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# Circulating and adipose tissue immune cells in tissue-specific insulin resistance in humans with overweight and obesity

Inez Trouwborst[1,2] | Kristiaan Wouters[3,4] | Johan W. Jocken[1] | Kelly M. Jardon[1,2] | Anouk Gijbels[2,5] | Pieter C. Dagnelie[3,4,6] | Marleen M. J. van Greevenbroek[3,4] | Carla J. van der Kallen[3,4] | Coen D. A. Stehouwer[3,4] | Casper G. Schalkwijk[3,4] | Nathalie Richard[7] | Igor Bendik[7] | Lydia A. Afman[5] | Ellen E. Blaak[1,2] | Gijs H. Goossens[1]

[1]Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, the Netherlands

[2]TI Food and Nutrition (TiFN), Wageningen, the Netherlands

[3]Department of Internal Medicine, Maastricht University Medical Center+, Maastricht, the Netherlands

[4]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands

[5]Division of Human Nutrition and Health, Wageningen University, Wageningen, the Netherlands

[6]School for Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, the Netherlands

[7]DSM Nutritional Products Ltd., Kaiseraugst, Switzerland

**Correspondence**
Gijs H. Goossens, Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+ Maastricht, the Netherlands.
Email: g.goossens@maastrichtuniversity.nl

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## Abstract

**Objective:** A proinflammatory adipose tissue (AT) microenvironment and systemic low-grade inflammation may differentially affect tissue-specific insulin sensitivity. This study investigated the relationships of abdominal subcutaneous AT (aSAT) and circulating immune cells, aSAT gene expression, and circulating inflammatory markers with liver and skeletal muscle insulin sensitivity in people with overweight and obesity.

**Methods:** Individuals with overweight and obesity from the PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) Study ($n = 219$) and the Maastricht Study (replication cohort; $n = 1256$) underwent a seven-point oral glucose tolerance test to assess liver and muscle insulin sensitivity, and circulating inflammatory markers were determined. In subgroups, flow cytometry was performed to identify circulating and aSAT immune cells, and aSAT gene expression was evaluated.

**Results:** The relative abundances of circulating T cells, nonclassical monocytes, and CD56dim CD16+ natural killer cells were inversely associated with liver, but not muscle, insulin sensitivity in the PERSON Study. The inverse association between circulating (classical) monocytes and liver insulin sensitivity was confirmed in the Maastricht Study. In aSAT, immune cell populations were not related to insulin sensitivity. Furthermore, aSAT gene expression of interleukin 6 and CD14 was positively associated with muscle, but not liver, insulin sensitivity.

**Conclusions:** The present findings demonstrate that circulating immune cell populations and inflammatory gene expression in aSAT show distinct associations with liver and muscle insulin sensitivity.

Inez Trouwborst and Kristiaan Wouters share first authorship.

## INTRODUCTION

Obesity is closely associated with the development of chronic diseases such as cardiovascular disease, type 2 diabetes (T2D), and certain types of cancer [1]. The excessive adipose tissue (AT) mass in obesity, resulting from a prolonged positive energy balance, is often accompanied by AT dysfunction [2–4]. AT dysfunction in obesity is characterized by a proinflammatory phenotype of both subcutaneous AT (SAT) and visceral AT (VAT), which may contribute to insulin resistance and cardiometabolic complications [2, 5].

AT dysfunction is characterized by increased production of proinflammatory factors by the enlarged adipocytes, as well as by dynamic changes in AT immune cell populations during the development of obesity [6, 7]. Indeed, a growing repertoire of innate and adaptive immune cells such as monocytes, macrophages, B and T cells, natural killer (NK) cells, and their subtypes have been reported to populate metabolic organs, including AT, in obesity [4, 8]. Previous studies in rodents and humans have shown that proinflammatory factors produced both by adipocytes and by resident and recruited immune cells in the enlarged AT impair metabolic pathways within the AT, which is linked to low-grade systemic inflammation and impaired lipid and glucose metabolism in organs such as the liver and skeletal muscle [2, 3, 5]. Thus, perturbations in the inflammatory AT phenotype may impair whole-body metabolic homeostasis in obesity, thereby contributing to the development of insulin resistance, cardiovascular disease, and T2D [8–11].

It has been well established that insulin resistance can develop simultaneously in multiple organs, but the severity may vary among organs [12–15]. Likewise, AT inflammation may have distinct effects on insulin sensitivity in different metabolic tissues [11, 14]. Indeed, findings from our laboratory demonstrated that individuals with predominant skeletal muscle insulin resistance (MIR) displayed higher inflammatory gene expression in abdominal SAT (aSAT) compared with individuals with primarily liver insulin resistance (LIR) [14]. In line with this, in two independent cohorts, plasma markers of low-grade inflammation were associated with MIR, but not with LIR [14]. However, importantly, aSAT and circulating immune cells were not determined in this study. Therefore, the relationships of immune cell subsets in aSAT and in the circulation with tissue-specific insulin resistance in individuals with overweight or obesity remain elusive.

In the present study, we investigated the relationships of aSAT and circulating immune cell populations, aSAT inflammation, and low-grade systemic inflammation with liver and skeletal muscle insulin sensitivity in individuals with overweight and obesity from the two-center PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) Study [16]. Moreover, we aimed to replicate our findings by performing complementary analyses in a large population-based cohort, i.e., the Maastricht Study [17].

### Study Importance

#### What is already known?

- Enlarged adipose tissue (AT) in obesity is often accompanied by a proinflammatory AT microenvironment, which may contribute to insulin resistance and cardiometabolic complications.
- Insulin resistance can develop simultaneously in multiple organs, but the severity may vary among organs. There are indications that the proinflammatory AT phenotype in obesity may have distinct effects on liver and skeletal muscle insulin sensitivity.

#### What does this study add?

- A higher relative abundance of several circulating immune cell populations (specifically classical monocytes), but not abdominal subcutaneous AT immune cells, was associated with liver, but not skeletal muscle, insulin resistance.
- Gene expression of inflammatory markers (interleukin 6 and CD14) in abdominal subcutaneous AT was positively associated with muscle, but not liver, insulin sensitivity.

#### How might these findings change the direction of research or the focus of clinical practice?

- The present findings contribute to a better understanding of the underlying etiology of liver and skeletal muscle insulin resistance, which may have implications for more personalized lifestyle and pharmacological interventions.

## METHODS

### Study design and participants

Data were collected from the two-center, randomized dietary intervention trial, i.e., the PERSON Study [16]. An extensive description of the PERSON Study design, objectives, and methods is available elsewhere [16]. We analyzed cross-sectional data collected at baseline from 219 individuals who participated in the PERSON Study. Individuals (age 40-75 years) with overweight or obesity (body mass index [BMI]: 25-40 kg/m$^2$) and no weight gain or loss >3 kg in the prior 3 months were included. Based on an oral glucose tolerance test (OGTT) performed at screening, individuals with either predominant LIR or MIR were included. Exclusion criteria included prediagnoses of diabetes, major cardiovascular disease, liver or kidney disease, medication affecting glucose and/or lipid metabolism, major

gastrointestinal disease, uncontrolled hypertension, alcohol abuse (>14 glasses/wk), smoking, and dietary restrictions interfering with the dietary study protocol. The PERSON Study was approved by the Medical Ethics Committee of Maastricht University Medical Center+ (NL63768.068.17) and registered at ClinicalTrials.gov (identifier: NCT03708419). The study was carried out in accordance with the principles of the Declaration of Helsinki.

To validate findings in the PERSON Study population, we used data from a population-based cohort, i.e., the Maastricht Study. The Maastricht Study focuses on the etiology of T2D, its classic complications, and its emerging comorbidities. The methodology and rationale for this study have been described previously [17]. The Maastricht Study was approved by the institutional Medical Ethical Committee (NL31329.068.10) and the Netherlands Health Council (Permit 131,088-105,234-PG). We selected participants from the Maastricht Study who were between 40 and 75 years old, had BMI between 25 and 40, did not have diabetes, did not use glucose-lowering medication, and did not smoke. The present analysis included 1256 participants from the Maastricht Study for whom data on tissue-specific insulin sensitivity and plasma inflammatory markers were available, of whom 273 participants had additional data on immune cells in whole blood, as described in more detail later in this paper.

## Anthropometrics and body composition

Body weight (kilograms), height (centimeters), and waist and hip circumferences (centimeters) were determined in duplicate to the closest 0.1 unit. Dual-energy x-ray absorptiometry was performed in both studies to assess whole-body fat percentage.

## Tissue-specific insulin sensitivity

A seven-point OGTT (75-g glucose/200-mL solution, Novolab) was performed after an overnight fast (>10 hours). Venous blood was drawn at $t = 0$, 15, 30, 45, 60, 90, and 120 minutes, and plasma glucose and insulin concentrations were determined for all time points. Tissue-specific insulin sensitivity was estimated using the hepatic insulin resistance index (HIRI) and the muscle insulin sensitivity index (MISI) for quantification of LIR and MIR, respectively. HIRI was calculated using the following formula: glucose 0 to 30 (area under the curve [AUC] in millimoles per liter × hours) × insulin 0 to 30 (AUC in picomoles per liter × hours). MISI was calculated as follows: (dGlucose/dt)/insulin (mean during OGTT in picomoles per liter). In the calculation for MISI, dGlucose/dt is the rate of decay of plasma glucose concentration (millimoles per liter) during the OGTT, calculated as the slope of the least-squares fit to the decline in plasma glucose concentration from peak to nadir [18]. The MISI calculation was optimized using the cubic spline method [19]. Importantly, a higher HIRI indicates worse hepatic insulin sensitivity, whereas a higher MISI indicates better skeletal muscle insulin sensitivity.

## aSAT biopsy

After an overnight fast, an aSAT biopsy (~1.5 g) was collected 6 to 10 cm lateral from the umbilicus under local anesthesia (lidocaine 1%, without adrenaline) in the PERSON Study only. The tissue samples were immediately rinsed with sterile saline. A minimum of 0.7 g of tissue was placed in 10 mL of DMEM/Nutrient Mixture F-12 solution for flow cytometry analysis, and another part was snap-frozen in liquid nitrogen and stored at −80 °C until analysis of aSAT gene expression.

## Flow cytometry analysis of aSAT and whole blood

Flow cytometry was performed in aSAT for the identification of immune cells by isolation of the stromal vascular fraction in the PERSON Study ($n = 81$). Fasted whole blood was used for analysis of circulating immune cells in both the PERSON Study and the Maastricht Study. The panels of antibodies used in the PERSON Study are reported in Supporting Information Table S1. The gating strategies for flow cytometry of whole blood and aSAT are reported in Supporting Information Figures S1 and S2, respectively. The antibodies and gating strategy for flow cytometry for the Maastricht Study were similar, as reported elsewhere [20]. All samples were measured with a FACS-Canto II (BD Biosciences) and analyzed with FACSdiva software (BD Biosciences). Fluorescence Minus One controls were performed during panel design, and an autofluorescence control was performed for each sample. Data are expressed as percentage of live immune cells.

## Gene expression in aSAT in the PERSON Study

aSAT gene expression analysis ($n = 91$) was performed in the PERSON Study using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), as described previously [21]. In short, RNA was precipitated and purified, and, subsequently, complementary DNA (cDNA) was synthesized and quantified by RT-qPCR using an iCycler (Bio-Rad Laboratories, Inc.). Gene expression was normalized to 18S, and the delta Ct method was used for calculating relative expression. Gene expression of several adipokines (adiponectin; dipeptidyl peptidase 4 [DPP4]; interleukin 6 [IL-6], leptin; plasminogen activator inhibitor 1 [PAI-1]; and tumor necrosis factor α [TNF-α]), lipolytic markers (1-acylglycerol-3-phosphate O-acyltransferase ABHD5/CGI-58; adipose triglyceride lipase [ATGL]; G0/G1 switch protein 2 [GOS2]; hormone-sensitive lipase [HSL]; and perilipin-1 [PLIN1]), oxidative metabolism markers (reduced nicotinamide adenine dinucleotide [NADH] dehydrogenase [ubiquinone] 1 beta subcomplex subunit B5 [NDUFB5]; NDUF alpha subcomplex subunit 1 [NDUFA1]; NDUF beta subcomplex subunit 3 [NDUFB3]; NDUF subunit C2 [NDUFC2]; V-type proton ATPase catalytic subunit A [ATP6V1A]; ATP6V1 subunit H [ATP6V1H]; medium-chain specific acyl-CoA dehydrogenase [ACADM]; very long-chain specific ACAD [ACADVL]; long-chain

**TABLE 1** Participant characteristics of the PERSON Study and the Maastricht Study

| | The PERSON Study | | The Maastricht Study | |
|---|---|---|---|---|
| | Total group ($n = 219$) | Subgroup with additional immune cell data ($n = 81$) | Total group ($n = 1256$) | Subgroup with additional immune cell data ($n = 273$) |
| Women, *n* (%) | 124 (57) | 48 (59) | 511 (41) | 129 (47) |
| Age (y) | 60.1 ± 7.8 | 59.6 ± 7.6 | 60.7 ± 8.0 | 61.3 ± 8.2 |
| BMI (kg/m$^2$) | 30.0 ± 3.5 | 31.6 ± 3.7 | 28.9 ± 3.1 | 29.2 ± 3.3 |
| Body fat (%) | 37.2 ± 7.5 | 38.7 ± 7.3 | 34.7 ± 7.0 | 35.6 ± 6.6 |
| Waist (cm) | 102.3 ± 9.5 | 103.6 ± 11.3 | 101.0 ± 10.0 | 101.2 ± 10.7 |
| Waist-hip ratio | 0.939 ± 0.088 | 0.935 ± 0.103 | 0.965 ± 0.085 | 0.960 ± 0.092 |
| Medication use, n (%) | | | | |
| Lipid-lowering | 15 (7) | 1 (1) | 494 (39) | 116 (43) |
| Antihypertensives | 37 (18) | 14 (17) | 525 (43) | 123 (45) |
| Antidepressants | 14 (6) | 8 (10) | 66 (5) | 15 (6) |
| Glucose status, n (%) | | | | |
| NGT | 166 (76) | 55 (68) | 940 (75) | 213 (78) |
| IFG | 9 (4) | 2 (3) | 62 (5) | 8 (3) |
| IGT | 30 (13) | 15 (19) | 154 (12) | 33 (12) |
| T2D, without glucose-lowering medication | 14 (6) | 9 (11) | 100 (8) | 19 (7) |
| Fasting glucose (mmol/L) | 5.3 (5.0-5.7) | 5.5 (5.2-5.9) | 5.7 (5.2-6.6) | 5.7 (5.3-6.8) |
| Fasting insulin (pmol/L) | 51.2 (39.0-63.8) | 54.7 (39.9-67.5) | 74.1 (52.1-108.9) | 78.0 (53.4-124.6) |
| HIRI (AU) | 389 (289-553) | 408 (308-539) | 458 (311-674) | 504 (330-754) |
| MISI (AU) | 0.121 (0.084-0.190) | 0.126 (0.088-0.190) | 0.118 (0.072-0.197) | 0.108 (0.068-0.184) |
| Adipocyte size (μm) | 73.0 ± 6.1 | 73.3 ± 6.3 | | |

*Note*: Data are mean ± SD for normally distributed values, median (IQR) for skewed variables, or *n* (%) for categorical data. Population characteristics are shown separately for the total groups and for individuals for whom immune cells were available because of the large difference in population size. Glucose status classification was based on World Health Organization criteria [22].
Abbreviations: AU, arbitrary unit; HIRI, hepatic insulin resistance index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MISI, muscle insulin sensitivity index; NGT, normal glucose tolerance; PERSON, PERSonalized Glucose Optimization Through Nutritional Intervention; T2D, type 2 diabetes.

specific ACAD [ACADL]; carnitine O-palmitoyltransferase 2 [CPT2]; citrate synthase [CS]; and uncoupling protein 2 [UCP2]), and immune cell markers (cluster of differentiation [CD] 11b; CD11c; human mannose receptor (hMR); CD14; CD64; and CD68) were determined. Primer sequences are provided in Supporting Information Table S2.

### Adipocyte size

One part of the aSAT biopsies taken in the PERSON Study was immediately embedded in paraffin. Thin (8 μm) histological sections were cut and placed on microscope glass slides. The samples were stained with hematoxylin and eosin for visualization of adipocyte cell membranes and nucleus. Analysis of adipocyte size was performed using computerized morphometric analysis (Leica QWin V3, Leica Camera AG) identifying and analyzing individual adipocytes (at least 200 adipocytes per sample).

### Plasma inflammatory markers

In the PERSON Study participants, the plasma inflammatory markers C-reactive protein (CRP), serum amyloid A (SAA), CD163, PAI-1, and TNF ligand superfamily member 12 (TNFSF12) were quantified using Luminex immunoassays (performed by Koninklijke DSM N.V.). In the Maastricht Study participants, circulating CRP and SAA concentrations were determined using Meso Scale Discovery multiplex assay.

### Statistical analysis

Participant characteristics are presented as mean ± standard deviation (SD) for normally distributed values. Non-normally distributed values are reported as median ± interquartile range (IQR). Multiple linear regression analyses were performed to assess the associations of immune cell populations, inflammatory markers, or aSAT gene expression (independent variables) with liver and skeletal muscle insulin sensitivity (as continuous dependent variables). The fully adjusted models are reported with adjustment for sex, age, BMI, and use of lipid-lowering medication, antihypertensives, and antidepressants. We performed a sensitivity analysis in the Maastricht Study to further adjust for potential differences between the PERSON Study and the Maastricht Study populations. We additionally adjusted for the presence of major cardiovascular disease, inflammatory bowel disease, chronic respiratory diseases, and cancer. Visual inspection of residual diagnostics was performed, and non-normality was corrected with log10 transformation. The independent variables were standardized by calculating a $z$ score to allow for direct comparison of effect sizes. Standardized βs (Std β) with 95% confidence intervals (CI) are reported. Only data of available cases were analyzed. The statistical analyses were performed using IBM SPSS Statistics software (version 25; IBM Corp.). Significance is set at $p < 0.05$.

## RESULTS

### Participant characteristics

From the PERSON Study, data on plasma inflammatory markers were available in 219 participants, and additional data on aSAT and circulating immune cell populations were available in a subgroup of 81 participants (Table 1). Because of the relatively large difference in group size, participant characteristics of both of these groups are reported. The subgroup showed comparable population characteristics with the total population, except for having a slightly higher BMI (mean [SD], 31.6 [3.7] vs. 30.0 [3.5], respectively). Mean age of the total PERSON Study population was 60.1 [7.8] years. The majority of participants were women (57%). The majority of study participants (76%) were characterized as having normal glucose tolerance according to the World Health Organization (WHO) criteria (fasting glucose <6.1 mmol/L and 2-hour glucose <7.8 mmol/L) [22].

In the Maastricht Study, data of 1256 participants were available for the present analysis (Table 1). Data on immune cells were available in a subgroup of this study population ($n = 273$). Participants were, on average, aged 60.7 [8.0] years, with BMI of 28.9 [3.1] (Table 1), which was comparable with the PERSON Study population. However, slightly fewer women compared with men were included in the analysis of the Maastricht Study (41% women). The Maastricht Study population showed a more unfavorable metabolic profile (higher fasting glucose and insulin concentrations, worse glucose status) compared with the PERSON Study population. The use of medication was overall higher in the Maastricht Study compared with the PERSON Study, specifically the use of lipid-lowering medication (39% vs. 7%, respectively) and antihypertensives (43% vs. 18%, respectively). The relative immune cell presence in the PERSON Study and the Maastricht Study is reported in Supporting Information Table S3. The relative abundances of immune cells were similar in the PERSON Study and the Maastricht Study for, among others, T cells, nonclassical monocytes, and NK cells, but higher median relative abundances of granulocytes (57% vs. 52%) and B cells (6% vs. 3%) and a lower abundance of classical monocytes (4% vs. 7%) were present in the Maastricht Study compared with the PERSON Study, respectively.

### Associations of AT and circulating immune cells and tissue-specific insulin sensitivity

The aSAT immune cell populations, as well as the ratio of pro- and anti-inflammatory macrophages (CD11C+/CD11C−), were not associated with either HIRI or MISI (all $p > 0.05$) in the PERSON Study (Table 2). Additional adjustment for adipocyte size (Table 2) yielded similar results. The relative abundances of several circulating immune cells, specifically T cells (Std β with 95% CI: 0.28 [0.06-0.49]), nonclassical monocytes (0.38 [0.17-0.60]), classical monocytes (0.40 [0.17-0.64]), and CD56dim CD16+ NK cells (0.28 [0.04-0.51]), were positively associated with HIRI in the PERSON Study. These associations indicate that a higher relative abundance of these immune cells in

**TABLE 2** Adjusted associations of whole blood and aSAT immune cell populations with tissue-specific insulin sensitivity in the PERSON Study and the Maastricht Study

| | HIRI | | | | | | MISI | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | The PERSON Study | | | | The Maastricht Study | | The PERSON Study | | | | The Maastricht Study | |
| | Std β (95% CI) | *p* value | Std β (95% CI) + adj. adipocyte size | *p* value | Std β (95% CI) | *p* value | Std β (95% CI) | *p* value | Std β (95% CI) + adj. adipocyte size | *p* value | Std β (95% CI) | *p* value |
| *Blood immune cells* | | | | | | | | | | | | |
| Granulocytes | −0.23 (−0.45 to −0.01) | **0.041** | | | 0.02 (−0.11 to 0.16) | 0.723 | 0.41 (0.16 to 0.66) | **0.002** | | | −0.05 (−0.22 to 0.11) | 0.546 |
| B cells | 0.20 (−0.03 to 0.42) | 0.090 | | | −0.15 (−0.27 to −0.03) | **0.018** | −0.11 (−0.39 to 0.16) | 0.414 | | | 0.00 (−0.16 to 0.16) | 0.998 |
| T cells | 0.28 (0.06 to 0.49) | **0.012** | | | −0.10 (−0.25 to 0.04) | 0.141 | −0.20 (−0.46 to 0.07) | 0.138 | | | 0.08 (−0.11 to 0.27) | 0.419 |
| Treg | | | | | −0.02 (−0.14 to 0.10) | 0.783 | | | | | −0.08 (−0.22 to 0.06) | 0.285 |
| Total monocytes | 0.38 (0.14 to 0.61) | **0.002** | | | 0.15 (0.03 to 0.28) | **0.014** | −0.03 (−0.33 to 0.27) | 0.850 | | | −0.04 (−0.20 to 0.12) | 0.631 |
| Nonclassical monocytes | 0.38 (0.17 to 0.60) | **0.001** | | | 0.10 (−0.03 to 0.23) | 0.127 | −0.06 (−0.34 to 0.23) | 0.702 | | | −0.01 (−0.18 to 0.15) | 0.874 |
| Intermediate monocytes | 0.20 (−0.04 to 0.43) | 0.098 | | | 0.11 (−0.01 to 0.23) | 0.067 | 0.04 (−0.25 to 0.33) | 0.762 | | | −0.02 (−0.17 to 0.14) | 0.842 |
| Classical monocytes | 0.40 (0.17 to 0.64) | **0.001** | | | 0.13 (0.00 to 0.26) | **0.044** | 0.03 (−0.28 to 0.34) | 0.852 | | | −0.04 (−0.21 to 0.12) | 0.590 |
| NK cells | 0.24 (0.01 to 0.47) | **0.038** | | | 0.03 (−0.08 to 0.15) | 0.560 | −0.23 (−0.51 to 0.04) | 0.096 | | | −0.05 (−0.20 to 0.10) | 0.474 |
| CD56bright CD16− NK cells | 0.10 (−0.13 to 0.33) | 0.376 | | | 0.00 (−0.12 to 0.12) | 1.000 | 0.04 (−0.24 to 0.31) | 0.779 | | | −0.03 (−0.18 to 0.13) | 0.731 |
| CD56dim CD16+ NK cells | 0.28 (0.04 to 0.51) | **0.024** | | | 0.00 (−0.12 to 0.13) | 0.945 | −0.23 (−0.52 to 0.06) | 0.124 | | | −0.03 (−0.19 to 0.13) | 0.706 |

(Continues)

**TABLE 2** (Continued)

| | HIRI | | | | | | MISI | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | The PERSON Study | | | | The Maastricht Study | | The PERSON Study | | | | The Maastricht Study | |
| | Std β (95% CI) | *p* value | Std β (95% CI) + adj. adipocyte size | *p* value | Std β (95% CI) | *p* value | Std β (95% CI) | *p* value | Std β (95% CI) + adj. adipocyte size | *p* value | Std β (95% CI) | *p* value |
| *aSAT immune cells* | | | | | | | | | | | | |
| Total macrophages + monocytes | 0.22 (−0.03 to 0.48) | 0.085 | 0.26 (−0.01 to 0.53) | 0.061 | | | −0.14 (−0.40 to 0.13) | 0.311 | −0.18 (−0.47 to 0.11) | 0.217 | | |
| CD11C-CD206+ macrophages | 0.02 (−0.25 to 0.30) | 0.876 | 0.01 (−0.29 to 0.27) | 0.931 | | | −0.07 (−0.35 to 0.22) | 0.648 | −0.08 (−0.39 to 0.22) | 0.587 | | |
| CD11C + CD206+ macrophages | −0.06 (−0.31 to 0.19) | 0.645 | −0.05 (−0.31 to 0.22) | 0.737 | | | −0.03 (−0.29 to 0.23) | 0.806 | −0.07 (−0.35 to 0.22) | 0.638 | | |
| CD11C+/CD11C− macrophages | −0.10 (−0.36 to 0.16) | 0.454 | −0.04 (−0.31 to 0.24) | 0.791 | | | 0.04 (−0.23 to 0.31) | 0.751 | 0.03 (−0.26 to 0.32) | 0.845 | | |
| NK cells | 0.09 (−0.16 to 0.34) | 0.487 | 0.04 (−0.23 to 0.30) | 0.781 | | | 0.07 (−0.19 to 0.33) | 0.575 | 0.07 (−0.21 to 0.34) | 0.631 | | |

*Note*: Standardized β values (95% CI) are reported for the associations among immune cells (expressed as percentage of live cells) and tissue-specific insulin sensitivity using data from the PERSON Study ($n = 81$) and the Maastricht Study ($n = 273$). A linear regression analysis was performed with adjustment for age, sex, BMI, and medication use (lipid-lowering, antihypertensives, and antidepressants). In the PERSON Study only, the models with additional adjustment for fat cell size are presented. Significant *p* values (<0.05) are bolded.

Abbreviations: aSAT, abdominal subcutaneous adipose tissue; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; NK, natural killer; PERSON, PERSonalized Glucose Optimization Through Nutritional Intervention; Std, standard; Treg, regulatory T cell.

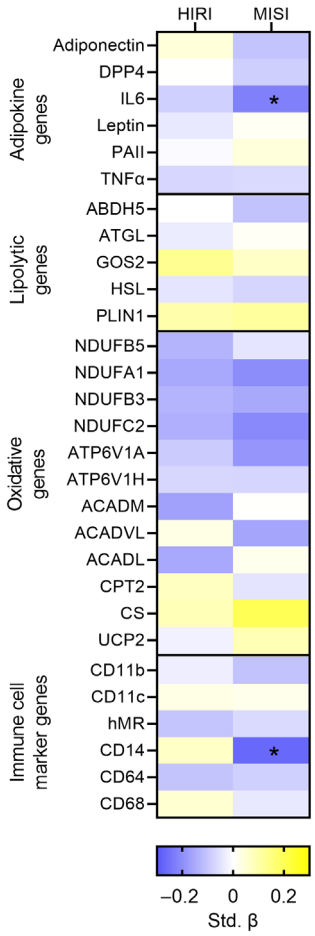


**FIGURE 1** Associations of abdominal subcutaneous adipose tissue gene expression with HIRI and MISI ($n = 91$) in the PERSON Study. Higher HIRI indicates lower liver insulin sensitivity, whereas lower MISI indicates lower muscle insulin sensitivity. Yellow indicates a positive Std β, whereas blue indicates a negative Std β. Gene expression is categorized into the expression of adipokines and lipolytic, oxidative metabolism, and immune cell markers. Data are adjusted for age, sex, BMI, and medication use (lipid-lowering, antihypertensives, and antidepressants). *$p < 0.05$. ABHD5, 1-acylglycerol-3-phosphate O-acyltransferase; ACADL, long-chain specific acyl-CoA dehydrogenase; ACADM, medium-chain specific acyl-CoA dehydrogenase; ACADVL, very long-chain specific acyl-CoA dehydrogenase; ATGL, adipose triglyceride lipase; ATP6V1A, V-type proton ATPase catalytic subunit A; ATP6V1H, ATP6V1 subunit H; CPT2, carnitine O-palmitoyltransferase 2; CS, citrate synthase; DPP4, dipeptidyl peptidase 4; GOS2, G0/G1 switch protein 2; HIRI, hepatic insulin resistance index; hMR, human mannose receptor; HSL, hormone-sensitive lipase; MISI, muscle insulin sensitivity index; NDUFA1, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit A1; NDUFB3, NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 3; NDUFB5, NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5; NDUFC2, NADH dehydrogenase [ubiquinone] 1 subunit C2; PAI-1, plasminogen activator inhibitor 1; PERSON, PERSonalized Glucose Optimization Through Nutritional Intervention; PLIN1, perilipin-1; Std, standard; UCP2, uncoupling protein 2 [Color figure can be viewed at wileyonlinelibrary.com]

whole blood was related to more pronounced hepatic insulin resistance (i.e., worse liver insulin sensitivity). Blood granulocytes were inversely associated with HIRI (i.e., a higher abundance of blood granulocytes was related to less LIR and, therefore, to better liver insulin sensitivity). Furthermore, a positive association between the relative abundance of granulocytes and MISI (0.41 [0.16-0.66]) was observed, whereas no significant associations were observed among any of the other blood immune cells and MISI. Further adjustment for MISI (in the model with HIRI as the dependent variable) and HIRI (in the model with MISI as the dependent variable; data not reported) yielded similar findings.

In the Maastricht Study, we also observed a significant positive association between circulating monocytes (0.15 [0.03 to 0.28]), specifically classical monocytes (0.13 [0.00 to 0.26]), and HIRI (Table 2), thereby confirming our findings in the PERSON Study population. In contrast, the relative abundance of circulating B cells was inversely associated with HIRI (−0.15 [−0.27 to −0.03]), indicating that a higher abundance of B cells was related to better liver insulin sensitivity. Other circulating immune cells were not associated with HIRI. Additionally, no significant associations were observed among the relative abundances of immune cells in whole blood and MISI (all $p > 0.05$).

## aSAT gene expression is related to muscle, but not liver, insulin sensitivity in the PERSON Study

Gene expression of adipokines and lipolytic, oxidative metabolism, and immune cell markers in aSAT was determined in the PERSON Study. In the fully adjusted model, we found inverse associations between aSAT expression of the proinflammatory marker IL-6 (−0.23 [−0.45 to −0.01], $p = 0.043$) and the immune cell marker CD14 (−0.27 [−0.50 to −0.04], $P = 0.022$) with MISI (Figure 1). These associations indicate that higher expression of these genes was associated with lower skeletal muscle insulin sensitivity. In contrast, none of the genes determined in aSAT was associated with HIRI.

## Association of plasma inflammatory markers and tissue-specific insulin sensitivity

Plasma concentration of the inflammatory marker PAI-1 was positively associated with HIRI (0.14 [0.00 to 0.28]). Plasma SAA (−0.18 [−0.31 to −0.05]) was inversely associated with HIRI, whereas a trend for inverse associations between plasma CRP (−0.14 [−0.28 to 0.00]) and TNFSF12 (−0.13 [−0.26 to 0.00]) concentrations with HIRI was observed (Table 3). However, in the Maastricht Study, we did not observe significant associations between plasma SAA or CRP and HIRI. None of the plasma inflammatory markers was associated with MISI in either the PERSON Study or the Maastricht Study.

# DISCUSSION

In the present study, we investigated the relationships of systemic and aSAT inflammation with liver and skeletal muscle insulin sensitivity in individuals with overweight and obesity. The present findings

**TABLE 3** Associations of inflammatory markers and tissue-specific insulin sensitivity in the PERSON Study and the Maastricht Study

| | HIRI | | | | MISI | | | |
|---|---|---|---|---|---|---|---|---|
| | The PERSON Study | | The Maastricht Study | | The PERSON Study | | The Maastricht Study | |
| | Std β (95% CI) | *p* value | Std β (95% CI) | *p* value | Std β (95% CI) | *p* value | Std β (95% CI) | *p* value |
| CRP | −0.137 (−0.279 to 0.004) | 0.056 | 0.030 (−0.099 to 0.159) | 0.646 | −0.109 (−0.251 to 0.034) | 0.134 | −0.065 (−0.233 to 0.102) | 0.443 |
| SAA | −0.183 (−0.312 to −0.053) | **0.006** | 0.062 (−0.072 to 0.196) | 0.361 | −0.052 (−0.188 to 0.085) | 0.457 | −0.145 (−0.320 to 0.030) | 0.105 |
| TNFSF12 | −0.127 (−0.258 to 0.004) | 0.057 | | | −0.068 (−0.205 to 0.068) | 0.325 | | |
| CD163 | 0.058 (−0.075 to 0.191) | 0.393 | | | 0.087 (−0.052 to 0.226) | 0.221 | | |
| PAI-1 | 0.141 (0.006 to 0.276) | **0.041** | | | −0.057 (−0.198 to 0.083) | 0.422 | | |

*Note*: Standardized effect sizes (95% CI) are reported. A linear regression analysis was performed with adjustment for age, sex, BMI, and medication use (lipid-lowering, antihypertensives, antidepressants, and glucose-lowering medication [the Maastricht Study]) using data from the PERSON Study ($n = 219$) and the Maastricht Study ($n = 1256$). Significant $p$ values (<0.05) are highlighted in bold.

Abbreviations: CRP, C-reactive protein; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; PAI-1, plasminogen activator inhibitor 1; PERSON, PERSonalized Glucose Optimization Through Nutritional Intervention; SAA, serum amyloid A; Std, standard; TNFSF12, tumor necrosis factor ligand superfamily member 12.

demonstrate that higher relative abundances of several circulating immune cell populations, specifically classical monocytes, were indicative of worse liver, but not skeletal, muscle insulin sensitivity. Furthermore, we found that inflammatory gene expression in aSAT was related to skeletal muscle insulin sensitivity, whereas aSAT immune cell populations were not associated with liver and skeletal muscle insulin sensitivity. Finally, plasma inflammatory markers showed distinct associations with liver and skeletal muscle insulin sensitivity, although this could not be validated in the Maastricht Study. Taken together, the present findings demonstrate that circulating immune cell populations, as well as inflammatory gene expression in aSAT, show distinct associations with liver and skeletal muscle insulin sensitivity, which may have implications for strategies to prevent and/or treat obesity-related complications (i.e., precision nutrition and pharmacological interventions).

Circulating T cells, nonclassical monocytes, classical monocytes, and CD56dim CD16+ NK cells were associated with worse liver, but not skeletal muscle, insulin sensitivity in the PERSON Study. We were able to confirm the association of monocytes, specifically classical monocytes, with worse LIR in the Maastricht Study. However, we could not confirm the associations among other immune cell populations and liver insulin sensitivity. Although the individuals from the Maastricht Study included in the present analysis were selected to resemble the PERSON Study participants with respect to age, BMI, nonsmoking status, and no use of glucose-lowering medication, and we adjusted for medication use, the participants of the Maastricht Study still had a slightly unhealthier metabolic profile. A sensitivity analysis in the Maastricht Study to further adjust for history of major cardiovascular disease, inflammatory bowel disease, chronic respiratory disease, and cancer did not alter the conclusion. The reason for these partly discrepant findings remains to be established but it may be related to inherent differences among the study populations and potential residual confounding when comparing a randomized trial (the PERSON Study) with a population-based cohort (the Maastricht Study).

Although we found associations among circulating immune cells and liver insulin sensitivity, aSAT immune cell populations were not associated with liver and/or skeletal muscle insulin sensitivity. The latter seems in line with the observation that immune cells in VAT, but not aSAT, were associated with whole-body insulin resistance and impaired glucose homeostasis [23, 24] and the higher abundances of M1 macrophages and NK cells in VAT, but not aSAT, of individuals with obesity compared with individuals with normal weight [25]. Importantly, the liver may be impacted to a larger extent by VAT than skeletal muscle. More specifically, in contrast to skeletal muscle, which predominantly drains from the systemic circulation and which is largely affected by SAT, the liver drains directly from the portal vein. Therefore, the liver is directly impacted by products released from VAT and the gut [26]. However, whether the (relative abundance of) immune cells in VAT rather than SAT are more closely linked to impaired liver insulin sensitivity remains elusive. Furthermore, some circulating immune cells (i.e., nonclassical monocytes and surface markers on NK cells) were associated with their counterparts in VAT

in previous studies [25, 27]. This may suggest that certain circulating immune cells included in the present analysis predominantly reflect VAT immune cells, which might explain the associations among circulating immune cells and liver insulin sensitivity in the present study. Unfortunately, data on VAT immune cells were not available to confirm this hypothesis. Therefore, although the underlying mechanism remains to be established, we show that circulating, but not aSAT, immune cells are specifically linked to LIR, but not MIR.

Another explanation for the observed associations among circulating immune cells, in particular classical monocytes, and impaired liver insulin sensitivity may be that the (fatty) liver, just like the AT, skeletal muscle, pancreas, and gut, may directly contribute to the composition and quantity of circulating immune cells as well as low-grade systemic inflammation in obesity [2, 8, 11]. Liver resident macrophages (Kupffer cells) often become activated when obesity develops, thereby activating inflammatory pathways in the liver that may, in turn, exert autocrine, paracrine, and/or endocrine effects [28]. Liver inflammation may be the result of AT inflammation or lipotoxicity in the liver [29]. Notably, these processes are closely related to LIR [11, 28, 30]. Together, these findings suggest that multiple (patho) physiological processes that can affect circulating immune cells, systemic low-grade inflammation, hepatic lipid accumulation and inflammation, and (liver) insulin sensitivity operate simultaneously. Therefore, we cannot exclude that the associations among circulating immune cells and LIR that we found in the present analyses may at least partly be due to liver inflammation.

Interestingly, we found that gene expression of IL-6 and CD14 in aSAT was positively associated with muscle, but not liver, insulin sensitivity in the PERSON Study. These findings are in agreement with results from the Diet, Obesity, and Genes (DiOGenes) study, showing that a proinflammatory gene expression profile in aSAT was present in individuals with MIR, but not with LIR, compared with individuals with normal insulin sensitivity [14]. Furthermore, we subsequently confirmed these findings in a post hoc analysis within the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) and the Maastricht Study, demonstrating that a low-grade inflammatory *z* score (based on plasma IL-6, IL-8, TNF-α, SAA, soluble intercellular adhesion molecule [sICAM], CRP, haptoglobin, and ceruloplasmin concentrations) was inversely associated with muscle, but not liver, insulin sensitivity [14]. We could not confirm an association of plasma inflammatory markers with tissue-specific insulin sensitivity in the present analyses, which may be a result of inclusion of different individual inflammatory markers and the smaller sample size of the PERSON Study. Our findings, together with previous observations, indicate that aSAT inflammation is more strongly related to muscle, rather than liver, insulin sensitivity. This may be related to the blood supply of skeletal muscle, which drains to a large extent from the systemic circulation, which, in turn, is largely affected by aSAT. Additionally, tissue-specific differences in molecular pathways affecting insulin signaling may determine to what extent inflammation affects insulin sensitivity. Indeed, obesity-related low-grade inflammation may impact insulin sensitivity via various mechanisms, including inhibition of insulin signaling by, among others, the activation of Jun N-terminal kinase (JNK) and nuclear factor (NF)-κβ [31, 32], suppression of AMP-activated protein kinase (AMPK) activity [33], and downregulation of peroxisome proliferator-activated receptor γ (PPAR-γ) [31]. It is tempting to speculate that SAT-derived proinflammatory factors may have more pronounced effects on insulin signaling in skeletal muscle compared with the liver. Clearly, further studies are warranted to examine this in more detail.

The strengths of the present study are that PERSON Study and the Maastricht Study participants have been phenotyped in detail, allowing for detailed investigation of tissue-specific insulin sensitivity and the inflammatory phenotype. Furthermore, next to data from the PERSON Study, we used a replication cohort (the Maastricht Study) to investigate the associations of circulating immune cells and systemic low-grade inflammation with tissue-specific insulin sensitivity and we were able to confirm the inverse associations among classical monocytes and liver insulin sensitivity in the Maastricht Study. The present study also has some limitations. Because the weight of the AT biopsies was not available, we were not able to quantify the absolute number of immune cells in aSAT; however, we were able to determine the relative abundances of immune cells. Nevertheless, quantitative expression of immune cells (i.e., per gram of AT) may lead to inaccuracies in determining immune cell numbers due to differences in adipocyte size. However, importantly, the present analysis demonstrated that the association between the relative abundance of aSAT immune cells and tissue-specific insulin sensitivity was independent of adipocyte size. Second, we did not collect VAT samples because of the invasiveness of this procedure and related medical/ethical issues. Future studies are warranted to explore the relationships of immune cells in VAT and circulating immune cells with tissue-specific insulin sensitivity. Finally, HIRI and MISI are OGTT-derived measures. Although HIRI and MISI have been validated against the gold-standard two-step hyperinsulinemic-euglycemic clamp, the postprandial glucose and insulin responses are also affected by interindividual differences in gastrointestinal factors, including the rate of glucose absorption and the incretin response [34]. Nevertheless, we have previously shown that OGTT-derived measurement of tissue-specific insulin sensitivity allows for the identification of distinct metabolic phenotypes [12, 14, 15], which, together with the fact that it is a simpler, more time efficient, and less invasive measurement compared with the gold-standard clamp technique, makes the seven-point OGTT a suitable method to assess tissue-specific insulin sensitivity, especially in larger human studies.

In conclusion, we demonstrated that circulating immune cell populations, specifically the abundance of (classical) monocytes, were associated with worse liver insulin sensitivity in individuals with overweight and obesity. No associations were found among immune cell populations in aSAT and tissue-specific insulin resistance. Moreover, proinflammatory aSAT gene expression was inversely associated with muscle, but not liver, insulin sensitivity. Collectively, the present findings show distinct associations of immune cells and inflammation with liver and skeletal muscle insulin sensitivity, which may have implications for more personalized lifestyle and pharmacological interventions.O

## AUTHOR CONTRIBUTIONS

Inez Trouwborst wrote the manuscript and performed the statistical analysis. Kristiaan Wouters performed the immune cell analysis in both studies. Inez Trouwborst, Kelly M. Jardon, and Anouk Gijbels were responsible for data collection in the PERSonalized Glucose Optimization Through Nutritional Intervention (PERSON) study. Nathalie Richard and Igor Bendik contributed to sample analysis. Inez Trouwborst, Kristiaan Wouters, Johan W. Jocken, Ellen E. Blaak, and Gijs H. Goossens conceptualized the manuscript. Lydia A. Afman, Gijs H. Goossens, and Ellen E. Blaak supervised the research and codesigned the study. Ellen E. Blaak was project leader and obtained funding for the PERSON Study. Pieter C. Dagnelie, Marleen M. J. van Greevenbroek, Carla J. van der Kallen, Coen D. A. Stehouwer, and Casper G. Schalkwijk obtained funding and organized the Maastricht Study. All authors read and approved the final version of the manuscript. Gijs H. Goossens takes full responsibility for the work as a whole.

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## CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data presented in this manuscript are available from the corresponding author on reasonable request.

## ORCID

*Inez Trouwborst* https://orcid.org/0000-0001-9538-7954
*Ellen E. Blaak* https://orcid.org/0000-0002-2496-3464
*Gijs H. Goossens* https://orcid.org/0000-0002-2092-3019

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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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