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Original article

Associations between blood markers of glucose metabolism and characteristics of circulating lymphocytes

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ABSTRACT

Aims: The pathophysiology of diabetes is not fully understood; recent research indicates close relations with immunological alterations. Therefore, the aim of this study was to investigate the associations between markers of glucose metabolism and characteristics of blood lymphocytes in a population-based cohort.

Methods: The analysis was based on data from 219 non-diabetic participants of the MEGA study in Augsburg, Germany, who were recruited between 2018 and 2021. The majority of participants were examined two different times with a time lag of 9 months. Fasting venous blood samples were taken and oral glucose tolerance tests (OGTT) were performed at both visits. Immune cells were analyzed from fresh blood using flow cytometry. The associations between fasting blood glucose levels, glucose levels at 2 h after oral glucose bolus and glycated hemoglobin (HbA1c) concentrations and the quantity of different lymphocyte subsets were analyzed using linear mixed regression models with random intercept. P values were FDR-adjusted.

Results: HbA1c was negatively associated with the marginal zone B cells (IgD + CD27+ B cells). Fasting glucose was positively associated with natural killer (NK) cells and 2-h OGTT glucose was positively associated with NKT cells. Finally, HbA1c showed significantly negative associations with the CD57-PD1- NKT cell subset.

Conclusion: Markers of glucose metabolism showed significant associations with B cell, NK cell and NKT cell subsets, which clearly indicates a relation between glucose metabolism and the adaptive immune system.

1. Introduction

Disturbed glucose tolerance and diabetes mellitus affects millions of people around the world; the estimated number of unrecorded cases is high [1]. Disorders of glucose metabolism and insulin resistance are not only a major cause of many subsequent diseases such as myocardial infarction and other cardiovascular diseases [2,3], they are also responsible for an elevated risk of mortality and premature death in the people affected [4,5]. To this date, the endeavor of many scientists around the world has led to a better understanding of the underlying pathophysiology and improved diagnostics and therapy. Nevertheless, the condition is far from being understood completely, including knowledge about risk factors, pathophysiological mechanisms and interconnection with other body systems like the immune system. While there is a variety of research on the role of the (adaptive) immune system and type 1 diabetes mellitus [6–13], there is only insufficient knowledge on the interconnection between type 2 diabetes and the immune system. Several prior studies and publications suggest a complex interplay between disturbances of glucose metabolism/type 2 diabetes and particularities of the immune system [14,15]. This for instance includes a suppression of cytokine production or release [14] and dysfunctions in immune cells, especially cells of the innate immune systems like neutrophils and monocytes/macrophages [14]. Although there are several prior studies that found associations between the adaptive immune system and type 2 diabetes [15], this topic remains far away from being understood sufficiently. Especially studies investigating the complex interplay between lymphocytes and glucose metabolism in non-diabetic individuals are extremely scarce. Contributing to a deeper and more comprehensive understanding of this complex topic was the

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objective of the present analysis. Therefore, we analyzed associations between lymphocytes and lymphocyte subsets and parameters of glucose metabolism in a population-based cohort of non-diabetic individuals.

2. Material and methods

2.1. Study population and data collection

The analysis was based on data from the MEGA study (German acronym for metabolic health study Augsburg). The MEGA study was conducted between 2018 and 2021 in Augsburg, Germany, and included participants between 25 and 65 years of age. The participants were examined up to 4 times within a time period of 9 months (baseline visit, follow-up visits after 1 and 6 months and a final visit after 9 months). The general aim of the MEGA study was to investigate immunological particularities and their association with anthropometric measures, diet, general lifestyle (e.g. smoking status etc.) and metabolic comorbidities such as dyslipidemia or glucose metabolism disorders. Therefore, normal-weight individuals and individuals with higher body mass index (BMI) from the general population were recruited. Participants were recruited by posters and flyers (e.g. at doctor's offices), direct recruitment of participants in public places (e.g. shopping mall), announcements in newspapers and on social media, and by word of mouth (in later stages). Amongst many other examinations like liver elastography or bioelectrical impedance analysis, the study protocol of the MEGA study included venous blood sampling, oral glucose tolerance test (OGTT, only participants without pre-known diabetes) and an extensive face-to-face interview about comorbidities, lifestyle, medications and other issues. All examinations were performed in an at least 12-h overnight fasting state. The following information, which was used for the present analysis, was obtained within the scope of the face-to-face interviews: diagnosed diabetes mellitus (yes, no), total alcohol consumption (beverages per day), and smoking status (current smoker, never smoker, previous smoker).

Only participants without fever (>38.5 °C in the last 24 h) and without antibiotics or immunosuppressant use in the last 3 months were included into this analysis. In total, 219 non-diabetic participants were examined and received an OGTT at least one time. Individuals with a pre-known diabetes and/or diabetes medication did not receive an OGTT and therefore were not part of the study sample. A total of 350 examinations including an OGTT were conducted (131 participants received two OGTTs at baseline and at the follow-up visit approximately 9 months later).

For 348 of these 350 examinations there was valid information derived from the B cell panel, for the NK cells and T cell panel there were 344 cases with valid information, and 333 cases for the Treg panel (the Treg panel was established with a certain delay, which causes a higher number of cases with missing information).

All study participants gave written informed consent. Methods of data and biosample collection have been approved by the ethics committee of the Ludwig-Maximilians Universität München and the study was performed in accordance with the Declaration of Helsinki. The study was registered at “Deutsches Register Klinischer Studien” (DRKS) with the project number DRKS00015784.

2.2. Fasting blood glucose, 2-h OGTT glucose levels, and HbA1c levels

Fasting blood glucose levels, 2-h OGTT glucose levels and HbA1c levels were determined at baseline and at the final visit, so for the majority of participants there were two measurements within a time period of approximately 9 months available. For the blood collection, NaF/citrate plasma tubes (GlucoEXACT) were used and the measurements were performed by the central laboratory of the University Hospital Augsburg immediately after collection. The method of the HbA1c measurements was reverse-phase cation-exchange high-pressure liquid chromatography (HPLC, Analyzer HA 8160; Menarini, Florence, Italy).

2.3. Flow cytometry

Venous EDTA blood samples were used for the fluorescence-based flow cytometry (Cytoflex LX flow cytometer, 6 lasers, Fa. Beckman Coulter). The blood samples were processed and measured immediately after blood collection. The general steps of the measurements are described in the following: In an initial step, erythrocytes were lysed using VersaLyse Lysing Solution (Beckman Coulter). Then, in several washing steps, the immune cells were isolated. In order to avoid non-specific antibody binding, the cells were treated with an FC receptor block. Subsequently, the antibody staining process was performed using fluorescence-labelled liquid antibodies. The T cell and NK cells panel (panel 1) contained the following antibodies: anti-CD3, anti-CD56, anti-CD4, anti-CD8, anti-CD45RA, anti-CD27, anti-CD197 and anti-CD279. The Treg panel (panel 2) included the antibodies anti-CD4, anti-CD25, anti-CD127 and anti-CD45RA. For the last panel (B cells), the following antibodies were used: anti-CD19, anti-CD21, anti-CD24, anti-CD27, anti-CD38, anti-IgM and anti-IgD. The best concentration of antibodies was predetermined by titration. Before the antibody-coupled immune cells were finally analyzed using flow cytometry, the cells were fixed using IO-Test 3 Fixative Solution (Beckman Coulter). For the subset analysis (gating), the Kaluza software from Beckman Coulter was used. Figures 1–3 display the gating strategy that was applied for each panel. As a template for the gating strategy, a publication by Streitz et al. was used [16] and for B cells and Tregs the same gating strategy was applied as in prior analyses based on the MEGA study [17–19]. The exact definition of each subpopulation according to the cell surface marker expression is displayed in Table S1, S2 and S3 of the supplementary material. For every cell subset, the relative frequency of the respective cells (as a share of the total cell count of the parent gate) was used for the statistical analysis.

2.4. Statistical analysis

For normally-distributed continuous variables, Student's t-tests were used; for non-normally distributed continuous variables, nonparametric tests were applied. The results were presented as mean and standard deviation (SD) or median and inter-quartiles range (IQR). Categorical variables were analyzed using Chi-square tests; the results were presented as absolute frequencies and column percentages.

There were repeated measurements for the majority of participants (baseline and follow-up visit). Nevertheless, the statistical analyses were characterized by a cross-sectional approach (no intervention or similar); the intention of the repeated measurements was to reduce the influence of short-term immune cell fluctuations (e.g. caused by infection) and not to identify any longitudinal changes.

Linear mixed regression models with random intercept were calculated to examine the associations between the exposure variables (fasting glucose, 2-h OGTT glucose and HbA1c levels) and the relative frequencies of different T cell, B cell and NK cell subsets (outcome). In order to ensure comparability of the effect sizes, both, the exposure as well as the outcome variables were standardized (the variables were centered and then divided by the SD so that the transformed variable had an expectancy value of 0 and a statistical variance of 1). Observations with a deviation from the mean of

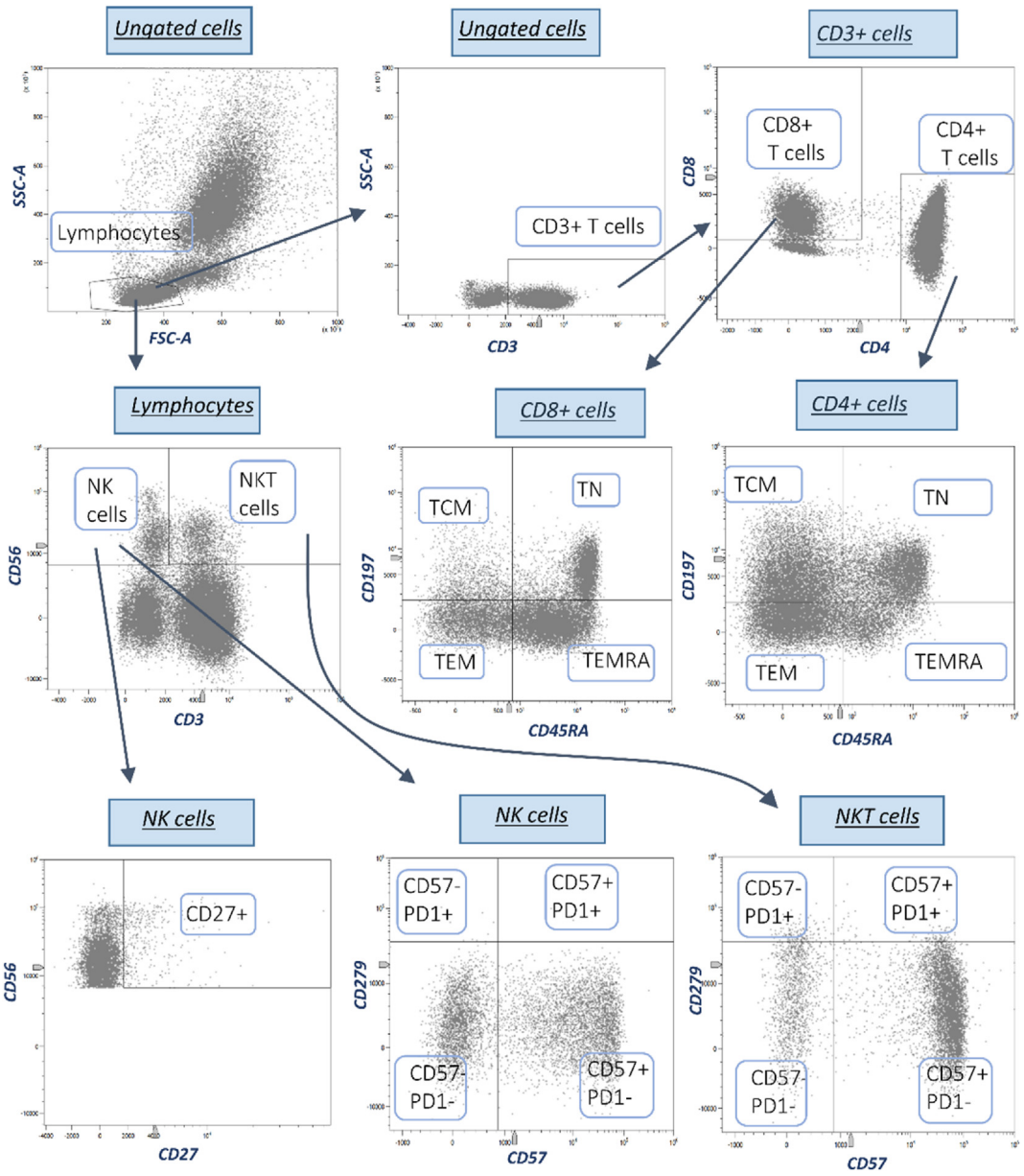

Fig. 1. Gating strategy for the T cell and NK cell panel. In the first step, T cells were identified by their CD3, CD4 and CD8 expression. T cell subsets were then analyzed using the following antibodies: anti-CD197 and anti-CD45RA. NK cells and NKT cells were identified by their cell surface expression of CD56 and CD3. Subpopulations were analyzed using anti-CD27, anti-CD57 and anti-CD279 antibodies. The identified cell subsets were quantified by calculating their proportion of the parent gate cells.

greater than three SD were removed to avoid excessive impact of single outliers.

According to literature review, the following potential confounder variables were included into the multivariable adjusted models: sex, age, smoking status, alcohol consumption and BMI. Normal distribution of the regression residuals was checked graphically.

For every major cell line (T cells, B cells, NK/NKT cells) the obtained p-values were adjusted to control the false discovery rate (FDR); 95 % confidence intervals (95 % CI) displayed in Figs. 4–7 were not FDR-adjusted. The displayed effect estimates (β-coefficient and 95%CI) represent the expected change in standardized outcome associated with one SD increase in the exposure variable.

Since loss to follow-up was low and missing observations were very scarce, a complete-case analysis was performed, meaning only participants without missing data on relevant variables were considered. Study participants with only one examination were included into the analysis.

All statistical analyses were performed using R version 4.3.2.

3. Results

Table 1 displays the baseline characteristics for the total sample (N = 219 individuals) and stratified for men and women. About two thirds of all study participants were female and the average age was 46.1 (11.9) years. While the median HbA1c was within the normal range for both genders, men had higher fasting glucose values, a higher BMI and more visceral fat mass than women. Moreover, men had more often a diagnosis of hypertension, drank more alcohol and were more likely to be current or former smokers.

The results of the linear mixed regression models are shown in Figs. 4–7.

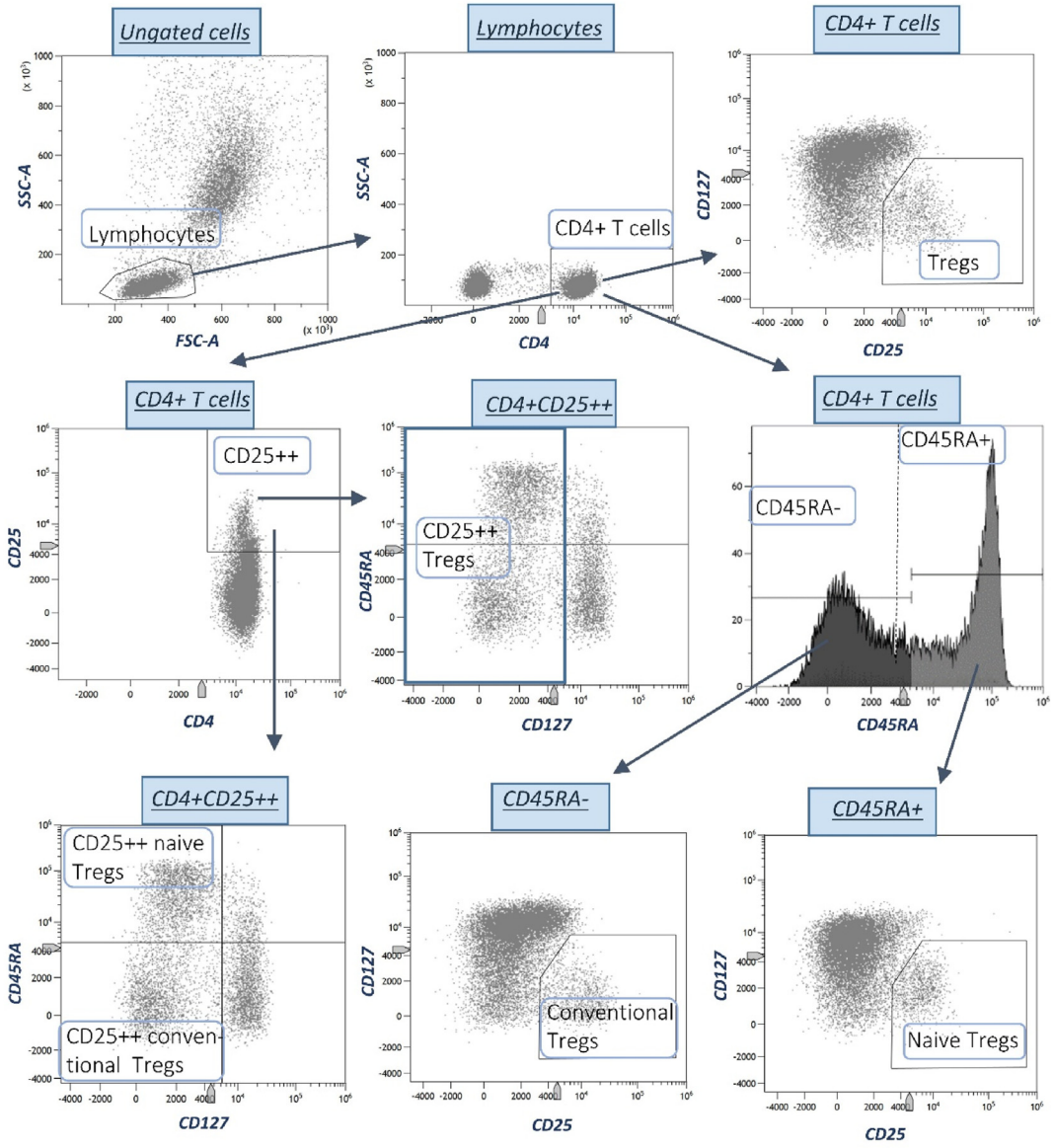

Fig. 2. Gating strategy for the Treg panel. In the first step, T helper cells were identified by their expression of CD4. Then, different CD4+ T cell subsets were analyzed using the following antibodies: anti-CD25, anti-CD127 and anti-CD45RA. The identified cell subsets were quantified by calculating their proportion of the parent gate cells. The applied gating strategy was also used in prior publications based on data from the MEGA study [17,18].

3.1. Glucose metabolism markers and T cells

Before FDR-adjustment, there was a positive association between HbA1c and CD8+TN cells (CD45RA + CD197+); and a negative association between HbA1c and the conventional Tregs and the CD25++ Tregs. Moreover, there was a positive association between naïve Tregs and fasting glucose. However, none of these associations remained significant after FDR-adjustment.

3.2. Glucose metabolism markers and B cells

We found a suggested positive association between fasting glucose and IgM-IgD- B cells (not significant after FDR-adjustment); and no association between B cells and glucose 2 h after OGTT. For HbA1c, there was a negative association with various B cell subsets, namely: IgD only B cells, memory B cells, marginal zone B cells and class non-switched memory B cells. After FDR-adjustment, only the association between HbA1c and marginal zone B cells (IgD + CD27+ B cells) remained significant.

3.3. Glucose metabolism markers and NK cells

Before FDR-adjustment, fasting glucose was positively associated with the general NK cell subset, the CD57+PD1-NK cells and the CD57+PD1-NKT cell subset; and negatively associated with CD27+ NK cells. For glucose 2 h after OGTT, we found a positive association with the general NKT cell subset and a negative association with CD57+PD1+ NKT cells. Finally, HbA1c was positively associated with NKT cells and CD57+PD1+ NK cells and negatively associated with CD57-PD1-NKT cells. After FDR-adjustment, there were still three significant association: positive associations between fasting glucose and NK cells and between glucose 2 h after OGTT and NKT cells respectively; and a negative association between HbA1c and the CD57-PD1-NKT cell subset.

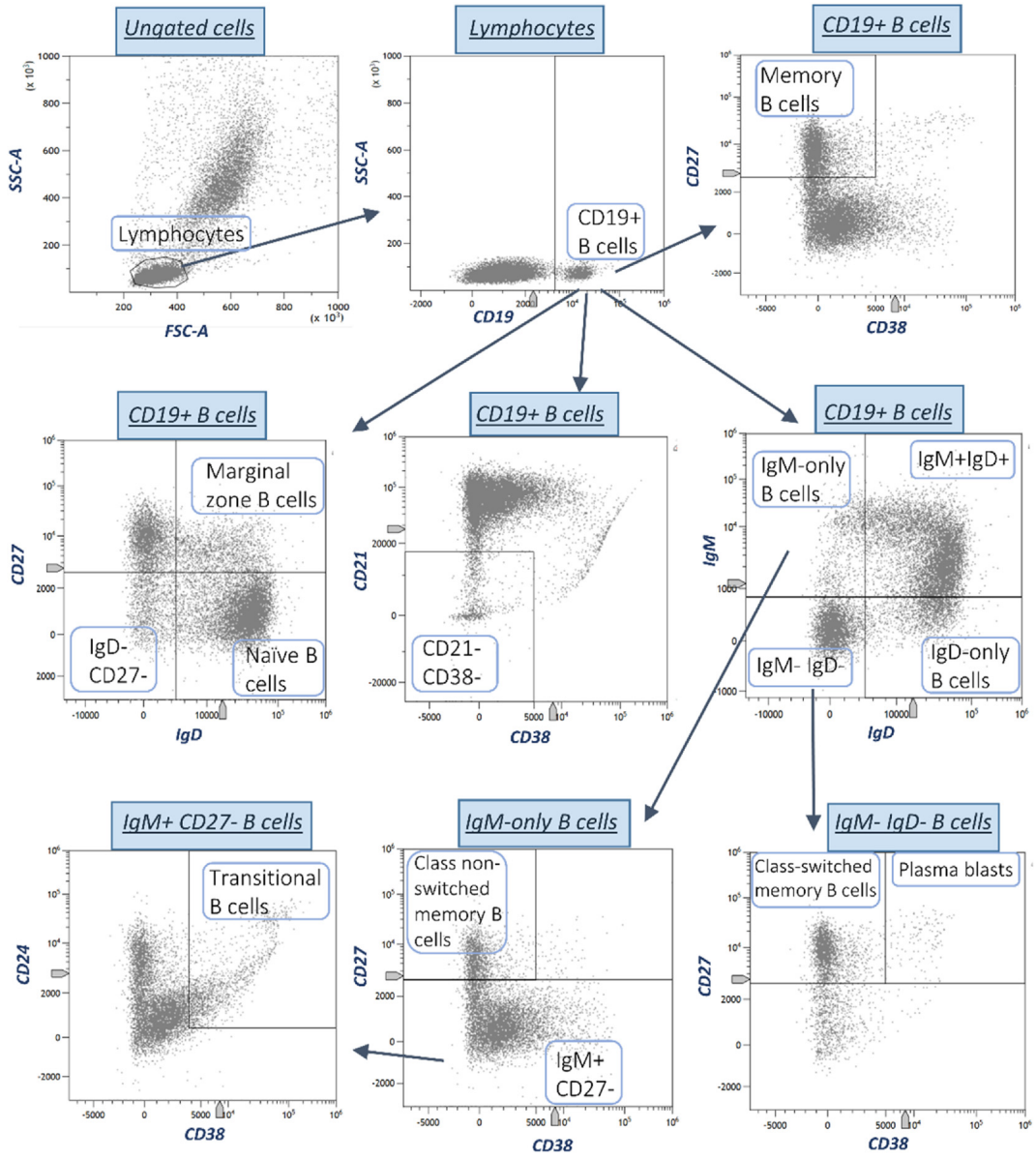

Fig. 3. Gating strategy for the B cell panel. After selecting the B cells using anti-CD19 antibodies, the B cells were further analyzed using anti-IgD, anti-IgM, anti-CD21, anti-CD24, anti-CD27 and anti-CD38 antibodies. The identified cell subsets were quantified by calculating their proportion of the parent gate cells. The applied gating strategy was also used in prior publications based on data from the MEGA study [17,19].

4. Discussion

In the present study, we investigated the associations between parameters of glucose metabolism and lymphocytes in a sample of non-diabetic individuals. After adjusting for multiple testing (FDR-adjustment), we found that HbA1c showed significant associations with B cell and NKT cell subsets. For fasting glucose levels and 2-h OGTT glucose levels we observed significantly positive associations with the general NK and NKT cells, respectively.

4.1. T cells and Tregs

No significant associations between markers of glucose metabolism and CD4+ or CD8+ T cells were found after FDR-adjustment. Nevertheless, there were some suggested associations (significant only before FDR-adjustment). HbA1c for example showed a suggested negative association with the conventional Tregs and the CD25++ Tregs. Interestingly, the results from a meta-analysis suggested that patients with Type 2 diabetes mellitus, and in particular those with diabetic complication, have decreased frequencies of immunosuppressive Treg cells compared to controls [20]. These results are difficult to compare to our findings, as we investigated only non-diabetic participants with HbA1c majorly in the normal range. However, the presents study indicates that there might be an interconnection between the frequency of Tregs and glucose metabolism in non-diabetic patients. Tregs are suspected to be involved in the pathophysiology of several diseases (e.g. autoimmune diseases) [21]. Recent results also indicated their involvement in the pathophysiological process of type 2 diabetes [14,15].

4.2. B cells

There was no significant association between fasting glucose and 2-h OGTT glucose with any B cell subset after FDR-adjustment. For HbA1c, there were several suggested negative association with

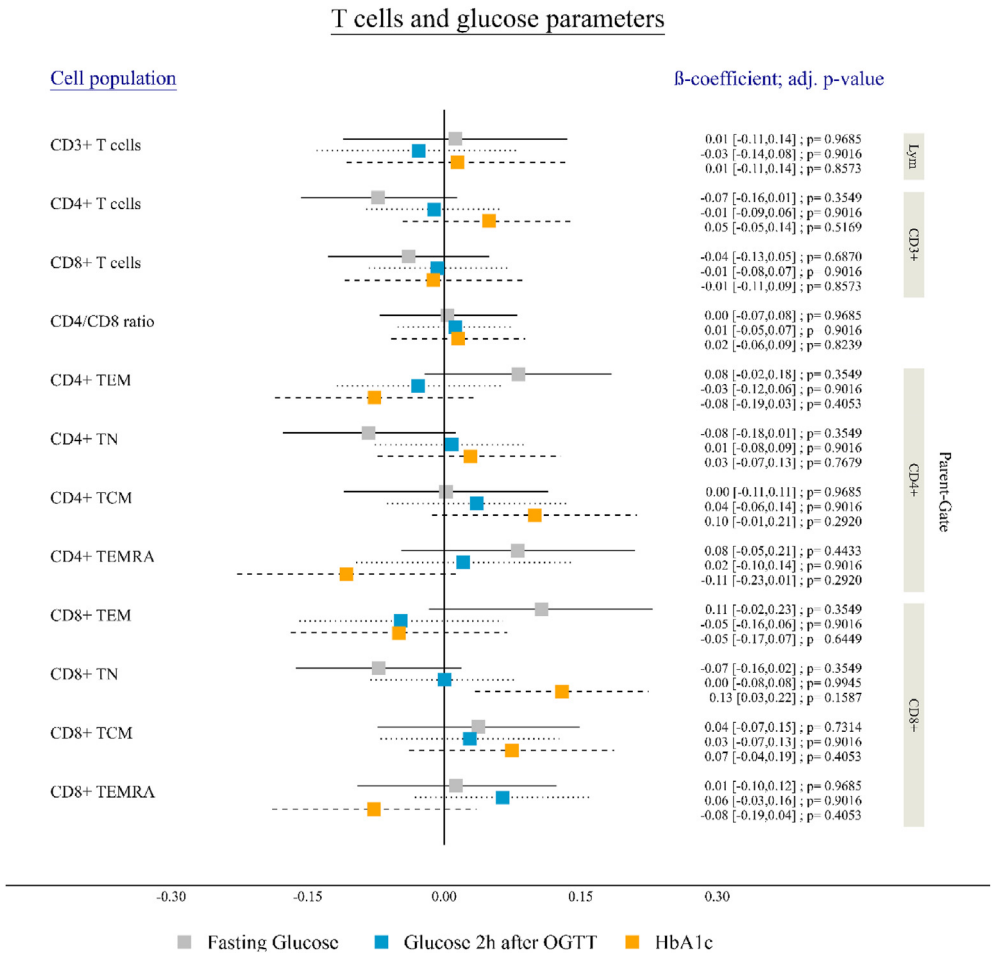

Fig. 4. Association between glucose markers and T cell subsets. Linear mixed models (random intercept) were adjusted for sex, age, smoking status, alcohol consumption and BMI. The exposure variables (fasting glucose levels, 2-h OGTT glucose levels and HbA1c) as well as the outcome variables (specific T cell subsets as proportion of parent gates) were standardized. The figure displays the estimated β-coefficients with 95%CI; p values were FDR-adjusted.

various B cell subsets (IgD only B cells, memory B cells, marginal zone B cells, class non-switched memory B cells); yet only the association with the marginal zone B cell subset remained significant after adjusting for multiple testing. Marginal zone B cells represent a mature B cell subset, which can be found in the marginal zone of the spleen, but also in lymph nodes and peripheral blood and can be described as innate-like B cells [22]. It is assumed that marginal zone B cells are important especially in the early stages of the immune response [23]. Marginal zone B cells are characterized by the expression of IgD and CD27. The latter can be described as a marker to distinguish between naïve (CD27-) and memory (CD27+) B cells [24]. Marginal zone B cells were shown to be increased in non-obese diabetic mice with type 1 diabetes [25], which somehow contradicts our findings. The pathophysiology of type 1 and type 2 diabetes differs in major parts, however, so the results are hardly comparable. Overall, there are only very few studies examining the association between glucose metabolism/type 2 diabetes and B cells [15,26]. A study by Winer et al. for example indicated a promotion of insulin resistance by B cells through the production of IgG antibodies and T cell activation [27].

Chronically elevated blood glucose levels, represented by elevated HbA1c levels, exert chronic cell stress on the immune cells [28]. The question arises as to whether this accelerates immunosenescence in the long term. Shi et al. demonstrated, that CD27+ memory B cells, particularly IgD + IgM + CD27+ memory B cells are decreased in elderly, which would be in line with our findings. Yet, B cell immunosenescence is a very complex process and other studies have shown a shift from naïve B cells to memory B cells during aging [29], which clearly demonstrates that further efforts must be made to get to a better understanding of these complex interaction between B cell function, glucose metabolism and immunosenescence.

4.3. NK cells and NKT cells

In the present study we observed significantly positive associations between NK and NKT cells with markers of glucose metabolism (fasting blood glucose and 2-h OGTT glucose respectively) after FDR-adjustment. NK cells are a part of the lymphocyte lineage, yet they belong to the innate immune system. NK cells play a major role in early immune response to infections and in tumor protection [30]. NKT cells, although CD56 positive, are actually a special T cell subset that share properties of both, NK cells and T cells [31]. A prior study reported a natural killer cell dysfunction in type 2 diabetes mellitus (e.g., a reduced NK degranulation capacity) [32], which could also contribute to a higher susceptibility to infections in diabetic individuals [33]. Nevertheless, other studies on NK and NKT cells and type 2 diabetes came to differing results and no specific conclusion can be drawn so far [15].

Some NK and NKT cells express the programmed cell death protein 1 (PD-1), also named CD279 [34–36]. PD-1 is often called an 'immune checkpoint' and provides immune modulatory

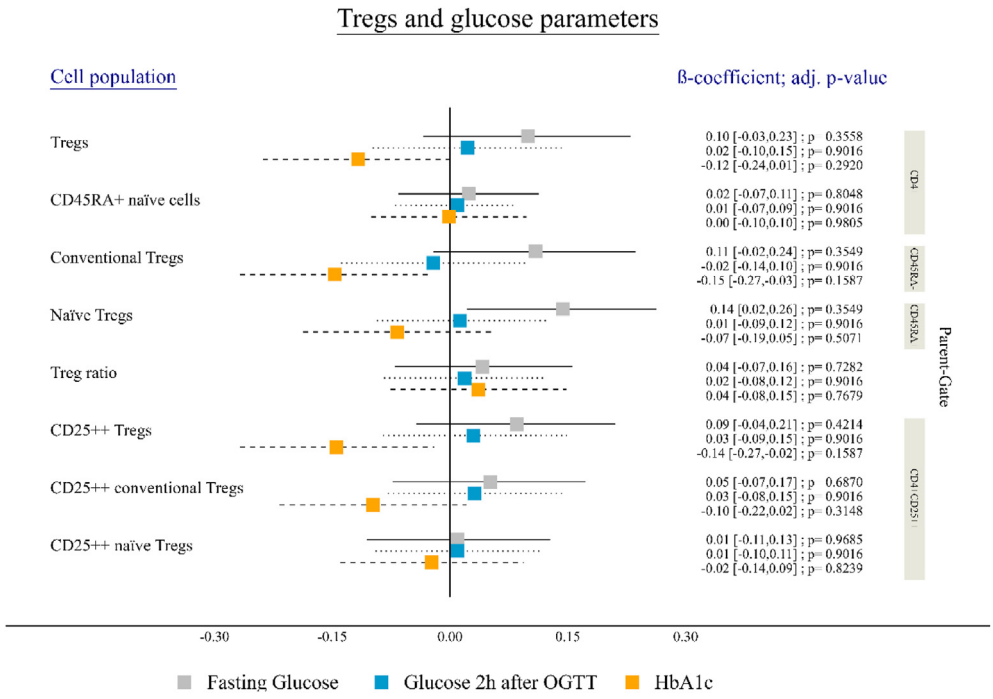

Fig. 5. Association between glucose markers and Tregs and Treg subsets. Linear mixed models (random intercept) were adjusted for sex, age, smoking status, alcohol consumption and BMI. The exposure variables (fasting glucose levels, 2-h OGTT glucose levels and HbA1c) as well as the outcome variables (specific Treg subsets as proportion of parent gates) were standardized. The figure displays the estimated β-coefficients with 95%CI; p values were FDR-adjusted.

properties [37]. In our study, we found an inverse association between HbA1c and PD-1 negative NKT cells that are characterized by not expressing CD57. The CD57 antigen is a marker of terminally differentiated NK and NKT cells [38]. The cell surface expression of this antigen indicates a reduced proliferative capacity, but they are also characterized by potent effector functions and a high cytotoxic potential [38]. While CD57 is expressed by cytotoxic NK cells, it is usually not seen in CD16negCD56bright regulatory NK cells [39]. It can be speculated whether the negative association between HbA1c and the CD57-PD1- NKT cells represents a reduced cytotoxicity of this cell subsets in the presence of chronic hyperglycemia, but this question must remain unclear at this point. Interestingly, aging and immunosenescence goes along with an increase of CD57 expression, but a reduced cytotoxicity in NK and NKT cells [40,41], which again could be compatible with our results found for NKT cells.

4.4. Causality between immune system and glucose metabolism

Summarizing, the issue of causality and, if existing, the direction of causality between lymphocytes and glucose metabolism remains unclear. The first main hypothesis would be an impact of an existing glucose disturbance/diabetes mellitus on immune cells and lymphocytes. Like all other body cells, lymphocytes metabolize glucose and Na + coupled glucose carriers ensure glucose uptake even at low extracellular glucose concentrations [42]. Nevertheless, several glucose transporters are upregulated by insulin and downregulated by hyperglycemia [42], clearly indicating an interplay between glucose metabolism and lymphocyte biology.

The main alternative presumption is that initial alterations in the immune system cause a disturbance of glucose metabolism. Prior studies have shown, that inflammation, for instance mediated by the inflammatory cytokine TNF-α, can cause insulin resistance [14]. In prior publications we have also reported about associations between body composition (BMI, body fast, visceral body fat etc.) and lymphocytes (B cells and Tregs) [18,19]. For example obesity and an elevated waist-to-hip ratio were inversely associated with the Treg and the conventional Treg subset [18]. In the present study, especially many men had an elevated BMI over 30 kg/m^2 and median visceral fat mass was more than three times higher than in women. Overweight and especially high amounts of visceral body fat are known risk factors for diabetes mellitus type 2. So, it could be hypothesized, whether specific immune cell particularities like a reduction of immunomodulatory Tregs are in some sense a mediator between diabetes risk factors (e.g., obesity) and a disturbed glucose metabolism.

4.5. Effect size an β coefficients

Regardless of possible causality, the effect sizes of the significant association described in this analysis are moderate. The strongest association that was identified was between marginal zone B cells and HbA1c with a beta of −0.25. The β-coefficients must be interpreted as changes of standard deviations in the outcome variable (frequency of lymphocyte subsets), for every change of one standard deviation in the exposure variable (HbA1c). That means that even the strongest associations are interfered by many other influential factors, which is not surprising considering the complexity of the immune system. However, since we analyzed HbA1c values only in the non-diabetic range, the effect sizes indicate a substantial interconnection between glucose metabolism and adaptive immune cells even in individuals without diabetes.

4.6. Strengths and limitations

Strengths of the present study are the relatively large number of examined non-diabetic individuals. For the majority of participants there are two measurements at baseline and approximately nine months later with an overall moderate drop-out rate. The repeated measurements limit the influence of short-term immune cell

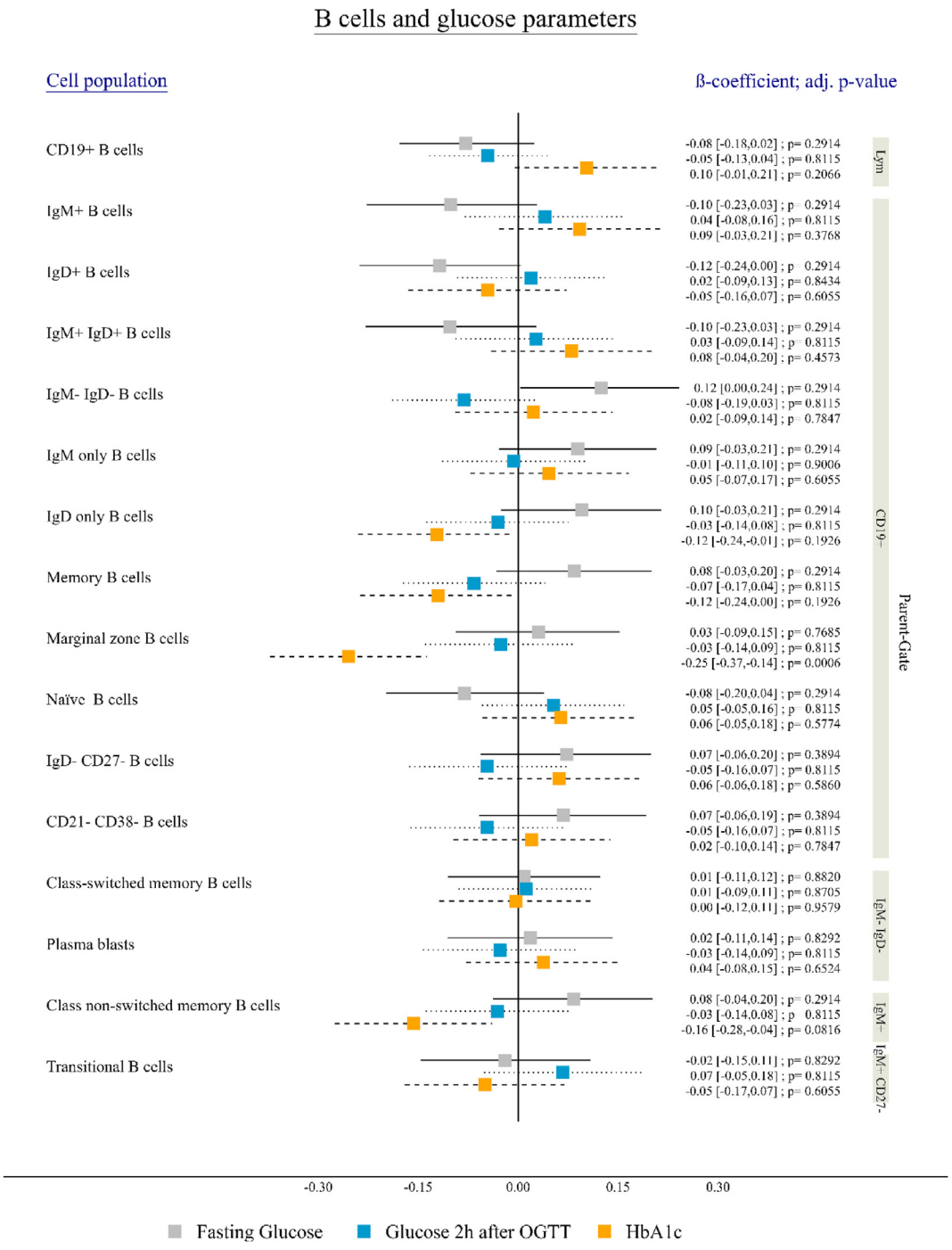

Fig. 6. Association between glucose markers and B cell subsets. Linear mixed models (random intercept) were adjusted for sex, age, smoking status, alcohol consumption and BMI. The exposure variables (fasting glucose levels, 2-h OGTT glucose levels and HbA1c) as well as the outcome variables (specific B cell subsets as proportion of parent gates) were standardized. The figure displays the estimated β-coefficients with 95%CI; p values were FDR-adjusted.

fluctuations e.g. caused by acute infection or illness. All measurements performed were highly standardized; the study personnel was trained according to standard operating procedures (SOP) and certified. The flow cytometry measurements were performed right after blood sampling. A wide range of data and information was collected for each participant that allowed to adjust for relevant potential confounders in the linear mixed regression models.

Of course, there are some limitations that must be considered. First to mention is the generally limited quantitative comparability of flow cytometry measurements among different laboratories. Although the processes and measurements were highly standardized, other laboratories and studies might slightly differ in methodological details. In this study, no negative controls were used for the flow cytometry measurements. Since the majority of our study sample consisted of a typical European population aged 25–65 years, the results may not be generalized to other ethnicities and age-groups. In our study sample, men were slightly underrepresented and had more comorbidities (hypertension, glucose

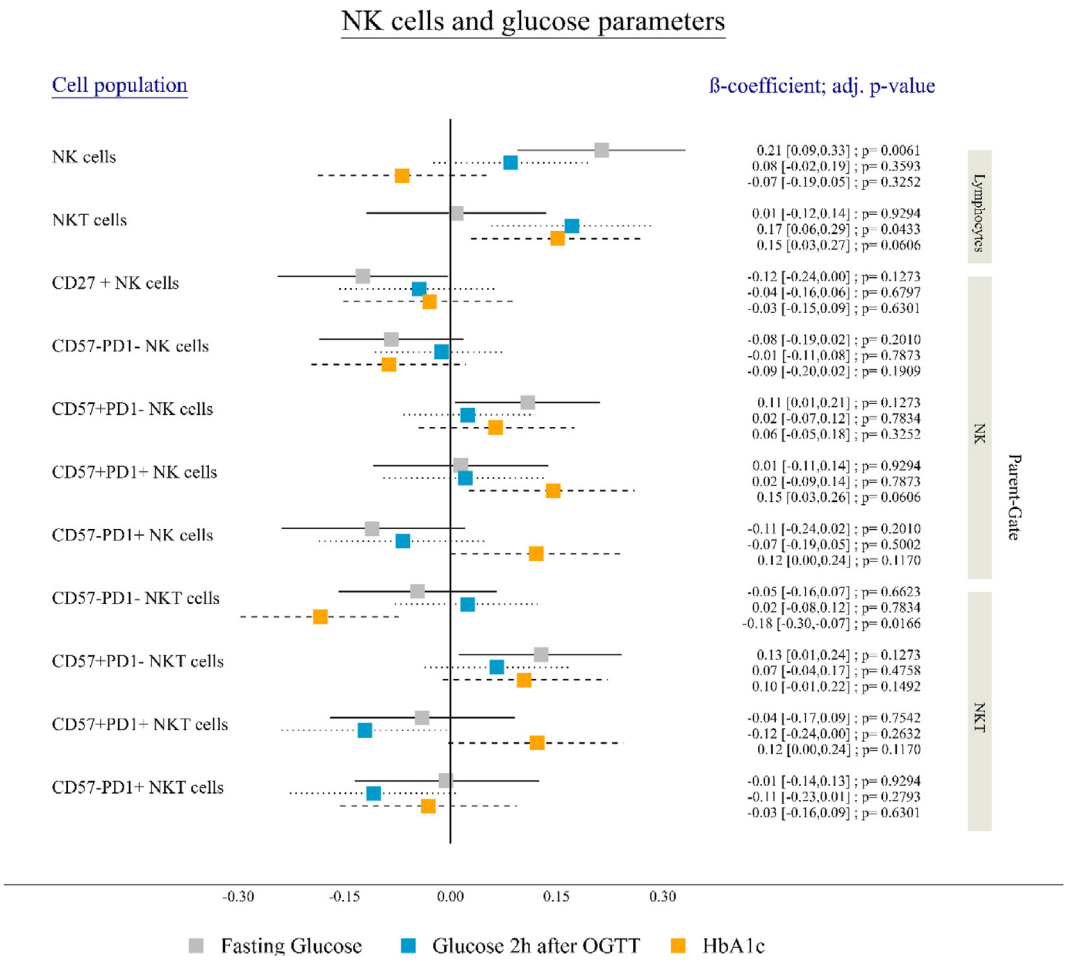

Fig. 7. Association between glucose markers and NK and NKT cell subsets. Linear mixed models (random intercept) were adjusted for sex, age, smoking status, alcohol consumption and BMI. The exposure variables (fasting glucose levels, 2-h OGTT glucose levels and HbA1c) as well as the outcome variables (specific NK and NKT cell subsets as proportion of parent gates) were standardized. The figure displays the estimated β-coefficients with 95%CI; p values were FDR-adjusted.

Table 1
Baseline characteristics for the total study sample. Categorical variables are represented as absolute numbers (%) and continuous variables are displayed as median (IQR).

	Total sample (N = 219)	Female (N = 151 [68.9 %])	Male (N = 68 [31.1 %])	P value
Age in years (mean, SD)	46.1 (11.9)	45.6 (11.3)	47.2 (13.1)	0.386
HbA1c (mmol/mol)	35.0 (32.0–37.0)	35.0 (32.0–37.0)	35.0 (32.0–38.0)	0.396
Fasting blood glucose levels (mg/dl)	97.0 (93.0–103.0)	96.0 (91.0–101.0)	100.0 (96.0–107.5)	<0.001
Glucose levels 2 h after OGTT (mg/dl)	104.0 (87.0–126.0)	103.0 (86.0–124.5)	107.5 (88.8–131.5)	0.528
BMI (kg/m^2)	25.5 (22.7–33.0)	24.6 (21.9–32.9)	30.7 (24.8–33.5)	0.001
Relative body fat (in %)	33.9 (27.0–41.2)	35.8 (29.8–45.1)	29.1 (17.8–35.7)	<0.001
Total visceral fat mass (kg)	1.4 (0.7–3.5)	1.1 (0.6–2.1)	3.9 (1.2–6.1)	<0.001
Hypertension (yes/no)	56 (25.6)	29 (19.2)	27 (39.7)	0.004
Total alcohol consumption (alcoholic beverages per day)	0.1 (0.1–0.5)	0.1 (0.0–0.5)	0.3 (0.1–0.6)	0.005
Smoking status				0.012
Current smoker	31 (14.2)	21 (13.9)	10 (14.7)	-
Never smoker	111 (50.7)	86 (57.0)	25 (36.8)	–
Former smoker	77 (35.2)	44 (29.1)	33 (48.5)	–

metabolism disorders), more unfavorable lifestyle habits (smoking, alcohol intake), a higher BMI and more visceral body fat mass than women. This is mainly in accordance with known differences in comorbidities and life-style habits in the general German population [43,44], but the variation between men and women might be more pronounced in our study sample. Finally, we might not have considered all relevant confounders (including unmeasured confounders) and we cannot make any statements about the causality of the observed associations.

5. Conclusion

Markers of glucose metabolism show significant associations with B cell, NK and NKT cell subsets in a population-based study sample of non-diabetic individuals. These associations indicate a relationship between glucose metabolism and the adaptive immune system. The results contribute to a more comprehensive understanding of the pathophysiological mechanisms underlying insulin resistance and diabetes mellitus.

Data availability statement

The datasets generated during and/or analyzed in the current study are not publicly available due to data protection aspects but are available in an anonymized form from the corresponding author on reasonable request.

Authors' contribution

CM and JL conceived the study. TS performed the statistical analysis and drafted the manuscript. CM supervised data analysis. CM and JL were responsible for the acquisition of the data. DF contributed to the statistical analysis and the general evaluation strategy. All authors approved the final manuscript.

Ethics approval and consent to participate

The study was registered at the DRKS (Deutsches Register Klinischer Studien) under the number DRKS00015784 and was approved by the Ethics Committee of the Ludwig-Maximilians-Universität München (project number 18–637).

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Conflict of interest

None.

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We thank all colleagues who were involved in the conduction of the study including examiners and study nurses, laboratory staff, secretaries, organization and documentation employees and student assistants. Our special thanks go to all study participants who enabled this study with their participation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.11.004.

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