-sample z test and χ^2 test, respectively. Significance was defined as $P < 0.05$ on a 2-sided test with 5% α error.

First, we performed bidirectional stepwise regression to determine clinical correlates of baseline sACE2 and Δ sACE2. Baseline covariables considered were age, sex, race, body height and weight, systolic and diastolic BP, heart rate, regular alcohol consumption, and circulating markers of metabolic health (HOMA-IR, triglycerides, HDL cholesterol, and LDL cholesterol). For Δ sACE2, stepwise models also included 6-mo changes in these covariables as well as the diet intervention (low-carbohydrate compared with low-fat). In a subsample analysis, stepwise regression models in addition considered DXA measurements (bone mineral content, lean mass, and fat mass) at baseline and changes therein. We set the P values for variables to enter and stay in the stepwise regression models at 0.10 and 0.05, respectively. Next, we compared the mean Δ sACE2 per dichotomous change in metabolic health (i.e., Δ sACE2 by improvement or worsening in HOMA-IR, triglycerides, and HDL cholesterol), while accounting for the important correlates identified in stepwise regression previously. In addition, we assessed in multiple logistic regression the adjusted risk of experiencing an sACE2 decrease during the intervention as associated with a favorable change in metabolic health profile.

To explore sACE2 connectivity within the protein network, we constructed 2 weighted networks using NetworkX 2.5: one with all 236 protein concentrations at baseline, another with the 6-mo protein changes. The R package “Weighted Gene Co-Expression Network Analysis” or WGCNA 1.69 was used for the scale-free analysis (25). We constructed the protein network with edges weighted by Pearson correlation coefficients produced from the `corr()` method in pandas 1.1.4 (26). Edges with a Pearson correlation coefficient >0.4 were used for the network construction.

Next, we applied a 2-step approach to identify from the pool of 235 proteins those related to sACE2 (at baseline and follow-up) and Δ sACE2: 1) protein selection based on overlap between 2 different feature selection techniques capable of handling complex network data and 2) verification of selected proteins by traditional regression statistics. By considering the overlap between the feature selection techniques, we partially accounted for intermethod variability overall and incidental protein selection in specific. For feature selection, we used partial least squares (PLS) analysis (20) and eXtreme Gradient Boosting (XGBoost) (21), 2 dimension reduction techniques capable of handling large sets of interrelated biomarkers. PLS constructs linear combinations that maximize the covariance between the proteins and the outcome (here: sACE2). These latent factors then replaced the original features (proteins) in the sACE2/ Δ sACE2 estimation. The number of latent factors was the number explaining a substantial proportion of the variation in features and outcome while not being significantly different from the model with the minimum predicted residual sum of squares. Per protein, we calculated the variable importance for

projection (VIP) scores of Wold, reflecting the importance of each biomarker in the construction of the final PLS model. In XGBoost, the final model was an additive combination of a number of trees, with each subsequent tree trained on a negative gradient of a loss function (21). This approach decreased both variance and bias, and thus increased prediction performance. XGBoost was optimized with a Tree-structured Parzen Estimator Approach using hyperopt 0.2.5. Feature importance was assessed as a mean increase in accuracy resulting from the tree splits per given protein.

We assessed associations between Δ sACE2 and the protein changes considered influential in both PLS analysis (VIP >1.5) and XGBoost modeling (feature importance >0.010) for prediction of Δ sACE2, while accounting for age and important clinical correlates of Δ sACE2 identified in stepwise regression (i.e., baseline sACE2, sex, diet, HOMA-IR, LDL cholesterol, and longitudinal changes in HOMA-IR, triglycerides, and HDL cholesterol). P values were corrected for multiple testing using the Holm–Bonferroni method.

Results

Characteristics of the DIETFITS cohort

Clinical characteristics of the 457 participants in the DIETFITS trial (mean \pm SD age: 39.8 ± 6.6 y; 59.1% women) are presented by examination phase in **Table 1** and by sex in **Supplemental Table 1**. On average, BP levels, body composition, and biochemical markers of metabolic health improved, whereas sACE2 concentrations declined significantly at 6 mo in the diet intervention ($P < 0.0001$ for all) (**Table 1**, **Figure 2A**). sACE2 dropped significantly in both the low-fat and the low-carbohydrate diet intervention groups ($P \leq 0.0091$ for both), but it declined stronger in the low-carbohydrate than in the low-fat diet group ($P = 0.0004$) (**Supplemental Table 2**).

sACE2 in relation to metabolic health and body composition

Clinical correlates of baseline sACE2.

At baseline, higher sACE2 concentrations were associated independently with male sex and with higher systolic BP, HOMA-IR, triglycerides, and HDL cholesterol in stepwise regression (adjusted $R^2 = 30.9\%$; $P \leq 0.0056$ for all) (**Supplemental Table 2**). In addition, no association was found between baseline sACE2 and total and LDL cholesterol ($P > 0.091$) (**Supplemental Table 3**). In subsample analysis considering DXA measurements, higher sACE2 concentrations were in addition correlated with lower fat mass at baseline (adjusted $R^2 = 31.6\%$) (**Supplemental Table 2**). The association between fat mass and sACE2 was inverse in both men and women ($P_{\text{sex interaction}} = 0.23$), but only reached statistical significance in women ($P < 0.0001$ in women, $P = 0.36$ in men) (**Supplemental Table 4**).

Clinical correlates of longitudinal change in sACE2.

Table 2 presents the correlates of the 6-mo change in sACE2 during the diet intervention. In stepwise regression, a stronger decline in sACE2 was associated with higher sACE2 at baseline, female sex, low-carbohydrate diet (as compared with low-fat), and lower HOMA-IR and LDL cholesterol at baseline as well

TABLE 1 Clinical characteristics of the 457 DIETFITS (Diet Intervention Examining the Factors Interacting with Treatment Success) study participants by examination phase¹

	Examination 1 (baseline)	Examination 2 (6-mo follow-up)	<i>P</i> value
Anthropometrics			
Age, y	39.8 ± 6.6	—	—
Body weight, kg	95.7 ± 14.8	88.4 ± 14.3	<0.0001
Body height, cm	169.3 ± 9.5	—	—
BMI, kg/m ²	33.3 ± 3.6	30.8 ± 3.7	<0.0001
Systolic BP, mm Hg	123.8 ± 12.5	119.3 ± 11.5	<0.0001
Diastolic BP, mm Hg	81.3 ± 7.6	78.3 ± 7.3	<0.0001
Heart rate, bpm	70.4 ± 10.1	67.5 ± 10.1	<0.0001
Biochemical data			
HOMA-IR	3.26 (1.71–7.26)	2.26 (1.43–6.39)	<0.0001
Total cholesterol, mg/dL	189.2 ± 33.9	183.6 ± 35.1	<0.0001
LDL cholesterol, mg/dL	114.2 ± 28.9	113.2 ± 29.7	0.32
HDL cholesterol, mg/dL	48.9 ± 9.8	49.2 ± 9.6	0.31
Triglycerides, mg/dL	113.1 (64.9–223.0)	92.5 (54.5–180.5)	<0.0001
sACE2, NPX	1.89 ± 0.63	1.74 ± 0.54	<0.0001
Race/ethnicity			
White	328 (71.8)	—	—
Black/African American	14 (3.1)	—	—
Asian	48 (10.5)	—	—
Other	67 (14.7)	—	—
DXA scan²			
BMC mass, kg	2.59 ± 0.44	2.59 ± 0.44	0.45
Fat mass, kg	33.4 ± 7.5	28.8 ± 8.0	<0.0001
Lean mass, kg	56.0 ± 11.3	54.1 ± 10.8	<0.0001
Fat percentage, %	36.5 ± 6.8	33.7 ± 7.6	<0.0001

¹Values are arithmetic mean ± SD, geometric mean (10th–90th percentile), or participant *n* (%). *P* values reflect the significance of 6-mo longitudinal changes according to a paired-sample *t* test. BMC, bone mineral content; BP, blood pressure; NPX, Normalized Protein eXpression; sACE2, soluble angiotensin-converting enzyme 2.

²Baseline and follow-up data for DXA scan measurements were available in 352 participants.

as with a stronger decline in HOMA-IR, triglycerides, and HDL cholesterol during the diet intervention (Table 2). In subsample analysis including the DXA measurements, a larger drop in sACE2 was in addition correlated with a larger drop in fat mass (Table 2). Multivariable-adjusted analyses confirmed the association between Δ sACE2 and the 6-mo changes in HOMA-IR, triglycerides, HDL cholesterol, and fat mass ($P \leq 0.019$ for all) (Supplemental Table 5). In support of this, after full adjustment, participants in whom HOMA-IR and triglycerides were decreased at month 6 of the diet intervention presented a significant drop in sACE2 (Figure 2B). In contrast, a significant rise in sACE2 was seen in those experiencing a 6-mo increase in HOMA-IR and triglycerides (Figure 2B). The multivariable-adjusted odds for experiencing a decrease in sACE2 during the diet intervention were significantly higher in participants with a 6-mo decrease in HOMA-IR (OR: 1.97; $P = 0.0073$) and triglycerides (OR: 2.71; $P < 0.0001$) than in those without (Figure 2C).

sACE2 and circulating proteins related to inflammation and cardiovascular disease

Network of diet-induced protein changes.

We constructed weighted networks of the 236 proteins at baseline (Supplemental Figure 1) and of their changes during the diet intervention (Supplemental Figure 2). Of

note, Δ sACE2 was directly connected to the 6-mo change in α -1-microglobulin/bikunin precursor (AMBP), chemokine C-C motif ligand 3, E-selectin, hydroxyacid oxidase 1 (HAOX1), osteoclast-associated immunoglobulin-like receptor, IL-16 and IL-18, kidney injury molecule 1 (KIM-1), tyrosine-protein kinase Mer (MERTK), placental growth factor (PGF), prostasin (PRSS8), spondin-2, thrombospondin-2, thrombomodulin, tyrosine-protein kinase receptor (TIE2), and TNF receptor superfamily member 10B (TRAIL-R2) (Supplemental Figure 2).

Proteins linked to sACE2 and Δ sACE2.

Figure 3 outlines the proteins identified by PLS and/or XGBoost as most likely to be associated with sACE2 and Δ sACE2. Of note, E-selectin, HAOX1, and KIM-1 consistently were selected as important proteins linked to baseline sACE2, follow-up sACE2, and Δ sACE2. The following 6-mo protein changes were related to Δ sACE2 in both PLS and XGBoost modeling: change in AMBP, E-selectin, HAOX-1, KIM-1, MERTK, PGF, thrombomodulin, and TRAIL-R2 (Figure 3). In support, Δ sACE2 remained independently associated with these protein changes in linear regression after adjustment for major clinical correlates identified previously (Supplemental Table 6). In addition, both baseline and follow-up sACE2 were found to be linked to aminopeptidase N (APN), decorin, heme oxygenase 1, matrix metalloproteinase 3, PRSS8, and soluble

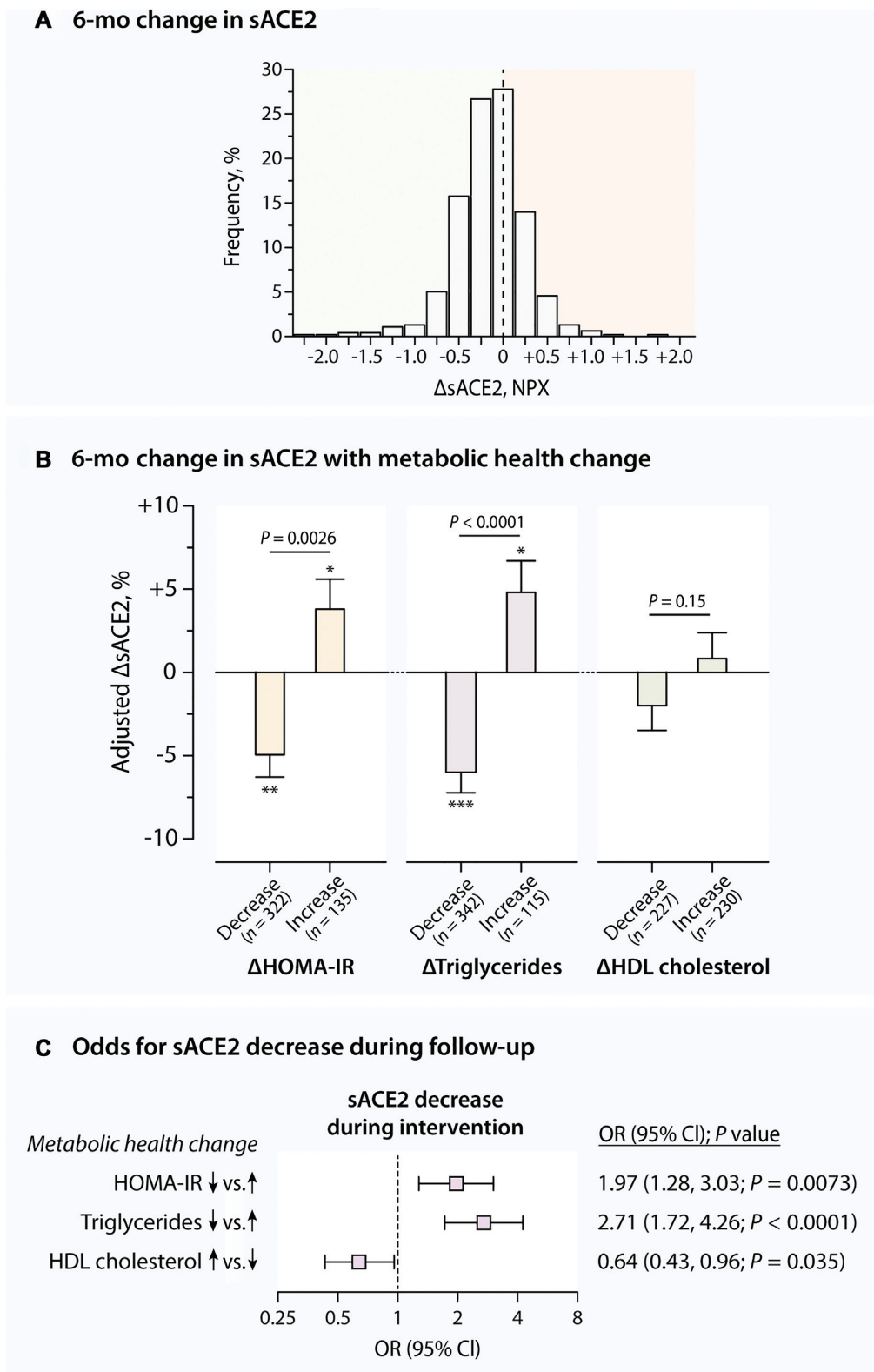


FIGURE 2 Multivariable-adjusted association between 6-mo changes (Δ) in sACE2 and metabolic health during a diet intervention. (A) Histogram of Δ sACE2 in the entire cohort ($n = 457$). (B) Multivariable-adjusted change in sACE2 by change in metabolic health profile. Mean \pm SE Δ sACE2 was adjusted for sACE2 at baseline, age, sex, and clinical correlates of Δ sACE2 (Table 2) in a linear mixed model and compared using an independent-samples t test. *,**,* Significant changes in sACE2 during follow-up according to paired-sample t test: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. (C) Multivariable-adjusted risk of decrease in sACE2 by change in metabolic health profile in logistic regression. NPX, normalized protein expression; sACE2, soluble angiotensin-converting enzyme 2.

TABLE 2 Clinical correlates of change in sACE2 during a diet intervention¹

	Δ sACE2		
	$\beta \pm$ SE	<i>P</i> value	Partial R^2
Full DIETFITS cohort (<i>n</i> = 457)			
Baseline correlates			
sACE2	− 56.3 ± 4.2	<0.0001	27.1%
Female sex	− 29.3 ± 8.3	0.0005	2.2%
Low-carbohydrate diet (vs. low-fat)	− 21.8 ± 7.8	0.0053	0.87%
HOMA-IR	10.8 ± 4.6	0.018	0.67%
LDL cholesterol	9.4 ± 3.7	0.011	0.74%
Change during intervention			
Δ HOMA-IR	19.1 ± 4.6	<0.0001	1.7%
Δ Triglycerides	17.7 ± 4.0	<0.0001	5.9%
Δ HDL cholesterol	14.0 ± 3.8	0.0003	1.4%
DIETFITS subsample with DXA measurements available (<i>n</i> = 352)			
Baseline correlates			
sACE2	− 31.1 ± 3.3	<0.0001	33.4%
Female sex	− 47.6 ± 9.2	<0.0001	3.0%
LDL cholesterol	10.3 ± 4.2	0.013	0.94%
Change during intervention			
Δ HOMA-IR	23.1 ± 6.6	0.0010	2.3%
Δ Triglycerides	30.1 ± 9.0	0.0006	4.8%
Δ HDL cholesterol	17.1 ± 4.1	<0.0001	2.6%
Δ Fat mass	10.6 ± 4.5	0.019	1.2%

¹Values were standardized partial regression coefficients ± SEs standardized for the distribution of both the predictor and sACE2 retrieved from bidirectional stepwise regression. Covariables considered were age, sex, race, diet (low-carbohydrate compared with low-fat diet), body height and weight, systolic and diastolic blood pressure, heart rate, regular alcohol consumption, HOMA-IR, triglycerides, HDL and LDL cholesterol, and, if available, BMC mass, fat mass, and lean mass (as assessed by DXA) as well as longitudinal changes in these variables and sex interaction terms. Total adjusted R^2 = 39.9% (full DIETFITS cohort) and 44.4% (DIETFITS subsample with DXA). None of the sex interaction terms were selected for the final stepwise regression models ($P > 0.05$ for all). DIETFITS, Diet Intervention Examining the Factors Interacting with Treatment Success; sACE2, soluble angiotensin-converting enzyme 2.

interleukin-1 receptor-like 1 (ST-2) (Figure 3). Summary data of the PLS models are presented in **Supplemental Figure 3** (for Δ ACE2) and **Supplemental Table 7** (for sACE2 at baseline and follow-up).

Discussion

We uncovered metabolic and proteomic profiles associated with sACE2 changes. In this randomized weight loss diet intervention trial, a stronger reduction in sACE2 was associated with improvements in body composition and metabolic health. Moreover, sACE2 concentrations were associated with proteins related to angiotensin peptide metabolism (APN), vascular injury and inflammation (PGF, E-selectin, MERTK), blood coagulation (thrombomodulin), renal function (KIM-1), liver function and oxalate metabolism (HAOX1), and chronic inflammation and oxidative stress (AMBP, TRAIL-R2).

sACE2 and biological sex

Previous cross-sectional population studies have consistently reported higher sACE2 concentrations in men than in women (13). Even with lower sACE2 at baseline, women experienced a stronger reduction in sACE2 concentration than men during the weight loss diet intervention. Our findings suggest that sACE2 is less affected by dietary interventions and fat mass changes

in men than in women. It remains to be studied to what extent sex differences in sACE2 dynamics may originate from sex differences in ACE2 gene regulation (a gene localized on the X chromosome). If true, such sex difference may encumber the benefits of a dietary intervention on cardiorenal health and COVID-19 susceptibility in men.

ACE2 as regulator of glucose and lipid metabolism

ACE2 catabolizes angiotensin II into angiotensin-(1–7) [Ang(1–7)], a peptide that exerts the protective actions of ACE2 on cardiorenal function and glucose and lipid metabolism. Of note, ACE2 may be upregulated in subjects with cardiometabolic risk factors to counteract the adverse effects of increased local or systemic angiotensin II activity. RAS and ACE2 have been linked experimentally and clinically to the metabolic syndrome and its associated aberrations in glucose and lipid metabolism (1, 17). As such, RAS overactivation and improper compensation by ACE2/Ang(1–7) could contribute to disorders associated with the metabolic syndrome, including pancreatic β -cell apoptosis, deficits in insulin secretion, and decreased insulin responsiveness in skeletal muscle and adipose tissue (1).

For instance, experimental studies demonstrated a protective role for ACE2 and Ang(1–7) in the regulation of pancreatic β -cell function and, in consequence, of insulin synthesis and secretion (18). Indeed, overexpression of ACE2 in the pancreas of db/db

Of note, we observed a direct relation between sACE2 and HDL cholesterol. Intuitively, one would expect an inverse relation: higher/worse sACE2 related to lower/worse HDL. As in another community-based study (12), sACE2 was inversely correlated to HDL cholesterol in our unadjusted analyses, but the relation shifted to a direct one in the adjusted models. We cannot exclude the possibility of a hidden confounder that biased the sACE2–HDL relation. For instance, alcohol consumption is known to raise HDL cholesterol concentrations while inducing low-grade hepatic and pancreatic injury (32). Self-reported alcohol consumption did not alter the sACE2–HDL relation in our analyses, but the alcohol reporting may have been biased by cognitive and sociocultural factors (33). The question remains whether the ACE2–HDL association is confounded by hepatic and metabolic risk factors.

sACE2 and protein markers of vascular, renal, hepatic, and inflammatory health

In the second part of our analysis, we identified protein dynamics associated with the sACE2 changes. These proteins may highlight molecular mechanisms linked to the aforementioned ACE2 activities.

sACE2 concentrations and sACE2 changes during the diet intervention were most strongly associated with KIM-1 and HAOX1, which may reflect the link between elevated sACE2 activity and impaired kidney, liver, and pancreas function (34). KIM-1 is a biomarker of renal proximal tubular injury. In a rat model, increased KIM-1 protein expression was associated with the development of RAS-mediated renal damage (35). HAOX1 is a peroxisomal enzyme that converts glycolate into the oxalate precursor glyoxylate in the liver and pancreas (36, 37). HAOX1 could be released in the bloodstream upon hepatic and pancreatic injury, which may explain its strong connection with sACE2, a regulator of lipid and glucose metabolism in the liver. In support of this, a cross-sectional community study found strong correlations between sACE and other circulating markers of liver and pancreas damage such as γ -glutamyl transferase (12).

The strong relation between sACE2 and APN at baseline and follow-up is rather straightforward: the metalloproteinase APN catabolizes angiotensin III into angiotensin IV and is thus a regulator of angiotensin peptide metabolism like ACE2 (38). Interestingly, several alphacoronaviruses use APN as a cell-entry receptor (39), whereas human betacoronaviruses like SARS-CoV-2 rely on ACE2 for cell entry (4).

The associated dynamics in sACE2, PGF, MERTK, E-selectin, and thrombomodulin suggest a tight connection between RAS/ACE2 and pathways related to vascular remodeling, vascular inflammation, and blood coagulation. PGF is a member of the vascular endothelial growth factor family of angiogenic cytokines that is enhanced during vascular injury (40). Angiotensin II stimulates PGF production in human endothelial and vascular smooth muscle cells (41). Moreover, experimental data link RAS overactivation and consequent PGF overexpression to the pathogenesis of atherosclerosis. Indeed, PGF mediates aldosterone-induced vascular remodeling, leading to smooth muscle cell proliferation and vascular fibrosis and inflammation at sites of endothelial injury (42, 43). Similarly, both MERTK and E-selectin—a cell adhesion molecule expressed solely on

activated endothelial cells—mediate the cascade of vascular inflammation leading to the formation of atherosclerotic lesions (44, 45). Thrombomodulin is an endothelial cell receptor with anticoagulatory, antifibrinolytic, and anti-inflammatory properties (46).

Lastly, the associations found between sACE2 dynamics and the biomarkers AMBP and TRAIL-R2 may reflect the counteracting role of ACE2 on systemic low-grade chronic inflammation and oxidative stress underlying cardiorenal and metabolic disorders. AMBP is a precursor of α 1-microglobulin, a heme and radical scavenger with antioxidative and immunosuppressive properties, and was found to be involved in erythropoiesis and erythrocyte homeostasis (47). TRAIL-R2—also known as TNFRSF10B—is a member of the death receptor superfamily and a key modulator of apoptosis. High TRAIL-R2 was associated with incident cardiometabolic morbidity in a large cohort of 4742 community-dwelling individuals (48). Future studies should deeper investigate the connections between sACE2 and the highlighted set of proteins and explore whether the associated proteins represent clinically relevant targets for detection and management of cardiovascular, renal, and COVID-19-related diseases.

Clinical implications

In particular, our findings may have implications on the risk stratification, prevention, and management of cardiometabolic and COVID-19-related disorders. For instance, improvements in insulin resistance and lipid profile may alleviate RAS contributions to vascular and metabolic pathologies. As such, weight loss diet interventions may be powerful prophylactic tools for both cardiovascular and COVID-19 risk reduction in the community. Improving body composition and metabolic health may particularly be an effective strategy for COVID-19 prophylaxis in people with poor cardiovascular disease risk profiles (7–10). In support, 33 patients with a prior history of metabolic surgery who tested positive for SARS-CoV-2 presented lower rates of hospital admission than SARS-CoV-2 positive patients with severe obesity (49). It remains to be studied whether an sACE2-reducing diet in individuals at high risk can lower SARS-CoV-2 incidence and disease severity. Future research should expand our analyses with genetic, environmental, and other metabolic factors to further unravel the mechanisms underlying sACE2 variability.

Study strengths and limitations

We acknowledge the study's strengths and limitations. First, a large number of proteins was measured in a large number of individuals. Technical variability may have influenced the proteomic measurements; however, the panels used in this study were validated extensively in terms of measurement ranges, assay specificity and precision, repeatability, reproducibility, and endogenous interference (<https://www.olink.com/resources-support/document-download-center/>). Second, although we determined a wide range of proteins linked to cardiovascular disease and inflammation, we may have missed hidden confounders or important proteins linked to sACE2. For instance, the protein panels did not include biochemical markers of overall liver and pancreas damage such as γ -glutamyl transferase, whereas such

markers were found to correlate strongly with sACE2 in the community previously (12). Third, extrapolation of our findings may be limited by the conduct of the study in a geographic area with individuals who have attained relatively high education levels and high accessibility to high-quality food. However, the study was broadly advertised and successfully enrolled participants with relatively good ethnic and racial diversity and with a range of educational attainment.

Conclusions

A decrease in sACE2 at 6 mo in a weight loss diet intervention was associated with improvements in metabolic health, fat mass, and markers of angiotensin peptide metabolism, hepatic and vascular injury and inflammation, renal function, chronic inflammation, and oxidative stress. Our findings highlight the need for further studies on diet interventions for cardiorenal and COVID-19 disease prophylaxis in the community. Future research should investigate to what extent dietary sACE2 alterations can counteract the incidence and severity of cardiorenal and SARS-CoV-2-related diseases.

The authors' responsibilities were as follows—NC, TK, and FH: performed the statistical analysis; NC, MP, and TK: drafted the manuscript; and all authors: contributed to the conception and design of the study, contributed to the data interpretation, revised the manuscript critically for important intellectual content, and read and approved the final manuscript. The authors report no conflicts of interest.

Data availability

Data included in the article, code book, and analytic code will be made available upon reasonable request.

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