**SUPPLEMENTAL MATERIALS**

**Sex-specific regulation of inflammation and metabolic syndrome in obesity**
Ter Horst – Immunometabolic regulation in obesity

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# Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

**Animals (in vivo studies)**

| Species | Vendor or Source | Background Strain | Sex | Persistent ID / URL |
|---|---|---|---|---|
| NA | | | | |
| | | | | |
| | | | | |

**Genetically Modified Animals**

| | Species | Vendor or Source | Background Strain | Other Information | Persistent ID / URL |
|---|---|---|---|---|---|
| **Parent - Male** | NA | | | | |
| **Parent - Female** | | | | | |

**Antibodies**

| Target antigen | Vendor or Source | Catalog # | Working concentration | Lot # (preferred but not required) | Persistent ID / URL |
|---|---|---|---|---|---|
| NA | | | | | |
| | | | | | |

**DNA/cDNA Clones**

| Clone Name | Sequence | Source / Repository | Persistent ID / URL |
|---|---|---|---|
| NA | | | |
| | | | |
| | | | |

**Cultured Cells**

| Name | Vendor or Source | Sex (F, M, or unknown) | Persistent ID / URL |
|---|---|---|---|
| NA | | | |
| | | | |
| | | | |

**Data & Code Availability**

| Description | Source / Repository | Persistent ID / URL |
|---|---|---|
| Data availability | BBMRI | https://hfgp.bbmri.nl/ |
| | | |
| | | |

**Other**

| Description | Source / Repository | Persistent ID / URL |
|---|---|---|
| Human IL-1β ELISA Kit | RD Systems | DY201 |
| Human IL-6 ELISA Kit | Sanquin | M9316 |
| Human TNF-α ELISA Kit | RD Systems | DY210 |

| | | |
|---|---|---|
| Human IL-22 ELISA Kit | RD Systems | DY782 |
| Human IL-17 ELISA Kit | RD Systems | DY317 |
| Human IFNγ ELISA Kit | Sanquin | M9333 |
| Human IL-1Ra ELISA Kit | RD Systems | DRA00B |
| Human Resistin ELISA Kit | R&D Systems ELISA | |
| Human Leptin ELISA Kit | R&D Systems ELISA | |
| Human Adiponectin ELISA Kit | R&D Systems ELISA | |
| Human AAT ELISA Kit | R&D Systems ELISA | |
| Human IL-1Ra ELISA Kit | R&D Systems ELISA | |
| Human IL-18 ELISA Kit | Simple Plex cartridges using the Ella apparatus (Protein Simple, San Jose, CA) | |
| Human IL-18BP ELISA Kit | R&D Duoset | |
| Plasma IL-6 ELISA Kit | Simple Plex cartridges using the Ella apparatus (Protein Simple, San Jose, CA) | |
| Plasma VEGF ELISA Kit | Simple Plex cartridges using the Ella apparatus (Protein Simple, San Jose, CA) | |
| Plasma hsCRP ELISA Kit | R&D Systems ELISA | |

**Supplementary methods**

**Measurements and sample collection details**

The 300-Obesity (300-OB) cohort study consists of 302 individuals between 55 and 80 years of age with a BMI > 27 kg/m$^2$ at screening. All participants were included between the year 2014 and 2016. This 300-OB cohort described in this study is part of the Human Functional Genomics Project (HFGP) at the Radboud university medical center, which contains a collection of cohorts with various backgrounds. Most of these participants previously took part in the Nijmegen Biomedical Study – Non-Invasive Measurements of Atherosclerosis 1 (NBS-NIMA1), a population-based survey of inhabitants of the municipality of Nijmegen, The Netherlands[1]. 949 of the 1,517 participants of the NBS-NIMA1 study (with a BMI > 25 kg/m$^2$ at the time of NBS-NIMA1) were invited to participate. 472 subjects were excluded, mainly because they did not fulfill the criterion BMI > 27 kg/m2. Another 250 subjects refused. This resulted in 227 individuals willing to participate and fulfilling all inclusion criteria. Additionally, we recruited 75 participants that were acquaintances of first 227 participants, fulfilling the inclusion criteria (age > 55 years and BMI > 27 kg/m$^2$). Exclusion criteria for the 300-OB cohort were:

- a recent cardiovascular event (MI, transient ischemic attack or stroke <6 months)
- prior bariatric surgery or bowel resection
- inflammatory bowel disease (IBD)
- renal dysfunction
- increased tendency for bleeding
- use of anti-coagulant therapy (oral or subcutaneous)
- use of thrombocyte aggregation inhibitors other than acetylsalicylic acid or carbasalate calcium

All subjects filled out questionnaires, which included questions about life style, environmental factors and medication usage. If used, participants temporarily discontinued lipid-lowering therapy four weeks prior to the measurements. Blood samples were taken in the morning following an overnight fast. All women were postmenopausal and were not receiving hormonal replacement therapy. All participants received detailed printed and oral information and subsequently gave written informed consent. The study was approved by the Ethical Committee of the Radboud University (nr. 46846.091.13). Experiments were conducted according to the principles expressed in the Declaration of Helsinki.

<u>The metabolic syndrome</u>

We used criteria defined in the National Cholesterol Education Program (NCEP) to diagnose the metabolic syndrome[2]. The metabolic syndrome at the time of this study is defined by the NCEP ATP-III criteria as the presence of any three of the following five traits:

- Abdominal obesity, defined as a waist circumference in men ≥102 cm (40 in) and in women ≥88 cm (35 in)
- Serum triglycerides (TG) ≥150 mg/dL (1.7 mmol/L) or drug treatment for elevated TG
- Serum high-density lipoprotein cholesterol (HDL-C) <40 mg/dL (1 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women or drug treatment for low HDL-C
- Blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure
- Fasting plasma glucose ≥100 mg/dL (5.6 mmol/L) or drug treatment for elevated blood glucose

Blood glucose, TG, total cholesterol and HDL-C were measured using standard laboratory procedures. Before measuring systolic and diastolic blood pressure, participants took 30 minutes of supine rest. Hypertension was defined as a systolic blood pressure (SBP) level > 140 mmHg and/or diastolic blood pressure (DBP) > 90 mmHg, or currently undergoing treatment for hypertension. Type 2 diabetes mellitus was defined as a glucose level over 7.0 mmol/L after an overnight fast or currently undergoing treatment for type 2 diabetes. Weight and height were determined during visitation, and this was used to calculate BMI. The BMI was calculated as body weight (kg) divided by the square of height (m). Waist circumference

was measured at the level of the umbilicus and hip circumference was measured at the level of the trochanter major. Both of there were rounded to the nearest first decimal.

**Circulating mediators**
Circulating mediators (cytokines and adipokines) were measured in human EDTA plasma using Enzyme Linked Immunosorbent Assay (ELISA). Concentrations of circulating mediators resistin, leptin, adiponectin, hsCRP and alpha-1 antitrypsin (AAT) were determined in EDTA plasma using the R&D Systems ELISA kits following the Manufacturer's protocol. Plasma interleukin(IL)-18, IL-6 and VEGF were measured in Simple Plex cartridges using the Ella apparatus (Protein Simple, San Jose, CA). IL-18 binding protein (IL-18BP) concentrations were measured using R&D Duoset kits following the manufacturer's standard protocol.

**Stimulation experiments**
PBMC stimulation experiments
At baseline, 20 mL EDTA venous blood was collected. Isolation of peripheral blood mononuclear cells (PBMCs) was performed as described in Oosting et al[3]. In short, the PBMCs were isolated within 6 hours by density gradient centrifugation of PBS diluted blood (1:1) over Ficoll-Paque, washed twice with PBS and subsequently resuspended in Dutch modified RPMI 1640 medium (Invitrogen) supplemented with 50 µg/mL gentamicin (Centrafarm), 2 mM GlutaMAX and 1 mM pyruvate (Life Technologies). PBMC stimulations were performed with $5x10^5$ cells/well in round-bottom 96-wells plates (Greiner) at 37 °C and 5% $CO_2$. Supernatants were collected after 24 hours and 7 days and stored at -20 °C until ELISA measurements were performed. The different stimuli and cytokines that were measured, are shown in Table 1 and 2.

Whole blood stimulation experiments
At baseline, 10 ml heparin blood was collected. A volume of 100 µl of heparin blood was added to a 48 well plate (Corning) containing 400 µl stimulus (final volume 500ul/well) for 48 hours at 37°C and 5% $CO_2$. Supernatants were collected and stored in -20°C until ELISA measurements were performed. The different stimuli and cytokines that were measured, are shown in Table 1 and 2.

ELISA analysis
Cytokine concentrations after stimulation were measured using commercially available ELISA kits. For the 24-hour PBMC stimulation and whole blood experiments the following kits were used:

- IL-1β: R&D systems, DY201
- IL-1Ra: R&D systems, DRA00B
- TNFα: R&D systems, DY210
- IL-6: Sanquin, M9316

For the 7-day PBMC stimulation experiments the following kits were used,

- IL-17: R&D systems, DY317
- IL-22: R&D systems, DY782
- IFNγ: Sanquin, M9333

All measurements we done according to the protocols supplied by the manufacturer.

**Cell count data**
Immune cell counts were determined in fresh whole blood EDTA samples using the Sysmex XE-5000. The Sysmex XE-5000 was used in a laboratory automation configuration part of a routine clinical setting. All analyses were preformed following the manufacturer's protocol. Details about the XE-5000 and automatic measurements can be found on the manufacturer's website.

**Metabolomics**
Untargeted 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 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.[4]. The total number of m/z signals that could be assigned to one or more metabolites was 1339 for the 300-OB cohort and 1979 for the 500FG cohort.

Lipidomics

A high-throughput Nuclear Magnetic Resonance (NMR) metabolomics platform (Nightingale's Biomarker Analysis Platform) was used to quantify a total of 231 lipid and metabolite measures in EDTA plasma. Most of these measures were very highly correlated to other measures from the same platform. Groups of lipoprotein particle characteristics were therefore made based on a correlation between variables of at least r>0.75 and expert knowledge. This led to 17 groups comprising of lipoprotein subclasses, lipoprotein particles sizes, apolipoproteins and cholesterol (Table 12). For each of these 17 groups a representative variable was selected to represent the whole group of measurements. This led to easier interpretation and less strict multiple testing correction.

**Adipose tissue analysis**

**Adipose tissue biopsies.** Subcutaneous adipose tissue biopsies were obtained under local anesthesia by needle biopsies performed 6-10cm lateral to the umbilicus in the right lower quadrant, after an overnight fast. Abdomen biopsies were rinsed with PBS after they were taken with a Sterican single-use needle (4665473) from B/braun. Specimens were placed in embedding cassettes and fixed in 4% paraformaldehyde (37%, VWR). After at least 48 hours, cassettes were processed overnight (o/n) with the Excelsior™ AS Tissue Processor (Thermo Scientific). Thereafter, samples were embedded in paraffin. Sectioning of specimens was done to make 8 µm thick coupes. Coupes were mounted per two on a glass slide and allowed to dry for 30 minutes. At last the coupes were incubated overnight at 37°C and stored at 4°C until later use

**Adipocyte size.** Haematoxylin and Eosin (H&E) staining was done to determine adipocyte size. The slides were first incubated two times in xylene (Depot) for 5 minutes and subsequently 5 minutes in 100%, 96% and 70% ethanol, respectively. The slides were then incubated for 20 minutes in haematoxylin (haematoxylin was prepared using the following protocol: first, 1 g haematoxylin (VWR) was added in 10 mL ethanol 96%. Then 200 mg sodiumlodate (VWR), 50 g potassiumaluin (VWR) and the complete dissolved haematoxylin(10 mL) was added to 1000 mL aquadest. Dissolved o/n at RT and 50 g chloralhydrate (VWR) and 1 g citric acid (Sigma) was added. Solution was mixed well, used after 2 days and filtered before use.) and rinsed in running tap water for 10 minutes. After that, the slides were incubated for 5 minutes in eosin (eosin was prepared using the following protocol: first, 1 g eosine-G (VWR) was added in 100 mL aquadest, then 100 mL 96% ethanol was added. Before used, solution was diluted 1:5 with aquadest), rinsed short in 70% ethanol and incubated for 5 minutes in 96% and 100% ethanol, respectively. At last the slides were incubated two times in xylene (Depot) for 5 minutes. Finally, the slides were sealed with Permount (Fischer Scientific) and a cover slide. After staining, adipocytes were scored on their size with the KS-400 software. This was done with the microscope Zeiss Axiophoto.

The morphometry of individual fat cells was afterwards assessed using digital image analyses as described previously [5]. For each participant, the adipocyte cell diameters of all adipocytes

in four microscopic fields of view were counted and measured. On average, 219 adipocytes (range 113 - 330) were measured per field.

**Presence of macrophages** To detect macrophages, adipose tissue sections were incubated with a CD68-monoclonal antibody (Serotec, Oxford, UK). Sections were preincubated with 20% normal horse serum followed by overnight incubation at 4 °C with the primary antibody diluted 1:40 in phosphate-buffered saline, 1% bovine serum albumin. After incubation with the primary antibody (mouse anti-human), a horse anti-mouse IgG conjugated to horseradish peroxidise (Vector labs brunschwig) was used as a secondary antibody. Visualization of the complex was done using 3,3'-diaminobenzidene for 12 min. Negative controls were used by omitting the primary antibody. Hematoxylin and eosin staining of sections was done using standard protocols. The percentage of macrophages was expressed as the total number of macrophages divided by the total number of adipocytes counted in 15 random microscopic fields of view. A crown-like structure was defined as an adipocyte surrounded by at least three macrophages [6].

**Quantification and statistical analysis**

Data preprocessing and scaling

For circulating markers and cytokines produced after stimulation, any value above or below a detection limit was set to that detection limit. Metabolomic data, lipidomic data, cytokine production capacity data, circulating inflammatory marker data were transformed using the rank based inverse normal transformation (INT) before analysis unless stated otherwise. The following R code was used (R programming language[7]):

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

where "transformed" is the transformed data and "original" the original data.

Metabolic syndrome and sex

Analysis was performed in the R programming language[7], using the 'lm' function from the "stats" package. A linear model was created with the following formula:

markerOfInterest ~ intercept + β1*age + β2*sex + β3*season + β4*metabolicSyndromeStatus

Details for the seasonality correction are provided below. The model was run 3 times, once for all data, once for just men and once for just women. In case the model was run for just one sex, the correction factor "sex" was removed. Each run p-values for regression coefficients of the relevant markers were corrected for multiple testing using the Benjamini-Hochberg FDR procedure[8], using FDR<0.05 after correction as the threshold for statistical significance.

To assess if any of our biological markers (circulating markers of inflammation, cell subsets, cytokine production capacity) showed significant differences between women and men when comparing individuals with and without metabolic syndrome, we calculated interaction effects between someone's "metabolic syndrome status" and sex:

markerOfInterest ~ intercept + β1*age + β2*sex + β3*season+ β4*metabolicSyndromeStatus + β5*sex:metabolicSyndromeStatus

The p-values were calculated for the estimate of the interaction term. Specifically, the "lm" function calculates a t-statistic to test whether the corresponding regression coefficient is different from 0.

The significance of the correlation between leptin and IL-6 was calculated using Spearman correlation and the "cor.test" function in the R programming language[7]. The null hypothesis in this test is that $\rho = 0$.

Similar approached were taken for each of the five factors that make up metabolic syndrome. In that case "metabolicSyndromeStatus" is replaced by one of these factors, for instance "highTriglycerides".

Associations between host factors, circulating markers, metabolomics, cell subtypes

Associations between parameters were calculated using the 'lm' function from the "stats" package in the R programming language[7]. The following data-types were associated to one another in this way for different analyses:

- age and sex with circulating markers of inflammation, cell subtypes (absolute and percentages), cytokine production capacity
- circulating markers of inflammation with circulating markers of inflammation and cell subtypes (absolute and percentages), metabolomics

A linear model was created with the following formula:

parameter1 ~ intercept + β1*age + β2*sex + β3*season + β4*parameter2

here, parameter1 and parameter2 are the parameters of interest. So, e.g. in the association between circulating markers of inflammation and cell subtypes, one comparison would be:

IL-6 ~ intercept + β1*age + β2*sex + β3*season + β4*neutrophils%

So, the association between IL-6 and neutrophils% is evaluated, whilst correcting for age, sex and season. Seasonality correction is explained below. The function "lm" in R provides the p-values. This function calculates a t-statistic to test whether the corresponding regression coefficient is different from 0. A hypothesis test using this t-statistic generates the p-value. Multiple testing correction was performed using the Benjamini-Hochberg FDR procedure[8].

Seasonality correction

The seasonality correction was performed similar to Ter Horst et al.[9]. This was introduced to correct for seasonal differences in levels of inflammatory markers and cytokine production capacity. In short, the following terms were added to the regression formula:

- sin(2 * pi * numDaysFromJan2014 / 365)
- cos(2 * pi * numDaysFromJan2014 / 365)
- numDaysFromJan2014

So, in the regression formula the following would be inserted:

β1*sin(2 * pi * numDaysFromJan2014 / 365) + β2*cos(2 * pi * numDaysFromJan2014 / 365) + β3*numDaysFromJan2014

where sin(2 * pi * numDaysFromJan2014 / 365) and cos(2 * pi * numDaysFromJan2014 / 365) combined capture seasonality patterns with a periodicity of one year. The linear term "numDaysFromJan2014", which indicates how many days after January 1st 2014 the sample was collected, was added to correct for potential sample storage degradation. For the 500FG cohort the same corrections were applied, though here numDaysFromJan2014 was replaced with numDaysFromJan2013, since samples were collected a year earlier.

PCA and tSNE

Principal component analysis (PCA) was applied to the inflammatory markers and circulating metabolite data. Any individuals with missing values for any marker were excluded from this analysis. The data was first inverse rank transformed followed by mean centering and standardization. PCA was performed in the R programming language using the "prcomp" function that is part of the "stats" package.

Metabolic association analysis

Associations between metabolite levels and circulating markers of inflammation were calculated using a linear model correcting for age, sex and seasonality. Seasonality corrections were performed as described in Ter Horst *et al.*[9]. The linear models were constructed using the "lm" function of the "stats" package in the R programming language. All p-values were calculated using linear regression by testing the null hypothesis that $\beta = 0$ for relationship between a metabolite and a marker of inflammation. Both the cytokine data and the metabolite data were transformed using a Rank-Based Inverse Normal Transformation. Associations were corrected for multiple testing using the Benjamini-Hochberg FDR-procedure[8].

Metabolic pathway analysis

Metabolic pathway analysis was performed using an adaptation of Gene Set Enrichment Analysis (GSEA), as originally developed by Subramanian et al.[10]. A fast implementation in the

R programming language called Fast Gene Set Enrichment Analysis (FGSEA)[11] was used, using the -log10(p-value) for each metabolite as the score.
Each mass/charge-ratio detected by the metabolomics could be the result of one or more metabolites. In the pathway analysis, all these metabolites were assigned the same score. However, if they mapped to any common pathways, the mass/charge-ratio was only counted once for that pathway, to avoid artificial enrichment. This was achieved by removing all but one of the metabolites with the same m/z value from each pathway.
The pathways provided by the KEGG pathway database[12] were used for enrichment analysis. Interesting pathways were visualized using Pathview[13], plotting the -log10(p-values) for the metabolites, setting any p-value smaller than 1e-4 to exactly 1e-4 for visualization purposes. Negative associations are indicated in blue in these plots and positive associations with red.
Top pathways for the circulating markers of inflammation were selected by taking the average of the lowest three p-values over all circulating markers and ordering them by these p-values.

## References


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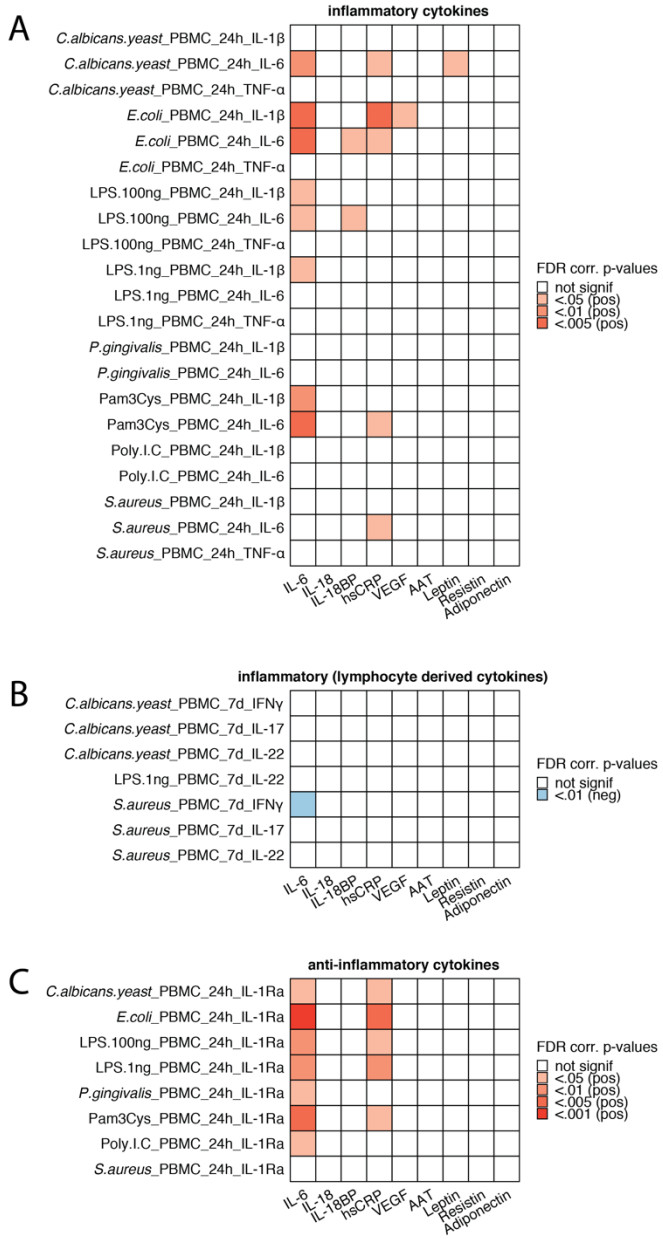
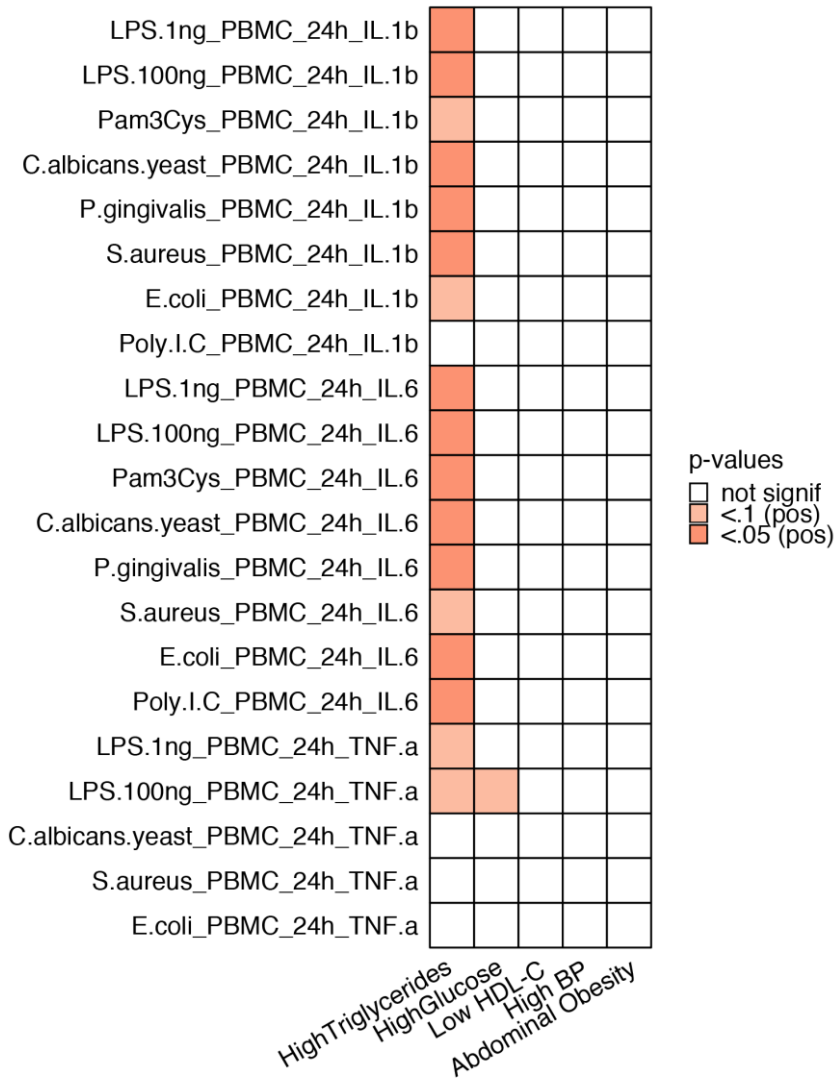
## Supplementary Figures




Supplemental Figure I – *Significance of the associations between circulating markers of inflammation and cytokine production capacity. (A) Pro-inflammatory monocyte derived cytokines. (B) Pro-inflammatory lymphocyte derived cytokines, (C) IL-1Ra (anti-inflammatory). P-values were calculated using linear regression by testing the null hypothesis that β = 0 for relationship between markers of inflammation (independent variable) and cytokine production capacity (dependent variable), see methods for details.*




Supplemental Figure II - *Significance of the interaction effect between sex and the 5 conditions that define metabolic syndrome. Red indicates that the increase of women with metabolic syndrome vs. those without was significantly lower than men with metabolic syndrome vs. those without, i.e. red means men show a stronger increase than women or women show a stronger decrease than men. The interaction effects were calculated using linear regression, with the null hypothesis that β = 0 for the interaction effect between sex and each of the 5 components making up metabolic syndrome (independent variables) and cytokine production capacity (dependent variables).*

**Supplementary Tables**

**24h PBMC stimulations + RPMI**

| **Stimulus 50uL/well** | **Final concentration** | |
|---|---|---|
| LPS low dose | 1,00E+00 | ng/mL |
| LPS high dose | 1,00E+02 | ng/mL |
| Pam3Cys | 1,00E+00 | ug/mL |
| *Candida Albicans* Yeast | 1,00E+06 | /mL |
| *Porphyromonas gingivalis* | 1,00E+06 | /mL |
| *Staphylococcus aureus* | 1,00E+06 | /mL |
| *Escheria coli* | 1,00E+06 | /mL |
| Poly I:C | 1,00E+01 | ug/mL |

**7d PBMC stimulations + RPMI + 10% human serum**

| **Stimulus 50uL/well** | **Final concentration** | |
|---|---|---|
| LPS | 1,00E+00 | ng/mL |
| *Candida albicans* Yeast | 1,00E+00 | ug/mL |
| *Staphylococcus aureus* | 1,00E+06 | /mL |

**24h Whole Blood stimulations + RPMI**

| **Stimulus 50uL/well** | **Final concentration** | |
|---|---|---|
| LPS | 1,00E+02 | ng/mL |
| PHA | 1,00E+01 | ug/mL |
| *Candida Albicans Yeast* | 1,00E+06 | /mL |
| *Staphylococcus Aureus* | 1,00E+06 | /mL |
| *Porphyromonas gingivalis* | 1,00E+06 | /mL |

Supplemental Table I - *Concentrations used for the cytokine production capacity experiments.*

| Group | Group representative | Metabolites |
|---|---|---|
| 1 | **XL-VLDL-L_mmol/l** | XXL-VLDL-P_mol/l |
| | | XXL-VLDL-L_mmol/l |
| | | XXL-VLDL-PL_mmol/l |
| | | XXL-VLDL-C_mmol/l |
| | | XXL-VLDL-CE_mmol/l |
| | | XXL-VLDL-FC_mmol/l |
| | | XXL-VLDL-TG_mmol/l |
| | | XL-VLDL-P_mol/l |
| | | XL-VLDL-L_mmol/l |
| | | XL-VLDL-PL_mmol/l |
| | | XL-VLDL-C_mmol/l |
| | | XL-VLDL-CE_mmol/l |
| | | XL-VLDL-FC_mmol/l |
| | | XL-VLDL-TG_mmol/l |
| 2 | **M-VLDL-L_mmol/l** | L-VLDL-P_mol/l |
| | | L-VLDL-L_mmol/l |
| | | L-VLDL-PL_mmol/l |
| | | L-VLDL-C_mmol/l |
| | | L-VLDL-CE_mmol/l |
| | | L-VLDL-FC_mmol/l |
| | | L-VLDL-TG_mmol/l |
| | | M-VLDL-P_mol/l |
| | | M-VLDL-L_mmol/l |
| 3 | **XS-VLDL-L_mmol/l** | S-VLDL-C_mmol/l |
| | | S-VLDL-CE_mmol/l |
| | | XS-VLDL-P_mol/l |
| | | XS-VLDL-L_mmol/l |
| | | XS-VLDL-PL_mmol/l |
| | | XS-VLDL-C_mmol/l |
| | | XS-VLDL-CE_mmol/l |
| | | XS-VLDL-FC_mmol/l |
| 4 | **L-LDL-L_mmol/l** | IDL-P_mol/l |
| | | IDL-L_mmol/l |
| | | IDL-PL_mmol/l |
| | | IDL-C_mmol/l |
| | | IDL-CE_mmol/l |
| | | IDL-FC_mmol/l |
| | | IDL-TG_mmol/l |
| | | L-LDL-P_mol/l |
| | | L-LDL-L_mmol/l |
| | | L-LDL-PL_mmol/l |
| | | L-LDL-C_mmol/l |
| | | L-LDL-CE_mmol/l |
| | | L-LDL-FC_mmol/l |
| | | L-LDL-TG_mmol/l |
| | | M-LDL-P_mol/l |
| | | EstC_mmol/l |
| | | FreeC_mmol/l |
| 6 | **ApoB_g/l** | ApoB_g/l |
| 7 | **XL-HDL-L_mmol/l** | XL-HDL-P_mol/l |
| | | XL-HDL-L_mmol/l |
| | | XL-HDL-PL_mmol/l |
| | | XL-HDL-C_mmol/l |
| | | XL-HDL-CE_mmol/l |
| | | XL-HDL-FC_mmol/l |
| 8 | **XL-HDL-TG_mmol/l** | XL-HDL-TG_mmol/l |
| 9 | **L-HDL-L_mmol/l** | L-HDL-P_mol/l |
| | | L-HDL-L_mmol/l |
| | | L-HDL-PL_mmol/l |
| | | L-HDL-C_mmol/l |
| | | L-HDL-CE_mmol/l |
| | | L-HDL-FC_mmol/l |
| | | HDL-D_nm |
| | | HDL-C_mmol/l |
| | | HDL2-C_mmol/l |
| 10 | **L-HDL-TG_mmol/l** | L-HDL-TG_mmol/l |
| 11 | **M-HDL-L_mmol/l** | M-HDL-P_mol/l |
| | | M-HDL-L_mmol/l |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | M-VLDL-PL_mmol/l | | | M-LDL-L_mmol/l | | | M-HDL-PL_mmol/l |
| | | M-VLDL-C_mmol/l | | | M-LDL-PL_mmol/l | | | M-HDL-C_mmol/l |
| | | M-VLDL-CE_mmol/l | | | M-LDL-C_mmol/l | | | M-HDL-CE_mmol/l |
| | | M-VLDL-FC_mmol/l | | | M-LDL-CE_mmol/l | | | M-HDL-FC_mmol/l |
| | | M-VLDL-TG_mmol/l | | | M-LDL-FC_mmol/l | **12** | **M-HDL-TG_mmol/l** | M-HDL-TG_mmol/l |
| | | S-VLDL-P_mol/l | | | M-LDL-TG_mmol/l | | | S-HDL-TG_mmol/l |
| | | S-VLDL-L_mmol/l | | | S-LDL-P_mol/l | | | HDL-TG_mmol/l |
| | | S-VLDL-PL_mmol/l | | | S-LDL-L_mmol/l | **13** | **S-HDL-L_mmol/l** | S-HDL-P_mol/l |
| | | S-VLDL-FC_mmol/l | | | S-LDL-PL_mmol/l | | | S-HDL-L_mmol/l |
| | | S-VLDL-TG_mmol/l | | | S-LDL-C_mmol/l | | | S-HDL-C_mmol/l |
| | | XS-VLDL-TG_mmol/l | | | S-LDL-CE_mmol/l | | | S-HDL-CE_mmol/l |
| | | VLDL-D_nm | | | S-LDL-FC_mmol/l | **14** | **S-HDL-PL_mmol/l** | S-HDL-PL_mmol/l |
| | | VLDL-C_mmol/l | | | S-LDL-TG_mmol/l | | | S-HDL-FC_mmol/l |
| | | Serum-TG_mmol/l | | | LDL-C_mmol/l | **15** | **LDL-D_nm** | LDL-D_nm |
| | | VLDL-TG_mmol/l | | | LDL-TG_mmol/l | **16** | **HDL3-C_mmol/l** | HDL3-C_mmol/l |
| | | | **5** | **Serum-C_mmol/l** | Serum-C_mmol/l | **17** | **ApoA1_g/l** | ApoA1_g/l |
| | | | | | Remnant-C_mmol/l | | | |

Supplemental Table II - *Representatives of different metabolite groups. The groups are created by clustering based on an intercorrelation >0.75 and lipoprotein physiology.* XXL-VLDL *Chylomicrons and extremely large VLDL particles*; XL- *Very large*; L- *Large*; M- *Medium*; S- *Small*; XS- *Extremely small*; VLDL *Very Low Density Lipoproteins*; IDL *Intermediate Density Lipoproteins*; LDL *Low Density Lipoproteins*; HDL *High Density Lipoproteins*;-P *Concentration of particles*; -L *Total lipids*; -PL *Phospholipids*; -C *Total cholesterol*; -CE *Cholesterol esters*; -FC *Free cholesterol*; -TG *Triglycerides*; -D *Mean diameter*; Serum-C *Serum cholesterol*; Remnant-C *Remnant cholesterol (non-HDL, non-LDL -cholesterol)*; Est *Esterified*; HDL2-C *Total cholesterol in HDL2*; HDL3-C *Total cholesterol in HDL3*-

| | Age | Sex |
|---|---|---|
| **AAT** | 0,142388 | 0,002371 |
| **Adiponectin** | 0,11097 | -0,4881 |
| **hsCRP** | 0,118479 | -0,19209 |
| **IL-18** | 0,074128 | 0,132304 |
| **IL-18BP** | 0,197454 | 0,026752 |
| **IL-6** | 0,204359 | -0,02637 |
| **Leptin** | 0,05913 | -0,6612 |
| **Resistin** | 0,132618 | 0,00887 |
| **VEGF** | -0,01743 | -0,05304 |

Supplemental Table III - *Standardized regression coefficients (betas) for the regression analysis between circulating markers of inflammation and age and sex. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless these were the factors for which the beta was being calculated).*

| | AAT | Adiponectin | hsCRP | IL-18 | IL-18BP | IL-6 | Leptin | Resistin | VEGF |
|---|---|---|---|---|---|---|---|---|---|
| **AAT** | | 0,02481405 | -0,04729 | 0,069933 | 0,063567 | 0,056892 | 0,065797 | -0,01318 | -0,0081 |
| **Adiponectin** | 0,024814 | | -0,05541 | -0,16879 | -0,1775 | -0,07114 | -0,13452 | -0,03397 | -0,20552 |
| **hsCRP** | -0,04729 | -0,0554144 | | 0,164025 | 0,163706 | 0,529467 | 0,263841 | 0,121842 | 0,163314 |
| **IL-18** | 0,069933 | -0,1687873 | 0,164025 | | 0,27935 | 0,186326 | 0,069173 | 0,030436 | 0,144312 |
| **IL-18BP** | 0,063567 | -0,1774967 | 0,163706 | 0,27935 | | 0,188381 | 0,165415 | 0,281947 | 0,131872 |
| **IL-6** | 0,056892 | -0,0711418 | 0,529467 | 0,186326 | 0,188381 | | 0,314167 | 0,073959 | 0,157365 |
| **Leptin** | 0,065797 | -0,1345161 | 0,263841 | 0,069173 | 0,165415 | 0,314167 | | 0,135124 | 0,145826 |
| **Resistin** | -0,01318 | -0,0339718 | 0,121842 | 0,030436 | 0,281947 | 0,073959 | 0,135124 | | 0,093136 |
| **VEGF** | -0,0081 | -0,2055238 | 0,163314 | 0,144312 | 0,131872 | 0,157365 | 0,145826 | 0,093136 | |

Supplemental Table IV - *Standardized regression coefficients (betas) for the regression analysis between circulating markers of inflammation. The betas were generated using a linear regression model. The data were corrected for age, sex and season.*

P-values

| | AAT | Adiponectin | hsCRP | IL-18 | IL-18BP | IL-6 | Leptin | Resistin | VEGF |
|---|---|---|---|---|---|---|---|---|---|
| **AAT** | | | | | | | | | |
| **Adiponectin** | 0,1462 | | | | | | | | |
| **hsCRP** | 0,3353 | -0,6718 | | | | | | | |
| **IL-18** | 0,6284 | 0,6146 | -0,5676 | | | | | | |
| **IL-18BP** | 0,6425 | -0,987 | -0,3314 | -0,6956 | | | | | |
| **IL-6** | 0,5403 | -0,0216 | 0,8371 | 0,1165 | 0,5525 | | | | |
| **Leptin** | -0,2731 | -0,7819 | 0,7311 | -0,857 | -0,4113 | 0,0098 | | | |
| **Resistin** | 0,4235 | -0,7861 | -0,6457 | 0,5942 | 0,9066 | 0,2006 | -0,8596 | | |
| **VEGF** | -0,8315 | 0,1487 | -0,4438 | -0,5628 | 0,8304 | 0,0098 | -0,9849 | 0,6818 | |

FDR corrected P-values

| | AAT | Adiponectin | hsCRP | IL-18 | IL-18BP | IL-6 | Leptin | Resistin | VEGF |
|---|---|---|---|---|---|---|---|---|---|
| **AAT** | | | | | | | | | |
| **Adiponectin** | 0,8922 | | | | | | | | |
| **hsCRP** | 0,937745 | -0,9377455 | | | | | | | |
| **IL-18** | 0,937745 | 0,93774545 | -0,93775 | | | | | | |
| **IL-18BP** | 0,937745 | -0,987 | -0,93775 | -0,93775 | | | | | |
| **IL-6** | 0,937745 | -0,2592 | 0,937745 | 0,8922 | 0,937745 | | | | |
| **Leptin** | -0,93775 | -0,9377455 | 0,937745 | -0,93775 | -0,93775 | 0,1764 | | | |
| **Resistin** | 0,937745 | -0,9377455 | -0,93775 | 0,937745 | 0,959929 | 0,937745 | -0,93775 | | |
| **VEGF** | -0,93775 | 0,8922 | -0,93775 | -0,93775 | 0,937745 | 0,1764 | -0,987 | 0,937745 | |

Supplemental Table V - *Significance for the correlation differences of men and women. The differences between the correlations of men and women were calculated, and significance was calculated using 10.000 permutations (see methods for details). The top matrix displays p-values and the bottom matrix FDR corrected p-values. The two most significant differences are marked in red. P-values are positive if men have a more positive association and p-values are negative if women have a more positive association.*

| | Age | Sex |
|---|---|---|
| **leukocytes_10^09/l** | 0,10008 | 0,079061 |
| **neutrophils_10^09/l** | 0,16533 | 0,122083 |
| **lymfocytes_10^09/l** | -0,08387 | -0,09722 |
| **monocytes_10^09/l** | 0,11768 | 0,282592 |
| **eosinophils_10^09/l** | 0,063722 | 0,129132 |
| **basophils_10^09/l** | 0,010639 | -0,01581 |

Supplemental Table VI - *Standardized regression coefficients (betas) for the regression analysis between cell counts, age and sex. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless these were the factors for which the beta was being calculated).*

| | leukocytes_10..09.l | neutrophils_10..09.l | lymfocytes_10..09.l | monocytes_10..09.l | eosinophils_10..09.l | basophils_10..09.l |
|---|---|---|---|---|---|---|
| **AAT** | -0,106799929 | -0,106301 | -0,089416029 | -0,104337029 | 0,058869277 | -0,015807622 |
| **Adiponectin** | -0,15913029 | -0,088281226 | -0,171636458 | -0,121281347 | -0,142980725 | -0,021309958 |
| **hsCRP** | 0,268894786 | 0,342364047 | -0,009584615 | 0,177873382 | 0,028989134 | 0,079550859 |
| **IL-18** | 0,161552083 | 0,102526431 | 0,163956202 | 0,074438841 | 0,06384509 | -0,005631775 |
| **IL-18BP** | 0,143424988 | 0,121091184 | 0,065979361 | 0,110600685 | 0,120151467 | -0,053489133 |
| **IL-6** | 0,291107038 | 0,373579499 | -0,003843082 | 0,296775619 | 0,070868252 | 0,099032983 |
| **Leptin** | 0,199061847 | 0,187907991 | 0,091491777 | 0,239448181 | 0,144084916 | 0,094806689 |
| **Resistin** | 0,303508969 | 0,355752982 | 0,0925476 | 0,207763479 | 0,136764055 | 0,10493164 |
| **VEGF** | 0,251514913 | 0,194262393 | 0,187919556 | 0,234679861 | 0,180318027 | 0,155313479 |

Supplemental Table VII - *Standardized regression coefficients (betas) for the regression analysis between circulating markers of inflammation and absolute cell counts. The betas were generated using a linear regression model. The data were corrected for age, sex and season.*

| | all | women | men |
|---|---|---|---|
| **AAT** | 0,059198 | 0,002913 | 0,105997 |
| **Adiponectin** | -0,28488 | -0,41527 | -0,194 |
| **hsCRP** | 0,13323 | 0,040551 | 0,211921 |
| **IL-18** | 0,213824 | 0,122627 | 0,280469 |
| **IL-18BP** | 0,17576 | 0,109682 | 0,229627 |
| **IL-6** | 0,210399 | -0,05782 | 0,405119 |
| **Leptin** | 0,224205 | 0,025587 | 0,376361 |
| **Resistin** | 0,018568 | -0,01897 | 0,059675 |
| **VEGF** | 0,14679 | 0,178749 | 0,120105 |

Supplemental Table VIII - *Standardized regression coefficients (betas) for the regression analysis between circulating markers of inflammation and the presence of the metabolic syndrome. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless the data was filtered for one of these factors). Betas are shown for all data, just women and just men.*

| | p-value | FDR |
|---|---|---|
| **AAT** | 0,354945 | 0,532418 |
| **Adiponectin** | 0,007438 | 0,022313 |
| **hsCRP** | 0,14424 | 0,259631 |
| **IL-18** | 0,112201 | 0,252452 |
| **IL-18BP** | 0,563441 | 0,724425 |
| **IL-6** | 2,04E-05 | 0,000183 |
| **Leptin** | 0,000499 | 0,002247 |
| **Resistin** | 0,915905 | 0,915905 |
| **VEGF** | -0,67618 | 0,760698 |

Supplemental Table IX - *Significance of the interaction effect between sex and the metabolic syndrome in predicting circulating cytokine levels. Both p-values and false discovery rates are reported. P-values are marked with a minus sign (negative) if the regression coefficient (beta) for the term was negative.*

| variable | sexSubgroup | metSSubgroup | mean_300OB | sd_300OB | mean_Nima1 | sd_Nima1 | pVal |
|---|---|---|---|---|---|---|---|
| **Age** | all | all | 67.10596026 | 5.407208573 | 62.72766512 | 5.5881198 | 2.98522E-17 |
| **Age** | all | MetSyndrYes | 67.4695122 | 5.659482276 | 63.38699521 | 5.63964568 | 1.6357E-06 |
| **Age** | all | MetSyndrNo | 66.67391304 | 5.077735922 | 62.42218399 | 5.54845989 | 3.08809E-10 |
| **Age** | male | all | 66.64670659 | 5.440895838 | 63.83102045 | 5.42589343 | 0.000154077 |
| **Age** | male | MetSyndrYes | 67.18888889 | 5.809512626 | 65.1847593 | 5.03677398 | 0.11341562 |
| **Age** | male | MetSyndrNo | 66.01298701 | 4.937753342 | 63.16524725 | 5.50573546 | 0.002492128 |
| **Age** | female | all | 67.67407407 | 5.330606683 | 61.70832161 | 5.55532422 | 1.7934E-15 |
| **Age** | female | MetSyndrYes | 67.81081081 | 5.491402186 | 61.58923112 | 5.67518488 | 2.55212E-07 |
| **Age** | female | MetSyndrNo | 67.50819672 | 5.169213192 | 61.76047802 | 5.52228082 | 1.05239E-08 |
| **BMI** | all | all | 30.72512477 | 3.461267172 | 30.25724586 | 3.43587305 | 0.008061416 |
| **BMI** | all | MetSyndrYes | 31.48913701 | 3.681269632 | 31.57811241 | 3.95644267 | 0.913805376 |
| **BMI** | all | MetSyndrNo | 29.81716819 | 2.944328363 | 29.64526136 | 2.98137028 | 0.264845584 |
| **BMI** | male | all | 30.53664695 | 3.219870484 | 29.73955397 | 2.72683506 | 0.005801527 |
| **BMI** | male | MetSyndrYes | 31.34351692 | 3.411765926 | 30.94101531 | 3.13231933 | 0.421575248 |
| **BMI** | male | MetSyndrNo | 29.59355219 | 2.709543142 | 29.14867133 | 2.29582619 | 0.190917651 |
| **BMI** | female | all | 30.95827881 | 3.737470968 | 30.73551959 | 3.9270684 | 0.244170751 |
| **BMI** | female | MetSyndrYes | 31.66624253 | 4.001253186 | 32.2152095 | 4.57550225 | 0.52665275 |
| **BMI** | female | MetSyndrNo | 30.09943757 | 3.217111911 | 30.08748021 | 3.42847611 | 0.561765717 |

Supplemental Table X – *Comparison of age and BMI between the 300-OB cohort and the NIMA1 cohort. Mean and standard deviations are calculated for each group and a t-test was used to evaluate the significance of the differences. Analyses are performed for several subgroups, split on sex and metabolic syndrome. Abbreviations: MetSyndrYes = group having the metabolic syndrome; MetSyndrNo = group not having the metabolic syndrome*

| | p-value | FDR |
|---|---|---|
| **leukocytes_10..09.l** | 0,61602533 | 0,61965105 |
| **neutrophils_10..09.l** | 0,23808552 | 0,61965105 |
| **lymfocytes_10..09.l** | -0,5062733 | 0,61965105 |
| **monocytes_10..09.l** | 0,40905009 | 0,61965105 |
| **eosinophils_10..09.l** | 0,61965105 | 0,61965105 |
| **basophils_10..09.l** | -0,3578378 | 0,61965105 |

Supplemental Table XI - *P-values and FDR corrected p-values for the interaction between sex and metabolic syndrome in predicting absolute cell numbers. All analyses were corrected for age, season, MetS and gender. P-values are marked with a minus sign (negative) if the regression coefficient (beta) for the term was negative.*

| | All | men | women |
|---|---|---|---|
| **LPS.1ng_PBMC_24h_IL-1b** | 0,093358 | 0,167764 | 0,006357 |
| **LPS.100ng_PBMC_24h_IL-1b** | 0,08928 | 0,219997 | -0,07106 |
| **Pam3Cys_PBMC_24h_IL-1b** | 0,086896 | 0,169691 | -0,01094 |
| **C.albicans.yeast_PBMC_24h_IL-1b** | 0,083158 | 0,074591 | 0,094077 |
| **P.gingivalis_PBMC_24h_IL-1b** | 0,099182 | 0,142683 | 0,044762 |
| **S.aureus_PBMC_24h_IL-1b** | 0,019474 | 0,032935 | 0,003615 |
| **E.coli_PBMC_24h_IL-1b** | 0,154471 | 0,199384 | 0,093057 |
| **Poly.I.C_PBMC_24h_IL-1b** | -0,0409 | 0,063468 | -0,16406 |
| **LPS.1ng_PBMC_24h_IL-6** | 0,07637 | 0,163185 | -0,02694 |
| **LPS.100ng_PBMC_24h_IL-6** | 0,054725 | 0,18954 | -0,1099 |
| **Pam3Cys_PBMC_24h_IL-6** | 0,082359 | 0,164079 | -0,01537 |
| **C.albicans.yeast_PBMC_24h_IL-6** | 0,093828 | 0,187974 | -0,02376 |
| **P.gingivalis_PBMC_24h_IL-6** | 0,082813 | 0,159237 | -0,02049 |
| **S.aureus_PBMC_24h_IL-6** | 0,03343 | 0,145565 | -0,08577 |
| **E.coli_PBMC_24h_IL-6** | 0,116317 | 0,198235 | 0,022655 |
| **Poly.I.C_PBMC_24h_IL-6** | -0,08469 | 0,022709 | -0,20073 |
| **LPS.1ng_PBMC_24h_TNF-a** | 0,026884 | 0,105668 | -0,06048 |
| **LPS.100ng_PBMC_24h_TNF-a** | -0,05035 | 0,057343 | -0,17827 |
| **C.albicans.yeast_PBMC_24h_TNF-a** | -0,02109 | -0,12686 | 0,084375 |
| **S.aureus_PBMC_24h_TNF-a** | -0,05098 | -0,07756 | -0,0157 |
| **E.coli_PBMC_24h_TNF-a** | 0,054424 | 0,091154 | 0,01195 |

Supplemental Table XII - *Standardized regression coefficients (betas) for the regression analysis between stimulated cytokines and the presence of the metabolic syndrome. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless the data was filtered for one of these factors). Betas are shown for all data, just women and just men.*

| | p-value | FDR |
|---|---|---|
| **LPS.1ng_PBMC_24h_IL-1b** | 0,009925 | 0,10421 |
| **LPS.100ng_PBMC_24h_IL-1b** | 0,007299 | 0,10421 |
| **Pam3Cys_PBMC_24h_IL-1b** | 0,022629 | 0,134396 |
| **C.albicans.yeast_PBMC_24h_IL-1b** | 0,025599 | 0,134396 |
| **P.gingivalis_PBMC_24h_IL-1b** | 0,045995 | 0,137984 |
| **S.aureus_PBMC_24h_IL-1b** | 0,044498 | 0,137984 |
| **E.coli_PBMC_24h_IL-1b** | 0,04472 | 0,137984 |
| **Poly.I.C_PBMC_24h_IL-1b** | 0,06214 | 0,151464 |
| **LPS.1ng_PBMC_24h_IL-6** | 0,071747 | 0,151464 |
| **LPS.100ng_PBMC_24h_IL-6** | 0,072126 | 0,151464 |
| **Pam3Cys_PBMC_24h_IL-6** | 0,082757 | 0,157991 |
| **C.albicans.yeast_PBMC_24h_IL-6** | 0,098876 | 0,173033 |
| **P.gingivalis_PBMC_24h_IL-6** | 0,113392 | 0,173236 |
| **S.aureus_PBMC_24h_IL-6** | 0,115491 | 0,173236 |
| **E.coli_PBMC_24h_IL-6** | -0,1429 | 0,200067 |
| **Poly.I.C_PBMC_24h_IL-6** | 0,304578 | 0,399758 |
| **LPS.1ng_PBMC_24h_TNF-a** | 0,348241 | 0,424699 |
| **LPS.100ng_PBMC_24h_TNF-a** | 0,364027 | 0,424699 |
| **C.albicans.yeast_PBMC_24h_TNF-a** | 0,655404 | 0,693751 |
| **S.aureus_PBMC_24h_TNF-a** | 0,660715 | 0,693751 |
| **E.coli_PBMC_24h_TNF-a** | -0,74103 | 0,741035 |

Supplemental Table XIII - *P-values and FDR corrected p-values for the interaction between sex and metabolic syndrome in predicting inflammatory cytokine production capacity. All analyses were corrected for age, season, MetS and gender. P-values are marked with a minus sign (negative) if the regression coefficient (beta) for the term was negative.*

| | All | Men | Women |
|---|---|---|---|
| **LPS.1ng_PBMC_24h_IL-1b** | 0,047904 | 0,161724 | -0,08477 |
| **LPS.100ng_PBMC_24h_IL-1b** | 0,04101 | 0,165069 | -0,11005 |
| **Pam3Cys_PBMC_24h_IL-1b** | 0,052559 | 0,140933 | -0,05161 |
| **C.albicans.yeast_PBMC_24h_IL-1b** | 0,05872 | 0,157075 | -0,0659 |
| **P.gingivalis_PBMC_24h_IL-1b** | 0,057605 | 0,186508 | -0,1027 |
| **S.aureus_PBMC_24h_IL-1b** | 0,086625 | 0,221743 | -0,07161 |
| **E.coli_PBMC_24h_IL-1b** | 0,047972 | 0,131819 | -0,0667 |
| **Poly.I.C_PBMC_24h_IL-1b** | -0,1008 | -0,0808 | -0,1245 |
| **LPS.1ng_PBMC_24h_IL-6** | 0,108847 | 0,262661 | -0,07175 |
| **LPS.100ng_PBMC_24h_IL-6** | 0,063365 | 0,210383 | -0,11406 |
| **Pam3Cys_PBMC_24h_IL-6** | 0,092954 | 0,217615 | -0,05396 |
| **C.albicans.yeast_PBMC_24h_IL-6** | 0,128281 | 0,262338 | -0,03784 |
| **P.gingivalis_PBMC_24h_IL-6** | 0,097313 | 0,242337 | -0,09707 |
| **S.aureus_PBMC_24h_IL-6** | 0,116797 | 0,239613 | -0,01016 |
| **E.coli_PBMC_24h_IL-6** | 0,093869 | 0,22477 | -0,05446 |
| **Poly.I.C_PBMC_24h_IL-6** | -0,1117 | -0,02329 | -0,207 |
| **LPS.1ng_PBMC_24h_TNF-a** | 0,006203 | 0,117904 | -0,11583 |
| **LPS.100ng_PBMC_24h_TNF-a** | -0,01202 | 0,092306 | -0,1344 |
| **C.albicans.yeast_PBMC_24h_TNF-a** | 0,090523 | 0,135106 | 0,047532 |
| **S.aureus_PBMC_24h_TNF-a** | 0,057764 | 0,129455 | -0,03498 |
| **E.coli_PBMC_24h_TNF-a** | -0,036 | 0,04298 | -0,12709 |

Supplemental Table XIV - *Standardized regression coefficients (betas) for the regression analysis between cytokine production capacity and the whether or not an individual has high triglycerides. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless the data was filtered for one of these factors). Betas are shown for all data, just women and just men.*

| | Sex | MetS_All | MetS_Women | MetS_Men |
|---|---|---|---|---|
| **XL.VLDL.L_mmol.l** | 0,076106 | 0,516152 | 0,535612945 | 0,50051483 |
| **M.VLDL.L_mmol.l** | 0,088442 | 0,524235 | 0,577648293 | 0,48342232 |
| **XS.VLDL.L_mmol.l** | -0,20134 | 0,251939 | 0,355656744 | 0,17395389 |
| **L.LDL.L_mmol.l** | -0,26583 | 0,126582 | 0,207918646 | 0,07842298 |
| **Serum.C_mmol.l** | -0,2934 | 0,114375 | 0,154418798 | 0,08869051 |
| **ApoB_g.l** | -0,13593 | 0,369622 | 0,414412447 | 0,33647203 |
| **XL.HDL.L_mmol.l** | -0,28633 | -0,32202 | -0,466917336 | -0,24562845 |
| **XL.HDL.TG_mmol.l** | -0,08058 | 0,256841 | 0,289553778 | 0,24082444 |
| **L.HDL.L_mmol.l** | -0,31998 | -0,47259 | -0,542431475 | -0,4322077 |
| **L.HDL.TG_mmol.l** | -0,29083 | -0,23638 | -0,203100426 | -0,25900503 |
| **M.HDL.L_mmol.l** | -0,31807 | -0,21314 | -0,317543284 | -0,15610895 |
| **M.HDL.TG_mmol.l** | -0,14338 | 0,382408 | 0,490211023 | 0,30967991 |
| **S.HDL.L_mmol.l** | -0,18191 | 0,038163 | 0,063351365 | 0,02283673 |
| **S.HDL.PL_mmol.l** | -0,08128 | -0,04113 | -0,08082433 | -0,01681145 |
| **LDL.D_nm** | 0,051065 | -0,21665 | -0,10645914 | -0,2790287 |
| **HDL3.C_mmol.l** | -0,34773 | -0,27926 | -0,33119696 | -0,24835423 |
| **ApoA1_g.l** | -0,41207 | -0,14321 | -0,302234948 | -0,03630078 |

Supplemental Table XV - *Standardized regression coefficients (betas) for the regression analysis between lipids and sex (left) and with the presence of the metabolic syndrome ("MetS"). For MetS the betas are shown for all individuals, for just women and for just men. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless these were the factors for which the beta was being calculated).*

Supplemental Table XVI - *FDR corrected p-values for the association between the metabolic syndrome (MetS) and all detected metabolite signals from an untargeted metabolomics platform. m/z ratios for the signal are listed in the first column, the significance of association with the MetS in the second and all possible assigned metabolites in the third column. P-values are negative if there is a negative association with the MetS and these are marked in blue, positive associations have a positive sign and are marked in red. The more significant, the darker the color.*

Supplemental Table XVII - *Pathway associations with the metabolic syndrome. The first column "pathway_metabolicSyndrome" shows the pathway names, the second and third column ("pval" and "padj") provide the raw and multiple testing corrected p-values. "size" is the size of the pathway after removing metabolites that are not present, "leadingEdge" is a vector with KEGG ids of "leading edge" metabolites that drive the enrichment, "pValsSignedIds" are the p-values of the metabolites in the pathway and "scoresUsed" are the -log10(p-values).*

| | MetS_beta | Sex_beta |
|---|---|---|
| **Valine** | 0,37690906 | 0,365626 |
| **Ketovaline** | 0,342751147 | 0,106758 |
| **I(so)Leucine** | 0,363376854 | 0,501542 |
| **4-Methyl-2-oxopentanoate** | 0,322945578 | 0,381866 |
| **(S)-2-Acetolactate** | 0,297703251 | 0,088 |
| **Pyruvate** | 0,140000037 | -0,09469 |
| **2-Methylmaleate** | 0,134819777 | -0,15773 |

Supplemental Table XVIII - *Standardized regression coefficients (betas) for the regression analysis between selected metabolites and sex and whether or not someone has the metabolic syndrome. The betas were generated using a linear regression model. The data were corrected for age, sex and season (unless these were the studied factors).*

| | meanMen (nmol/l) | sdMen (nmol/l) | meanWomen (nmol/l) | sdWomen (nmol/l) | pVal |
|---|---|---|---|---|---|
| **cortisol** | 339.7798616 | 114.9468335 | 388.7365338 | 132.7863937 | 0.00117984 |
| **11-deoxy-cortisol** | 1.365901007 | 0.747490477 | 1.331280797 | 0.898876855 | 0.301044577 |
| **androsteendion** | 2.590804123 | 0.960785934 | 2.287458723 | 1.112823397 | 0.000730601 |
| **testosterone** | 16.30003088 | 5.04974097 | 0.918736969 | 0.459992479 | 5.3802E-141 |
| **17-hydroxy-progesterone** | 2.515032657 | 0.887784965 | 1.011294197 | 0.595935244 | 9.79578E-47 |

Supplemental Table XIX – *Hormone level differences between men and women in the 300-OB cohort. The p-value was calculated using a t-test.*

Supplemental Table XX - *FDR corrected p-values for the associations between markers of inflammation and metabolites detected using untargeted metabolomics. m/z ratios for the signal are listed in the first column and all possible assigned metabolites in the second column. P-values are negative if there is a negative association with the MetS and these are marked in blue, positive associations have a positive sign and are marked in red. The more significant, the darker the color.*

Supplemental Table XXI - *Significance (p-values) of differences in males vs females in correlations between circulating markers of inflammation and metabolites from an untargeted metabolomics analysis. P-values have a negative sign (minus) if the association is negative. Based on the significance the cells are coloured blue for negative associations and red for positive associations. The first column provides the m/z values and the second column all possible metabolites that were assigned to that m/z value.*