**SUPPLEMENTAL DATA**

**The effect of spironolactone in patients with obesity at risk for heart failure: proteomic insights from the HOMAGE trial**

**Supplemental Material.** *Assays and studied biomarkers using the Olink® methods*

**Supplemental Table 1.** *Protein names and respective Olink® panel sorted in alphabetical order*

**Supplemental Table 2.** *Baseline of the patients according to body mass index*

**Supplemental Table 3.** *Baseline of the patients according to waist conference and treatment group*

**Supplemental Table 4.** *Baseline of the patients according to body mass index and treatment group*

**Supplemental Table 5.** *Proteins associated with an increased body mass index in the blood sample at baseline (before spironolactone treatment)*

**Supplemental Table 6.** *Change of proteins after 1 month and 9 months of spironolactone treatment*

*Assays and studied biomarkers using the Olink® methods*

Proteins were measured using the Olink® CVDII, CVDIII, and Inflammation panels\* (Olink Proteomics AB, Uppsala, Sweden) according to the manufacturer's instructions. The Proximity Extension Assay (PEA) technology used for the Olink protocol has been well described (Assarsson et al, 2014; accessible at: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0095192), and enables 92 analytes to be analyzed simultaneously, using 1µL of each sample. In brief, pairs of oligonucleotide-labeled antibody probes bind to their targeted protein, and if the two probes are brought in close proximity the oligonucleotides will hybridize in a pair-wise manner. The addition of a DNA polymerase leads to a proximity-dependent DNA polymerization event, generating a unique PCR target sequence. The resulting DNA sequence is subsequently detected and quantified using a microfluidic real-time PCR instrument (Biomark HD, Fluidigm). The resulting Ct-data is then quality controlled and normalized using a set of internal and external controls. The final assay read-out is presented in Normalized Protein eXpression (NPX) values, which is an arbitrary unit on a log2-scale where a high value corresponds to a higher protein expression. The four internal controls are designed to mimic and monitor the different steps of the PEA. They consist of two non-human proteins with matching antibody-probes that functions as incubation/immuno controls, an IgG antibody with two matching probes attached to it that functions as an extension control and a complete double-stranded amplicon that function as detection control. The internal controls are introduced to all samples as well as to the external controls. The external controls consist of a triplicate of negative controls used to calculate the limit of detection (LOD), as well as a triplicate of interplate controls (IPCs) containing 92 sets of antibodies with both matching probes for each assay attached to them that are used for normalization. For each sample and assay NPX is calculated by the following equations: 1. Ct(analyte) – Ct(extension control) =dCt(analyte) (to decrease technical variation) 2. dCt(analyte) – Ct(median IPC)=ddCt(analyte) (to improve inter plate variation) 3. Correction factor(analyte) – ddCt(analyte) =NPX(analyte) (for more intuitive data). The correction factor is a set variable unique for each assay and reagent lot. Quality control of the data is performed in two steps: First, the standard deviation for each of the incubation/immuno controls and the detection control is calculated for each run. A run will only pass quality control if the standard deviation for each of the controls is below 0.2. Secondly, each sample is quality controlled using incubation control 2 and the detection control. The run median of each of the controls is calculated and all samples within the run is compared to that. Samples that fall more than +/- 0.3 NPX from the plate median with regards to these two controls will fail the quality control and receive a QC warning in the data output file. All assay validation data (detection limits, intra- and inter-assay precision data, etc.) are available on manufacturer's website (www.olink.com).

**Supplemental Table 1.** *Protein names and respective Olink® panel sorted in alphabetical order*

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein full name** | **Entry name** | **Olink® Panel\*** | **Uniprot ID\*\*** |
| Angiotensin-converting enzyme 2 | ACE2 | CVD II | Q9BYF1 |
| Adenosine Deaminase | ADA | INF | P00813 |
| Adisintegrin and metalloproteinase with thrombospondin motifs 13 | ADAMTS13 | CVD II | Q76LX8 |
| ADM | ADM | CVD II | P35318 |
| Agouti-related protein | AGRP | CVD II | O00253 |
| CD166 antigen | ALCAM | CVD III | Q13740 |
| Protein AMBP | AMBP | CVD II | P02760 |
| Angiopoietin-1 | ANG1 | CVD II | Q15389 |
| Aminopeptidase N | APN | CVD III | P15144 |
| Axin-1 | AXIN1 | INF | O15169 |
| Tyrosine-protein kinase receptor UFO | AXL | CVD III | P30530 |
| Azurocidin | AZU1 | CVD III | P20160 |
| Brain-derived neurotrophic factor | BDNF | INF | P23560 |
| Beta-nerve growth factor | BETANGF | INF | P01138 |
| Bleomycin hydrolase | BLMHYDROLASE | CVD III | Q13867 |
| Bone morphogenetic protein 6 | BMP6 | CVD II | P22004 |
| Natriuretic peptides B | BNP | CVD II | P16860 |
| Eukaryotic translation initiation factor 4E-binding protein 1 | BP1\_4E | INF | Q13541 |
| Carbonic anhydrase 5A, mitochondrial | CA5A | CVD II | P35218 |
| Caspase-3 | CASP3 | CVD III | P42574 |
| Caspase 8 | CASP8 | INF | Q14790 |
| Eotaxin-1 | CCL11 | INF | P51671 |
| C-C motif chemokine 15 | CCL15 | CVD III | Q16663 |
| C-C motif chemokine 16 | CCL16 | CVD III | O15467 |
| C-C motif chemokine 17 | CCL17 | CVD II | Q92583 |
| C-C motif chemokine 19 | CCL19 | INF | Q99731 |
| C-C motif chemokine 20 | CCL20 | INF | P78556 |
| C-C motif chemikine 22 | CCL22 | CVD III | O00626 |
| C-C motif chemokine 23 | CCL23 | INF | P55773 |
| C-C motif chemokine 24 | CCL24 | CVD III | O00175 |
| C-C motif chemokine 25 | CCL25 | INF | O15444 |
| C-C motif chemokine 28 | CCL28 | INF | Q9NRJ3 |
| C-C motif chemokine 3 | CCL3 | CVD II | P10147 |
| C-C motif chemokine 4 | CCL4 | INF | P13236 |
| Scavenger receptor cysteine-rich type 1 protein M130 | CD163 | CVD III | Q86VB7 |
| Natural killer cell receptor 2B4 | CD244 | INF | Q9BZW8 |
| T-cell surface glycoprotein CD4 | CD4 | CVD II | P01730 |
| CD40L receptor | CD40 | INF | P25942 |
| CD40 ligand | CD40L | CVD II | P29965 |
| T-cell surface glycoprotein CD5 | CD5 | INF | P06127 |
| T cell surface glycoprotein CD6 | CD6 | INF | P30203 |
| SLAM family member 5 | CD84 | CVD II | Q9UIB8 |
| Complement component C1q receptor | CD93 | CVD III | Q9NPY3 |
| CUB domain-containing protein 1 | CDCP1 | INF | Q9H5V8 |
| Cadherin-5 | CDH5 | CVD III | P33151 |
| Carcinoembryonic antigenrelated cell adhesion molecule 8 | CEACAM8 | CVD II | P31997 |
| Chitinase-3-like protein 1 | CHI3L1 | CVD III | P36222 |
| Chitotriosidase-1 | CHIT1 | CVD III | Q13231 |
| Contactin-1 | CNTN1 | CVD III | Q12860 |
| Collagen alpha-1(I) chain | COL1A1 | CVD III | P02452 |
| Carboxypeptidase A1 | CPA1 | CVD III | P15085 |
| Carboxypeptidase B | CPB1 | CVD III | P15086 |
| Macrophage colony-stimulating factor 1 | CSF1 | INF | P09603 |
| Cystatin D | CST5 | INF | P28325 |
| Cystatin-B | CSTB | CVD III | P04080 |
| Chymotrypsin C | CTRC | CVD II | Q99895 |
| Cathepsin D | CTSD | CVD III | P07339 |
| Cathepsin L1 | CTSL1 | CVD II | P07711 |
| Cathepsin Z | CTSZ | CVD III | Q9UBR2 |
| Fractalkine | CX3CL1 | INF | P78423 |
| C-X-C motif chemokine 1 (CVD2) | CXCL1 | CVD II | P09341 |
| C-X-C motif chemokine 10 | CXCL10 | INF | P02778 |
| C-X-C motif chemokine 11 | CXCL11 | INF | O14625 |
| C-X-C motif chemokine 16 | CXCL16 | CVD III | Q9H2A7 |
| C-X-C motif chemokine 5 | CXCL5 | INF | P42830 |
| C-X-C motif chemokine 6 | CXCL6 | INF | P80162 |
| C-X-C motif chemokine 9 | CXCL9 | INF | Q07325 |
| Decorin | DCN | CVD II | P07585 |
| 2,4-dienoyl-CoA reductase, mitochondrial | DECR1 | CVD II | Q16698 |
| Dickkopf-related protein 1 | DKK1 | CVD II | O94907 |
| Azurocidin | DLK1 | CVD III | P80370 |
| Delta and Notch-like epidermal growth factor-related receptor | DNER | INF | Q8NFT8 |
| Epidermal growth factor receptor | EGFR | CVD III | P00533 |
| Protein S100-A12 | ENRAGE | INF | P80511 |
| Epithelial cell adhesion molecule | EPCAM | CVD III | P16422 |
| Ephrin type-B receptor 4 | EPHB4 | CVD III | P54760 |
| Fatty acid-binding protein, intestinal | FABP2 | CVD II | P12104 |
| Fatty acid-binding protein, adipocyte | FABP4 | CVD III | P15090 |
| Tumor necrosis factor receptor superfamily member 6 | FAS | CVD III | P25445 |
| Fibroblast growth factor 19 | FGF19 | INF | O95750 |
| Fibroblast growth factor 21 (CVD2) | FGF21 | CVD II | Q9NSA1 |
| Fibroblast growth factor 23 (CVD2) | FGF23 | CVD II | Q9GZV9 |
| Fibroblast growth factor 5 | FGF5 | INF | P12034 |
| Fms-related tyrosine kinase 3 ligand | FLT3L | INF | P49771 |
| Follistatin | FS | CVD II | P19883 |
| Galectin-3 | GAL3 | CVD III | P17931 |
| Galectin-4 | GAL4 | CVD III | P56470 |
| Galectin-9 | GAL9 | CVD II | O00182 |
| Growth/differentiation factor 15 | GDF15 | CVD III | Q99988 |
| Growth/differentiation factor 2 | GDF2 | CVD II | Q9UK05 |
| Growth hormone | GH | CVD II | P01241 |
| Gastric intrinsic factor | GIF | CVD II | P27352 |
| Lactoylglutathione lyase | GLO1 | CVD II | Q04760 |
| Granulins | GRN | CVD III | P28799 |
| Gastrotropin | GT | CVD II | P51161 |
| Hydroxyacid oxidase 1 | HAOX1 | CVD II | Q9UJM8 |
| Proheparin-binding EGF-like growth factor | HBEGF | CVD II | Q99075 |
| Glial cell line-derived neurotrophic factor | HGDNF | INF | P39905 |
| Hepatocyte growth factor | HGF | INF | P14210 |
| Heme oxygenase 1 | HO1 | CVD II | P09601 |
| Osteoclast-associated immunoglobulin-like receptor | HOSCAR | CVD II | Q8IYS5 |
| Heat shock 27 kDa protein | HSP27 | CVD II | P04792 |
| Intercellular adhesion molecule 2 | ICAM2 | CVD III | P13598 |
| Alpha-L-iduronidase | IDUA | CVD II | P35475 |
| Insulin-like growth factor-binding protein 1 | IGFBP1 | CVD III | P08833 |
| Insulin-like Growth Factor-Binding Protein 2 | IGFBP2 | CVD III | P18065 |
| Insulin-like growth factor-binding protein 7 | IGFBP7 | CVD III | Q16270 |
| Low affinity immunoglobulin gamma Fc region receptor II-b | IGGFCRECEPTORIIB | CVD II | P31994 |
| Interleukin-10 | IL10 | INF | P22301 |
| Interleukin-10 receptor subunit alpha | IL10RA | INF | Q13651 |
| Interleukin-10 receptor subunit beta | IL10RB | INF | Q08334 |
| Interleukin-12 subunit beta | IL12B | INF | P29460 |
| Interleukin-13 | IL13 | INF | P35225 |
| Interleukin-15 receptor subunit alpha | IL15RA | INF | Q13261 |
| Pro-interleukin-16 | IL16 | CVD II | Q14005 |
| Interleukin-17A | IL17A | INF | Q16552 |
| Interleukin-17C | IL17C | INF | Q9P0M4 |
| Interleukin-17D | IL17D | CVD II | Q8TAD2 |
| Interleukin-17 receptor A | IL17RA | CVD III | Q96F46 |
| Interleukin-18 (CVD2) | IL18 | CVD II | Q14116 |
| Interleukin-18-binding protein | IL18BP | CVD III | O95998 |
| Interleukin-18 receptor 1 | IL18R1 | INF | Q13478 |
| Interleukin-1 receptor antagonist protein | IL1RA | CVD II | P18510 |
| Interleukin-1 receptor-like 2 | IL1RL2 | CVD II | Q9HB29 |
| Interleukin-1 receptor type 1 | IL1RT1 | CVD III | P14778 |
| Interleukin-1 receptor type 2 | IL1RT2 | CVD III | P27930 |
| Interleukin-20 receptor subunit alpha | IL20RA | INF | Q9UHF4 |
| Interleukin-27 | IL27 | CVD II | Q8NEV9 |
| Interleukin-2 receptor subunit alpha | IL2RA | CVD III | P01589 |
| Interleukin-4 receptor subunit alpha | IL4RA | CVD II | P24394 |
| Interleukin-6 (CVD2) | IL6 | CVD II | P05231 |
| Interleukin-6 receptor subunit alpha | IL6RA | CVD III | P08887 |
| Interleukin-7 | IL7 | INF | P13232 |
| Interleukin-8 | IL8 | INF | P10145 |
| Melusin | ITGB1BP2 | CVD II | Q9UKP3 |
| Integrin beta-2 | ITGB2 | CVD III | P05107 |
| Junctional adhesion molecule A | JAMA | CVD III | Q9Y624 |
| Kidney injury molecule 1 | KIM1 | CVD II | Q96D42 |
| Kallikrein-6 | KLK6 | CVD III | Q92876 |
| Latency-associated peptide transforming growth factor beta 1 | LAPTGFBETA1 | INF | P01137 |
| Low-density lipoprotein receptor | LDLRECEPTOR | CVD III | P01130 |
| Leptin | LEP | CVD II | P41159 |
| Leukemia inhibitory factor receptor | LIFR | INF | P42702 |
| Lectin-like oxidized LDL receptor 1 | LOX1 | CVD II | P78380 |
| Lipoprotein lipase | LPL | CVD II | P06858 |
| Lymphotoxin-beta receptor | LTBR | CVD III | P36941 |
| Macrophage receptor MARCO | MARCO | CVD II | Q9UEW3 |
| Myoglobin | MB | CVD III | P02144 |
| Monocyte chemotactic protein 1 | MCP1\_CVD3 | CVD III | P13500 |
| Monocyte chemotactic protein 2 | MCP2 | INF | P80075 |
| Monocyte chemotactic protein 3 | MCP3 | INF | P80098 |
| Monocyte chemotactic protein 4 | MCP4 | INF | Q99616 |
| Matrix extracellular phosphoglycoprotein | MEPE | CVD III | Q9NQ76 |
| Tyrosine-protein kinase Mer | MERTK | CVD II | Q12866 |
| Matrix metalloproteinase-1 | MMP1 | INF | P03956 |
| Matrix metalloproteinase-10 | MMP10 | INF | P09238 |
| Matrix metalloproteinase-12 | MMP12 | CVD II | P39900 |
| Matrix metalloproteinase-2 | MMP2 | CVD III | P08253 |
| Matrix metalloproteinase-3 | MMP3 | CVD III | P08254 |
| Matrix metalloproteinase-7 | MMP7 | CVD II | P09237 |
| Matrix metalloproteinase-9 | MMP9 | CVD III | P14780 |
| Myeloperoxidase | MPO | CVD III | P05164 |
| NF-kappa-B essential modulator | NEMO | CVD II | Q9Y6K9 |
| Neurogenic locus notch homolog protein 3 | NOTCH3 | CVD III | Q9UM47 |
| Neurotrophin-3 | NT3 | INF | P20783 |
| N-terminal prohormone brain natriuretic peptide | NTPROBNP | CVDII | P16860 |
| Osteoprotegerin | OPG\_CVD3 | CVD III | O00300 |
| Osteopontin | OPN | CVD III | P10451 |
| Oncostatin-M | OSM | INF | P13725 |
| Plasminogen activator inhibitor 1 | PAI | CVD III | P05121 |
| Pappalysin-1 | PAPPA | CVD II | Q13219 |
| Proteinase-activated receptor 1 | PAR1 | CVD II | P25116 |
| Poly [ADP-ribose] polymerase 1 | PARP1 | CVD II | P09874 |
| Proprotein convertase subtilisin/kexin type 9 | PCSK9 | CVD III | Q8NBP7 |
| Platelet-derived growth factor subunit A | PDGFSUBUNITA | CVD III | P04085 |
| Platelet-derived growth factor subunit B | PDGFSUBUNITB | CVD II | P01127 |
| Programmed cell death 1 ligand 1 | PDL1 | INF | Q9NZQ7 |
| Programmed cell death 1 ligand 2 | PDL2 | CVD II | Q9BQ51 |
| Platelet endothelial cell adhesion molecule | PECAM1 | CVD III | P16284 |
| Peptidoglycan recognition protein 1 | PGLYRP1 | CVD III | O75594 |
| Elafin | PI3 | CVD III | P19957 |
| Polymeric immunoglobulin receptor | PIGR | CVD II | P01833 |
| Perlecan | PLC | CVD III | P98160 |
| Placenta growth factor | PLGF | CVD II | P49763 |
| Paraoxonase (PON 3) | PON3 | CVD III | Q15166 |
| Prolargin | PRELP | CVD II | P51888 |
| Brother of CDO | PROTEINBOC | CVD II | Q9BWV1 |
| Serine protease 27 | PRSS27 | CVD II | Q9BQR3 |
| Prostasin | PRSS8 | CVD II | Q16651 |
| Myeloblastin | PRTN3 | CVD III | P24158 |
| P-selectin glycoprotein ligand 1 | PSGL1 | CVD II | Q14242 |
| Pulmonary surfactant-associated protein D | PSPD | CVD III | P35247 |
| Pentraxin-related protein PTX3 | PTX3 | CVD II | P26022 |
| Receptor for advanced glycosylation end products | RAGE | CVD II | Q15109 |
| Retinoic acid receptor responder protein 2 | RARRES2 | CVD III | Q99969 |
| Renin | REN | CVD II | P00797 |
| Resistin | RETN | CVD III | Q9HD89 |
| Stem cell factor (CVD2) | SCF | CVD II | P21583 |
| Secretoglobin family 3A member 2 | SCGB3A2 | CVD III | Q96PL1 |
| E-selectin | SELE | CVD III | P16581 |
| P-selectin | SELP | CVD III | P16109 |
| Serpin A12 | SERPINA12 | CVD II | Q8IW75 |
| Tyrosine-protein phosphatase non-receptor type substrate 1 | SHPS1 | CVD III | P78324 |
| SIR2-like protein 2 | SIRT2 | INF | Q8IXJ6 |
| Signaling lymphocytic activation molecule | SLAMF1 | INF | Q13291 |
| SLAM family member 7 | SLAMF7 | CVD II | Q9NQ25 |
| Superoxide dismutase [Mn], mitochondrial | SOD2 | CVD II | P04179 |
| Sortilin | SORT1 | CVD II | Q99523 |
| Spondin-1 | SPON1 | CVD III | Q9HCB6 |
| Spondin-2 | SPON2 | CVD II | Q9BUD6 |
| Proto-oncogene tyrosine-protein kinase Src | SRC | CVD II | P12931 |
| Sulfotransferase 1A1 | ST1A1 | INF | P50225 |
| ST2 protein | ST2 | CVD III | Q01638 |
| STAM-binding protein | STAMPB | INF | O95630 |
| Serine/threonine-protein kinase 4 | STK4 | CVD II | Q13043 |
| Tissue factor | TF | CVD II | P13726 |
| Trefoil factor 3 | TFF3 | CVD III | Q07654 |
| Tissue factor pathway inhibitor | TFPI | CVD III | P10646 |
| Transforming growth factor alpha | TGFALPHA | INF | P01135 |
| Protein-glutamine gamma-glutamyltransferase 2 | TGM2 | CVD II | P21980 |
| Thrombospondin-2 | THBS2 | CVD II | P35442 |
| Thrombopoietin | THPO | CVD II | P40225 |
| Angiopoietin-1 receptor | TIE2 | CVD II | Q02763 |
| Metalloproteinase inhibitor 4 | TIMP4 | CVD III | Q99727 |
| Trem-like transcript 2 protein | TLT2 | CVD III | Q5T2D2 |
| Thrombomodulin | TM | CVD II | P07204 |
| Tumor necrosis factor | TNF | INF | P01375 |
| TNF-beta | TNFB | INF | P01374 |
| Tumor necrosis factor receptor 1 | TNFR1 | CVD III | P19438 |
| Tumor necrosis factor receptor 2 | TNFR2 | CVD III | P20333 |
| Tumor necrosis factor receptor superfamily member 10A | TNFRSF10A | CVD II | O00220 |
| Tumor necrosis factor receptor superfamily member 10C | TNFRSF10C | CVD III | O14798 |
| Tumor necrosis factor receptor superfamily member 11A | TNFRSF11A | CVD II | Q9Y6Q6 |
| Tumor necrosis factor receptor superfamily member 13B | TNFRSF13B | CVD II | O14836 |
| Tumor necrosis factor receptor superfamily member 14 | TNFRSF14 | CVD III | Q92956 |
| Tumor necrosis factor receptor superfamily member 9 | TNFRSF9 | INF | Q07011 |
| Tumor necrosis factor ligand superfamily member 13B | TNFSF13B | CVD III | Q9Y275 |
| Tumor necrosis factor ligand superfamily member 14 | TNFSF14 | INF | O43557 |
| Tissue-type plasminogen activator | TPA | CVD III | P00750 |
| Transferrin receptor protein 1 | TR | CVD III | P02786 |
| TNF-related apoptosis-inducing ligand | TRAIL | INF | P50591 |
| TNF-related apoptosis-inducing ligand receptor 2 | TRAILR2 | CVD II | O14763 |
| TNF-related activation-induced cytokine | TRANCE | INF | O14788 |
| Tartrate-resistant acid phosphatase type 5 | TRAP | CVD III | P13686 |
| Tumor necrosis factor (Ligand) superfamily, member 12 | TWEAK | INF | O43508 |
| Urokinase-type plasminogen activator | UPA\_CVD3 | CVD III | P00749 |
| Urokinase plasminogen activator surface receptor | UPAR | CVD III | Q03405 |
| Vascular endothelial growth factor A | VEGFA | INF | P15692 |
| Vascular endothelial growth factor D | VEGFD | CVD II | O43915 |
| V-set and immunoglobulin domain-containing protein 2 | VSIG2 | CVD II | Q96IQ7 |
| von Willebrand factor | VWF | CVD III | P04275 |
| Lymphotactin | XCL1 | CVD II | P47992 |

**Supplemental Table 2.** *Baseline of the patients according to body mass index*

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **BMI <30kg/m2**  *n=347* | **BMI ≥30kg/m2**  *n=178* | **p-value** |
| Age (years) | 74 (69 – 79) | 71 (67 – 76) | **<0.001** |
| Male gender (%) | 259 (75) | 132 (74) | 0.9 |
| BMI (kg/m2) | 26.3 (24.2 – 28) | 33.5 (31.6 – 36.2) | NA |
| WC (cm) | 97 (92 – 103) | 113 (108 – 121) | **<0.001** |
| Heart rate (b.p.m.) | 60 (55 – 67) | 63 (56 – 69) | 0.11 |
| Systolic blood pressure (mmHg) | 140 (127 – 154) | 141 (131 – 157) | 0.16 |
| Diastolic blood pressure (mmHg) | 78 (71 – 85) | 79 (72 – 85) | 0.19 |
| *Medical history (%)* | | | |
| Hypertension | 263 (76) | 149 (84) | **0.037** |
| Diabetes mellitus | 113 (33) | 103 (58) | **<0.001** |
| Coronary artery disease | 263 (76) | 114 (64) | **0.005** |
| Stroke/TIA | 18 (5) | 10 (6) | 0.84 |
| COPD | 20 (6) | 12 (7) | 0.66 |
| Echocardiography | | | |
| LV ejection fraction (%) | 63 (57 – 66) | 64 (59 – 67) | 0.12 |
| Indexed LV mass (g/m2) | 92 (79 – 109) | 100 (84 – 117) | **0.002** |
| LV hypertrophy | 83 (26) | 56 (36) | **0.025** |
| Indexed LA volume (ml/m2) | 31 (26 – 37) | 28 (24 – 35) | **0.003** |
| E/e’ | 9.3 (7.4 – 11.5) | 9.4 (7.7 – 11.6) | 0.43 |
|  | | | |
| eGFR (ml/min/1.73 m2) | 72 (61 – 84) | 73 (61 – 85) | 0.88 |
| Urea (mmol/L) | 9 (6 – 14) | 9 (6 – 14) | 0.94 |
| NT-proBNP (pg/mL) | 259 (168 – 428) | 229 (147 – 395) | 0.06 |
| Hemoglobin (g/dL) | 14 (13 – 15) | 14 (13 – 15) | 0.88 |
| Sodium (mmol/L) | 140 (138 – 141) | 139 (138 – 141) | 0.25 |
| Potassium (mmol/L) | 4.3 (4.1 – 4.5) | 4.3 (4.1 – 4.6) | 0.39 |
|  | | | |
| Antiplatelet | 277 (80) | 135 (76) | 0.29 |
| Beta-blocker | 242 (70) | 123 (69) | 0.88 |
| ACE inhibitor or ARB | 266 (77) | 149 (84) | 0.06 |
| Thiazides | 48 (14) | 39 (22) | **0.018** |
| Lipid-lowering agent | 291 (84) | 142 (80) | 0.24 |
| *Values are median (interquartile range)*  *ACE, angiotensin-converting enzyme; ARB, angiotensin receptor type II blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; LA, left atrium; LV, left ventricle; NA, not applicable; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; WC, waist circumference.* | | | |

**Supplemental Table 3.** *Baseline of the patients according to waist conference and treatment group*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Characteristics** | **Normal Waist Cirmcumference**  *n=211* | | **p-value** | **High Waist Circumference**  *n=299* | | **p-value** |
|  | **Spironolactone**  *n=103* | **Control**  *n=108* |  | **Spironolactone**  *n=155* | **Control**  *n=144* |  |
| Age (years) | 73 (70 – 79) | 72 (68 – 79) | 0.42 | 72 (68 – 78) | 73 (69 – 79) | 0.39 |
| Male gender (%) | 91 (88) | 89 (82) | 0.22 | 108 (70) | 92 (64) | 0.29 |
| BMI (kg/m2) | 25 (23 – 27) | 25 (24 – 27) | 0.67 | 31 (28 – 34) | 31 (28 – 34) | 0.55 |
| WC (cm) | 95 (88 – 99) | 94 (88 – 99) | 0.39 | 110 (104 – 116) | 108 (103 – 116) | 0.46 |
| Heart rate (b.p.m.) | 59 (55 – 67) | 59 (53 – 65) | 0.22 | 61 (56 – 67) | 63 (56 – 70) | 0.56 |
| Systolic blood pressure (mmHg) | 140 (123 – 154) | 139 (126 – 153) | 0.98 | 141 (130 – 157) | 141 (130 – 158) | 0.65 |
| Diastolic blood pressure (mmHg) | 80 (71 – 85) | 78 (71 – 88) | 0.47 | 80 (73 – 84) | 77 (71 – 84) | 0.11 |
| *Medical history (%)* | | | | | | |
| Hypertension | 76 (74) | 72 (67) | 0.26 | 134 (87) | 119 (83) | 0.36 |
| Diabetes mellitus | 27 (26) | 28 (26) | 0.96 | 76 (49) | 77 (54) | 0.44 |
| Coronary artery disease | 81 (79) | 91 (84) | 0.29 | 103 (67) | 90 (63) | 0.48 |
| Stroke/TIA | 3 (3) | 4 (4) | 0.75 | 12 (8) | 8 (6) | 0.45 |
| COPD | 5 (5) | 5 (5) | 0.94 | 14 (9) | 7 (5) | 0.16 |
| Echocardiography | | | | | | |
| LV ejection fraction (%) | 62 (57 – 66) | 62 (56 – 66) | 0.61 | 61 (59 – 68) | 64 (57 – 67) | 0.7 |
| Indexed LV mass (g/m2) | 92 (82 – 103) | 91 (79 – 114) | 0.91 | 97 (81 – 115) | 99 (84 – 113) | 0.72 |
| LV hypertrophy | 17 (18) | 25 (25) | 0.23 | 47 (36) | 44 (34) | 0.84 |
| Indexed LA volume (ml/m2) | 31 (27 – 38) | 32 (26 – 37) | 0.92 | 29 (24 – 35) | 28 (24 – 36) | 0.73 |
| E/e’ | 9 (7.3 – 10.9) | 8.7 (7.2 – 10.4) | 0.52 | 9.3 (7.6 – 11.3) | 9.6 (7.9 – 12.8) | 0.13 |
| *Lab* | | | | | | |
| eGFR (ml/min/1.73 m2) | 73 (61 – 87) | 74 (65 – 85) | 0.48 | 74 (63 – 89) | 70 (58 – 79) | **0.008** |
| Urea (mmol/L) | 8 (6 – 15) | 8 (6 – 14) | 0.59 | 9 (6 – 15) | 10 (6 – 15) | 0.54 |
| NT-proBNP (pg/mL) | 259 (166 – 404) | 227 (140 – 325) | 0.1 | 173 (129 – 340) | 203 (133 – 308) | 0.7 |
| Hemoglobin (g/dL) | 14 (13 – 15) | 14 (13 – 15) | 0.85 | 14 (13 – 15) | 14 (13 – 15) | 0.56 |
| Sodium (mmol/L) | 139 (138 – 142) | 140 (139 – 141) | 0.32 | 140 (137 – 141) | 139 (138 – 141) | 0.39 |
| Potassium (mmol/L) | 4.3 (4.1 – 4.5) | 4.3 (4.1 – 4.5) | 0.51 | 4.3 (4 – 4.6) | 4.3 (4.1 – 4.6) | 0.62 |
| *Medication (%)* | | | | | | |
| Antiplatelet | 86 (84) | 87 (81) | 0.58 | 118 (76) | 110 (76) | 0.96 |
| Beta-blocker | 74 (72) | 81 (75) | 0.6 | 107 (69) | 91 (63) | 0.29 |
| ACE inhibitor or ARB | 76 (74) | 82 (76) | 0.72 | 126 (81) | 116 (81) | 0.87 |
| Thiazides | 13 (13) | 10 (9) | 0.43 | 33 (21) | 31 (22) | 0.96 |
| Lipid-lowering agent | 82 (80) | 94 (87) | 0.15 | 129 (83) | 114 (79) | 0.37 |
| *Values are median (interquartile range)*  *ACE, angiotensin-converting enzyme; ARB, angiotensin receptor type II blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; LA, left atrium; LV, left ventricle; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; WC, waist circumference.*  *High waist circumference (≥102 cm for men and ≥88 cm for women characterizing abdominal obesity); NWC, normal waist circumference (<102 cm for men and <88 cm for women)* | | | | | | |

**Supplemental Table 4.** *Baseline of the patients according to body mass index and treatment group*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Characteristics** | **BMI <30kg/m2**  *n=347* | | **p-value** | **BMI ≥30kg/m2**  *n=178* | | **p-value** |
|  | **Spironolactone**  *n=169* | **Control**  *n=178* |  | **Spironolactone**  *n=95* | **Control**  *n=83* |  |
| Age (years) | 75 (70 – 80) | 73 (69 – 79) | 0.18 | 71 (66 – 75) | 72 (68 – 77) | 0.24 |
| Male gender (%) | 131 (78) | 128 (72) | 0.23 | 73 (77) | 59 (71) | 0.38 |
| BMI (kg/m2) | 27 (24 – 28) | 26 (24 – 28) | 0.37 | 33 (31 – 36) | 34 (32 – 36) | 0.6 |
| WC (cm) | 97 (92 – 103) | 96 (91 – 103) | 0.46 | 113 (109 – 120) | 113 (107 – 123) | 0.98 |
| Heart rate (b.p.m.) | 60 (55 – 66) | 61 (54 – 67) | 0.86 | 62 (57 – 69) | 63 (54 – 69) | 0.6 |
| Systolic blood pressure (mmHg) | 140 (125 – 154) | 140 (128 – 155) | 0.48 | 141 (131 – 157) | 141 (131 – 156) | 0.85 |
| Diastolic blood pressure (mmHg) | 78 (70 – 83) | 78 (71 – 86) | 0.27 | 81 (76 – 85) | 76 (71 – 84) | **0.013** |
| *Medical history (%)* | | | | | | |
| Hypertension | 133 (79) | 130 (73) | 0.22 | 80 (84) | 69 (83) | 0.85 |
| Diabetes mellitus | 52 (31) | 61 (34) | 0.49 | 55 (58) | 48 (58) | 0.99 |
| Coronary artery disease | 127 (75) | 136 (76) | 0.78 | 62 (65) | 52 (63) | 0.72 |
| Stroke/TIA | 9 (5) | 9 (5) | 0.91 | 6 (6) | 4 (5) | 0.67 |
| COPD | 11 (7) | 9 (5) | 0.56 | 10 (11) | 2 (2) | **0.031** |
| Echocardiography | | | | | | |
| LV ejection fraction (%) | 63 (57 – 67) | 63 (56 – 66) | 0.4 | 63 (59 – 67) | 64 (58 – 68) | 0.57 |
| Indexed LV mass (g/m2) | 92 (81 – 106) | 92 (78 – 112) | 0.8 | 100 (82 – 119) | 100 (88 – 115) | 0.76 |
| LV hypertrophy | 37 (24) | 46 (28) | 0.45 | 31 (38) | 25 (34) | 0.65 |
| Indexed LA volume (ml/m2) | 30 (26 – 37) | 32 (26 – 37) | 0.5 | 29 (25 – 34) | 28 (21 – 35) | 0.65 |
| E/e’ | 9.1 (7.3 – 11.3) | 9.4 (7.5 – 11.3) | 0.63 | 9.4 (7.8 – 11.1) | 9.4 (7.5 – 12.3) | 0.85 |
| *Lab* | | | | | | |
| eGFR (ml/min/1.73 m2) | 73 (62 – 87) | 71 (61 – 83) | 0.3 | 74 (62 – 89) | 72 (59 – 80) | 0.16 |
| Urea (mmol/L) | 8 (6 – 15) | 9 (6 – 14) | 0.76 | 9 (6 – 13) | 9 (6 – 16) | 0.58 |
| NT-proBNP (pg/mL) | 218 (145 – 369) | 220 (140 – 354) | 0.58 | 179 (124 – 362) | 206 (119 – 301) | 0.58 |
| Hemoglobin (g/dL) | 14 (13 – 15) | 14 (13 – 15) | 0.56 | 14 (13 – 15) | 14 (13 – 15) | 0.89 |
| Sodium (mmol/L) | 140 (138 – 141) | 140 (138 – 141) | 0.6 | 140 (137 – 141) | 139 (138 – 141) | 0.72 |
| Potassium (mmol/L) | 4.3 (4.1 – 4.5) | 4.3 (4.1 – 4.6) | 0.51 | 4.3 (4 – 4.6) | 4.4 (4.1 – 4.6) | 0.56 |
| *Medication (%)* | | | | | | |
| Antiplatelet | 138 (82) | 139 (78) | 0.41 | 70 (74) | 65 (78) | 0.47 |
| Beta-blocker | 120 (71) | 122 (69) | 0.62 | 65 (68) | 58 (70) | 0.83 |
| ACE inhibitor or ARB | 128 (76) | 138 (78) | 0.69 | 79 (83) | 70 (84) | 0.83 |
| Thiazides | 24 (14) | 24 (14) | 0.85 | 22 (23) | 17 (20) | 0.67 |
| Lipid-lowering agent | 140 (83) | 151 (85) | 0.61 | 77 (81) | 65 (78) | 0.65 |
| *Values are median (interquartile range)*  *ACE, angiotensin-converting enzyme; ARB, angiotensin receptor type II blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; LA, left atrium; LV, left ventricle; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; WC, waist circumference.* | | | | | | |

**Supplemental Table 5.** *Proteins associated with an increased body mass index in the blood sample at baseline (before spironolactone treatment)*

|  |  |  |
| --- | --- | --- |
| **Protein** |  | **Body mass index >30** |
| ***Proteins associated with a high BMI and a high WC*** | | |
| Adrenomedullin | adm | 3.32 (2.23 – 4.41) |
| Thrombospondin-2 | thbs2 | 2.93 (1.27 – 4.59) |
| Retinoic acid receptor responder protein-2 | rarres2 | 3.11 (1.85 – 4.38) |
| Fatty-acid binding protein-4 | fabp4 | 3.07 (2.54 – 3.59) |
| Perlecan | plc | 3.18 (1.8 – 4.56) |
| Leptin | lep | 3.11 (2.79 – 3.43) |
| Interleukin receptor 1 receptor antagonist | il1ra | 2.73 (2.09 – 3.37) |
| Procollagen III amino terminal propeptide | p3np | 1.32 (0.62 – 2.02) |
| Plasminogen activator inhibitor-1 | pai | 0.92 (0.52 – 1.31) |
| Insulin-like growth factor-binding protein-1 | igfbp1 | -1.48 (-1.88 - -1.08) |
| Insulin-like growth factor-binding protein-2 | igfbp2 | -2.13 (-2.82 - -1.45) |
| Paraoxonase-3 | pon3 | -1.9 (-2.63 - -1.18) |
| ***Proteins associated with a high BMI but not with a high WC*** | | |
| LDL-receptor | ldlr | 1.55 (0.75 – 2.36) |
| Tissue plasminogen activator | tpa | 0.96 (0.45 – 1.47) |
| Delta/Notch like epidermal growth factor-related receptor | dner | -2.71 (-4.07 - -1.35) |
| c-c motif chemokine ligand 28 | ccl28 | -2.28 (-3.45 - -1.11) |
| c-terminal telopeptide of collagen I | citp | 1.43 (0.69 – 2.17) |
| *Values are the β-coefficient (95% confidence interval). All reported values were significant using a false discovery rate (FDR) of 5%. The β-coefficients represent the changes in Log2 NPX values, as per Olink® standard reporting of protein concentrations.* | | |

**Supplemental Table 6.** *Change of proteins after 1 month and 9 months of spironolactone treatment*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **MONTH 1** | | **MONTH 9** | |
| **Biomarker** | ***β-coefficient (95% CI)*** | **p-value** | ***β-coefficient (95% CI)*** | **p-value** |
| lep | 0.15 (0.06-0.24) | **0.0009** | 0.07 (-0.02-0.17) | 0.14 |
| fabp4 | 0.12 (0.03-0.22) | **0.01** | 0.12 (0.02-0.23) | **0.02** |
| rarres2 | 0.05 (0-0.1) | 0.06 | 0.09 (0.04-0.14) | **0.0002** |
| thbs2 | -0.04 (-0.08-0) | 0.08 | -0.04 (-0.08--0.01) | **0.02** |
| igfbp2 | -0.05 (-0.12-0.02) | 0.17 | 0 (-0.08-0.08) | 0.96 |
| igfbp1 | -0.09 (-0.23-0.05) | 0.21 | -0.07 (-0.22-0.09) | 0.4 |
| plc | 0.02 (-0.03-0.07) | 0.39 | 0.06 (0.02-0.11) | **0.01** |
| pon3 | 0.03 (-0.04-0.1) | 0.43 | 0.03 (-0.04-0.1) | 0.38 |
| pai | 0.03 (-0.12-0.18) | 0.69 | 0.08 (-0.07-0.23) | 0.3 |
| il1ra | -0.01 (-0.08-0.06) | 0.81 | -0.03 (-0.12-0.06) | 0.5 |
| adm | 0 (-0.06-0.05) | 0.91 | 0.06 (0-0.11) | **0.046** |
| PIIINP | -0.01 (-0.27-0.25) | 0.92 | -0.05 (-0.35-0.25) | 0.76 |
|  | | | | |