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CHANGES IN PROTEOMIC PROFILES ARE RELATED TO CHANGES IN BODY MASS INDEX AND FAT DISTRIBUTION DURING TEN YEARS OF AGING

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Abstract

Objective—We investigated how changes in 84 proteins over a ten-year period of aging were related to changes in measures of body fat and distribution over the same period.

Methods—Cardiovascular candidate proteins were measured using the proximal extension assay (PEA) technique, along with BMI and WHR at ages 70, 75 and 80 in 1,016 participants of the PIVUS cohort. Associations of changes in plasma protein levels, BMI and WHR over time were analyzed using linear mixed models.

Results—Changes in 19 and 16 proteins were significantly associated with changes in BMI and WHR, respectively ($P < 0.00059$), over the investigated ten-year period. Leptin and FABP4 were among the proteins most strongly associated with changes in both BMI and WHR. Four of the proteins significantly tracked with change in BMI ($P < 0.00059$) but not WHR ($P > 0.05$): ESM-1, PTX3, ST2 and spondin-1. Five proteins tracked with change in WHR ($P < 0.00059$), but not BMI

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($P>0.05$): caspase-8, cathepsin L1, lectin-like oxidized LDL receptor 1, IL6RA, and C-C motif chemokine 20.

Conclusions—This is the first large longitudinal study of how changes in plasma protein signatures are associated with changes in measures of body fat and distribution over 10 years of aging.

Keywords

proteomics; body mass index; waist-hip ratio; obesity; longitudinal changes; aging

INTRODUCTION

During the last decade, genome-wide association studies (GWAS) have identified a large number of loci associated with body mass index (BMI), an estimate of relative weight; and waist-hip ratio (WHR), reflecting body fat distribution (1, 2). Loci associated with these different obesity-related phenotypes are only partly overlapping. Hence, we hypothesized that circulating proteins would also show different patterns of associations with these two measures, and that knowledge about how such associations change over a critical period of aging can provide important information about potential mechanisms underlying changes in body weight and fat distribution during aging to learn more about obesity.

Using the proximal extension assay (PEA) technique for measurement of multiple proteins on a targeted proteomics assay, we have recently discovered a range of proteins to be related to several cardiovascular and metabolic traits in the community-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (3–6). We have also recently shown that the majority of 84 cardiovascular candidate proteins increase over time when measured at ages 70, 75 and 80 in the PIVUS cohort (7).

In the present study, we related changes in these 84 cardiovascular candidate proteins to changes in BMI or WHR between age 70 and 80 in the PIVUS cohort. Such a longitudinal design is superior to using cross-sectional data collected at one time point, as it increases statistical power, and decreases risk of reverse causation explaining concomitant changes of protein levels and measures of body fat and distribution. Our aim was to relate changes in these 84 proteins to changes in BMI or WHR between age 70 and 80 combined with different orthogonal data sources to increase understanding of changes in body weight and fat distribution during aging.

METHODS

Study sample

Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) is a longitudinal cohort study drawn from the general population of Uppsala, Sweden, initiated in 2001 to study cardiovascular and metabolic disease. At the baseline investigation in 2001–2004, 1,016 70-year old participants were enrolled (50% women). Follow-up investigations have been performed after five ($n=826$) and ten years ($n=602$). A detailed description of the cohort, including clinical characteristics, has been published previously (8).

Examination and measurements

The participants were investigated in the morning after an overnight fast. Measurements of traditional lipids and fasting glucose were done at the Department of Clinical Chemistry in fresh samples. For other biomarkers, plasma was stored in a freezer (−80°C) for subsequent analyses. BMI and WHR were calculated using standard procedures. Information on diseases, drug use and lifestyle were collected using a questionnaire. Exercise habits were given on a four-graded scale, with the lowest being sedentary, and the highest being endurance training three times or more per week. Plasma creatinine and cystatin C were measured by a standard enzymatic method and by an enhanced turbidimetric method, respectively; and a validated formula to calculate glomerular filtration rate (GFR) using both of these markers was used (9).

Proteins were measured using the proximal extension assay (PEA) technique (10) on a commercial proteomics assay with 92 preselected proteins proposed to be associated with cardiovascular disease (CVD-I, Olink, Uppsala, Sweden). All measurements were performed in the same fashion from plasma collected at the three occasions. Of the 92 proteins, 84 passed our strict quality control criteria (call rate >75% at all three time points), and were included in downstream analyses. Details regarding the protein arrays, quality control and processing of data in the PIVUS study has been published previously (4, 5).

Statistical analysis

The distributions of all protein levels were skewed to the right, and protein levels were therefore log₂-transformed to achieve a normal distribution. In the final analysis, the log₂-transformed values for the proteins were transformed to a SD-scale with proteins from all time points combined, so that the regression coefficients (betas) were comparable between the proteins.

First, changes in BMI and WHR over 10 years (three measurements, at ages 70, 75 and 80) were evaluated by mixed models with random intercepts using BMI and WHR as dependent variables in two separate models, and time as the independent variable and sex as covariate (age same in all subjects). Then, the relationships between changes over 10 years in levels of the 84 proteins and changes over 10 years in BMI or WHR were examined. Mixed models with random intercept were used for this purpose as well, with protein levels as dependent variables, and BMI or WHR as independent variables along with potential confounders. Mixed random effect modeling uses all available data from all participants, but compensates for missing data points, which is one of the advantages of this technique (11). One model was analyzed for each protein, and separately for BMI and WHR. The independent variable was split into a between-individual component, based on the first observations for each individual; and a within-individual component, which is the difference between the first measurement and measurements at subsequent time points. Thus, the between-individual component is related to the mean of the three measurements of the proteins, while the within-individual component, as a single term, relates the change in each protein to the change in BMI (or WHR). The general formula for these analyses is; $Y_{ij} = Z_i\beta_{\alpha_0} - X_{i1}\beta_{\alpha_C} + (X_{ij}-X_{i1})\beta_{\alpha_L} + e_{ij}$; where Y is a protein, X is BMI (or WHR), i is the individual, j the time, β_{α_C} is the coefficient for the first observation and β_{α_L} is the coefficient for

change over time. Confounders and the intercept are given as $Z_i\beta_0$ (11). We used only a random intercept, since inclusion of a random slope in addition made the models not to converge in many cases (due to collinearity). The random part of the model is the identity of the study participants and the rest of the model are fixed effects.

All models were adjusted for the following potential confounders: time in freezer, exercise habits, glomerular filtration rate, smoking, and use of beta-blockers, diuretics, statins, insulin and oral antidiabetic drugs. All of these variables are related to obesity and a preliminary evaluation showed these variables also to be related to several of the proteins. These variables deemed unlikely to be on the causal pathway between change in BMI (or WHR) and change in protein levels, and therefore considered as confounders rather than mediators.

In addition, to evaluate sex-specific associations of protein changes with changes in BMI and WHR, we included interaction terms (BMI*sex and WHR*sex) in a separate set of models (using the same framework as described above). We regarded a Bonferroni-corrected P-value of 0.00059 (0.05/84) as significant in each of the analyses. Since many of the proteins are highly correlated, we did not adjust for analyzing two outcomes (BMI and WHR), to strike a reasonable balance between risk for false negatives and false positives. STATA14 was used for analysis (Stata Inc, College Station, TX, USA). The kernel density plots for BMI and WHR was drawn using the Lattice package in R 3.4.4.

In silico lookups

We used data from the Genotype-Tissue Expression (GTEx) project (<https://gtexportal.org/home/>) (12) to explore expression patterns of transcripts encoding selected proteins associated with BMI or WHR in physiologically relevant tissues, specifically subcutaneous and visceral adipose tissue, liver, skeletal muscle (tissues involved in obesity and insulin resistance) and whole blood (as the proteins were measured in blood samples). Further, we searched the GWAS catalog (<https://www.ebi.ac.uk/gwas>) of published genome-wide association studies (13) inquiring gene names corresponding to the proteins. We included results from studies with a total sample size of at least 1,000 individuals.

RESULTS

BMI did not change significantly during the 10-year period (age 70: 27.0 [SD 4.3] kg/m²; age 80: 26.9 [SD 4.5], $P=0.22$), while WHR increased from 0.90 (SD 0.074) to 0.95 (SD 0.074, $p<0.0001$). The trajectories over time of these two obesity measures are depicted in Figure 1.

Tracking of protein levels and measures of body fat and distribution

Taking multiple testing into account ($P<0.00059$), changes of 19 protein levels were significantly associated with changes in BMI between age 70 and 80 (Table 1). The proteins most strongly correlated with change of BMI were leptin, fatty acid-binding protein 4 (FABP4) (Figure 2), interleukin-1 receptor antagonist protein (IL-1RA), tissue-type plasminogen activator (t-PA), and endothelial cell-specific molecule 1 (ESM-1). In the corresponding analysis for concomitant changes in protein levels and WHR, 16 proteins

were significantly associated (Table 2). The proteins who tracked most closely with change in WHR were leptin, caspase-8 (CASP-8), cathepsin L1 (CTSL1), FABP4 and t-PA.

Changes of eight protein levels were significantly associated with changes in both BMI and WHR: leptin, FABP4, t-PA, growth hormone (GH), N-terminal pro-B-type natriuretic peptide (NT-pro-BNP), cathepsin D (CTSD), e-selectin (SELE) and tumor necrosis factor ligand superfamily member 14 (TNFSF14; Table 1 and 2). Changes of leptin and FABP4 over ten years were among the proteins most strongly correlated with changes of both BMI and WHR. Both of these proteins were positively associated with BMI and WHR change (i.e. increases of adiposity were associated with increasing protein levels; Table 1 and 2). However, the patterns of the two proteins were different due to the fact that leptin *decreased* over time, while FABP4 *increased* over time (Figure 2). Despite this difference, individuals who showed the largest positive changes in BMI or WHR over time (represented by the 75th percentile in Figure 2) demonstrated the largest positive changes in leptin and FABP4, and subjects who showed the smallest positive changes in BMI or WHR over time (represented by the 25th percentile in Figure 2) showed the least positive changes in leptin and FABP4.

Changes in protein levels exclusively associated with changes in BMI

Four of the proteins significantly tracked with change in BMI ($P<0.00059$) in a negative fashion, but showed no evidence of association with change in WHR ($P>0.05$): endothelial cell-specific molecule 1 (ESM-1), pentraxin-related protein PTX3 (PTX3), ST2 protein (ST2) and spondin-1 (SPON1; the two most strongly associated with BMI shown in Figure 3). Individuals who showed the largest increases in BMI over time (represented by the 75th percentile in Figure 3) showed the least positive changes in the protein levels, while those who showed the least positive changes in BMI over time (represented by the 25th percentile in Figure 3) showed the largest positive changes in protein levels.

In silico findings of BMI-associated proteins in expression and GWAS databases

According to GTEx, ST2 expression was detectable in liver, but not in other examined tissues of high relevance for insulin resistance and obesity; and transcript levels corresponding to the other three proteins exclusively associated with BMI were not detectable in these tissues (Figure 4). According to the GWAS catalog, there are many studies relating variants in or near *IL1RL1* (encoding the ST2 protein) with autoimmune and inflammatory disease phenotypes, such as asthma, allergy, eosinophils, Crohn's disease and celiac disease (Supplementary Table 1). Further, the GWAS catalogue shows that variants in *SPON1* have been associated with vitamin D levels, glucagon levels in response to oral glucose tolerance test, HDL cholesterol, inflammatory biomarkers and common carotid intima-media thickness (Supplementary Table 1).

Changes in protein levels exclusively associated with changes in WHR

In contrast, five proteins tracked with change in WHR ($P<0.00059$) in a positive fashion, but lacked evidence of association with change in BMI ($P>0.05$): caspase-8 (CASP-8), cathepsin L1 (CTSL1), lectin-like oxidized LDL receptor 1 (LOX-1), interleukin-6 receptor subunit alpha (IL-6RA), and C-C motif chemokine 20 (CCL20; the two most strongly associated with WHR shown Figure 5). Both CASP-8 and CTSL1 declined between age 70 and 80.

Individuals who showed the largest positive changes in WHR over time (represented by the 75th percentile in Figure 5) showed the smallest negative changes in CASP-8 and CTSL1, while individuals who showed the least positive changes in WHR over time (represented by the 25th percentile in Figure 5) showed the largest negative changes in CASP-8 and CTSL1.

In silico findings of WHR-associated proteins in expression and GWAS databases

According to GTEx data, CSTL1 is expressed in liver and blood, IL-6RA is expressed in liver, skeletal muscle and blood; and CASP-8 is expressed in blood (Figure 4). Variants in or near *IL6R*, *CCL20* and *CASP8* have been associated with various inflammatory diseases (Supplementary Table 1). According to the GWAS catalogue, there is a genetic variant in *IL6R* associated with coronary heart disease, and a variant in *CCL20* associated with blood pressure ($P < 7 \times 10^{-7}$); Supplementary Table 1). This orthogonal data suggest that these proteins might be relevant not only for WHR, but also directly involved in development of cardiovascular disease.

Sex differences in relations of protein levels and body fat and distribution over time

Since body fat distribution is different in males and females, we analyzed if correlations of changes of BMI and WHR with protein levels were different between sexes by including interaction terms between changes of BMI or WHR and sex. No significant interactions were observed for BMI, but correlations of change in three proteins with change in WHR were significantly different between men and women following adjustment for multiple testing. The association of change in leptin and change in WHR was significantly larger in males than females (beta, 4.0 (SE, 0.4) in males; beta, 2.1 (SE, 0.3) in females, $p_{\text{interaction}} = 4.9 \times 10^{-10}$ for interaction). Significant interactions of WHR and sex were found also for MMP-3 and prolactin, but the associations of changes in these two proteins with changes in WHR were non-significant in both sexes when analyzed separately (MMP-3: beta, 0.25 (SE, 0.58) in males; beta, -0.93 (SE, 0.47) in females, $p_{\text{interaction}} = 0.000022$; prolactin: beta, 1.0 (SE, 0.6) in males; beta, -1.7 (SE, 0.6) in females, $p_{\text{interaction}} = 0.000010$).

DISCUSSION

We have previously shown that the majority of the investigated 84 cardiovascular-related proteins increase over a 10-year period (7). We now extend those observations by identifying eight proteins for which the changes over time was related to changes in both BMI and WHR; of these, the strongest associations were observed for leptin and FABP4. On the other hand, we also identified nine proteins whose changes were related to only one of these measurements of body weight and fat distribution.

Proteins tracking with body weight and fat distribution

Prior studies of associations of protein levels with changes in body weight or fat distribution during ageing have typically focused on one or a few proteins, and typically on well-studied biomarkers, such as CRP, IL-6, NT-proBNP and Troponin I (14–16). In the present study, we evaluated for the first time if changes of a large number of proteins between age 70 and 80 were related to changes in measures body weight and fat distribution during the same time

period. Further, we investigated whether such correlations differed between BMI and WHR, which can give insights to biology underlying changes in body weight and fat distribution during this critical period of aging.

Given that the adipokines leptin and FABP4 are secreted by adipocytes and have well-established roles in adipocyte biology, it is not surprising that changes of these proteins were amongst the most strongly correlated with changes in both BMI and WHR. Both proteins tracked with BMI and WHR; even though leptin levels generally declined, while FABP4 levels increased over time. These two adipokines are among the most studied biomarkers in relation to obesity, and have repeatedly been shown to be cross-sectionally associated with these obesity measures, and other metabolic factors (6, 17–19). Further, we observed that the correlation of changes in leptin and WHR was significantly larger in males than females. There is a well-known sex difference in leptin levels between men and women,(20–22) but we are unaware of prior reports of sex differences regarding longitudinal changes of leptin and their relation to obesity.

Proteins primarily associated with body mass index

The increases in levels of endothelial cell-specific molecule 1 (ESM-1; also frequently called endocan) and ST-2 were amongst the proteins most closely linked to increases in BMI, while not being significantly associated to changes of WHR. ESM-1 is a soluble sulfate proteoglycan involved in neoangiogenesis. This protein has previously been linked to smoking in a negative fashion (23), and to prevalent myocardial infarction in a positive fashion (24) in cross-sectional studies. Expression of ESM-1 has been detected in adipocytes, but contrary to our findings it has been reported that plasma levels of ESM-1 were reduced in obese women and increased following weight reduction in a small cross-sectional study (25). Several differences exist between that study and the present, such as sex distribution, age and degree of weight loss that could explain the discrepant findings.

Soluble suppression of tumorigenicity 2 (sST2) mediates the effect of interleukin-33 (IL-33), and increased circulating levels of ST2 have been linked to coronary heart disease (26). Studies on ST2 in obesity are rare and point to increased levels in morbid obesity (27), but experimental data in obese Zucker rats have demonstrated decreased expression of ST2 in many tissues, including adipocytes (28). Based on another study in rats, it has been suggested that ST2 could be involved in the vascular remodeling associated with obesity (29).

Proteins primarily associated with waist-hip ratio

The changes of caspase-8 (CASP-8) and cathepsin L1 (CTSL1) levels were most closely linked to changes in WHR in a positive fashion, while not significantly related to changes in BMI. Caspase-8 is a cysteine protease involved in apoptosis and cell death, and this protein has been implicated in the remodeling of the heart following myocardial injury, such as myocardial infarction (30). In subcutaneous, but not visceral, biopsies taken from humans with varying degrees of obesity, the expression of caspase-8 has been inversely related to the degree of obesity (31); but apart from that single prior study, human data relating caspase-8 and obesity are lacking. Since cell cycle abnormalities, such as apoptosis regulation, have

been postulated to play a major role in obesity-related complications (32), we believe that the connection between WHR and caspase-8 found in the present study is worthwhile to explore further.

Cathepsin L1 is also a cysteine proteinase being involved in the degradation of endocytosed proteins and intra-cellular proteins, being expressed in adipose tissue (33). Cathepsin L levels have been found to be elevated in patients with acute coronary syndromes (34), and higher levels have been associated with cardiovascular mortality.(35) A lifestyle intervention program with increased physical activity and more healthy food aiming to reduce body weight reduced cathepsin L levels (36). In contrast, cathepsin L levels were not changed in obese patients undergoing bariatric surgery or a weight loss program in another study (33).

One study comparing the untargeted proteome before and following a weight loss program found changes in several proteins, like sex hormone-binding globulin, adiponectin, C-reactive protein, calprotectin, serum amyloid A, and proteoglycan 4 (PRG4) (37). However, none of the reported proteins were analyzed in the present analysis; so no comparison with this prior study could be performed. In another recent study evaluating the effect of intentional weight loss on the untargeted proteome, 93 proteins changed significantly, but only the well-known adipokine leptin were shared with the present study (38).

Strength and limitations

A major strength of the present study is the repeated measurements of a large number of proteins three times during a ten-year period, which allowed us to investigate the individual changes in those protein levels, as well as to relate them to changes in measures of body weight and fat distribution in a longitudinal fashion with high statistical power. Even though a longitudinal approach like ours may facilitate studies of mechanisms, we acknowledge that causality still never be established using observational data. Our study also had several limitations. First, since we used a target proteomic array designed with proteins known or suspected to be linked to cardiovascular disease, we cannot exclude that other proteins would display different longitudinal patterns and associations with examined variables. An untargeted proteomics approach would have given a wider assay of proteins investigated, but such approaches usually have lower throughput making it harder to analyze many samples in a repeated fashion. Second, since we studied elderly individuals from Sweden, the generalizability to other age groups and populations is unknown. Third, we had no information about whether changes in body weight and fat distribution were intentional (e.g. weight loss due to improved lifestyle) or non-intentional (e.g. due to illness). Fourth, the PEA technique provides relative rather than absolute levels. Fifth, we are unaware of any other studies with repeated measurements of these proteins along with BMI and WHR measurements that could be used for validation. Therefore, all results should be taken with caution until confirmed using orthogonal evidence.

Conclusions

In conclusion, changes in BMI and WHR between age 70 and 80 are related to changes in different proteins, emphasizing that mechanisms underlying general body size and obesity (as reflected by BMI) are different from those underlying fat distribution (as reflected by

WHR). Longitudinal studies of how protein signatures track with different measures of body fat and distribution can inform about underlying mechanisms associated with obesity and cachexia, and help prioritize targets for further functional follow-up studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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WHAT IS ALREADY KNOWN ON THIS SUBJECT?

- Genetic loci associated with body mass index and waist-hip ratio are only partly overlapping, and we hypothesized that circulating proteins would also show different patterns of associations with these two measures.
- Knowledge about how such associations change over a critical period of aging can provide important information about potential mechanisms underlying changes in body weight and fat distribution during aging to learn more about obesity.

WHAT DOES THIS STUDY ADD?

- Changes in 19 and 16 proteins were significantly associated with changes in BMI and WHR, respectively ($P < 0.00059$), over the investigated ten-year period.
- Eight of the studied proteins were related to changes in both BMI and WHR – of these, the strongest associations were observed for leptin and FABP4.
- Nine were related to only one of these measurements of body weight and fat distribution – these included endocan, ST2, caspase-8 and cathepsin L1.

Figure 1. Kernel density plots for body mass index (BMI, kg/m^2 , top panel) and waist-hip ratio (WHR, lower panel) at ages 70, 75 and 80 years.

Figure 2.

Associations of changes in BMI (left) or waist-hip ratio (WHR; right) and changes in leptin (upper) and fatty acid-binding protein 4 (FABP4; lower). The graphs show the changes over time of proteins for the 25th, 50th and 75th percentile of change in BMI or WHR. The value of the protein at age 70 is set at the median for all three groups of change in BMI or WHR to help interpretation of the graphs. Thus, the general direction of change of the protein levels over time is given by the 50th percentile change in BMI or WHR. The 75th percentile change in BMI or WHR represents those with a larger increase in BMI or WHR over time, while the 25th percentile represents those with a smaller BMI/WHR change. In this case, subjects with a larger increase in BMI or WHR showed the smallest decline in leptin and the largest increase in FABP4. This corresponds to positive relationships between changes in BMI (or WHR) and changes in both leptin and FABP4.

Figure 3.

Associations of changes in BMI and changes in the top two proteins tracking with BMI ($P < 0.00059$), but not WHR ($P > 0.05$) – endothelial cell-specific molecule 1 (ESM-1) and pentraxin-related protein PTX3 (PTX3). The graphs show the changes over time in the proteins for the 25th, 50th and 75th percentile change in BMI. The value of the protein at age 70 is set at the median for all three groups of change in BMI to help interpretation of the graphs. Thus, the general direction of change of the protein over time is given by the 50th percentile change in BMI or WHR. The 25th percentile change in BMI or WHR represents those with the smallest increase in BMI or WHR over time, while the 75th percentile represent those with the largest increase. In this case, subjects with smallest increase in BMI or WHR showed the largest increase in ESM-1 and PTX3. This corresponds to inverse relationships between changes in BMI (or WHR) and changes in both ESM-1 and PTX3.

Figure 4.

Gene expression in subcutaneous and visceral fat, liver, muscle and whole blood from GTEx of 11 transcripts encoding the proteins most strongly associated with both BMI and WHR (LEP and FABP4); those specific to BMI (ESM-1, PTX3, ST2 and SPON1); and those specific to WHR (CASP-8, CTSL1, LOX-1, IL-6RA and CCL20).

Figure 5.

Associations of changes in waist-hip ratio (WHR) and changes in the top two proteins tracking with WHR ($P < 0.00059$), but not BMI ($P > 0.05$) – caspase-8 (CASP-8) and cathepsin L1 (CTSL1). The graphs show the changes over time in the metabolites for the 25th, 50th and 75th percentile change in WHR. The value of the protein at age 70 is set at the median for all three groups of change in WHR to help interpretation of the graphs. Thus, the general direction of change of the protein over time is given by the 50th percentile change in BMI or WHR. The 75th percentile change in BMI or WHR represents those with the largest increase in BMI or WHR over time, while the 25th percentile represent the smallest increase. In this case, subjects with a large increase in BMI or WHR showed the least decline in CASP-8 and CTSL1. This corresponds to positive relationships between changes in BMI (or WHR) and changes in both CASP-8 and CTSL1.

Table 1.

Associations of changes in BMI and changes in 19 protein levels with significant associations ($P < 0.00059$; Bonferroni-correction for 84 proteins) *

Protein	Beta	SE	P-value
Leptin (LEP)	.161	.007	4.21e-133
Fatty acid-binding protein 4 (FABP4)	.1	.009	2.21e-28
Interleukin-1 receptor antagonist protein (IL-1RA)	.103	.011	1.90e-21
Tissue-type plasminogen activator (t-PA)	.086	.011	1.32e-15
Endothelial cell-specific molecule 1 (ESM-1)	-.081	.011	2.69e-14
Growth hormone (GH)	-.093	.013	4.09e-12
Pentraxin-related protein PTX3 (PTX3)	-.081	.012	4.40e-12
N-terminal pro-B-type natriuretic peptide (NT-pro-BNP)	-.054	.009	4.84e-09
Cathepsin D (CTSD)	.056	.01	6.84e-08
ST2 protein (ST2)	-.05	.01	3.93e-07
Interleukin-27 subunit alpha (IL27-A)	-.044	.009	2.03e-06
Interleukin-18 (IL-18)	.046	.01	3.60e-06
Vascular endothelial growth factor D (VEGF-D)	-.044	.01	4.38e-06
Agouti-related protein (AGRP)	-.046	.011	.000025
E-selectin (SELE)	.038	.01	.000052
Growth/differentiation factor 15 (GDF-15)	-.037	.009	.00010
Spondin-1 (SPON1)	-.042	.011	.00023
Pappalysin-1 (PAPPA)	-.039	.011	.00025
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	.047	.013	.00026

* Beta coefficients represent change in protein level (on SD-scale to increase cross-comparability) per one year of aging. Models were adjusted for the following potential confounders: time in freezer, exercise habits, glomerular filtration rate, smoking, and use of beta-blockers, diuretics, statins, insulin and oral antidiabetic drugs.

Table 2.

Associations of changes in waist-hip ratio (WHR) and changes in 16 protein levels with significant associations ($P < 0.00059$; Bonferroni-correction for 84 proteins)*

Protein	Beta	SE	P-value
Leptin (LEP)	2.659	.262	3.99e-24
Caspase-8 (CASP-8)	3.238	.351	2.59e-20
Cathepsin L1 (CTSL1)	3.607	.461	5.44e-15
Fatty acid-binding protein 4 (FABP4)	2.126	.326	7.02e-11
Tissue-type plasminogen activator (t-PA)	2.192	.371	3.56e-09
Cathepsin D (CTSD)	1.848	.36	2.76e-07
E-selectin (SELE)	1.605	.326	8.68e-07
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	2.123	.443	1.61e-06
Lectin-like oxidized LDL receptor 1 (LOX-1)	2.156	.453	1.99e-06
N-terminal pro-B-type natriuretic peptide (NT-pro-BNP)	-1.47	.315	3.11e-06
Protein S100-A12 (EN-RAGE)	2.187	.47	3.21e-06
Growth hormone (GH)	-1.956	.46	.000021
Matrix metalloproteinase-12 (MMP-12)	-1.355	.325	.000029
Interleukin-6 receptor subunit alpha (IL-6RA)	1.296	.336	.00011
Hepatocyte growth factor (HGF)	1.396	.369	.00015
C-C motif chemokine 20 (CCL20)	1.596	.44	.00028

* Beta coefficients represent change in protein level (on SD-scale to increase cross-comparability) per one year of aging. Models were adjusted for the following potential confounders: time in freezer, exercise habits, glomerular filtration rate, smoking, and use of beta-blockers, diuretics, statins, insulin and oral antidiabetic drugs.