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Obesity is Associated with More Activated Neutrophils in African American Male Youth

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Abstract

Background—There is emerging evidence suggesting the role of peripheral blood leukocytes in the pathogenesis of obesity and related diseases. However, few studies have taken a genome wide approach to investigating gene expression profiles in peripheral leukocytes between obese and lean individuals with the consideration of obesity related shifts in leukocyte types.

Method—We conducted this study in 95 African Americans of both genders (age 14-20, 46 lean and 49 obese). Complete blood count with differential test (CBC) was performed in whole blood. Genome wide gene expression analysis was obtained using Illumina HumanHT-12 V4 Beadchip with RNA extracted from peripheral leukocytes. Out of the 95 participants, 64 had neutrophils stored. The validation study was based on Real-time polymerase chain reaction with RNA extracted from purified neutrophils.

Results—CBC test suggested that in males, obesity was associated with increased neutrophil percentage ($p=0.03$). Genome wide gene expression analysis showed that in males, the majority of the most differentially expressed genes were related to neutrophil activation. Validation of the gene expression levels of *ELANE* (*neutrophil elastase*) and *MPO* (*myeloperoxidase*) in purified neutrophils demonstrated that the expression of these two genes – important biomarkers of neutrophils activation – were significantly elevated in obese males ($p=0.01$ and $p=0.02$, respectively).

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Conflicts

There is no conflict of interest.

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Conclusion—The identification of increased neutrophil percentage and activation in obese African American males suggests that neutrophils play an essential role in the pathogenesis of obesity related disease. Further functional and mechanistic studies on neutrophils may contribute to the development of novel intervention strategies reducing the burden associated with obesity-related health problems.

Keywords

Obesity; gene expression; African American; neutrophil; leukocyte

Introduction

Obesity is one of the major public health problems worldwide. It significantly increases the risk of various diseases, such as diabetes, cardiovascular diseases and certain types of cancer^{1, 2}. Low-grade systemic inflammation has been widely shown to accompany obesity and is thought to etiologically contribute to its related comorbidities³. The basis for this view mainly comes from studies on adipose tissues and targeted metabolic organs (e.g., muscle, liver, etc.) in which local productions of cytokines and immune cell infiltrations are discovered^{4, 5}. However, because of the not-easy availability of intra-abdominal fat or target metabolic organs in human studies, these findings have remained of limited use in clinical practice and development of prevention strategies. Since adipose-infiltrating immune cells in obesity are largely derived from bone marrows, a major gap of knowledge is whether changes in circulating leukocytes or leukocyte populations could provide etiological clues, pharmaceutical targets or as markers of the chronic, low-grade inflammatory response to obesity.

With the rapid development of genome wide technologies, it is of great interest to use genome-wide approaches to investigate obesity induced gene expression changes in circulating leukocytes. To our best knowledge, there have only been two studies exploring the gene expression differences between obese cases and lean controls using genome wide gene expression approaches in peripheral blood cells^{6, 7}. However, both studies were performed in whole blood rather than leukocytes, for which the influence of the high abundance of globin transcripts cannot be neglected. Moreover, both studies did not consider the potential influence of leukocyte composition changes related to obesity.

In this study, based on the complete blood count with differential counts and the genome-wide gene expression profiles on peripheral leukocytes from 46 obese cases and 49 lean controls, we aimed to identify obesity related gene expression changes after considering alterations in leukocyte composition. Gene ontology analysis was then performed to provide functional interpretations of the differentially expressed genes. Additionally, we conducted further replication in purified neutrophils to demonstrate that the genome wide findings in males were not driven by the increased percentage of neutrophils in obese cases. To our best knowledge, this is the first study investigating obesity induced gene expression profiling using peripheral leukocytes and purified neutrophils in African American youth and young adults with consideration of cell compositions.

Methods

Subjects

We selected 46 African American (AA) male participants (22 obese vs. 24 lean) and 49 AA female participants (24 obese vs. 25 lean) from the EpiGO (EpiGenetic basis of Obesity induced cardiovascular disease and type 2 diabetes) study. The EpiGO study was established in 2011 with the goal of identifying methylation changes involved in the pathogenesis of obesity and its related co-morbidities. Currently it is still ongoing and will in total enroll 400 obese and 400 lean youth aged 14-20 years with roughly equal number of AAs and European Americans (EA) as well as males and females. The detail information of the EpiGO study has been described previously ⁸.

This study was approved by the Institutional Review Board of Georgia Regents University, and performed under the guide of Declaration of Helsinki. Written informed consent was provided by all participants (if age ≥ 18 years) or by their parents (if age < 18 years).

For all participants, height and weight were measured by standard methods using a wall-mounted stadiometer and a scale, respectively. Body mass index (BMI) was calculated as weight/height², and BMI percentile was calculated according to their age, sex, height and weight ⁹. The inclusion criteria of the EpiGO study are as followed: (1) age between 14 (with equal) to 21 (less than) years old; (2) both parents of the participants reported being of European or African ancestry; (3) obese participants with BMI ≥ 30 kg/m² or BMI percentile ≥ 95% if age ≥ 20; (4) lean participants with BMI < 25 kg/m² or BMI percentile < 50% if age ≥ 20; (5) free of any chronic or acute disease; (6) no daily medication controls for illnesses.

Measurements

Metabolic traits—Blood pressure (BP) was obtained at the 11th, 13th, 15th minute during a 15 minute supine relaxation period. The average of the three records was used to represent BP levels. Fasting serum triglyceride (TG), high-density lipoprotein cholesterol (HDL), insulin and glucose levels were measured in the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) supported Clinical Nutrition Research Unit Core Laboratory at the University of Alabama. TG, HDL and glucoses were measured using colorimetric method on Sirus analyzer (Stanbio Laboratory, Boerne, TX), while insulin was measured using an immunoenzymatic method on a TOSOH AIA-600 II analyzer (TOSOH Bioscience, S. San Fran, CA). The inter-assay CV from this lab is 3.63%, 1.61% and 2.34%, and the intra-assay CV is 2.53%, 1.99% and 2.93%, for TG, HDL, and glucose, respectively. The assay sensitivity is 1.0 uU/ml for insulin and the intra-assay CV is 1.49%.

Complete blood count with differential test (CBC test)—Peripheral blood was collected and brought to the clinical pathology core lab within 2 hours for the complete blood count with differential count, which includes the total leukocyte count and percentages of peripheral blood cell types including neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Leukocyte isolation and RNA extraction—Leukocytes were obtained after removal of plasma and lysis of all the red blood cells. The cell pellet was dissolved in RNA protect Cell Reagent (QIAGEN, Inc.) immediately and stored at -80°C . For the RNA extraction, the frozen cells were brought back to the room temperature and repelleted. After removal of the RNA protect Cell Reagent, RNA was extracted using QIAamp RNA mini Kit (QIAGEN, Inc.). RNA concentration and purity were evaluated on NanoDrop spectrophotometer (Thermo Scientific Inc.). RNA integrity (RIN) was evaluated on Bioanalyzer 2100 (Agilent Technologies, Inc.). The RIN score of all these 95 samples were greater than 8, indicating good RNA qualities.

Neutrophil isolation and RNA extraction—We used the approach developed by De et al.¹⁰. With a high purity of 97%, this method is simple for rapid isolation of functional neutrophils using commercially available cell preparation tube (CPT). This tube is produced by BD Biosciences Corp (San Jose, CA), which contains a gel lock to maintain the gradient between the Ficoll-hypaque and the blood. The red blood cells/neutrophil mixture was collected. After lysis of the red blood cells, the neutrophil cells were pelleted and washed with ice cold phosphate buffered saline (PBS). Similarly to the leukocytes, the neutrophil cell pellet was dissolved in RNA protect Cell Reagent (QIAGEN, Inc.) immediately and stored at -80°C . Neutrophil RNA was extracted using the same approach as the one used for leukocytes.

Genome wide gene expression profiling in leukocyte—The Illumina HumanHT-12 v4 Expression BeadChip (Illumina, Inc.), a direct hybridization assay, was used for genome wide gene expression analysis with 1 μg total RNA. This chip targets more than 48,000 probes that provide genome-wide coverage of well-characterized genes, gene candidates and splice variants. All of these 95 samples were tested in one batch at the Genomics Core Facility of the University of Chicago.

Real-Time polymerase chain reaction (RT-PCR) in neutrophils—Out of the 95 participants, 64 had frozen neutrophils stored (33 obese vs. 31 lean, 27 male vs. 37 female, details see online table S1). To validate the microarray findings, the expression levels of ELANE and MPO genes in purified neutrophils were quantified relative to endogenous control gene, beta actin (β -actin) using pre-designed BIO-RAD gene expression assays (BIO-RAD, Hercules, CA) following the instructions.

Statistical analysis

All the statistical analyses, with the exception of the genome wide gene expression analysis, were conducted using Stata SE Version 12 (StataCorp). Linear regression was performed to test whether the percentages of cell types in peripheral leukocyte were different between obese and lean subjects with age and sex included as covariates. The percentage of monocyte was log-transformed and the percentage of eosinophil was square root transformed to obtain normal distribution. For the percentage of basophil which cannot be transformed to normal distribution, the nonparametric Wilcoxon rank-sum test was further conducted. Similar results were obtained, so results for the regression analysis were reported here. We further tested whether this difference was dependent on sex by including the group

(obese vs. lean) \times sex interaction term. This is, leukocyte subtype percentages were used as dependent variable, group, sex and group \times sex interaction as independent variables, and age as covariate. Linear regression was also used to test whether the expression levels of *ELANE* and *MPO* genes in purified neutrophils were different between obese cases and lean controls in each sex with age adjusted. Log transformation was applied to the expression levels of these two genes to obtain normal distribution.

For the genome wide gene expression analysis, the background subtracted signals were imported into R-environment. Probes with detection P-value less than 0.05 in more than 50% of the samples were defined as “present” and a total of 19,066 presented probes harbored 11,776 known genes from all the 95 subjects were selected for data analysis. Quartile normalization and log transform was performed before the analysis. The Limma package ¹¹ was used to test the mean difference and to evaluate obesity-related differentially expressed genes in males and females separately with age adjustment. Raw P-values were assigned based on the empirical Bayes shrinkage from the designed linear model. To correct for multiple testing, the set of raw p values were converted to false discovery rates (FDR) according to Benjamini and Hochberg ¹².

Gene ontology analysis was performed using DAVID (the Database for annotation visualization and Integrated Discovery v6.7) with GOTERM biological process selected (<http://david.abcc.ncifcrf.gov>). The human genome was used as background, and the enrichment P-values were derived from a modified Fisher's exact test. The most significant probe was selected to present the gene if one transcript has more than one probe. The genes with p-value<0.01 and absolute log₂ fold change>0.5 were selected for both genders and imported into the analysis. The top ten enriched pathways were exported from the output.

Results

The general characteristic of the 95 subjects are presented by group and sex in Table 1. The average age of all participants was around 17 years, and there were no significant age differences between males and females or between obese cases and lean controls. In both males and females, obesity was associated with higher systolic blood pressure (p<0.001) higher fasting insulin (p<0.001) levels and lower high-density lipoprotein cholesterol levels.

In terms of the percentages of the leukocyte subtypes, we observed a significant sex \times group (obese vs. lean) interaction for percentage of neutrophils (p=0.037), therefore, we conducted the stratified analyses in each gender. As shown in Table 2, in the male participants, obesity was associated with significantly increase in the percentage of neutrophils (52.95 \pm 9.74 vs. 46.45 \pm 11.15, p=0.03). In females, none of the percentages of these major leukocyte types showed significant differences between obese cases and lean controls. In terms of the cell counts, there was a significant increase in the total number of white blood cells in male obese subjects (6.03 \pm 1.62 vs. 4.97 \pm 1.57, p=0.05). Similar trend was observed in females but did not reach significance (6.20 \pm 1.47 vs. 5.51 \pm 1.41, p=0.13). There were also significant increases of lymphocytes counts in female obese subjects (2.13 \pm 1.34 vs. 1.80 \pm 0.55, p=0.03) and significant increases of neutrophil counts in male obese subjects (3.33 \pm 1.62 vs.

2.39±1.14, $p=0.04$), which were due to the increases of the total number of white blood cells in obese cases.

Because of the different effects of obesity on peripheral leukocyte cell compositions in males and females, we performed the following gene expression analysis in males and females separately. Figure 1 is the volcano plot showing the raw p-values versus mean gene expression difference for all genes between the case and the control group in males and females separately. The most significant gene was *LCN2* in males showing a raw p value of 3.43×10^{-5} and a FDR of 0.42 and the most significant gene was *FAAH* in females showing a raw p value of 1.79×10^{-5} and a FDR of 0.34. Among the 11,776 unique genes, we identified in total 226 genes as obesity related differentially expressed genes in males and 222 genes in females with p-value <0.01. Out of the 226 genes, 73 (32.30%) genes were up-regulated and 153 (67.70%) were down-regulated in obese males. For females, there were 57 (25.68%) up-regulated genes and 165 (74.32%) down-regulated genes in obese.

The top twenty differentially expressed genes ranked by the smallest p-value or the largest log₂ fold change were presented in Table 3. Among the top lists in males, *LCN2* (*lipocalin-2*), *CEACAM6* (carcinoembryonic antigen-related cell adhesion molecule 6, CD66c), *ELANE* and *AZU1* (*azurocidin 1*) were directly related with neutrophil activation. Interestingly, there were more genes associated with neutrophil activations in the top list ranked by the largest fold change. Not only *LCN2*, *CEACAM6* and *ELANE* gene, another 8 genes (*LYZ*-lysozyme, *OLFM4*-olfactomedin 4, *DEFA1B*-defensin alpha 1b, *DEFA3*-defensin alpha 3, *DEFA4*-defensin alpha 4, *DEFA1* defensin alpha 1, *CTSG*-cathepsin G and *CEACAM8*- carcinoembryonic antigen-related cell adhesion molecule 8) all have functions closely linked to neutrophil activation. Different from the findings in males, several of the genes in the top lists in females are involved in the activation of natural killer cells including *KIR3DL1*, *KIR3DL3*, *KIR2DL1*, *KIR2DL3*, *KIR2DL4* and *KIR2DS5* (killer cell immunoglobulin-like receptor, three domains long cytoplasmic tail 1; three domains long cytoplasmic tail 3; two domains long cytoplasmic tail 1; two domains long cytoplasmic tail 3; two domains long cytoplasmic tail 4; two domains short cytoplasmic tail 5) and *KLRC1* (killer cell lectin-like receptor subfamily C, member 1).

Gene ontology analysis using DAVID showed the similar results with innate immune system including defense response to fungus and defense response to bacterium among the top GO categories in males (Table 4). Similarly, defense response is also one of the top GO categories in females (Table 4).

Due to the fact that in this study obesity was only associated with increased percentage of neutrophils in males, we conducted further replication in purified neutrophils to exclude the possibility that the findings in males were driven by the increased percentage of neutrophils in obese cases. We performed RT-PCR for two genes closely related with neutrophil activation and extensively studied in the literature (*ELANE*, one of the genes in our top list, and *MPO*, also showing significantly increased expression levels in our study [$\text{LogFC}=0.44$, $P=0.005$]) in 64 out of the 95 subjects for whom we have purified neutrophil stored (obese group: 15M/18F; lean group: 12M/19F; online table S1). Results were shown in Figure 2. Similar to the results in the microarray analysis using the leukocytes, obesity was associated

with significantly higher expression levels of both genes in neutrophils in males (*ELANE*, $p=0.01$; *MPO*, $p=0.02$) but not in females.

Discussion

In this study, we not only demonstrated the gene expression differences in peripheral leukocytes from African American obese and lean subjects, in both males and females, but also observed that in African American males obesity was associated with increased percentage of neutrophils and increased expression of neutrophil activation related genes.

Obesity has been found to be associated with high leukocyte count, serving as one piece of evidence of the involvement of inflammation^{13, 14}. The major interests on circulating leukocyte subtypes are monocytes and T cells since the discovery of enhanced adipose tissue macrophage and T cells infiltration in obesity^{15, 16}. While several studies suggest a mild increase in the total circulating numbers of these subtypes, this has not been a universal finding in other studies with even reports of negative association between the percentage of these cell types and BMI^{17, 18}. In contrast with the inconsistent finding in these leukocyte subtypes, obesity is consistently associated with persistent neutrophilia, manifesting not only by elevated neutrophil counts but also increased percentages^{14, 17, 19, 20}. All of these studies were conducted in Caucasians. In this study, we observed the same pattern in African American males, that is, obese cases have significantly higher peripheral neutrophil percentages and total cell numbers.

The importance of neutrophils in the pathogenesis of obesity related disease was further emphasized recently. In 2012 and 2013 a series of studies were published demonstrating the importance of neutrophil infiltration in obesity induced immune dysfunction²¹⁻²³. For example, Talukdar *et al*²² found that the neutrophil was the first immune cell responding to inflammation and its infiltration into the adipose tissue can occur as early as after 3 days of high fat feeding and can be maintained for up to 90 days on a high fat diet. Treatment with neutrophil elastase (*ELANE*, a key enzyme secreted by activated neutrophil) causes increased cellular insulin resistance and the deletion of *ELANE* in high fat diet induced obese mice leads to less tissue inflammation associated with lower adipose tissue neutrophil and macrophage infiltrations. Using an unbiased proteomic approach in a mouse model, Mansuy-Aubert *et al*²¹ observed that obesity was associated with an imbalance between *ELANE* and its inhibitor $\alpha 1$ -Antitrypsin and this imbalance was linked with insulin sensitivity and inflammation in target organs. Previous studies in humans also observed that serum *ELANE* activity was positively and significantly correlated with BMI²⁴⁻²⁷. In consistent with these two animal studies and previous human studies on *ELANE*, in the current study we also observed that the expression of *ELANE* was significantly increased in the peripheral leukocytes of obese AA males. To exclude the possibility that this finding was driven by the different cell compositions observed in obese and lean AA males, we further validated the findings of two genes, *ELANE* and *MPO* in purified neutrophils. The *MPO* gene encodes a peroxidase enzyme which is a major component of the azurophilic granules of neutrophils. *MPO* is one of the keys in the initiation and progression of both acute and chronic inflammatory diseases, including cardiovascular disease, and has been identified as a marker of neutrophil infiltration into tissues. A recent study²⁸ in 446 Caucasian

prepubertal children (age 6-12 years, 223 normal weight vs. 223 obese) found that plasma *MPO* level was not only elevated in obese cases but also associated with pro-inflammatory and cardiovascular risk biomarkers. In the current study, the expression of *MPO* was also significantly increased in the peripheral leukocytes of obese males. The results from our validation study in purified neutrophils are consistent with our genome wide microarray findings and with these previous studies. In males, the obese participants showed increased expression levels of both the *MPO* and *ELANE* genes in purified neutrophils.

The other top signals related to neutrophil activation in males included *LCN2*, *CEACAM6*, *CEACAM8*, *LYZ*, *OLFM4*, *AZU1* and alpha defensin families. *LCN2*, lipocalin-2, also known as neutrophil gelatinase-associated lipocalin, is involved in innate immunity and mainly expressed by neutrophils [3]. *CEACAM6* and *CEACAM8*, also known as CD66c and CD66b (Cluster of Differentiation 66c and 66b), come from the same family which encode the subunits of carcinoembryonic antigen-related cell adhesion molecule 6 and are cell-adhesion proteins on neutrophils [4]. *LYZ*, also known as muramidase or N-acetylmuramide glycanhydrolase, is mainly present in cytoplasmic granules of neutrophils with the major function of damaging bacterial cell walls. A recent study suggested that *OLFM4* was associated with neutrophil-specific granules and defines a subset of human neutrophils [5]. *AZU1* gene, azurocidin 1, is mainly expressed in azurophil granules which is one of the specialized lysosomes of the neutrophils [6]. The alpha defensin families, including *DEFA1B*, *DEFA3*, *DEFA4*, *DEFA1*, are particularly abundant in neutrophils with the activations of against bacteria, fungi and viruses.

Our results were also consistent with a recently study of bariatric surgery²⁹. Berisha et al performed a genome-wide gene expression analysis using whole blood samples from 11 obese subjects prior to and 6-12 months after bariatric surgery. The *LCN2* mRNA level decreased with -46% changes following bariatric surgery. Besides, several other genes, including *DEFA1*, *DEFA3*, *CEACAM8*, which were increased in obese subjects in our study, were found to be significantly decreased after bariatric surgery. These consistent findings suggested that these differentially expressed genes are closely related to obesity status and their expression levels can be reversed following weight change.

Different from the findings in males, we did not observe that obesity was associated with increased percentage of neutrophil, nor did the gene expression analysis show increased expression of neutrophil activation related genes in females. On the contrary, several genes in the top lists of the gene expression analysis in females were involved in the activation of nature killer cells. Interestingly, in Ilavska et al's study in which the potential sex difference was explored, a significantly negative correlation between the percentage of NK cells and BMI was observed only in females but not in males¹⁷. The other two studies which observed a decreased percentage of NK cells in obese cases vs. lean controls also have samples dominantly by females (65%-70%)^{30, 31}. In consistent with the lower percentage of NK cells in obese females, the NK cell activation related genes are down-regulated in our gene expression analysis in females. It will be of great interest to know whether this observation is due to changes in NK cell percentages or obesity is indeed associated with impaired NK functions including decreased percentage and decreased activation in females. Unfortunately, neither the percentage of NK cells was measured, nor was purified NK cell

kept in the current study. Nevertheless, both of neutrophils and nature killer cells are essential parts of innate immune system, which is the first line of defense response in a non-specific manner. This is consistent with the results of gene ontology analysis in the current study. This is, defense response is the top GO term in both males and females.

The strength of current study includes: 1) the focus on youth, which reduces the probability that gene expression changes are hidden or biased by the coexistence of obesity related comorbidities and the use of various medications, which are commonly required by obese adults. Youth are a population frequently showing obesity related metabolic risks but not yet manifesting overt clinical disease; 2) the availability of complete blood counts with differentials, which enabled us to consider the potential influence of major leukocyte composition changes; 3) the availability of purified neutrophils, which enabled us to exclude the possibility that the findings in males were driven by the increased percentage of neutrophils in obese cases.

Nevertheless, several limitations of this study need to be recognized. First, genome-wide gene expression analysis on purified neutrophils is needed to provide further confirmation of the relationship between obesity and neutrophil activation related genes. Second, this study was based on African American populations. Further replication in Caucasians as well as other ethnicities will be needed.

In conclusion, we found that in young AA males, obese status was not only associated with increased percentage of peripheral neutrophil, but also with increased neutrophil gene activation. Further functional and mechanistic studies on neutrophils could contribute to the development of novel intervention strategies reducing the burden associated with obesity-related health problems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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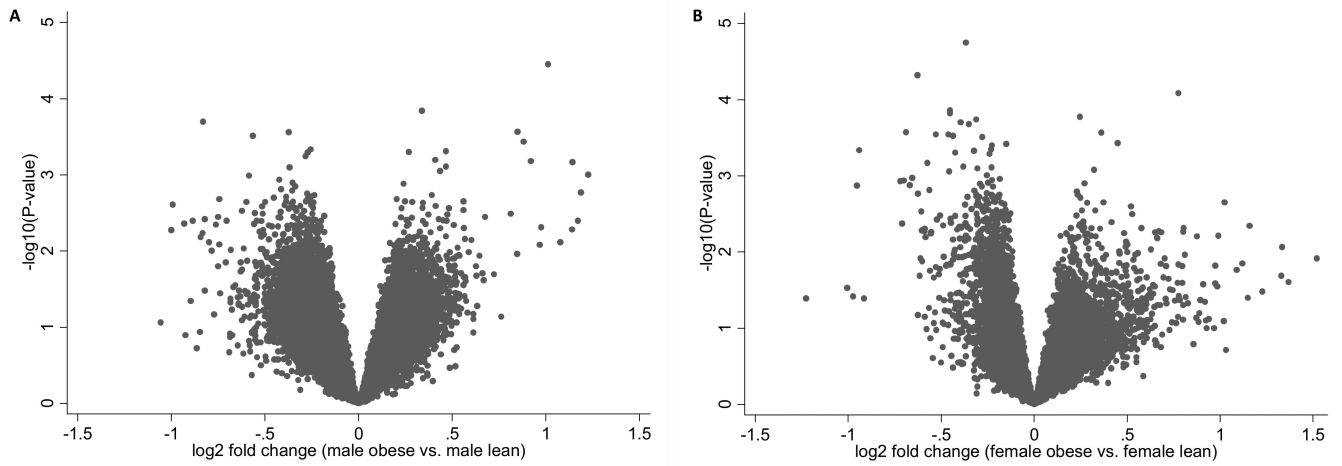


Figure 1. Volcano plot of obesity related differentially expressed genes in males and females
(A) Volcano plot of the \log_2 fold change of gene expression levels (X-axis) against the $-\log_{10}$ P-value (Y-axis) comparing obese and lean males.
(B) Volcano plot of the \log_2 fold change of gene expression levels (X-axis) against the $-\log_{10}$ P-value (Y-axis) comparing obese and lean females.

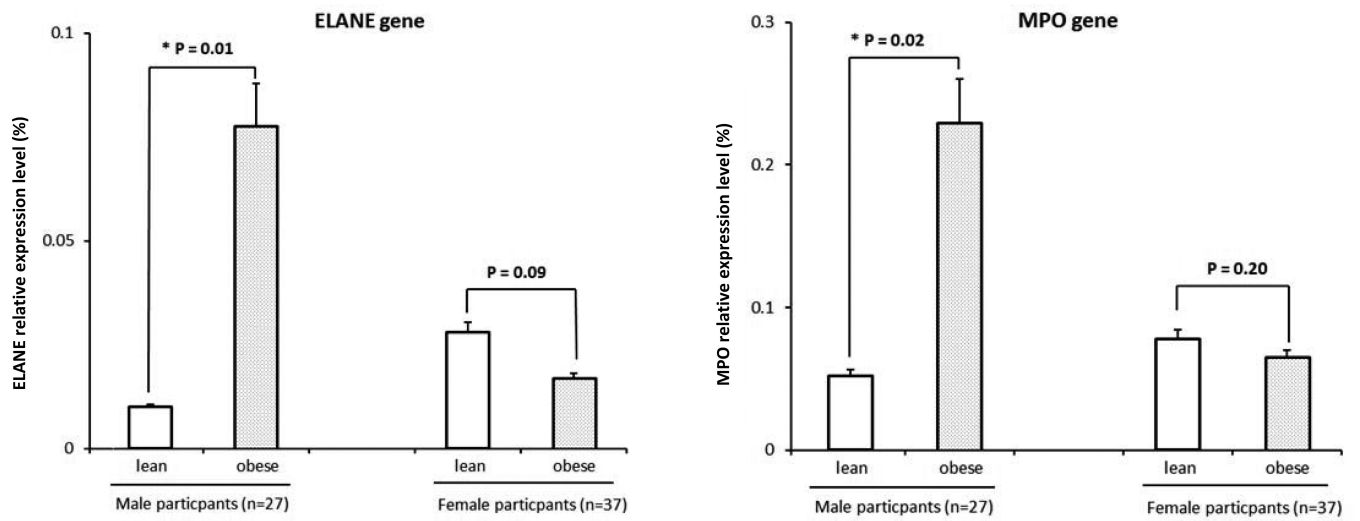


Figure 2. Real time PCR replication of ELANE and MPO in neutrophils

(A) Histogram showing the gene expression levels of ELANE relative to β -actin (Y-axis, %) comparing obese and lean males ($p=0.01$ with age adjusted) and females ($p=0.09$ with age adjusted), respectively

(B) Histogram showing the gene expression levels of MPO relative to β -actin (Y-axis, %) comparing obese and lean males ($p=0.02$ with age adjusted) and females ($p=0.20$ with age adjusted), respectively

General characteristics of all participants in genome-wide gene expression analysis using mRNA from peripheral leukocytes (n=95)

Table 1

General character	Male participants		Female participants		P-value *
	Lean (n=24)	Obese (n=22)	Lean (n=25)	Obese (n=24)	
Age (years)	17.42±1.91	17.58±1.96	17.91±1.72	17.35±1.49	0.23
BMI (kg/m ²)	19.34±1.34	39.11±6.95	18.57±1.16	44.43±7.52	<0.001
BMI percentile (%)	22.47±12.57	99.15±0.73	19.05±9.84	99.07±0.46	<0.001
SBP (mmHg)	110.78±9.63	127.24±14.02	103.69±5.81	129.16±19.69	<0.001
DBP (mmHg)	64.54±7.74	63.88±6.57	63.27±5.09	67.19±10.77	0.06
TG (mg/dl)	60.57±26.94	67.76±24.54	54.60±22.99	61.54±19.40	0.15
HDLc (mg/dl)	56.09±13.17	44.67±11.53	61.24±15.01	47.67±8.84	<0.001
Fasting insulin (uU/ml)	8.23±3.40	19.44±9.94	12.71±7.25	30.52±14.95	<0.001
Fasting glucose (mg/dl)	88.83±7.70	90.48±7.71	83.80±6.99	88.08±9.07	0.11

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDLC, high-density lipoprotein cholesterol.

* P-value adjusted for age (if applicable)

Table 2

Complete blood count with differential (mean ± standard deviation)

Blood cell composition	Male participants			Female participants		
	Lean	Obese	P-value*	Lean	Obese	P-value*
Neutrophils (%)	46.45±11.15	52.95±9.74	0.03	55.92±10.77	54.18±10.30	0.63
Lymphocytes (%)	40.63±10.70	35.72±9.32	0.11	33.72±9.47	35.68±8.65	0.50
Monocytes (%)	9.29±2.79	7.95±1.53	0.06	7.80±3.20	7.64±2.11	0.81
Eosinophils (%)	3.29±1.78	2.55±1.79	0.18	1.96±1.90	2.27±2.43	0.55
Basophils (%)	0.63±0.49	0.81±0.66	0.29	0.60±0.58	0.55±0.60	0.85
White blood cell (1000/mm ³)	4.97±1.57	6.03±1.62	0.05	5.51±1.41	6.20±1.47	0.13
Neutrophils (1000/mm ³)	2.39±1.14	3.33±1.62	0.04	3.18±1.19	3.48±1.34	0.46
Lymphocytes (1000/mm ³)	1.95±0.71	2.03±0.59	0.51	1.80±0.55	2.13±0.33	0.03
Monocytes (1000/mm ³)	0.45±0.16	0.46±0.12	0.80	0.40±0.16	0.48±0.16	0.15
Eosinophils (1000/mm ³)	0.19±0.15	0.17±0.13	0.81	0.11±0.10	0.13±0.11	0.34
Basophils (1000/mm ³)	0.01±0.04	0.05±0.08	0.08	0.03±0.05	0.03±0.05	0.86

* P-value adjusted for age

Table 3

Top 20 differentially expressed genes sorted by P-value or Log2 fold changes

Sorted by P-value			Sorted by Log2 fold changes		
Gene Symbol	Log2 fold change	P-value *	Gene Symbol	Log2 fold change	P-value *
<i>Obese (n=22) vs. lean (n=24) males</i>					
LCN2 **	1.01	3.43E-05	DEFA1B	1.19	1.66E-03
PLEKHJ1	0.34	1.40E-04	ALAS2	1.14	6.58E-04
PID1	-0.83	2.95E-04	DEFA3	1.14	5.10E-03
CEACAM6	0.85	2.65E-04	LCN2	1.01	3.43E-05
C6orf97	-0.37	2.68E-04	IGJ	-1.00	5.16E-03
HSD17B12	-0.56	2.96E-04	LYZ	-0.99	2.39E-03
ELANE	0.88	3.59E-04	DEFA4	0.98	4.70E-03
SLC7A6OS	-0.26	4.51E-04	DEFA1	0.97	8.13E-03
AZU1	0.47	4.77E-04	OLFM4	0.92	6.47E-04
FGF18	0.27	4.88E-04	ELANE	0.88	3.59E-04
MRPL45	-0.27	4.94E-04	CEACAM6	0.85	2.65E-04
TESSP5	-0.28	5.56E-04	PID1	-0.83	1.95E-04
WASH1	0.41	6.21E-04	TNFRSF17	-0.82	3.71E-03
OLFM4	0.92	6.47E-04	CEACAM8	0.81	3.16E-03
ALAS2	1.14	6.58E-04	TXNDC5	-0.80	7.41E-03
C17orf67	0.47	7.51E-04	RPS23	-0.78	9.65E-03
BRCC3	-0.37	7.73E-04	GLDC	-0.74	8.03E-03
NCRNA00092	0.44	8.64E-04	RPL12P6	-0.68	9.43E-03
CEP68	-0.42	1.14E-03	CTSG	0.68	3.50E-03
ANKRD37	-0.34	1.37E-03	KIAA0101	-0.61	9.05E-03
<i>Obese (n=24) vs. lean (n=25) females</i>					
FAAH	-0.37	1.79E-05	SLPI	1.34	8.66E-03
KIR3DL1	-0.63	4.76E-05	IL1B	1.16	4.58E-03
NRG1	0.78	8.22E-05	CASP5	1.02	2.23E-03
FLJ42627	-0.45	1.38E-04	TREML3	0.99	6.11E-03
ITIH4	-0.45	1.50E-04	KIR2DL3	-0.95	1.34E-03
SYTL3	-0.40	1.99E-04	KIR2DL4	-0.94	4.62E-04
LIMD2	-0.35	2.09E-04	NAIP	0.88	6.24E-03
KLHL5	0.36	2.71E-04	CCR1	0.80	4.83E-03
ABCA2	-0.46	2.88E-04	DSC2	0.80	5.38E-03
NCAM1	-0.44	2.97E-04	NRG1	0.78	8.22E-05
GTF3C1	-0.28	3.09E-04	KLRC1	-0.71	4.23E-03
DACT1	0.45	3.73E-04	THBD	0.68	5.56E-03
SNX1	-0.23	4.01E-04	MPL	0.66	6.58E-03
KIAA2026	-0.23	4.51E-04	C17orf97	0.66	6.51E-03
KIR2DL4	-0.94	4.61E-04	PROS1	0.65	5.52E-03

Sorted by P-value			Sorted by Log ₂ fold changes		
Gene Symbol	Log ₂ fold change	P-value *	Gene Symbol	Log ₂ fold change	P-value *
STAG3L4	-0.32	4.66E-04	EMR2	0.63	9.26E-03
ZCCHC14	-0.24	5.15E-04	KIR3DL1	-0.63	4.76E-05
ANKRD20A1	-0.57	6.77E-04	KIR2DS5	-0.63	1.70E-03
GSTM2	-0.38	7.56E-04	KIR2DL1	-0.61	2.94E-03
CLK2	-0.23	7.69E-04	KIR3DL3	-0.60	5.19E-03

* P-value adjusted age

** Bold genes were closely related to neutrophil activation

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Table 4

Pathway analysis of obesity related differentially expressed genes*

Terms	Genes	P-value
<i>Obese (n=22) vs. lean (n=24) males</i>		
Defense response to bacterium	CTSG, DEFA1B, DEFA3, DEFA4, LYZ	1.0E-04
Defense response to fungus	DEFA1B, DEFA3, DEFA4	3.6E-04
Response to other organism	CTSG, DEFA1B, DEFA3, DEFA4, LCN2, LYZ	3.9E-04
Killing of cells of another organism	DEFA1B, DEFA3, DEFA4	4.2E-04
Response to bacterium	CTSG, DEFA1B, DEFA3, DEFA4, LYZ	8.0E-04
Immune system process	ALAS2, CEACAM8, CTSG, DEFA1B, ELANE, IGF, IL4R, TNFRSF17	1.1E-03
Response to fungus	DEFA1B, DEFA3, DEFA4	1.3E-03
Cell killing	DEFA1B, DEFA3, DEFA4	1.3E-03
Response to biotic stimulus	CTSG, DEFA1B, DEFA3, DEFA4, LCN2, LYZ	1.4E-03
Translational elongation	RPL12P6, RPL21, RPS21, RPS23	1.4E-03
<i>Obese (n=24) vs. lean (n=25) females</i>		
Immune response	BMP6, CCRI, IL1B, KIR3DL1, KIR2DL3, KIR2DS5, SPON2	4.1E-05
Immune system process	BMP6, CCRI, IL1B, KIR3DL1, KIR2DL3, KIR2DS5, SPON2	4.2E-04
Response to wounding	BMP6, CCRI, IL1B, NRG1, PROS1, THBD	9.0E-04
Response to stimulus	BMP6, CCRI, IL1B, KIR3DL2, KIR3DL1, KIR2DL1, KIR2DL3, KIR2DL4, KIR2DS5, NRG1, PROS1, SPON2, THBD	1.6E-03
Defense response	BMP6, CCRI, IL1B, KIR3DL2, KIR2DL4, KIR2DS5	1.8E-03
Response to stress	BMP6, CCRI, IL1B, KIR3DL2, KIR2DL4, KIR2DS5, NRG1, PROS1, THBD	2.0E-03
Wound healing	IL1B, NRG1, PROS1, THBD	2.7E-03
Cellular defense response	KIR3DL2, KIR2DL4, KIR2DS5	3.7E-03
Response to external stimulus	BMP6, CCRI, IL1B, NRG1, PROS1, THBD	9.6E-03
Anti-apoptosis	NAIP, IL1B, NRG1	3.7E-02

* Genes were selected with p-value<0.01 and absolute log2 fold change > 0.5, and the human genome was chosen as background in the analysis