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Associations of gene expression in blood with BMI and weight changes among women in the Norwegian Women and Cancer postgenome cohort

Nikita Baiju[1] | Charlotta Rylander[1] | Pål Sætrom[2,3,4,5] | Torkjel M. Sandanger[1] | Therese H. Nøst[1,5]

[1]Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

[2]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[3]Department of Computer Science, Norwegian University of Science and Technology, Trondheim, Norway

[4]Bioinformatics Core Facility, Norwegian University of Science and Technology, Trondheim, Norway

[5]Department of Public Health and Nursing, K.G. Jebsen Center for Genetic Epidemiology, Norwegian University of Science and Technology, Trondheim, Norway

Correspondence
Nikita Baiju, Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway.
Email: nikita.baiju@uit.no

Abstract

Objective: This study aimed to evaluate associations between blood gene expression profiles and (1) current BMI and (2) past weight changes (WCs) among women who had never been diagnosed with cancer in the Norwegian Women and Cancer (NOWAC) postgenome cohort.

Methods: This cross-sectional study ($N = 1694$) used gene expression profiles and information from three questionnaires: Q1 (baseline), Q2 (follow-up), and Q3 (blood collection). The authors performed gene-wise linear regression models to identify differentially expressed genes (DEGs) and functional enrichment analyses to identify their biological functions.

Results: When assessing BMI_{Q3}, the study observed 2394, 769, and 768 DEGs for the obesity-versus-normal weight, obesity-versus-overweight, and overweight-versus-normal weight comparisons, respectively. Up to 169 DEGs were observed when investigating $WC_{Q3\text{-}Q1}$ (mean = 7 years, range = 5.5–14 years) and $WC_{Q3\text{-}Q2}$ (mean = 1 year, range = <1 month–9 years) in interaction models with BMI categories, of which 1 to 169 genes were associated with WCs and 0 to 9 were associated with interaction effects of BMI and WCs. Biological functions of BMI-associated DEGs were linked to metabolism, erythrocytes, oxidative stress, and immune processes, whereas WC-associated DEGs were linked to signal transduction.

Conclusions: Many BMI-associated but few WC-associated DEGs were identified in the blood of women in Norway. The biological functions of BMI-associated DEGs likely reflect systemic impacts of obesity, especially blood reticulocyte-erythrocyte ratio shifts.

INTRODUCTION

Overweight and obesity are states of abnormal or excessive fat accumulation that cause risks to health [1]. These states are widespread, and global projections indicate that more than 2.16 and 1.12 billion adults will have overweight and obesity by 2030, respectively [2]. Although the primary causes of obesity are considered to be excess dietary intake and inadequate physical activity, other factors (e.g., endocrine disruptions, smoking cessation) may also contribute [3, 4]. Obesity and overweight are major risk factors for

noncommunicable diseases such as diabetes, cardiovascular diseases, musculoskeletal disorders, and several cancers [1, 5]. Additionally, independent of body composition, weight gain in adulthood is a risk factor for several cancers, including postmenopausal breast cancer [5, 6].

Gene expression profiles can improve our understanding of the molecular mechanisms of multifactorial conditions like obesity [7]. In cross-sectional studies, increased body mass index (BMI) has been associated with differences in the expression of several genes [8, 9] that indicated alterations in biological functions related to the regulation of body mass, metabolism, and cellular functions [8, 10]. Insulin resistance, oxidative stress, and liver damage markers are overexpressed in people with obesity compared with those without obesity [11]. Gene expression profiles in adipose tissue have been associated with obesity and weight loss in several studies [9, 12–19]. This is expected as, biologically, adipose tissue is most relevant to obesity [10]. Few studies have examined gene expression related to obesity either in whole blood [10, 20] or peripheral blood mononuclear cells (PBMCs) [21] and found differences. However, to our knowledge, no study has assessed this relationship in a large population-based sample. Furthermore, no study has yet evaluated differences in blood gene expression related to weight changes (WCs).

We aimed to evaluate associations between gene expression profiles in whole blood and (1) current BMI and (2) past WCs in a large population-based sample of women who had never been diagnosed with cancer and to assess the biological functions of differentially expressed genes (DEGs).

Study Importance

What is already known?

- Obesity has been linked to altered gene expression in whole blood, yet few studies have investigated the association between blood gene expression and BMI in a large sample of women.
- No study, to our knowledge, has evaluated the associations between blood gene expression and past weight changes (WCs).

What does this study add?

- A large number of BMI-associated differentially expressed genes (DEGs) but few WC-associated DEGs (i.e., >700 and <168 DEGs, respectively) were identified in blood of women in Norway.
- The biological functions of BMI-associated DEGs were linked to general metabolism, erythrocyte functions, oxidative stress, and immune processes, whereas WC-associated DEGs were linked to signal transduction.
- This is the first study, to our knowledge, to conclude that blood gene expression reflects current BMI more than past WCs.

How might these results change the direction of research?

- The study results likely reflect systemic impacts of obesity, especially reticulocyte-erythrocyte ratio shifts in blood, as these functions coincide with its known physiological effects. This knowledge is relevant for further research related to the health effects of BMI and WCs, especially those that focus on blood-based markers.

METHODS

Study design and sample

We used a cross-sectional study design based on microarray data from women participating in the prospective population-based Norwegian Women and Cancer (NOWAC) postgenome cohort. This subcohort of the NOWAC study consists of approximately 50,000 women (mean age: 49.78 years; mean BMI: 23.38 kg/m^2) who had blood samples collected from 2003 to 2006 for gene expression analysis. Samples were collected in PreAnalytiX (PAX) gene tubes, and details about the study sample and women selected for gene expression analyses are available in Baiju et al. [22]. Several case-control studies have investigated gene expression profiles in the NOWAC postgenome cohort; here, we included only the controls from these studies and further excluded women who had later been diagnosed with cancer, so that the study sample included only women who had never been diagnosed with cancer ($N = 1694$; details about inclusion and exclusion criteria are presented in Figure 1A). All included women completed a baseline questionnaire (Q1), many completed a follow-up questionnaire (Q2), and all completed a third questionnaire at the time of blood collection (Q3). The mean interval between Q1 and Q2 (Q1-Q2) was approximately 6 years, between Q2 and Q3 (Q2-Q3) was approximately 1 year, and between Q1 and Q3 (Q1-Q3) was approximately 7 years (Figure 1B). We obtained relevant questionnaire and registry information from the NOWAC study databases. The Regional Ethical Committee of North Norway (REK) has approved the collection and storage of data and human biological material in the NOWAC cohort and biobank (NOWAC postgenome cohort) (reference numbers: 2010/2075/REK Nord and 2014/1605/REK Nord, respectively).

Laboratory analyses and preprocessing of gene expression data

Laboratory analyses were performed between January 2011 and January 2015 at the Genomics Core Facility, Norwegian University of Science and Technology, Trondheim. Total RNA was extracted and

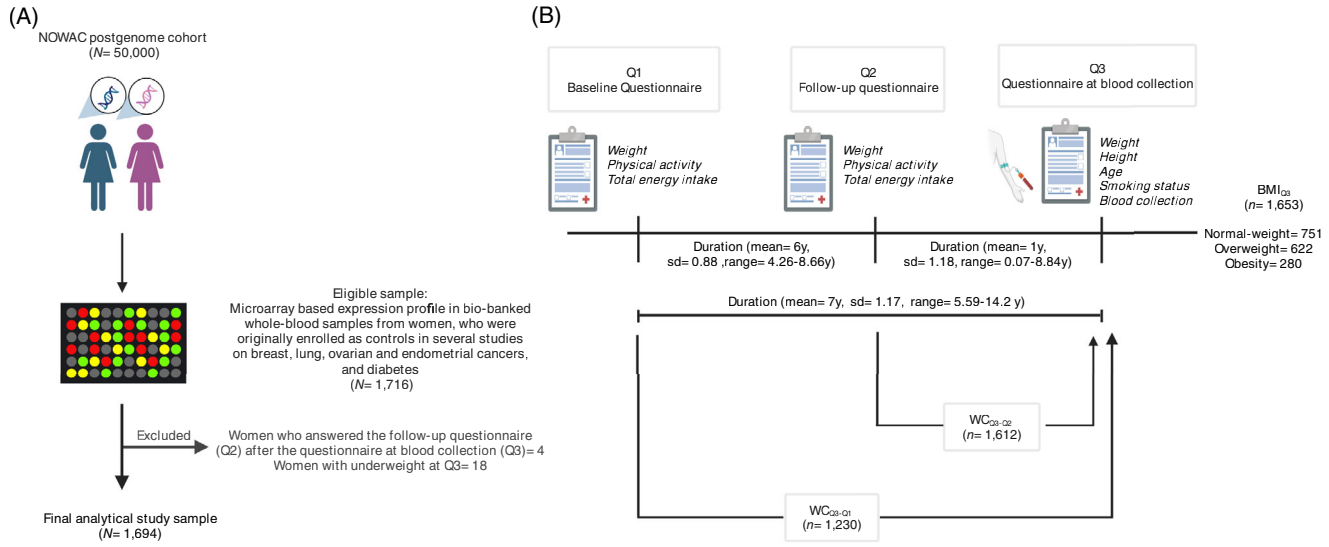

FIGURE 1 (A) Flowchart of study sample and (B) timeline of questionnaires in the NOWAC study (created with BioRender.com). BMI_{Q3}, body mass index categories at Q3; NOWAC, The Norwegian Women and Cancer study; Q1, baseline questionnaire; Q2, follow-up questionnaire; Q3, questionnaire at blood collection; $WC_{Q3\text{-}Q1}$, weight change between Q1 and Q3; $WC_{Q3\text{-}Q2}$, weight change between Q2 and Q3 [Color figure can be viewed at wileyonlinelibrary.com]

purified from PAX gene-tube samples following the PAX gene blood RNA kit protocol. RNA purity and RNA integrity were assessed using a NanoDrop ND 8000 spectrophotometer (ThermoFisher Scientific) and bioanalyzer capillary electrophoresis (Agilent Technologies), respectively. Complementary DNA (cDNA) was prepared using the Illumina TotalPrepT-96 RNA amplification kit and hybridized to Illumina HumanWG-3 or HumanHT-12 Gene Expression BeadChip microarrays. The raw microarray images were processed in Illumina Genome Studio.

Details about the preprocessing of gene expression data are available in Baiju et al. [22]. Briefly, we performed background correction, removed bad-quality probes, and filtered probes detected in <20% of samples. Furthermore, we performed $\log_2$ transformation and quantile normalization before all data were combined and inspected for batch effects using principal component analysis plots. These stringent filtering criteria rendered 9095 probes, and the probe with the highest interquartile range was selected per gene, which resulted in 7713 unique genes in our data set.

BMI and WCs

BMI at Q1 (BMI_{Q1}), Q2 (BMI_{Q2}), and Q3 (BMI_{Q3}) was calculated by dividing weight in kilograms by height in meters squared and then categorized according to the World Health Organization standard (underweight: <18.5, normal weight: 18.5–24.9, overweight: 25.0–29.9, obesity: ≥30.0).

We calculated WCs between Q1 and Q3 ($WC_{Q3\text{-}Q1}$, mean interval 7 years) and between Q2 and Q3 ($WC_{Q3\text{-}Q2}$, mean interval 1 year). We also defined WC categories based on patterns of WCs between Q1 and Q2 and between Q2 and Q3: consistent stable weight (CSW, women with stable weight [−2 to +2 kg] at Q1-Q2 and Q2-Q3); consistent weight gain (CWG, women with weight gain [above +2 kg] at Q1-Q2 and Q2-Q3); consistent weight loss (CWL, women with weight loss [below −2 kg] at Q1-Q2 and Q2-Q3); former weight gain (FWG, women with weight gain at Q1-Q2 and stable weight at Q2-Q3); former weight loss (FWL, women with weight loss at Q1-Q2 and stable weight at Q2-Q3); recent weight gain (RWG, women with stable weight at Q1-Q2 and weight gain at Q2-Q3); and recent weight loss (RWL, women with stable weight at Q1-Q2 and weight loss at Q2-Q3).

Statistical analyses

We performed all analyses using R version 4.0.5. We used the Bioconductor package *limma* for gene-wise linear models to identify DEGs and considered a significance threshold of Benjamini-Hochberg false discovery rate (FDR) ≤ 0.05.

BMI analyses

We evaluated associations between blood gene expression and BMI_{Q3} modeled as a categorical variable (categorical BMI analyses) in three comparisons: obesity versus normal weight, obesity versus overweight, and overweight versus normal weight. To assess incremental associations, we also modeled BMI_{Q3} as a continuous standardized metric (continuous BMI analyses) and scaled it using the R function *scale*, which for each observation subtracts the mean and divides by the standard deviation. Forty-one women had missing information on BMI_{Q3}, resulting in an analytical sample of 1653 women for these analyses.

WC analyses

We evaluated associations between blood gene expression and past WCs modeled as a categorical variable (categorical WC analyses) in six comparisons: CWG versus CSW, CWL versus CSW, FWG versus CSW, FWL versus CSW, RWG versus CSW, and RWL versus CSW. We then conducted sensitivity analyses restricted to women with <1 year between Q2 and Q3. We excluded 160 women who reported decreased weight at Q1 to Q2 and increased weight at Q2 to Q3 and vice versa, that is, weight-cyclers, and 499 women with missing values, resulting in an analytical sample of 1035 women in these analyses.

We also evaluated associations between gene expression and past WCs modeled as a continuous metric in two interaction models that included BMI category to assess trends across these categories (WC-BMI interaction analyses). To account for differences in the intervals of $WC_{Q3\text{-}Q1}$ and $WC_{Q3\text{-}Q2}$, we divided the absolute values of WCs (kilograms) by the number of years between Q3 and Q1 or Q2 (kilograms/year) before scaling it (R function *scale*). The first interaction model included BMI_{Q1} or BMI_{Q2} and succeeding WCs (i.e., BMI_{Q1}* $WC_{Q3\text{-}Q1}$ or BMI_{Q2}* $WC_{Q3\text{-}Q2}$); the second included current BMI (BMI_{Q3}) and preceding WCs (i.e., BMI_{Q3}* $WC_{Q3\text{-}Q1}$ or BMI_{Q3}* $WC_{Q3\text{-}Q2}$). We excluded 464 and 82 women with missing values for $WC_{Q3\text{-}Q1}$ and $WC_{Q3\text{-}Q2}$, respectively, resulting in respective analytical samples of 1230 and 1612 women.

To evaluate the influence of extreme WC values, we performed sensitivity analyses in which we assigned WC values that were under the 5th percentile and over the 95th percentile to the values of the 5th and 95th percentiles, respectively. Additionally, we performed sensitivity analyses using the WC unit of BMI/year instead of kilograms/year.

White blood cell proportions

Blood cell type composition affects gene expression profiles [23], and reticulocyte, erythrocyte, and white blood cell (WBC) counts were not available for our study sample. However, we estimated the proportions of 22 populations of WBCs in the samples using an *in silico* gene expression deconvolution method CIBERSORT and the LM22 signature matrix [24]. To distinguish changes in gene expression related to WBC composition from those related to BMI, we adjusted for WBC proportions that were significantly associated with BMI_{Q3} according to the Kruskal-Wallis test and with overall gene expression data according to the *global test* from the Bioconductor package *global test*.

Covariates

We assessed the distribution of the following self-reported covariates by BMI_{Q3} categories: age at Q3 (years), physical activity/day at either Q2 or Q1 (not available from Q3; stated on a scale of 1 to 10, in which 1 represented "not active" and 10 represented "extremely active"), total energy intake at either Q2 or Q1 (not available from Q3; kilojoules/day), and smoking status at Q3 (current/former/never smokers). We considered laboratory batch (laboratory plates) and sample storage time as technical covariates. We employed two adjustment models (minimally adjusted and fully adjusted) for all analyses. Minimally adjusted models included technical covariates only, whereas fully adjusted models included technical covariates, selected WBC proportions (described earlier), age, and smoking status at Q3. In categorical WC analyses, we additionally adjusted for BMI_{Q1} in the fully adjusted models. Furthermore, sensitivity analyses related to BMI analyses were additionally adjusted for physical activity and total energy intake in the fully adjusted models.

Functional enrichment analyses

We investigated the biological functions of the BMI- and WC-associated DEGs identified in the fully adjusted models by functional enrichment analyses. Analyses were conducted separately for overexpressed (log_2 fold-change [logFC] > 0) and underexpressed genes (logFC < 0) using the Bioconductor packages *clusterProfiler* and *ReactomePA* of Gene Ontology (GO) biological processes (BP), GO molecular functions (MF), GO cellular components (CC), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and REACTOME pathways.

Quantitative replication

To assess whether our results were in line with previous results or novel findings, we compared our BMI-associated DEG results with results of analyses in external/independent transcriptomic data sets in whole blood and other relevant tissues.

RESULTS

The BMI_{Q3} distribution in our study sample was 751 (45%), 622 (38%), and 280 (17%) for normal weight, overweight, and obesity, respectively. There were no substantial differences in the distribution of most covariates across BMI_{Q3} categories, but women with obesity were older, and normal weight women reported the highest mean physical activity level and total energy intake (Figure 2, Supporting Information Table S1). WC category distribution was 263 (25%), 138 (13%), and 14 (1%) for CSW, CWG, and CWL; and 396 (38%), 91 (9%), 101 (10%), and 32 (3%) for FWG, FWL, RWG, and RWL, respectively.

Estimated WBC proportions

Among the estimated WBC proportions, naive B cells, memory B cells, naive CD4 T cells, and memory-activated CD4 T cells were significantly associated with both BMI_{Q3} category and overall gene

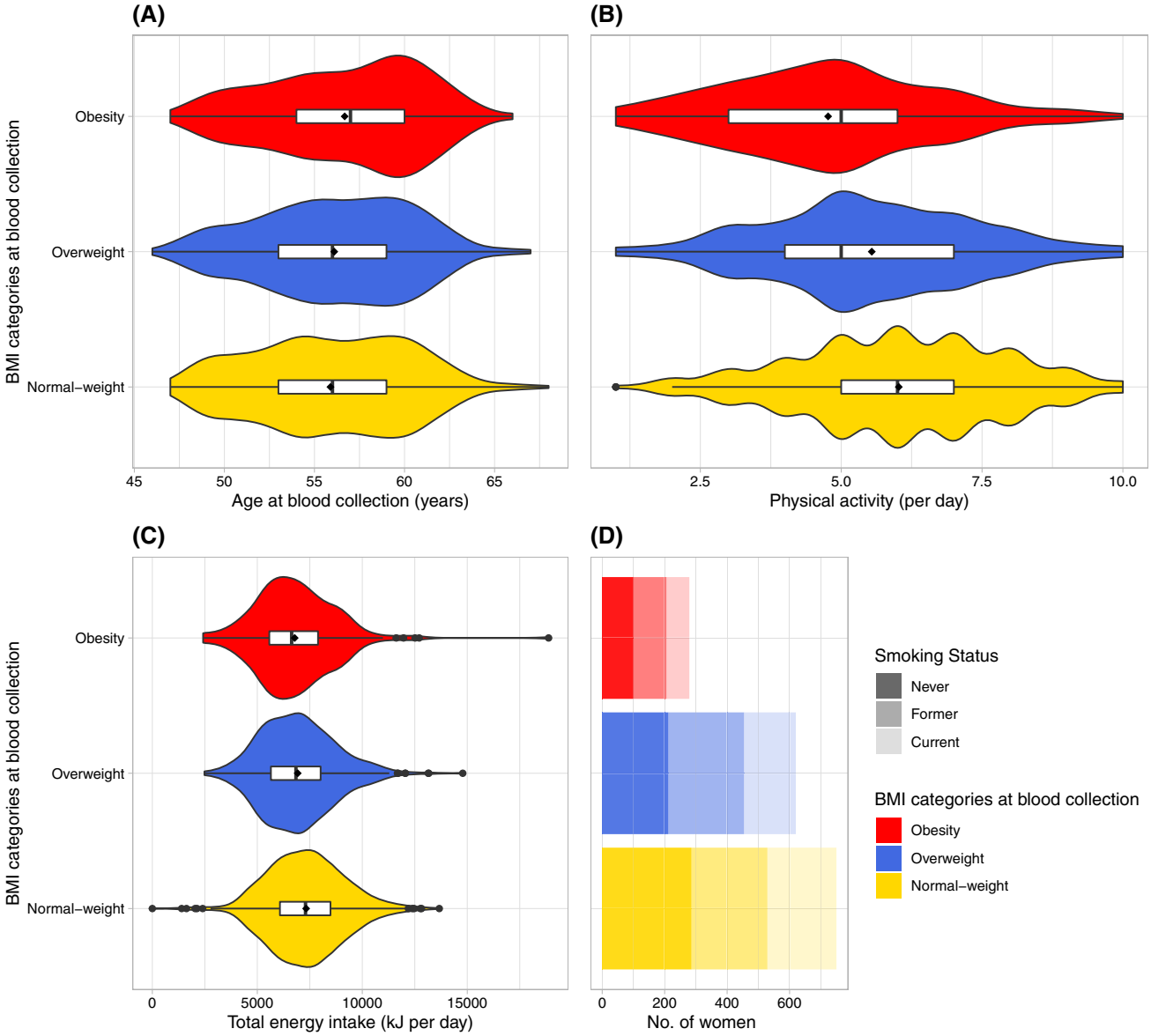

FIGURE 2 BMI category at blood collection by (A) age at blood collection (years), (B) physical activity (per day), (C) total energy intake (kJ per day), and (D) smoking status at blood collection. Violin plots represent the kernel density estimates for women with obesity, overweight, and normal weight. White boxes extend from the 25th to the 75th percentile, vertical bars inside the box represent the median, whiskers extend 1.5 times the length of the interquartile range to the right and left side of the 75th and 25th percentiles, respectively, and outliers are represented as black dots. The black diamond-shaped dot represents the respective mean. In panel D, the color lightness represents smoking status (never, former, current smokers) at blood collection. Here, physical activity and total energy intake were from either Q2 or Q1 (not available for Q3). Q1, baseline questionnaire; Q2, follow-up questionnaire; Q3, questionnaire at blood collection [Color figure can be viewed at wileyonlinelibrary.com]

expression (Supporting Information Table S2). Women with obesity had slightly higher mean proportions of naive B cells and lower mean proportions of the three other cell types listed compared with women with normal weight (Supporting Information Figure S1).

BMI-associated DEGs

Table 1 presents the number of BMI-associated DEGs in the minimally and fully adjusted models. In the fully adjusted model of categorical BMI analyses, the top-ranked gene (i.e., the gene with the lowest FDR-adjusted *p* value) in the obesity-versus-normal weight comparison (Figure 3A,D) and the obesity-versus-overweight comparison (Figure 3B,E) was *FAM46C* (renamed: *TENT5C*; logFC = 0.86, FDR = 6×10^{-32}). *FAM46C* (logFC = 0.34, FDR = 1×10^{-45}; Supporting Information Figure S2) was also the top-ranked gene in continuous BMI analyses. *FAM46C* expression was higher in women with higher BMI (Supporting Information Figure S3). In the overweight-versus-normal weight comparison (Figure 3C,F), the top-ranked gene was *SLC45A3* (logFC = 0.28, FDR = 1×10^{-14}), and its expression was lower in women with higher BMI (Supporting Information Figure S3).

TABLE 1 Number of DEGs identified in BMI and WC analyses

	Minimally adjusted models[a]			Fully adjusted models[b]		
	DEGs (FDR ≤ 0.05)	Overexpressed (logFC > 0)	Underexpressed (logFC < 0)	DEGs (FDR ≤ 0.05)	Overexpressed (logFC > 0)	Underexpressed (logFC < 0)
BMI models						
Obesity vs. normal weight	2294	1011	1283	2394	1057	1337
Obesity vs. overweight	553	370	183	769	475	294
Overweight vs. normal weight	629	285	344	768	315	453
BMI[c]	2970	1257	1713	3106	1293	1813
WC models						
First interaction model (BMI_{Q1orQ2}*WC)						
$WC^{d,e}_{Q3\text{-}Q1}$	4	34	9	3	1	2
$BMI_{Q1\text{-}Overweight}$: $WC^{d,f}_{Q3\text{-}Q1}$	0	0	0	0	0	0
$BMI_{Q1\text{-}Obesity}$: $WC^{d,f}_{Q3\text{-}Q1}$	0	0	0	0	0	0
$WC^{d,e}_{Q3\text{-}Q2}$	217	31	186	169	23	146
$BMI_{Q2\text{-}Overweight}$: $WC^{d,e}_{Q3\text{-}Q2}$	0	0	0	0	0	0
$BMI_{Q2\text{-}Obesity}$: $WC^{d,f}_{Q3\text{-}Q2}$	0	0	0	0	0	0
Second interaction model (BMI_{Q3}*WC)						
$WC^{d,e}_{Q3\text{-}Q1}$	17	3	14	5	0	5
$BMI_{Q3\text{-}Overweight}$: $WC^{d,f}_{Q3\text{-}Q1}$	0	0	0	0	0	0
$BMI_{Q3\text{-}Obesity}$: $WC^{d,f}_{Q3\text{-}Q1}$	27	25	2	9	9	0
$WC^{d,e}_{Q3\text{-}Q2}$	4	2	2	1	0	1
$BMI_{Q3\text{-}Overweight}$: $WC^{d,f}_{Q3\text{-}Q2}$	0	0	0	0	0	0
$BMI_{Q3\text{-}Obesity}$: $WC^{d,f}_{Q3\text{-}Q2}$	1	0	1	1	0	1

Note: No DEGs were observed in models comparing WC categories (CWG vs. CSW, CWL vs. CSW, FWG vs. CSW, FWL vs. CSW, RWG vs. CSW, and RWL vs. CSW), and hence they are not presented here.

Abbreviations: CSW, consistent stable weight; CWG, consistent weight gain; CWL, consistent weight loss; DEGs, differentially expressed genes; FDR, false discovery rate; FWG, former weight gain; FWL, former weight loss; logFC, $\log_2$ fold-change; Q1, baseline questionnaire; Q2, follow-up questionnaire; Q3, questionnaire at blood collection; RWG, recent weight gain; RWL, recent weight loss; WC, weight change.

[a]Adjusted for laboratory batch (laboratory plates) and sample storage time.

[b]Adjusted for minimally adjusted model plus selected white blood cell proportions, age, and smoking status at Q3.

[c]BMI was included in the model as a scaled continuous metric.

[d]WCs were included in the model as a scaled continuous metric.

[e]These results represent those for the main effect of WCs in the interaction model.

[f]These results represent those for the interaction effect of BMI and WCs in the interaction model.

The distributions of *p* values varied across comparison groups, and the observed and expected distributions deviated the most for the obesity-versus-normal weight comparison (Supporting Information Figure S4). Many DEGs overlapped in the minimally and fully adjusted models (2080, 522, 580, and 2705 overlapping DEGs in the obesity-versus-normal weight, obesity-versus-overweight, overweight-versus-normal weight comparisons and continuous BMI analyses, respectively; Supporting Information Tables S3–S6). Furthermore, results from sensitivity analyses, which were additionally adjusted for physical activity and total energy intake, did not alter the overall results (99%, 94%, 96%, and 99% of DEGs overlapped in the fully adjusted models for the obesity-versus-normal weight, obesity-versus-overweight, overweight-versus-normal weight comparisons and continuous BMI analyses, respectively); and the logFC

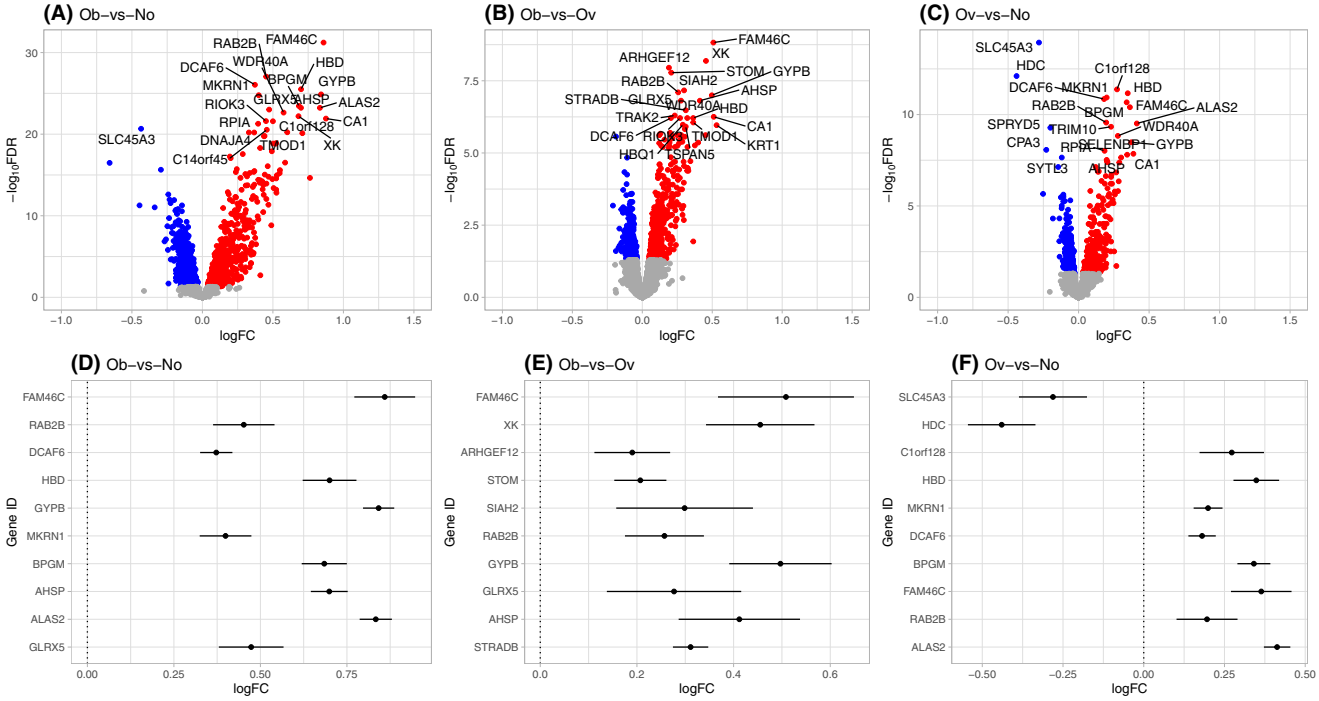

FIGURE 3 (A–C) Volcano plots of the test statistics for BMI categories at blood collection in fully adjusted models from the tests of DEGs and (D–F) forest plots of the 10 top-ranked genes in the tests of DEGs. In volcano plots, red dots display overexpressed genes (FDR ≤ 0.05 and logFC > 0) and blue dots display underexpressed genes (FDR ≤ 0.05 and logFC < 0), whereas gray dots display genes with FDR > 0.05; gene names displayed are the top 20 DEGs in the respective tests. In forest plots, the gene names of DEGs are presented for those with the lowest FDR-adjusted *p* values ranked from the top; the horizontal line for each gene represents the confidence interval; and the vertical dotted line represents the line of no difference. DEGs, differentially expressed genes; FDR, false discovery rate; logFC, $\log_2$ fold-change; Ob-vs.-No, comparison of women with obesity versus normal weight; Ob-vs.-Ov, comparison of women with obesity versus overweight; Ov-vs.-No, comparison of women with overweight versus normal weight [Color figure can be viewed at wileyonlinelibrary.com]

for the top-ranked *FAM46C* changed ≤2%, whereas it did not change for *SLC45A3* (results not shown). Fully adjusted categorical BMI analyses revealed a cumulative total of 169 overexpressed and 72 underexpressed genes that overlapped in the different comparisons.

Furthermore, 525 DEGs overlapped across all models testing association with BMI (obesity-vs.-normal weight and overweight-vs.-normal weight comparisons and continuous BMI analyses; both adjustment models; Supporting Information Tables S3–S6). The overall gene expression of these 525 DEGs largely clustered according to BMI status (Supporting Information Figure S5). Among the 50 genes with the lowest *p* values in the same models, 33 DEGs overlapped.

WC-associated DEGs

No WC-associated DEGs were identified in any of the categorical WC analyses, be it the minimally adjusted model, the fully adjusted model, or the sensitivity analyses restricted to women with <1 year between Q2 and Q3 ($n = 657$). However, a few DEGs were identified in the WC-BMI interaction analyses (Table 1). In the first interaction model (BMI_{Q1orQ2}*WC), the main effect of $WC_{Q3\text{-}Q1}$ or $WC_{Q3\text{-}Q2}$ had 3 and 168 overlapping DEGs between minimally and fully adjusted models, respectively (Supporting Information Table S7); in the second interaction model (BMI_{Q3}*WC) they had 5 and 1 overlapping DEGs, respectively (Supporting Information Table S8). The interaction effect of BMI and WCs was not significant in the first interaction model (Table 1, Supporting Information Table S7), but it was significant in the second, indicating DEGs for each 1-unit increase in $WC_{Q3\text{-}Q1}$ or $WC_{Q3\text{-}Q2}$, but only among women with obesity at Q3 (Table 1, Supporting Information Table S8). The top-ranked genes from the interaction effect of BMI and WCs in the second interaction model were *CECR6* (renamed: *TMEM121B*; logFC = 0.19, FDR = 9.91×10^{-3}; Figure 4A) for $WC_{Q3\text{-}Q1}$ and *STT3A* for $WC_{Q3\text{-}Q2}$ (logFC = −0.09, FDR = 1.35×10^{-2}; Figure 4B). All DEGs identified in the minimally adjusted second interaction model overlapped with those in the fully adjusted model (Supporting Information Table S8). Among the 169 DEGs from the main effect of $WC_{Q3\text{-}Q2}$ in the first interaction model (fully adjusted), 21 (12%) overlapped with the 525 DEGs across all BMI models (Supporting Information Table S7). The overall gene expression of the 169 DEGs did not show apparent clustering in relation to WCs (Supporting Information Figure S6), which could be because of the low logFC-values observed for these genes. Differing trends in expression of the top-ranked genes across BMI_{Q3} categories could indicate slightly increasing expression with increasing weight from Q1 to Q3 for *CECR6* (Supporting Information Figure S7A–C) and

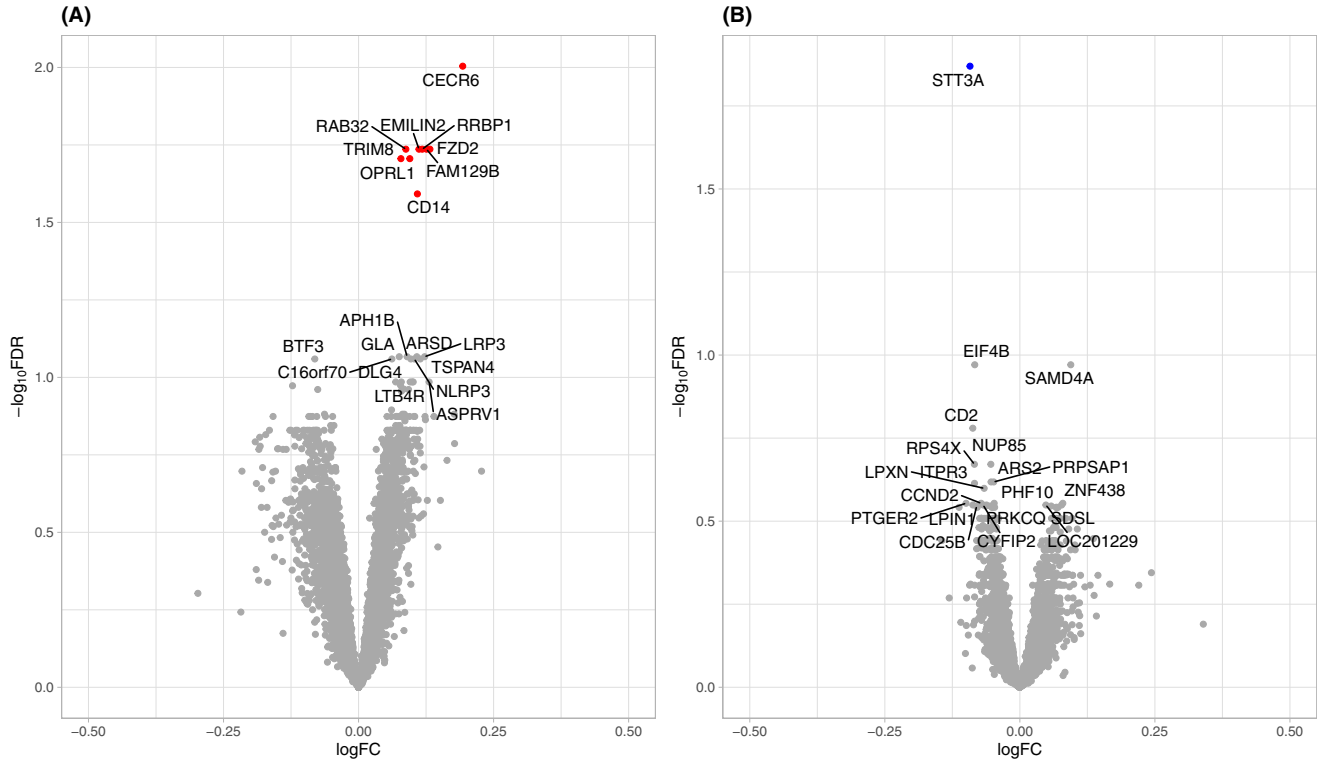

FIGURE 4 Volcano plots of the test statistics of DEGs in fully adjusted models from WC-BMI interaction analyses according to WCs (A) from Q1 to Q3 and (B) from Q2 to Q3. Red dots display overexpressed genes (FDR ≤ 0.05 and logFC > 0) and blue dots display underexpressed genes (FDR ≤ 0.05 and logFC < 0), whereas gray dots display genes with FDR > 0.05; gene names displayed are the significant DEGs in the respective tests. WCs are modeled as a scaled continuous metric. The results are of the interaction effect of WCs and BMI from the second interaction model (i.e., BMI_{Q3}* $WC_{Q3\text{-}Q1}$ or BMI_{Q3}* $WC_{Q3\text{-}Q2}$). DEGs, differentially expressed genes; FDR, false discovery rate; logFC, log_2 fold-change; Q1, baseline questionnaire; Q2, follow-up questionnaire; Q3, questionnaire at blood collection; WC, weight change; $WC_{Q3\text{-}Q1}$, weight change between Q1 and Q3; $WC_{Q3\text{-}Q2}$, weight change between Q2 and Q3 [Color figure can be viewed at wileyonlinelibrary.com]

decreasing expression with increasing weight from Q2 to Q3 for *STT3A* (Supporting Information Figure S7D–F) for women with obesity. The sensitivity analyses for WC-BMI interaction analyses, one that replaced extreme WC values with values of the 5th and 95th percentiles and one that included WCs as BMI/year, rendered the same results as the fully adjusted models (results not shown). There were high correlations (>0.93) between WC variables measured in kilograms/year and BMI/year. Further *p* values were distributed uniformly in all comparisons of WCs as a continuous metric (Supporting Information Figure S8).

Functional enrichment analyses

Overexpressed genes identified in the categorical BMI analyses were enriched for terms largely related to metabolic and catabolic processes, cellular response to toxic substances, erythrocyte homeostasis, and development (GO-BP); cellular oxidant detoxification and blood protein bindings (GO-MF, GO-CC, and KEGG); ribosome structure and hemoglobin complexes (GO-CC); and metabolism of amino acids and translation (REACTOME). Underexpressed genes were enriched in fewer categories but included peptide and antigen bindings (GO-MF, KEGG); lysosome and vacuoles components (GO-CC); and asthma, tuberculosis, and influenza A (KEGG; Figure 5, Supporting Information Table S9). The enriched terms for BMI-associated DEGs in continuous BMI analyses (Supporting Information Table S10 and Figure S9) and for the 525 DEGs across all BMI models (Supporting Information Table S11 and Figure S10) were largely like those indicated for the categorical BMI analyses. The enriched terms for the 33 DEGs overlapping across the 50 genes with the lowest *p* values in BMI models were related to erythrocyte functions (Supporting Information Table S12).

There were few WC-associated DEGs in the WC-BMI interaction analyses. Still, the terms signaling receptor and molecular transducer activities (GO-MF) were overrepresented by 4/9 overexpressed genes identified in the $WC_{Q3\text{-}Q1}$ interaction model (Supporting Information Table S13).

Quantitative replication

Comparing the 525 DEGs across BMI models with DEGs reported in similar published studies in whole blood (reporting 3762 [20] and 144 [10] DEGs), in PBMCs (1864 DEGs [21]), and in adipose tissue (only

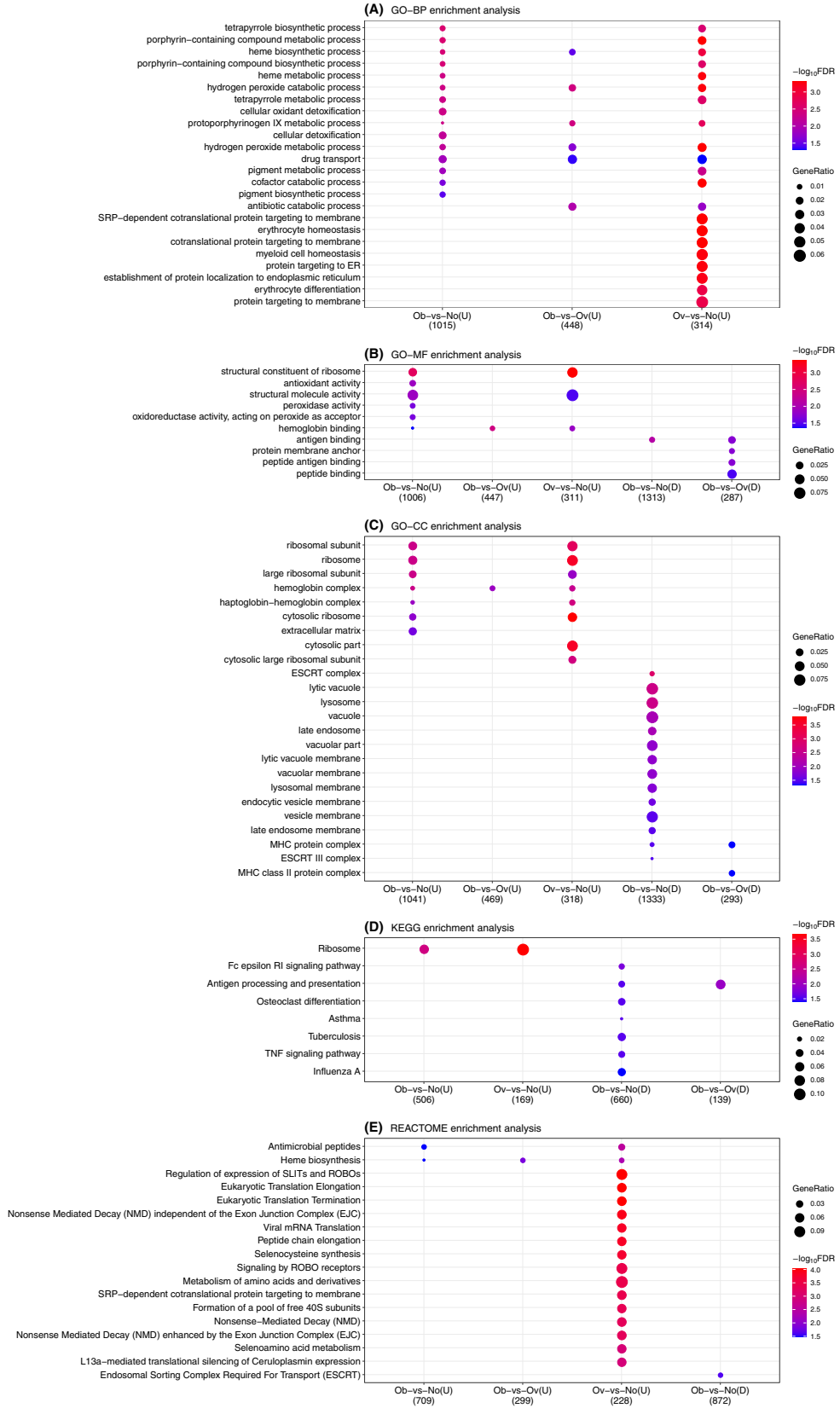

FIGURE 5 Legend on next page.

males, reporting 2936 DEGs [9]), there were 396 (75.42%), 19 (3.6%), 77 (14.66%), and 93 (17.71%) DEGs overlapping, respectively. The corresponding effect directions were 99.74%, 100%, 85.71%, and 25.8% overlapping, respectively. Furthermore, among the 3106 DEGs in the continuous BMI analyses (fully adjusted), 1552 (49.96%), 42 (1.35%), 337 (10.84%), and 538 (17.32%) DEGs overlapped with the previously mentioned studies (of which 97.29%, 95.23%, 73.59%, and 39.77% had corresponding effect directions), respectively (Supporting Information Table S14). Finally, the effect estimates for BMI association showed strong positive correlations with those from previous studies in whole blood [10, 20] and PBMCs [21] but negative correlation with those from adipose tissue [9] (Supporting Information Figure S11).

DISCUSSION

This was the first study to extensively investigate associations between blood gene expression and (1) current BMI and (2) past WCs in a large sample of women. We showed that blood gene expression was a good reflection of current BMI (here represented by BMI_{Q3}, i.e., at blood collection), but not of past WCs. BMI_{Q3} was clearly associated with blood gene expression, as >2000 DEGs were identified in the obesity-versus-normal weight comparison. Furthermore, >700 DEGs were identified in the obesity-versus-overweight and overweight-versus-normal weight comparisons. Across the models testing associations with BMI, 525 DEGs overlapped. *FAM46C* was the top-ranked gene in all BMI analyses, except in the overweight-versus-normal weight comparison, in which the top-ranked gene was *SLC45A3*. Our results showed that *FAM46C* was positively associated with increasing BMI, whereas *SLC45A3* was negatively associated. In contrast, when focusing on WCs within the preceding 7 years (range = 5.5–14 years) or 1 year (range = <1 month–9 years) and accounting for interactions with BMI categories, we observed limited associations with current gene expression, as between 1 and 169 genes were associated with the main effect of past WCs and between 0 and 9 genes were associated with the interaction effect of past WCs and current BMI.

Our categorical WC analyses did not reveal any DEGs, but after introducing an interaction with BMI categories (WC-BMI interaction analyses), up to 169 DEGs were identified. The assumption for the two interaction models was that current BMI was a stronger predictor of current gene expression than past WC/BMI, and thus the second interaction model (BMI_{Q3}*WC) would be more accurate than the first (BMI_{Q1orQ2}*WC). Our results agreed with these predictions, as the interaction effect of WCs and BMI was not significant in the first interaction model, whereas up to nine genes were significant in the second interaction model. The expression of top-ranked genes from the WC-BMI interaction analyses could indicate a positive and negative association of $WC_{Q3\text{-}Q1}$ and $WC_{Q3\text{-}Q2}$ among women with obesity for *CECR6* and *STT3A*, respectively; however, the trend was not very strong. The 21 DEGs among the WC models ($n = 169$) that overlapped with 525 DEGs across all BMI models indicated that past WCs were represented in current BMI models to some extent; however, they were not among the top-ranked genes.

Comparing the 525 DEGs across BMI models and 3106 DEGs from continuous BMI analyses (fully adjusted) with similar previous studies conducted in whole blood [10, 20] and PBMCs [21] showed that these and our results were largely consistent, although our top-ranked genes *FAM46C* and *SLC45A3* were identified only in Homuth et al. [20]. The overlap with the study in adipose tissue [9] was less than expected by chance (p value $= 4.1 \times 10^{-3}$; odds ratio = 0.58; Supporting Information Table S14). Furthermore, correlations between the estimates for associations were strongly positive with other studies in whole blood [10, 20] and in PBMCs [21], but negative in adipose tissue [9], indicating that systemic signals in blood related to BMI differed from those in adipose tissues. Thus, DEGs in whole blood related to BMI in women in this study could be generalizable to both sexes and other blood samples, but gene expression profiles were differently regulated in adipose tissue.

Functional enrichment analyses of BMI-associated DEGs indicated a broad range of functions in enriched pathways. For genes overexpressed in women with obesity, terms describing various catabolic (e.g., cofactor catabolic processes) and metabolic processes (e.g., hydrogen peroxide, heme, tetrapyrrole metabolic processes), as well as erythrocyte homeostasis, hemoglobin binding, and ribosome structures, were enriched. These findings aligned with previous studies in whole blood [10, 20] and PBMCs [21]. The enriched terms erythrocyte differentiation, myeloid cell homeostasis, erythrocyte homeostasis, and heme biosynthetic/metabolic process indicated overexpression of genes in processes in erythrocytes or their precursors (reticulocytes) [25]. Notably, the BMI-associated top-ranked gene, *FAM46C*, and several other top-ranked genes (*HBD*, *GYPB*, and *ALAS2*) are primarily expressed in bone marrow, blood, and early erythroid cells [25]. Erythrocyte indices have been observed as positively associated with obesity [26, 27], and they could be explained by

FIGURE 5 Summary of functional enrichment analyses for BMI categories at blood collection for overexpressed (upregulated) and underexpressed (downregulated) genes for the (A) GO-BP, (B) GO-MF, (C) GO-CC, (D) KEGG, and (E) REACTOME pathway databases. The color of the dots indicates $-\log_{10}$FDR, where red dots represent the most enriched categories (i.e., ones with the lowest $-\log_{10}$FDR); "GeneRatio" indicates the proportion of genes overlapping between lists of differentially expressed genes and the genes in GO categories. BP, biological processes; CC, cellular components; FDR, false discovery rate; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular functions; Ob-vs.-No, comparison of women with obesity versus normal weight; Ob-vs.-Ov, comparison of women with obesity versus overweight; Ov-vs.-No, comparison of women with overweight versus normal weight; U, overexpressed genes (upregulated, FDR ≤ 0.05, logFC > 0); D, underexpressed genes (downregulated, FDR ≤ 0.05 and logFC < 0) [Color figure can be viewed at wileyonlinelibrary.com]

proliferation of reticulocytes in the bone marrow [28, 29] induced by the hormone leptin, released by bone marrow-resident adipocytes. In contrast, erythrocytes in people with obesity have a shorter half-life in circulation because of impaired insulin resistance and pronounced oxidative stress resulting from hyperglycemia [30]. This reticulocyte-erythrocyte ratio shift is expected to be reflected in the whole blood transcriptome because reticulocytes are also transcriptionally active [20]. Therefore, DEGs identified in this study likely reflect a shift in the reticulocyte-erythrocyte ratio associated with higher BMI. However, as erythrocyte/reticulocyte cell counts were not available in NOWAC, such adjustments in the statistical analyses were not feasible.

Enriched terms for overexpressed genes further included the terms peptide chain elongation and eukaryotic translation termination/elongation which appeared related to protein synthesis [31]. In line with these terms, another study investigating gene expression related to BMI in whole blood observed ribosome and protein synthesis pathways as top-ranked among women [10]. These enriched terms indicated physiological changes previously observed for people with obesity, for example, higher levels of oxidative stress [30, 32], hemoglobin [33, 34], and disrupted protein synthesis [35].

Enriched terms among underexpressed genes included antigen binding, processing, and presentation, peptide binding, and tumor necrosis factor signaling pathways, which suggest there could be altered blood immune responses in women with obesity, something that has been observed among participants with obesity in previous transcriptomic studies [9–11]. Furthermore, altered immune response/function (e.g., related to the terms influenza, asthma, antigen binding) in persons with obesity could explain previously observed associations between obesity and increased risk of comorbidities and infectious diseases, like influenza and COVID-19, and increased viral shedding and transmission [36, 37]. Overall, general metabolism and blood processes were enriched, which likely reflects the broad influence of BMI on systemic gene expression.

The evaluation of past WCs and gene expression profiles in blood was novel but it indicated few DEGs, and thus related biological functions were not strongly indicated. Still, the overexpressed genes *RBP1/FZD2/OPRL1/CD14* indicated a relation between past WCs and genes involved in signal transduction.

In general, our results indicated that current BMI and past WCs had, respectively, large and small effects on blood gene expression. This could be expected, as blood gene expression represents a snapshot, and past exposures such as WCs are generally not strongly reflected [22, 38]. Still, until now, no study had investigated the association between blood gene expression and past WCs. Previous studies have reported that weight reduction in individuals with obesity after diet interventions is associated with gene expression profiles in adipose tissue before and after the interventions [13–19]. However, as follow-up time in these studies (4 weeks to 9 months) was shorter than the time intervals in our study (range = <1 month–14 years), the WCs we observed could be too far in the past to have a major influence on blood gene expression. Still, sensitivity analyses restricted to women with <1 year between Q2 and Q3 did not show any significant DEGs. Future studies focusing on systemic signatures related to WCs should likely include blood samples taken within months of the WCs occurring for transcriptomic signals to be detectable. DEGs related to obesity/WC might be expected in adipose/muscle tissue, but a study demonstrated that blood samples can be another informative, accessible tissue to explore circulating features of the state of obesity [10].

We observed an association between current BMI and naive B cells, memory B cells, naive CD4 T cells, and memory-activated CD4 T cells, possibly because BMI and body weight were positively correlated with WBC counts in apparently healthy young adults (higher in women) [39]. As skewed WBC proportions due to differences in BMI could have influenced our BMI analyses, we included these estimated cell proportions in our fully adjusted models [40]. The estimated proportions of WBCs in our study deviated from the expected range, but this deviation also has been observed in other recent studies based on the NOWAC postgenome cohort [41, 42]. This indicated a bias that could be explained by the deconvolution technique or data preprocessing [41, 43]. Still, absolute differences in estimated WBCs across BMI categories were modest, and the top-ranked genes identified in our models were very similar, indicating that these genes were not substantially influenced by distributions of WBCs. Last, erythrocyte/reticulocyte counts were not available, and their adjustment was thus not possible.

The main strength of this study was the large study sample (1694 women). Indeed, previous studies on BMI and blood gene expression have been rather small (32–190 participants) [8–11], with the exception of one large population-based cohort study (1048 participants, 53% women) [20]. Another strength was that our study was based on repeated measurements; thus we were able to generate BMI and WC variables for all women at different time points. Still, individual intervals varied, and we standardized WCs by dividing them by individual follow-up times. Additionally, this study was based on self-reported questionnaire information, which could be influenced by measurement and recall bias. A validation study of self-reported BMI among NOWAC study participants found a slight, but statistically significant, underreporting of weight and self-reported BMI, especially among women with overweight and obesity, but the authors concluded that, for middle-aged Norwegian women, self-reported weight and height provide a valid ranking of BMI [44]. The present study included only women who had never been diagnosed with cancer, but we cannot disregard the influence of other common chronic diseases. Furthermore, the current cross-sectional study results only represent snapshots of blood gene expression and cannot indicate causality. Last, although RNA-sequencing has become a routinely used technology, results from microarray technology, like those in this study, are still reliable and overall comparable to RNA-sequencing results [45], although non-coding RNAs and splice variants cannot be detected. Future studies could validate gene expression findings, especially related to WCs, using alternative targeted technologies (e.g., quantiative polymerase chain reaction or NanoString) or investigate cell type-specific gene expression using single cell RNA-sequencing, but that would require new sample collection.

CONCLUSION

Many BMI-associated DEGs, but few WC-associated DEGs, were identified in blood of women in Norway. This is the first study to our knowledge to conclude that blood gene expression reflects current BMI more than past WCs. The biological functions of BMI-associated DEGs were linked to metabolism, erythrocytes, oxidative stress, and immune processes. These likely reflect systemic impacts of obesity, especially reticulocyte-erythrocyte ratio shifts in blood, as these functions coincide with its known physiological effects. Furthermore, the biological functions of WC-associated DEGs were linked to signal transduction. This knowledge is relevant for further research related to the health effects of BMI and WCs, especially focusing on blood-based markers.O

AUTHOR CONTRIBUTIONS

Therese H. Nøst and Charlotta Rylander conceptualized the main research idea. Therese H. Nøst curated the data. Therese H. Nøst, Pål Sætrom, Charlotta Rylander, Torkjel M. Sandanger, and Nikita Baiju designed the research methodology. Nikita Baiju performed the formal data analyses and wrote the manuscript and the supplementary information with significant contribution from Therese H. Nøst. All coauthors discussed the results and reviewed the manuscript and supplementary information.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

Data cannot be shared publicly because of local and national ethical and security policies. Data access for researchers will be conditional on adherence to both the data access procedures of the NOWAC study and UiT The Arctic University of Norway (contact: Tonje Braaten, tonje.braaten@uit.no) in addition to approval from the local ethical committee.

ORCID

Nikita Baiju https://orcid.org/0000-0003-4373-8526

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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