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ORIGINAL RESEARCH ARTICLE

Novel biomarkers associated with incident heart failure in 10 106 Finnish men

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Abstract

Aims There are only a few studies on novel biomarkers for incident heart failure (HF). We investigated the association of multiple circulating biomarkers with incident HF in a large prospective population-based study.

Methods and results Conventional risk factors and inflammatory biomarkers were measured, and systemic metabolic measures determined by a high-throughput serum nuclear magnetic resonance platform in a population-based Metabolic Syndrome in Men study including 10 106 Finnish men without HF at baseline. During an 8.8 year follow-up, 172 (1.7%) participants developed HF. Adiponectin, high-sensitivity C-reactive protein (hs-CRP), glycoprotein acetyls, alanine, phenylalanine, glycerol, and pyruvate were associated with incident HF in unadjusted Cox regression analyses, in addition to age, systolic blood pressure, body mass index (BMI), waist circumference, fasting plasma glucose and insulin, haemoglobin A1c (HbA1c), and urinary albumin excretion rate (UAER). After adjustment for age, BMI, diabetes, and statin medication, only adiponectin [hazard ratio (HR) 1.18 (1.10–1.26, P = 4.1E-08)], pyruvate [HR 1.38 (1.28–1.50, P = 8.2E-05)], and UAER [HR 1.15 (1.11—1.18, P = 7.8E-06)] remained statistically significant. In principal component analysis of biomarkers associated with HF in univariate Cox regression analysis, we identified six components, explaining 61.7% of total variance. Four principal components, one with significant loadings on waist, BMI, fasting plasma insulin, interleukin 1 receptor antagonist, and hs-CRP; another on pyruvate, glycoprotein acetyls, alanine, glycerol and HbA1c; third on age and glomerular filtration rate; and fourth on systolic blood pressure, UAER, and adiponectin, significantly associated with incident HF.

Conclusions Several novel metabolic and inflammatory biomarkers were associated with incident HF, suggesting early activation of respective pathways in the pathogenesis of HF.

Keywords Heart failure; Hypertension; Biomarkers; Metabolomics; Inflammation; Principal component analysis

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Introduction

Heart failure (HF), caused by abnormal cardiac structure and function, represents the end stage of several cardiac diseases, and significantly worsens both quality of life and life expectancy with a 5 year survival rate of approximately 50%. Lifetime risk of the development of HF is 20% and overall prevalence 2%. There are over 30 million cases of HF worldwide resulting in an overall annual economic cost of over $100 billion.[1–3]

Natriuretic peptides and troponins have been used in clinical diagnosis and treatment of HF, and they also predict incident HF.[4–7] There are, however, only few studies on other biomarkers of incident HF,[8–17] and very few of them have investigated large panels of biomarkers.[10,13–15] Several pathogenic mechanisms are suggested to be operative in HF, but they are only partially understood.[1,7] Transition from current practice of treating HF to biomarker-based precise medicine targeted to prevent HF requires investigation of multiple biomarkers reflective of pathophysiological pathways resulting in HF.[1,7]

Inflammatory biomarkers are elevated in HF,[1,7] but there are only few prospective studies on the subject showing that

high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 predict HF.[8,14,15,17] Metabolomics, or metabolic profiling, permits a large-scale analysis of many metabolic alterations occurring in HF.[1,10,13] There are only few studies on metabolic biomarkers associated with incident HF.[10,13,16] Elevated phenylalanine concentrations and four other metabolites of amino acid metabolism have been associated with incident HF.[10,13] Recently, we have used systems genetics approach to identify a new biomarker glycoprotein NMB, playing a role in tissue regeneration by regulation of inflammatory response and suppressing fibrosis, which was negatively associated with HF.[16]

The aim of the present study was to investigate the association of a large panel of inflammatory biomarkers and metabolites, in addition to anthropometric measures and conventional risk factors, with incident HF in 10 106 Finnish men.

Methods

Subjects and clinical measurements at baseline

The baseline study of the Metabolic Syndrome in Men (METSIM) included 10 197 men, aged from 45 to 73 years, randomly selected from the population register of Kuopio, Eastern Finland (population 95 000). At baseline, every study subject participating in the study gave written consent, and had a 1 day outpatient visit to the Clinical Research Unit at the University of Kuopio,[18] where blood samples for proton nuclear magnetic resonance (NMR) analysis, metabolic, inflammatory, and other biomarkers were taken. The design and methods of the METSIM study have been previously described in detail.[19] In the present study, all participants with prevalent HF before the baseline study (n = 91) were excluded from statistical analyses, giving the final study population of 10 106 men. The baseline measurements are available practically in all 10 106 subjects (*Table 1*).

Anthropometric measurements at baseline

Height and weight were measured to the nearest 0.5 cm and .1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured at the midpoint between the lateral iliac crest and lowest rib. Smoking status was defined as current smoking (yes vs. no). Diagnosis of hypertension was based on the use of antihypertensive medication (information obtained from the National Drug Reimbursement registry).

Oral glucose tolerance test at baseline

A 2 h oral glucose tolerance test (75 g of glucose) was performed, and samples for plasma glucose and insulin were

Table 1 Cox regression analysis of demographic and anthropometric variables and biomarkers as predictors for heart failure in 10 106 participants of an 8.8 year follow-up study of the METSIM cohort

Variable	*N*, non-case/case	HR	95% CI Lower	95% CI Upper	*P*	*P**	*P***	*P****	*P*****
Age (years)	9934/172	1.12	1.09	1.14	**1.4E-19**				
Type 2 diabetes (yes/no)	9910/171	3.33	2.43	4.57	**9.2E-14**	**6.8E-09**	**1.5E-04**		
Systolic blood pressure (mmHg)	9933/172	1.36	1.19	1.56	**5.1E-06**	0.014	0.162	0.391	0.347
Body mass index (kg/m^2)	9931/172	1.50	1.34	1.67	**1.8E-12**	**1.9E-13**			
Waist circumference (cm)	9929/172	1.63	1.45	1.83	**5.9E-16**	**3.6E-15**	0.002	0.011	0.010
Fasting plasma glucose (mmol/L)	9934/172	1.22	1.13	1.33	**1.1E-06**	**3.5E-05**	0.044	0.569	0.602
Fasting plasma insulin (mU/L)	9930/172	1.13	1.07	1.19	**9.0E-06**	**1.8E-04**	0.449	0.815	0.681
HbA1c (%)	9911/172	1.33	1.25	1.43	**8.3E-17**	**3.9E-11**	**1.1E-05**	0.038	0.056
Glomerular filtration rate	9934/172	0.73	0.63	0.86	**8.6E-05**	0.256	0.297	0.198	0.217
Urinary albumin excretion rate (μg/min)	9798/167	1.15	1.11	1.18	**8.7E-17**	**5.8E-13**	**1.3E-09**	**3.8E-06**	**7.8E-06**
Plasma adiponectin (μg/mL)	9932/172	1.18	1.10	1.26	**3.9E-06**	**5.0E-04**	**1.7E-06**	**2.3E-07**	**4.1E-08**
High sensitivity CRP (mg/L)	9933/172	1.09	1.04	1.15	**4.5E-04**	0.002	0.038	0.047	0.032
IL1 receptor antagonist (pg/L)	9933/172	1.15	1.05	1.26	0.003	0.003	0.931	0.707	0.683
Glycoprotein acetyls (mmol/L)	9878/170	1.25	1.11	1.41	**3.1E-04**	**7.2E-05**	0.036	0.186	0.223
Alanine (mmol/L)	9885/170	1.31	1.15	1.49	**5.5E-05**	**0.001**	0.081	0.448	0.537
Histidine (mmol/L)	9891/170	0.75	0.64	0.88	**0.001**	0.017	0.058	0.122	0.163
Phenylalanine (mmol/L)	9878/168	1.21	1.09	1.33	**1.6E-04**	0.006	0.357	0.396	0.393
Tyrosine (mmol/L)	9890/170	1.22	1.07	1.39	0.003	0.007	0.419	0.458	0.438
Acetoacetate (mmol/L)	9891/170	1.14	1.05	1.24	0.002	0.008	0.006	0.035	0.027
Glycerol (mmol/L)	9657/166	1.36	1.21	1.53	**4.1E-07**	**5.0E-06**	0.012	0.187	0.188
Pyruvate (mmol/L)	9891/170	1.38	1.28	1.50	**9.5E-15**	**1.0E-13**	**9.4E-08**	**5.5E-05**	**8.2E-05**

CI, confidence interval; CRP, C-reactive protein; HbA1c, haemoglobin A1c.
Analysis includes 10 106 participants without heart failure (HF) at baseline, of whom 172 participants developed incident HF during the follow-up period. Hazard ratios (HR) are standardized. $P < 0.002$ (0.05/21) is considered as statistically significant (bold). $P < 0.05$ is considered as nominally significant (underlined). *P* unadjusted. *P** adjusted for age. *P*** adjusted for age and body mass index. *P**** adjusted for age, body mass index and diabetes. *P***** adjusted for age, body mass index, diabetes and statin medication.

drawn at 0, 30, and 120 min. Glucose tolerance based on an oral glucose tolerance test was classified according to ADA 2003 criteria, including type 2 diabetes (fasting glucose tolerance test $\geq$ 7.0 mmol/L and/or 2 h plasma glucose $\geq$ 11.1 mmol/L).[20]

Laboratory measurements

Levels of plasma glucose, insulin, haemoglobin A1c (HbA1c), glomerular filtration rate and urinary albumin excretions rate were measured as previously described.[18,21]

Inflammatory biomarkers

Serum concentrations of hs-CRP were assayed using kinetic immunoturbidimetry (near-infrared particle immunoassay; IMMAGE Immunochemistry System; Beckman Coulter, Fullerton, CA) and plasma interleukin 1 receptor antagonist (IL-1RA) using a photometric immunoassay [enzyme-linked immunosorbent assay (ELISA)] method (Quantikine DRA00 Human IL-1RA; R&D Systems, Inc., Minneapolis, MN). Plasma adiponectin was measured using ELISA (human adiponectin ELISA kit; Linco Research, St. Charles, MI). Concentrations of glycoprotein acetyls (including mainly alpha-1-acid glycoprotein) were quantified from serum samples using a high-throughput proton nuclear magnetic resonance metabolomics platform, as previously described.[21]

Proton nuclear magnetic resonance analysis

For all METSIM study participants, proton NMR analysis was performed at the same time from the baseline serum samples. Altogether, 27 serum metabolic measures, including amino acids, fatty acids, ketone bodies, and lipoprotein lipids (Supporting Information, *Table S3*), were determined using a high-throughput serum NMR platform operating at 500 MHz. Fasting serum samples collected at baseline were stored at −80°C and thawed overnight in a refrigerator before sample preparation. Aliquots of each sample (300 μL) were mixed with sodium phosphate buffer (300 μL). The NMR data were measured using a Bruker AVANCE III spectrometer operating at 500.36 MHz (^{1}H observation frequency; 11.74 T) and equipped with an inverse selective SEI probehead, including an automatic tuning and matching unit and a *z*-axis gradient coil for automated shimming. A BTO-2000 thermocouple serves to temperature stabilization, the sample at the level of approximately 0.01°C. The NMR technology is widely used, standardized, and accredited (https://nightingalehealth.com/about/technology). Details of the NMR experimentation have been described previously.[22,23]

Diagnosis of cardiovascular disease

Diagnoses of cardiovascular disease were obtained from the hospital registry of Kuopio University Hospital, which includes all diagnoses of inpatients and outpatients. Kuopio University Hospital is the only hospital and cardiology outpatient clinic treating patients with HF in the Kuopio region. All patients with HF from the Kuopio region are referred to the Kuopio University Hospital for echocardiography and diagnostic cardiac work-up.

Diagnosis of heart failure

Medical records of all participants with hospital discharge or outpatient clinic diagnoses suggestive of HF (International Classification of Diseases, 10th Revision [ICD-10]: I11.0, I11.9, and I50) before or after the baseline examination were reviewed in detail by a medical student (M.J.) and a cardiologist (J.K.). Diagnosis and aetiology of HF was based on clinical findings, N-terminal pro brain natriuretic peptide (NT-proBNP) measurements, electrocardiogram, chest X-ray, and echocardiography.[4] The type of HF was classified either as HF with preserved ejection fraction (left ventricular ejection fraction $\geq$ 40% on echocardiography) or HF with reduced ejection fraction (left ventricular ejection fraction $<$ 40%). Hypertensive HF was defined as HF in the presence of uncontrolled hypertension (ICD-10: I10), and/or hypertensive heart disease (ICD-10: I11.9) characterized by posterior wall (PW) $>$ 12 mm, and/or left ventricle end-diastolic diameter $>$ 60 mm and/or left ventricle end-systolic diameter $>$ 35 mm on echocardiography, and no other heart disease severe enough to cause HF. Other aetiologies of HF were obtained from the medical records derived clinical diagnoses made by a cardiologist or internist and were verified by a cardiologist with a 20 year experience in clinical cardiology (J.K.). Medical records of all subjects with a diagnosis of acute coronary syndrome were reviewed by a cardiology fellow (R.J.) and a cardiologist (J.K.).

Study follow-up and time to the diagnosis of incident heart failure

The study participants were followed-up to a mean of 8.8 years (median 6.9 years, range 0.1–9.6 years), and incident cases of HF were recorded until 31 May 2017. Mean time from the baseline examination to the diagnosis of incident HF was 4.4 years (median 4.5 years, range 0.1–9.2 years).

Mortality data

Of the 172 study participants who developed HF, 27 died during the follow-up. Causes of death were confirmed from

medical records, and death certificates were obtained from the National Causes-of-Death Register of Statistics Finland, which records and reviews all deaths in Finland.

Statistical analysis

Statistical analyses were conducted using the SPSS version 21 (SPSS, Chicago, IL). Data are presented as mean ± SD. Independent samples *t*-test and Pearson χ^2 test were used to assess statistical significance of the differences in clinical characteristics, inflammatory biomarkers, and proton NMR metabolic measures between the participants with and without incident HF. All variables except for age were log-transformed to correct for their skewed distributions. Cox regression analysis was applied to investigate the association of the baseline variables with incident HF. The anthropometric parameters and biomarkers, which differed significantly in independent samples *t*-test between the subjects with and without incident HF, were included in Cox regression analyses. *P* value 0.05, divided by the number of comparisons, was considered as statistically significant. Principal component (PC) analysis was applied to analyse the baseline variables associated with incident HF in unadjusted Cox regression analysis. Associations between the PCs and incident HF was investigated with Cox regression analysis.

Ethics statement

Study protocol was approved by the Ethics Committee of University of Eastern Finland and the Kuopio University Hospital. The study was conducted in accordance with the Helsinki Declaration. All study participants gave written informed consent.

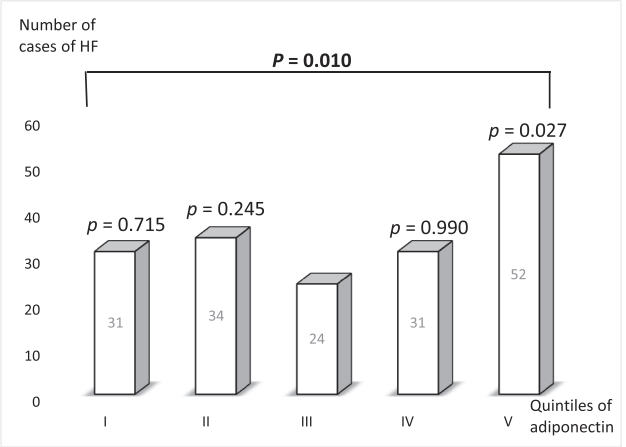
Results

Altogether 172 (1.7%) men of the METSIM study developed incident HF during the follow-up. *Table S1* gives the baseline anthropometric, metabolic, and inflammatory marker measurements of the METSIM study participants who did and did not develop incident HF. Participants with incident HF were older, had more often type 2 diabetes, reimbursement for hypertension, and a history of myocardial infarction, compared with those without incident HF. Systolic blood pressure, BMI, waist circumference, levels of fasting plasma glucose and insulin, HbA1c, and urinary albumin excretion rate (UAER) were higher in subjects with incident HF compared with those without incident HF. Glomerular filtration rate was lower in subjects with incident HF compared with those without incident HF. Subjects with incident HF had higher concentrations of adiponectin, hs-CRP, IL-1RA, and glycoprotein acetyls. We investigated the number of cases of incident HF across the quintiles of plasma adiponectin concentration (*Figure 1*). The number of incident cases was lowest in the third quintile and highest in the fifth quintile. There was a statistically significant difference between the third quintile and the fifth quintile in incident HF cases. In the linearity test, there was a significant deviation from linearity (*P* = 2.2E-17).

Table S2 shows the levels of baseline serum metabolic measures in proton NMR analysis in participants with and without incident HF. The concentrations of alanine, phenylalanine, tyrosine, acetoacetate, glycerol, and pyruvate were significantly higher, and the concentration of histidine lower among the participants with incident HF compared with participants without HF.

Table S3 shows hospital medical records derived aetiology and echocardiographic, NT-proBNP and electrocardiogram characteristics of the subjects with incident HF at the time of the diagnosis of HF, and outcomes during the follow-up. Of subjects with HF, 68 had hypertension as the aetiology of HF. In 62, the aetiology of HF was coronary artery disease. Of them, 36 had suffered from a myocardial infarction before the baseline examination, 19 patients had HF due to an acute myocardial infarction during the follow-up, and 7 subjects had ischemic HF without myocardial infarction. Cardiomyopathy was diagnosed in 31 (18.0%) of the subjects with incident HF. Altogether 13 (12.5%) patients had HF due to valvular heart disease. On echocardiography performed at the time of HF diagnosis, about half of the patients had preserved ejection fraction. The mean left ventricular ejection fraction was 42.9 ± 15.0%, left ventricular end-diastolic diameter 57.3 ± 9.4 mm, and left ventricular end-systolic diameter 44.1 ± 11.3 mm. Mean NT-proBNP level in patients with incident HF was 4815.0 ± 8115.7 ng/L. Altogether, 82 (47.7%) participants were in sinus rhythm, and 73 (42.4%) had atrial

Figure 1 Quintiles of adiponectin level contain the following adiponectin levels: I, 1.00–4.70; II, 4.80–6.10; III, 6.20–7.70; IV, 7.80–10.10; V, 10.20–112.60. *P*, Pearson χ^2 test over all quintiles. *p*, Pearson χ^2 test with the lowest quintile as the reference category.



fibrillation. During the follow-up, implantable cardioverter-defibrillator or cardiac resynchronization therapy device was implanted in 15 (8.7%) and 16 (9.3%) of subjects with incident HF, respectively. Altogether, 27 (15.7%) of those who developed incident HF were deceased by the end of the follow-up period.

The clinical characteristics of incident HF by hypertensive vs. other aetiology are shown in *Table S4*. Participants with hypertensive HF had more often preserved left ventricular ejection fraction and atrial fibrillation, and less often diagnosis of coronary artery disease including previous myocardial infarction, compared with those with non-hypertensive HF.

Table 1 presents univariate Cox regression analyses of the associations of baseline anthropometric factors and biomarkers with incident HF. In unadjusted analyses, all inflammatory and metabolomic biomarkers were associated with incident HF with statistically significant hazard ratios ranging from 1.09 to 3.33, except for IL-1RA, tyrosine, and acetoacetate, which had nominally significant associations with HF. When adjusted for age, the following biomarkers were associated with incident HF: type 2 diabetes, BMI, waist, fasting plasma glucose, fasting plasma insulin, HbA1c, UAER, adiponectin, glycoprotein acetyls, alanine, glycerol, and pyruvate. When adjusted for both age and BMI, the following parameters were significantly associated with incident HF: type 2 diabetes, blood HbA1c, UAER, plasma adiponectin, and pyruvate. When adjusted for age, BMI, diabetes and statin medication, only UAER, plasma adiponectin, and pyruvate remained statistically significant.

As many of the variables associated with incident HF in our study are intercorrelated, we used PC analysis to further study the baseline variables associated with incident HF. The components, their loadings, and percentages explained of the total variance are shown in *Table 2*. Six components, accounting for 61.7% of the total variance and reflecting (1) obesity, hyperinsulinemia, and low-grade inflammation; (2) small molecule metabolites, low-grade inflammation, and hyperglycemia; (3) amino acids; (4) aging and declining kidney function; (5) high systolic blood pressure, albuminuria, and adiponectin levels; and (6) metabolites associated with myocardial energy metabolism, were identified (*Table 2* and *Figure 2*).

Table 3 and *Figure 2* show Cox regression analysis of PCs and incident HF. PCs 1, 2, 4, and 5 were significantly associated with HF with hazard ratios ranging from 1.44 to 1.87. PC 3 had nominal negative association with HF, and PC 6 had a borderline significant association with HF.

Variables associating with hypertensive HF in unadjusted Cox models were very similar to those associating with incident HF caused by other aetiologies (*Table S5*). Systolic blood pressure, waist, adiponectin, and acetoacetate remained significant after adjustment for age, BMI, diabetes, and statin medication. Of six PCs (*Table 2*), all except for PC 3, which reflects amino acid levels, were associated with hypertensive HF in univariate Cox regression analysis (*Table S6*). We also compared biomarkers in subjects with myocardial infarction before baseline, who did (n = 36) or did not develop HF during the follow-up (n = 424). Subjects with previous myocardial infarction who developed HF during the follow-up had, in addition to more advanced age, higher prevalence of type 2 diabetes, higher GHbA1c and lower glomerular filtration rate, also higher levels of adiponectin than those who did not develop HF (data not shown). However, these differences were only nominally significant, preventing more detailed subgroup analysis.

Discussion

In the context of current literature

Aetiology of HF

Hypertension, coronary artery disease, particularly myocardial infarction, cardiomyopathies, and valvular heart disease were the most common causal etiologies of HF in our study, in agreement with current literature.[1] Age, type 2 diabetes, obesity, hyperinsulinemia, declining kidney function, and microalbuminuria, which are well-established comorbidities predisposing to the development of HF, were associated with incident HF.

Novel biomarkers for incident HF

In the present study, several novel inflammatory and metabolic biomarkers—adiponectin, glycoprotein acetyls, glycerol, pyruvate, acetoacetate, alanine, and phenylalanine—were associated with incident HF in unadjusted models. Adiponectin is an anti-inflammatory, insulin-sensitizing, and antiatherogenic adipocytokine that enhances endothelial function. In few previous studies, adiponectin concentrations have been implicated in HF.[11,12] A J-shaped association between adiponectin and HF has been suggested in one previous study.[12] Glycoprotein acetyls, biomarkers of low-grade inflammation, have been associated with HF in one metabolomics study in unadjusted but not in adjusted models.[13] In our previous study, glycoprotein acetyls increased the risk of type 2 diabetes and cardiovascular events and correlated strongly with several cardiovascular risk factors.[21] Pyruvate is an intermediary metabolite, which enhances free energy of ATP hydrolysis in myocardium and has anti-inflammatory properties.[24] There is only one previous publication on pyruvate in HF,[25] and there are no previous studies showing that pyruvate increases the risk of HF. Acetoacetate, a ketone body, and glycerol may be used as energy substrate by cardiac myocytes, particularly in the failing heart. Increased ketone levels have been reported in HF patients,[26] but there are no previous studies showing that acetoacetate or glycerol levels are associated with incident HF. Thus, our study is the first to show that pyruvate, acetoacetate, and glycerol are

Table 2 Principal component analysis of baseline variables, which were associated with incident heart failure in unadjusted Cox regression analyses

Component	Variable	Loading	Percent variance explained	Component	Variable	Loading	Percent variance explained
PC 1	Waist	**0.876**	25.77	PC 2	Pyruvate	**0.798**	9.46
	Body mass index	**0.876**			Glycoprotein acetyls	**0.660**	
	Fasting plasma insulin	**0.680**			Alanine	**0.649**	
	IL1 receptor antagonist	**0.600**			Glycerol	**0.493**	
	High sensitivity CRP	**0.536**			Blood GHbA1C	**0.426**	
	Tyrosine	0.315			Fasting plasma insulin	0.363	
	Plasma adiponectin	−0.335			Phenylalanine	0.276	
	Phenylalanine	0.301			Plasma adiponectin	−0.214	
	Glycoprotein acetyls	0.284			Urinary albumin excretion	0.212	
	Blood GHbA1C	0.293			IL1 receptor antagonist	0.189	
	Glycerol	0.249			High sensitivity CRP	0.170	
	Histidine	−0.178			Tyrosine	0.145	
	Urinary albumin excretion	0.146			Systolic blood pressure	0.110	
	Pyruvate	0.146			Waist	0.092	
	Systolic blood pressure	0.132			Histidine	−0.070	
	Alanine	0.086			Body mass index	0.061	
	Age	−0.021			Acetoacetate	−0.052	
	Glomerular filtration rate	0.009			Glomerular filtration rate	−0.026	
	Acetoacetate	0.000			Age	0.011	
PC 3	Histidine	**0.798**	8.26	PC 4	Age	**0.769**	6.70
	Tyrosine	**0.750**			Glomerular filtration rate	**−0.768**	
	Phenylalanine	**0.649**			Blood GHbA1C	0.296	
	Alanine	**0.423**			Phenylalanine	0.257	
	Glycoprotein acetyls	0.208			High sensitivity CRP	0.177	
	Glycerol	0.164			Histidine	−0.158	
	Blood GHbA1C	−0.157			Alanine	0.134	
	Fasting plasma insulin	0.149			Systolic blood pressure	0.104	
	Body mass index	0.139			Tyrosine	0.078	
	Plasma adiponectin	−0.137			Acetoacetate	0.067	
	Waist	0.122			IL1 receptor antagonist	−0.061	
	Systolic blood pressure	0.109			Pyruvate	−0.053	
	Urinary albumin excretion	−0.105			Plasma adiponectin	0.050	
	Glomerular filtration rate	−0.064			Glycerol	−0.036	
	Acetoacetate	−0.037			Fasting plasma insulin	0.035	
	IL1 receptor antagonist	−0.033			Glycoprotein acetyls	0.027	
	Pyruvate	−0.027			Body mass index	−0.02	
	High sensitivity CRP	−0.025			Urinary albumin ecxretion	0.023	
	Age	−0.011			Waist	0.008	
PC 5	Systolic blood pressure	**0.741**	6.21	PC 6	Acetoacetate	**0.834**	
	Urinary albumin excretion	**0.531**			Glycerol	**0.503**	5.29
	Plasma adiponectin	**0.410**			Alanine	−0.330	
	Age	0.318			High sensitivity CRP	0.300	
	Glycerol	0.220			Glycoprotein acetyls	0.210	
	Body mass index	0.211			IL1 receptor antagonist	0.128	
	Waist	0.209			Plasma adiponectin	0.171	
	High sensitivity CRP	−0.143			Fasting plasma insulin	−0.171	
	Fasting plasma insulin	0.128			Tyrosine	−0.156	
	Pyruvate	0.123			Phenylalanine	0.137	
	Blood GHbA1C	0.123			Glomerular filtration rate	−0.089	
	Alanine	0.092			Blood GHbA1C	−0.076	
	Glomerular filtration rate	0.090			Histidine	0.055	
	Phenylalanine	−0.066			Systolic blood pressure	0.033	
	Histidine	−0.063			Body mass index	−0.030	
	IL1 receptor antagonist	−0.060			Age	−0.022	
	Acetoacetate	0.048			Urinary albumin excretion	−0.005	
	Tyrosine	0.045			Waist	−0.003	
	Glycoprotein acetyls	0.014			Pyruvate	−0.001	

CRP, C-reactive protein; HbA1c, haemoglobin A1c; PC, principal component.
Analysis includes 10 106 participants without heart failure (HF) at baseline, of whom 172 participants developed incident HF during the follow-up period. All variables except for age were log-transformed for statistical analysis. Bold indicates variables with significant (>0.400) loadings.

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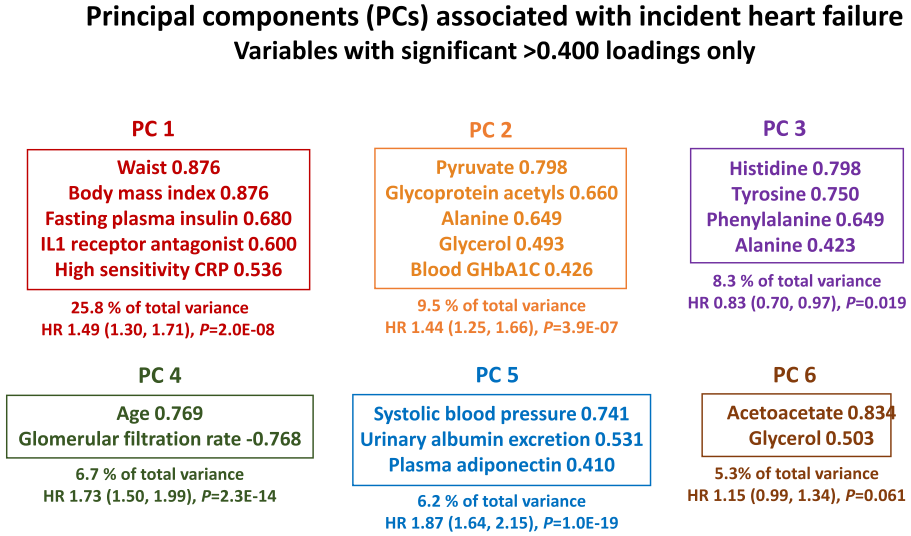
Figure 2 In a non-selected population of 10 106 Finnish men without heart failure (HF) at baseline, biomarkers including novel small molecule energy metabolites and inflammatory markers, clustered in six principal components, which were associated with incident heart failure with high or borderline statistical significance, suggesting early activation of multiple pathogenic pathways in heart failure. HR, hazard ratio; PC, principal component.



associated with incident HF. Of amino acids, elevated phenylalanine concentrations were associated with incident HF hospitalizations in the PROSPER and FINRISK 1997 studies.[13] In our previous study, alanine, leucine, isoleucine, tyrosine, and glutamine increased the risk of incident type 2 diabetes.[27] A catabolic defect in the metabolism of proteins and impaired uptake and utilization of amino acids are possible mechanisms.

Novel biomarkers of hypertensive heart failure and heart failure due to previous myocardial infarction

In hypertensive HF, adiponectin, glycerol, pyruvate, and acetoacetate were associated with incident HF in unadjusted models. In HF due to previous myocardial infarction, of novel biomarkers, only adiponectin was associated with incident HF with nominal significance. Biomarker profiles of incident HF may differ between diversing etiologies of HF, but larger studies are required to investigate this question.

Table 3 Principal components (*Table 2*) associated with incident heart failure in the METSIM cohort in adjusted Cox regression analysis

PC number	HR	95% CI	*P*
PC 1	1.49	1.30, 1.71	**2.0E-08**
PC 2	1.44	1.25, 1.66	**3.9E-07**
PC 3	0.83	0.70, 0.97	0.019
PC 4	1.73	1.50, 1.99	**2.3E-14**
PC 5	1.87	1.64, 2.15	**1.0E-19**
PC 6	1.15	0.99, 1.34	0.061

CI, confidence interval; HR, hazard ratio; PC, principal component. Cox regression includes 10 106 participants without heart failure (HF) at baseline, of whom 172 participants developed incident HF during the follow-up period. $P < 0.008$ (0.05/6) is considered statistically significant (bold), and $P < 0.05$ is considered nominally significant (underlined).

Principal component analysis

Principal component analysis is a statistical procedure that converts a set of observations of correlated variables into a set of values of linearly uncorrelated variables called PCs, which can be investigated with respect to cardiovascular endpoints, and may suggest pathophysiological pathways. In the present study, PC analysis identified several components including inflammatory and metabolic biomarkers, which were associated with incident HF in addition to obesity, age, and blood pressure-related components, suggesting that novel biomarkers have incremental effect in the pathogenesis of HF. To the best of our knowledge, PC analysis has not been used to investigate biomarkers in incident HF. In our previous study on elderly Finnish subjects, however, we applied factor analysis, which is a statistical method very similar to PC analysis, and identified insulin resistance factor, which predicted coronary events in elderly nondiabetic men.[28]

Possible mechanisms

Chronic inflammation

Inflammation is implicated in HF pathophysiology by promoting cardiac injury, fibrosis, and dysfunction.[29] In the present study, adiponectin and pyruvate, both related to inflammation, were associated with incident HF in adjusted Cox regression analyses. Furthermore, in PC analysis, multiple

inflammatory markers loaded on three different PCs associated with the risk of HF. Hs-CRP and IL-1RA loaded on the obesity-hyperinsulinemia component, pyruvate and glycoprotein acetyls on the metabolite component, and adiponectin on the vascular component, suggesting several and diverse inflammatory cascades in HF, activated well before clinical HF.

Impairment in metabolic pathways

HF is associated with impairment in glucose, amino acid, and ketone metabolic pathways.[30] Ketone body oxidation is increased, and amino acid metabolism is deranged in HF.[31] In animal models, metabolic remodelling precedes, initiates, and sustains functional and structural heart remodelling.[31] In the present study, small molecular metabolites pyruvate, glycerol, and acetoacetate were associated with incident HF, and loaded on three PCs, one of which was significantly associated with incident HF, suggesting that metabolic dysfunction precedes clinical HF also in humans.

Strengths and limitations

The strengths of our study include a large prospective, population-based study with carefully investigated phenotype at baseline, a comprehensive and detailed diagnostics of HF and its aetiology, and a long follow-up period. Furthermore, the present study is among the first studies analysing a large panel of novel biomarkers, including proton NMR-derived metabolic measures.

There are also limitations in our study. First, conventional biomarkers for HF natriuretic peptides and troponin T were not measured. In a recent study, several novel circulating biomarkers, including biomarkers of inflammation and intermediary metabolism, added the predictive value of HF-related hospital admisissions on the top of NT-proBNP and TnT in patients with HF and preserved ejection fraction,[32] suggesting that measuring large panels of biomarkers may give additional insight in the pathogenesis and progression of HF. Second, the present study included only middle-aged and elderly Finnish men, and the number of participants with incident HF was rather low. Therefore, further studies in other populations are warranted to confirm our results and to assess their applicability to women and other populations and age groups.

Clinical implications

We identified several novel biomarkers for incident HF, including markers of inflammation and energy metabolism. Our findings give new insights into the pathophysiology of HF. Measuring inflammatory and metabolic biomarkers may help to predict HF and facilitate early diagnosis. Finally, targeting multiple pathophysiological pathways might be useful in the prevention and treatment of HF.

Conclusions

Plasma adiponectin and pyruvate were independently associated with HF in the 8.8 year follow-up of the METSIM cohort. In addition, we identified six PCs of biomarkers, including novel small molecule energy metabolites and inflammatory biomarkers. Four PCs were significantly associated with indicent HF, suggesting an early activation of multiple pathophysiological pathways, including inflammation and metabolic dearrangement, in the pathogenesis of HF.

Conflict of interest

The authors declare no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Baseline anthropometric, metabolic, and inflammatory marker measurements of the study participants without and with incident heart failure

Table S2. Serum metabolic measures in proton NMR analysis at baseline in the participants with and without incident heart failure during the METSIM study follow-up

Table S3. Clinical characteristics and outcomes of the METSIM study participants with incident heart failure.

Table S4. Clinical characteristics and outcomes by the etiology of heart failure in the METSIM study participants with incident heart failure

Table S5. Cox regression analysis of demographic and anthropometric variables and biomarkers as predictors of hypertensive heart failure in 10,002 study participants in an 8.8-year follow-up study of the METSIM cohort

Table S6. Cox regression analysis of the principal components shown in Table 2 as predictors of hypertensive HF in 10,002 study participants in an 8.8-year follow-up study of the METSIM cohort.

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