Supplementary material

VASCULOMETABOLIC AND INFLAMMATORY EFFECTS OF ALDOSTERONE IN OBESITY

SHORT TITLE: ALDOSTERONE IN THE OBESE

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EXTENDED METHODS

Baseline characteristics and sample collection

All subjects filled out questionnaires about life style, previous diagnosis of hypertension and diabetes and medication use. Waist circumference was measured at the level of the umbilicus to the nearest 0.1cm. Hip circumference was measured at the level of the trochanter major. Systolic and diastolic blood pressure were measured after 30 minutes of supine rest. Hypertension was defined when by systolic blood pressure (SBP) level was ≥140 mmHg and/or diastolic blood pressure (DBP) was ≥ 90 mmHg or if the participant was currently under treatment for hypertension. Diabetes mellitus type 2 (DM 2) was defined as a fasting glucose level > 7.0 mmol/L or if the participant was currently under treatment for diabetes mellitus. The metabolic syndrome was defined following the criteria of the National Cholesterol Education Program (NCEP). We used the sum of metabolic syndrome factors (MetS) in our analysis, since the presence of the metabolic syndrome greatly enhances the risk for the development of atherosclerosis (15).

Blood samples were obtained in the morning following an overnight fast, plasma and serum were frozen at -80°C until further analysis. Blood glucose, triglycerides (TG), total cholesterol, high density lipoprotein cholesterol (HDL-C) and Apolipoprotein B (ApoB) were measured using standard laboratory procedures.

Vascular measurements

Vascular measurements were performed after an overnight fast or in the afternoon six hours after a standardized breakfast. Participants abstained from caffeinated products and smoking for twelve hours before the visit. All measurements were performed in a quiet, temperature-controlled room with the patients in supine position. After a resting period of at least 30 minutes, baseline resting diameter, distensibility and wall thickness of the carotid artery were
assessed by a well-trained sonographer. A 7.5-MHz transducer of a Mylab Class C ultrasound device (Esaote Biomedica, Genoa, Italy) connected to a computer with a data acquisition board (Art.lab). Ultrasound parameters were set to optimize longitudinal B-mode images of the lumen/arterial wall interface. The cIMT and diameter measurements were performed in the proximal 1cm straight portion of the carotid artery in three different angles (90°, 120° and 180°) for 6 seconds. The measurements were recorded during the diastolic phase.

Measurement of the cIMT was performed using an automatic boundary detection system based on RF processing-based measurement (Art.lab, Esaote Europe BV, Maastricht, Netherlands) (1). Analysis of the cIMT and diameter was performed by an independent blinded researcher. The primary outcome variable was defined as the mean cIMT of the 3 different angles (18). Subsequently the presence of plaque and the thickness of plaques in the common carotid, internal carotid, or external carotid artery or at the carotid bulbus were measured. The presence of plaque was defined as focal thickening of the wall of at least 1.5x the mean cIMT or a cIMT >1.5mm, according to the Mannheim intima-media thickness consensus (2).

Ex vivo stimulation

Whole blood stimulation was performed using 100ul of heparin blood and 400ul of stimuli in flat-bottom 48-well plates (Greiner). Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque density gradient (Oosting et al., 2015). Cells were resuspended in medium (RPMI 1640) supplemented with gentamicin 10 mg/mL, glutamine 10 mM and pyruvate 10 mM. PBMC stimulations were performed in round-bottom 96-wells plates (Greiner) with 5x10^5 cells/well for either 24 hours or 7 days in the presence of 10% human pool serum at 37°C and 5% CO2. Supernatants were collected and stored in −20°C until used for ELISA. TNF-α, IL-1β, IL-6, IL-1Ra, IL-17 and IL-22 were measured using kits from R&D systems, the IFN-γ and IL-10 kits were obtained from Sanquin.
Circulating mediators

Cytokines and circulating mediators were measured in human EDTA plasma using Enzyme Linked Immunosorbent Assay (ELISA). Adiponectin, leptin, resistin, AAT, hsCRP, and IL-18BP were measured using Set kits from R&D Systems following manufacturer’s instructions. IL-6 and IL-18 were measured by Simple Plex cartridges using the ELLA (Protein Simple, San Jose).

Lipodomics

We used a high-throughput Nuclear Magnetic Resonance (NMR) metabolomics platform (Nightingale's Biomarker Analysis Platform) (3) for the quantification of 231 lipid and metabolite measures. The NMR metabolomics platform has previously been used in various epidemiological studies (4, 5). In this study we focused on the lipoproteins with total lipid concentrations of 14 lipoprotein subclasses, lipoprotein particles sizes, apolipoproteins and cholesterol. Groups of lipoprotein particle characteristics were made based on a correlation between variables of r>0.75.

Metabolomics

Blood was collected in EDTA tubes and plasma was extracted. Plasma samples were frozen and stored at -80°C before extraction. Prior to extraction plasma samples were allowed to thaw on ice for 30-60 minutes. 20 µL of serum/plasma was aliquoted into a labeled 2 mL microtube and then 180 µL of aq. 80% LCMS-grade methanol was added. The samples were thoroughly mixed on a vortex mixer for 15 seconds to precipitate protein and afterwards allowed to incubate for 1 hour at 4°C. Samples were centrifuged (room temperature)
at > 14,000g for 15 minutes to pellet the precipitate. 100 µL of the supernatant was transferred to a fresh microtube tube. Samples were stored at -80°C prior to shipping.

Flow injection electrospray time-of-flight mass spectrometry was performed by General Metabolomics (1 Broadway, Cambridge MA 02142) to identify metabolic features based on m/z. Details of the procedure can be found in Fuhrer et al. The total number of m/z signals that could be assigned to one or more metabolites was 1339 for the 300-OB cohort.

**Assessment of fat distribution and hepatic steatosis**

Abdominal fat distribution and liver fat content were determined by magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (MRS), respectively. Both MR examinations were performed on a 3.0 T Magnetom Skyra or Trio (Siemens, Erlangen, Germany). Subjects were scanned in a supine position with their arms positioned parallel to the lateral sides of the body. At the L4-L5 level, sixteen axial T1-weighted MRI slices of 0.5 cm were acquired for each subject, with breathing commands to avoid motion-induced artifacts.

To determine liver fat content, a voxel of 27 cm³ was positioned in the right lobe of the liver, avoiding the biliary tree and large blood vessels. A STEAM(6) localization sequence without water suppression was used for data acquisition. To minimize relaxation effects on signal intensity, long repetition time (TR = 3s) and short echo time (TE = 20 ms) were used. Six scans were averaged during for 15 seconds.

**VAT, SAT, sSAT and dSAT analysis**

After data acquisition, data were analyzed with HIPPO FAT (version 1.3, V. Positano) software developed in the IDL 6.0 environment. Due to the T1-weighting, fatty tissues are represented with signal intensity in these images.
VAT, SAT, dSAT and sSAT volumes were measured on 8 separate slices, with an interslice distance of 5 mm, around the L4-L5 intervertebrate level. HIPPO FAT enables the automatic separation of SAT from VAT by generating three contour lines at each image provided by an active fuzzy clustering algorithm (8): (1) one line along the outer margin of the SAT, (2) one line along the inner margin of the SAT and (3) one line around the smallest possible region in the visceral region that included all VAT. A histogram of signal intensities in the VAT region was provided, in which a Gaussian curve automatically fitted the high-intensity peak, which identified the visceral fat.

After automatic segmentation, the analyst checked and -when needed- manually adjusted both the contour lines and the shape of Gaussian curve by eyeballing. The MRI scan allows visualization of the scarpa fascia as a fine black line. To divide sSAT from dSAT, a line was drawn manually over the scarpa fascia. Adipose tissue pixels between this line and the outer margin of the SAT were defined as sSAT. dSAT was defined by the total subcutaneous adipose tissue pixels minus the superficial subcutaneous adipose tissue pixels (dSAT = SAT – sSAT).

Interclass correlation coefficients for inter-observer comparisons were 0.799 for VAT, 0.999 for SAT, 0.998 for dSAT and 0.999 for sSAT based on N=11.

Quantification of hepatic fat content

Using the jMRUI software v3.0 package and the AMARES algorithm(9) the MR spectra were post processed to determine water (4.7 ppm) and methylene (1.3 ppm) resonance areas. Intrahepatic triglyceride content was expressed as the fraction of the methylene signal in the combined signal of methylene and water. Based on the European guidelines (10) we considered NAFLD to be present when the ratio methylene to methylene and water was higher than 5.6%.

Statistics

Regression analysis
Prior to analysis, aldosterone and renin levels were normalized using the rank-based inverse normal transformation (INT). Circulating markers, blood cell composition, cytokine production capacity, lipidomics, metabolomics, fat distribution and liver fat were transformed using the same technique. The following R code was used (R programming language):

```
transformed = rank(original)
transformed = qnorm(transformed / (length(transformed) + 0.5)),
```

where “transformed” indicated the transformed data and the original data is coded as “original”.

To evaluate associations between aldosterone or renin and various other measurements, a rank-based regression technique was applied using the “Rfit” package (Kloke and McKean, The R Journal Vol. 4/2, December 2012) in the “R” programming language. Rfit is a regression method that is more robust to outliers in response space than the standard linear models. Aldosterone or renin levels were compared to sets of measurements (e.g. metabolomics, circulating cytokine levels) or individual measurements (e.g. insulin levels). P-values were calculated by testing the null hypothesis that $\beta = 0$. All comparisons were corrected for multiple testing within each set of measurements using the Benjamini Hochberg False Discovery Rate (FDR) procedure (Benjamini, Yoav; Hochberg, Yosef (1995). Journal of the Royal Statistical Society, Series B. 57 (1): 289–300. MR 1325392). The covariates included in the analysis were age, sex, BMI, smoking, systolic and diastolic blood pressure (SBP and DBP) and season (correction of seasonality is described in Ter Horst et al (11)). The resulting formula looks like:

```
parameter1 ~ intercept + $\beta_1$*age + $\beta_2$*sex + $\beta_3$*smoking + $\beta_4$*SBP + $\beta_5$*DBP + $\beta_6$*season + $\beta_7$*parameter2. Here, parameter1 and parameter2 are the two parameters we want to associate.
```
When analyzing for associations with uric acid and white blood cell counts we added creatinine and the use of diuretic drugs as covariates to the analysis, as a surrogate marker for low circulating volume.

To evaluate the strength of the significant associations, the correlations coefficients of renin and aldosterone with the different parameters of interest were calculated using the “cor” function of the “stats” package in R. The data were corrected for the different covariates listed above using a linear regression model. The linear assumptions of this model are different from the rank based model mentioned above, however the correlation coefficients should still give a good impression of the strength of the associations. Standardized Regression Coefficients (beta) were calculated based on objects of class ‘lm’ using the ‘lm.beta’ function part of the ‘QuantPsyc’ package in the R programming language. The covariates previously described to be corrected for in the regression were included.

**Metabolite set enrichment analysis**

Using metaboanalyst.ca v4.0 (12) we performed an enrichment analysis of the metabolites showing a significant (FDR<0.05), positive association with aldosterone levels.

**SUPPLEMENTARY TABLE 1. Associations of aldosterone (and renin) with inflammatory and metabolic parameters in individuals not using antihypertensive medication.**

| Circulating markers of inflammation | No antihypertensives (55% of cohort) | No diuretic antihypertensives (80% of cohort) |
|-------------------------------------|--------------------------------------|---------------------------------------------|
|                                     | FDR (aldo)₁ | FDR (renin)₁ | FDR (aldo)₁ | FDR (renin)₁ |
| VEGF                                | ns          | ns           | ns (p=0.20) | ns           |
| IL-6                                | ns          | ns           | ns (p=0.20) | ns (p=0.09)  |
| Adiponectin                         | ns          | ns           | ns          | ns (p=-0.13) |
| AAT                                 | ns          | ns           | ns          | ns           |
| IL-18   | ns     | ns     | ns     | ns     |
|---------|--------|--------|--------|--------|
| IL-18BP | ns     | ns     | ns     | ns (p=0.09) |
| CRP     | ns     | ns     | ns     | ns     |
| Resistin| ns     | ns     | ns     | ns     |
| Leptin  | ns     | ns     | ns     | ns     |
| Cell subtypes | p<0.01 | p<0.05 | p<0.01 | p<0.05 |
| leukocytes |         |        |         |         |
| lymfocytes | p<0.05 | p<0.05 | p<0.05 | ns (p=0.06) |
| neutrofils | ns (p=0.07) | p<0.05 | p<0.05 | ns (p=0.06) |
| monocytes | ns     | ns     | ns     | ns (p=0.06) |
| trombocytes | ns     | ns (p=0.14) | ns     | ns (p=0.06) |
| Metabolic syndrome components | ns (p=0.05) | ns | p<0.05 | ns (p=0.11) |
| triglycerides |        |        |         |         |
| glucose | ns     | ns     | p<0.05 | p<0.05 |
| waist (cm) | ns     | ns     | ns     | ns     |
| HDL cholesterol | ns | ns | ns | ns |
| systolic blood pressure | ns | ns | ns | ns |
| Lipodomics | ns (p=0.12) | ns | p<0.05 | ns (p=0.14) |
| Large and XL VLDL particles | ns (p=0.19) | ns | ns | ns |
| Fat distribution | ns (p=0.12) | ns (p=0.08) | ns (p=0.09) | p<0.05 |
| Liver fat | ns     | ns     | ns     | ns (p=0.08) |
| VAT      | ns (p=0.12) | ns (p=0.08) | ns | p<0.05 |
| SAT      | ns     | ns (p=0.08) | ns | ns |

FDR < 0.05 is considered significant, FDR ≤ 0.20 is described as ‘ns’ with p-value, FDR > 0.20 is noted ‘ns’ without p-value.
SUPPLEMENTARY TABLE 2. Association of aldosterone with various metabolites.

After Benjamini FDR correction for multiple testing, aldosterone positively correlated with 165 metabolites.

| METABOLITE                                                                 | FDR         |
|---------------------------------------------------------------------------|-------------|
| X676_DOCOSATRIENOIC.ACID_333.279039                                       | 1.87E-06    |
| X845_3S.5R.6S.7E.9X..7.M_389.217866                                        | 4.39E-05    |
| X470_CYCLOHEXANEUNDECANOL_267.232787                                       | 4.39E-05    |
| X401_3METHYLCYCLOPENTADE_237.222054                                        | 6.11E-05    |
| X749_C24.5_357.27974                                                        | 9.11E-05    |
| X772_3B.ALLOTETRAHYDROCOR_365.233253                                       | 9.21E-05    |
| X532_ESTRIOL_287.164659                                                     | 9.21E-05    |
| X734_PROPOFOL.GLUCURONIDE_353.160592                                       | 9.89E-05    |
| X751_NORENDOXIFEN_358.181114                                               | 0.000105    |
| X753_C24.4_359.295167                                                      | 0.000119    |
| X609_.R..2.HYDROXYSTERCUL_309.242757                                       | 0.000148    |
| X672_C22.4_331.263905                                                      | 0.000168    |
| X448_HYPOGEIC.ACID_253.217675                                              | 0.000179    |
| X1244_GLYCEROL.1..9Z.OCTAD_645.450669                                      | 0.000202    |
| X670_CARNOSIC.ACID_331.190751                                              | 0.000209    |
| X378_C14.0_227.20125                                                       | 0.000216    |
| X744_C24.6_355.264024                                                      | 0.000216    |
| X581_2.HYDROXYESTRADIOL.3_301.179366                                       | 0.000265    |
| X864_3..HYDROXY.T2.TRIOL_397.186691                                        | 0.000287    |
| X944_LYSOPE.0.0.14.0_424.247398                                            | 0.000296    |
| X951_TARAXACOLIDE.1.O.B.D_427.197037                                       | 0.000349    |
| X663_PICROCROCIN_329.160714                                                | 0.000462    |
| X587_ARACHIDONIC.ACID_303.232849                                            | 0.000477    |
| X735_PROSTAGLANDIN.F2A_353.233159                                           | 0.00049     |
| X595_8.11.14.EICOSATRIENO_305.248262                                       | 0.00049     |
| X562_NONADECA.10.Z..ENOIC_295.264072                                       | 0.00065     |
| X320_SEDOHEPTULOSE..1.3.7_209.067473                                       | 0.000658    |
| X625_13.HDOHE_313.25304                                                    | 0.000707    |
| X1123_DIDE.O.METHYL.4.O.AL_508.166513                                       | 0.00072     |
| X634_L.CITRONELLOL.GLUCOS_317.196812                                       | 0.000878    |
| X945_PALIPERIDONE_425.2004                                                 | 0.000898    |
| X369_C14.1_225.185602                                                      | 0.000917    |
| X509_MILTIRONE_281.155681                                                  | 0.001001    |
| X521_ANDROSTENEDIONE_285.186208                                            | 0.001268    |
| X792_6.EPI.7.ISOCUCURBIC_373.186837                                        | 0.001268    |
| X969_POLYSORBATE.60_433.280878                                              | 0.001377    |
| X504_12S.HHT_279.196541                                                    | 0.001377    |
| X742_GAMMA.CROCETIN_355.191595                                             | 0.0016      |
| X441_C16.2_251.201338                                                      | 0.001851    |
| Compound                          | RT (min) |
|----------------------------------|----------|
| X1166_ESTRIOL.3.SULFATE.16_543.153378 | 0.008553 |
| X771_20.CARBOXY.LEUKOTRIE_365.197182   | 0.008893 |
| X1129_O.6.DEOXY.A.L.GALACT_510.183575 | 0.008893 |
| X912_BETA.D.XYLOPYRANOSYL_413.129765  | 0.00924  |
| X770_2R.6X_7.METHYL.3_M_365.181688     | 0.009361 |
| X895_ACIDISSIMINOL.EPOXID_408.218027  | 0.009361 |
| X707_AMP_346.055773                 | 0.0094   |
| X877_ACETYL.TRIBUTYL.CITR_401.218813 | 0.009738 |
| X1081_7.METHYL.1.4.5.NAPHT_483.15143 | 0.009738 |
| X314_ETHYL.BETA.D.GLUCOPY_207.086994  | 0.009803 |
| X85_DEOXYRIBOSE_133.050605           | 0.00999  |
| X1108_LIMONEXIC.ACID_501.175786      | 0.010234 |
| X461_Z..Z..5.TETRADE_263.23791       | 0.01069  |
| X1034_MANGOSTENONE.B_461.197012      | 0.01086  |
| X282_C12.1_197.154167                | 0.010916 |
| X791_WIKSTROMOL_373.129971           | 0.011156 |
| X674_2.6.DIMETHYL.7.OCTEN_333.191873 | 0.011156 |
| X1089_GLIMEPIRIDE_489.216394         | 0.011903 |
| X949_ADN_426.021894                 | 0.011903 |
| X867_PIPAZETHATE_398.15361           | 0.012532 |
| X928_KANZONOL.F_419.185383           | 0.013384 |
| X333_GLYCYL.HISTIDINE_211.08369      | 0.013415 |
| X508_5.7.DIMETHoxyISOFLAV_281.08259  | 0.013581 |
| X946_CINNZEYLANINE_425.217945       | 0.013929 |
| X671_9.10.13.TRIHYDROYST_331.248955 | 0.013962 |
| X927_KUWANON.A_419.149139            | 0.013962 |
| X817_MISOPROSTOL_381.264409          | 0.015178 |
| X1150_16.17.DIHYDRO.16A.17_527.213262 | 0.015533 |
| X1005_ACUMINOSIDE_447.221834        | 0.015577 |
| X922_SIMVASTATIN_417.264717          | 0.015769 |
| X659_POLYOXYETHYLENE.40.M_327.290188 | 0.016927 |
| X1075_11.OXO.AN DrosterONE_479.227988 | 0.017419 |
| X1012_SOFA Lcone_449.196978         | 0.019298 |
| X1085_EGONOL.GLUCOSIDE_487.161916   | 0.019564 |
| X1186_APIUMOSIDE_569.164211         | 0.021414 |
| X1223_CHOLESTANE.3.7.12.25_611.378678 | 0.022429 |
| X720_8.ISO.15.KETO.PGE2_349.202571  | 0.022429 |
| X1025_ATROVIRINONE_455.169676        | 0.023684 |
| X705_METHYL..10..SHOGAOL_345.243847 | 0.023949 |
| X790_GENIPOSIDIC.ACID_373.113821    | 0.024705 |
| X855_VESNARINONE_394.177271         | 0.024774 |
| X920_CUDRAFLAVONE.A_417.135896      | 0.025718 |
| X526_R.S..NORLAUDANOSOLI_286.11081  | 0.026107 |
| X1218_PRODELPHINIDIN.A1_607.109878  | 0.026474 |
| X876_CORTISONE.ACETATE_401.197138   | 0.027009 |
| X643_1.ACETOXY.2.HYDROXY_323.222702 | 0.027724 |
| Compound                  | m/z      | DP    |
|---------------------------|----------|-------|
| X863_METHYL_3.4.DIHYDROXY | 397.150484 | 0.027785  |
| X610_C20.1_309.279734     |          | 0.02807  |
| X743_5.7.MEGASTIGMADIEN_9 | 355.21242 | 0.02807  |
| X644_C21.1_323.295321     |          | 0.028623 |
| X911_5...3...45_TRIHYDRO | 413.110295 | 0.028623 |
| X524_AVOCADENE_285.242691 |          | 0.029042 |
| X669_HEMIARIENSIN_399.144852 |        | 0.029713 |
| X859_1.HEXANOL.ARABINOSYL | 395.191322 | 0.030295 |
| X741_XANTHOXYLOL_355.120233 |           | 0.030955 |
| X788_NEODIOSPYRIN_373.07392 |         | 0.030955 |
| X176_10.UNDECENAL_167.143917 |       | 0.031641 |
| X799_RESOLVIN.D2_375.217104 |           | 0.031641 |
| X968_1.1..ETHYLIDENEBISTR | 433.188126 | 0.032405 |
| X1015_ENTEROCIN.900_450.24159 |       | 0.032836 |
| X1018_8.BUTANOYLNEOSOLANIO | 451.197995 | 0.033171 |
| X685_LISURIDE_337.203174   |          | 0.033171 |
| X586_3A.16B.DIHYDROXYANDR | 303.198069 | 0.033172 |
| X973_E.3174_435.134048     |          | 0.035177 |
| X602_OBTUSILACTONE.A_307.227411 |       | 0.035591 |
| X1291_PC.15.0.18.4.6Z.9Z.1 | 738.508  | 0.037546 |
| X591_SYNDESINE_304.152334 |          | 0.037754 |
| X506_N..1.DEOXY.1.FRUCTOS | 280.102811 | 0.040345 |
| X1102_TAUROURSODEOXYCHOLIC | 498.288985 | 0.041402 |
| X505_C18.2_279.233414 (LINOLEIC ACID) | | 0.043274 |
| X1044_DOLICHLY.B.D.GLUCOSY | 465.226528 | 0.044345 |
| X833_CORCHOIONOL.C.9.GLUC | 385.186653 | 0.045711 |
| X433_C16.3_249.185841     |          | 0.0462  |
| X486_3HYDROXYTETRADECANE_ | 273.169985 | 0.046356 |
| X673_1.5.DIBUTYL METHYL.H_333.155429 |       | 0.046912 |
| X807_6.GINGERDIOL.3.5.D_379.212944 |       | 0.047408 |
| X808_BISNORCHOLIC.Acid_379.250323 |       | 0.047408 |
| X541_GLYCEROL.1.5.HYDROX | 289.201368 | 0.048199 |
| X1045_ANDROSTERONE.GLUCURO_465.248721 |       | 0.048416 |
| X1050_DUKUNOLIDE.D_467.170444 |          | 0.049692 |

1. Brands PJ, Hoeks AP, Willigers J, Willekes C, Reneman RS. An integrated system for the non-invasive assessment of vessel wall and hemodynamic properties of large arteries by means of ultrasound. European journal of ultrasound : official journal of the European Federation of Societies for Ultrasound in Medicine and Biology. 1999;9(3):257-66.
2. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovascular diseases. 2012;34(4):290-6.
3. Soininen P, Kangas AJ, Wurtz P, Tukiainen T, Tynkkynen T, Laatikainen R, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst. 2009;134(9):1781-5.
4. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation. 2015;131(9):774-85.
5. Kettunen J, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikainen LP, et al. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nature genetics. 2012;44(3):269-76.
6. Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, Sauter R. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. Magnetic resonance in medicine. 1989;9(1):79-93.
7. Positano V, Gastaldelli A, Sironi AM, Santarelli MF, Lombardi M, Landini L. An accurate and robust method for unsupervised assessment of abdominal fat by MRI. Journal of magnetic resonance imaging : JMRI. 2004;20(4):684-9.
8. Positano V, Cusi K, Santarelli MF, Sironi A, Petz R, Defronzo R, et al. Automatic correction of intensity inhomogeneities improves unsupervised assessment of abdominal fat by MRI. Journal of magnetic resonance imaging : JMRI. 2008;28(2):403-10.
9. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson. 1997;129(1):35-43.
10. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of hepatology. 2016;64(6):1388-402.
11. Ter Horst R, Jaeger M, Smeekens SP, Oosting M, Swertz MA, Li Y, et al. Host and Environmental Factors Influencing Individual Human Cytokine Responses. Cell. 2016;167(4):1111-24 e13.
12. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486-W94.