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Differential white cell count and incident type 2 diabetes: the Insulin Resistance Atherosclerosis Study

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Abstract

Aims/hypothesis—White cell count has been shown to predict incident type 2 diabetes, but differential white cell count has received scant attention. We examined the risk of developing diabetes associated with differential white cell count and neutrophil:lymphocyte ratio and the effect of insulin sensitivity and subclinical inflammation on white cell associations.

Methods—Incident diabetes was ascertained in 866 participants aged 40–69 years in the Insulin Resistance Atherosclerosis Study after a 5 year follow-up period. The insulin sensitivity index (S_I) was measured by the frequently sampled IVGTT.

Results—C-reactive protein was directly and independently associated with neutrophil ($p<0.001$) and monocyte counts ($p<0.01$) and neutrophil:lymphocyte ratio ($p<0.001$), whereas S_I was inversely and independently related to lymphocyte count ($p<0.05$). There were 138 (15.9%) incident cases of diabetes. Demographically adjusted ORs for incident diabetes, comparing the top and bottom tertiles of white cell (1.80 [95% CI 1.10, 2.92]), neutrophil (1.67 [1.04, 2.71]) and lymphocyte counts (2.30 [1.41, 3.76]), were statistically significant. No association was demonstrated for monocyte count (1.18 [0.73, 1.90]) or neutrophil:lymphocyte ratio (0.89 [0.55, 1.45]). White cell and neutrophil associations were no longer significant after further adjusting for family history of diabetes, fasting glucose and smoking, but the OR comparing the top and bottom tertiles of lymphocyte count remained significant (1.92 [1.12, 3.29]). This last relationship was better explained by S_I rather than C-reactive protein.

Conclusions/interpretation—A lymphocyte association with incident diabetes, which was the strongest association among the major white cell types, was partially explained by insulin sensitivity rather than subclinical inflammation.

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Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

CL contributed to the conception and design of the study, analysis and interpretation of data and drafting the article, and gave final approval of the version to be published. AJH contributed to the analysis and interpretation of data and revised the manuscript critically for important intellectual content, and gave final approval of the version to be published. SMH contributed to the acquisition of data, analysis and interpretation of data and drafting the article, and gave final approval of the version to be published.

Keywords

Clinical science; Epidemiology; Human; Insulin sensitivity and resistance; Pathogenic mechanisms; Prediction and prevention of type 2 diabetes

Introduction

Low-grade inflammation is a key component in the pathophysiology of type 2 diabetes [1], particularly in the development of obesity-related insulin resistance [2]. Obesity increases the number of macrophages in adipose tissue and upregulates the production of inflammatory factors [3]. In patients with type 2 diabetes, treatment with an IL-1 receptor antagonist and salsalate (a non-acetylated form of salicylate) has been shown to improve glycaemic control and/or beta cell secretory function [4, 5]. Increased diabetic risk [6] and insulin resistance [7] have been described in patients with chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. Treatment of these conditions with anti-TNF-α blockers ameliorates disease activity, inflammatory mediators and insulin resistance [8, 9].

White cell count, a marker of subclinical inflammation, is directly associated with insulin resistance [10–13] and inversely with insulin secretion [11]. White cell count has been shown to predict both worsening insulin sensitivity [10] and incident type 2 diabetes [10, 14–19], although there is controversy on its usefulness in risk prediction [19–21]. Data on the ability to predict type 2 diabetes by major white cell types are scant [10, 15, 16]. A significant association has been reported for both neutrophil and lymphocyte counts, but not for monocyte count [15, 16]. However, the incidence of diabetes was predicted by white cell count, but not by any major white cell type, in a relatively small study among Pima Indians [10]. Distinct metabolic traits may account (at least partially) for the relationship between white cell subfractions and diabetic risk. Neutrophil count has been shown to correlate with high-sensitivity C-reactive protein (hsCRP) concentration better than any other major white cell type in non-diabetic individuals [22]. Lymphocytes are expanded in obese adipose tissue [3] and regulate macrophage production of inflammatory mediators [1]. Raised levels of neutrophils, lymphocytes and the neutrophil:lymphocyte ratio have been linked to the metabolic syndrome [23, 24]. However, whether major white cell types are associated with the future development of type 2 diabetes beyond the effect of insulin sensitivity and subclinical inflammation is not known [16].

The aims of this study were twofold: (1) to examine the risk of developing diabetes associated with total and differential white cell counts and neutrophil:lymphocyte ratio; and (2) to assess the effects of glucose tolerance, insulin sensitivity, insulin secretion and low-grade inflammation on white cell associations. We analysed these issues in 866 participants who were non-diabetic at baseline [25]. Incident diabetes was ascertained after a 5.2 year follow-up using the 2003 ADA diagnostic criteria. The insulin sensitivity index (S_I) and acute insulin response (AIR) were directly measured using the frequently sampled IVGTT (FSIVGTT).

Methods

Study sample

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicentre observational epidemiologic study of the relationships between insulin resistance, cardiovascular disease and the known risk factors for insulin resistance in different ethnic groups and varying states of glucose tolerance. The design and methods of this study have previously been described in detail [25]. Briefly, the study was conducted at four clinical centres. At centres in

Oakland and Los Angeles, California, non-Hispanic white and African-American participants were recruited from Kaiser Permanente, a non-profit health maintenance organisation. Centres in San Antonio, Texas, and San Luis Valley, Colorado, recruited non-Hispanic white and Hispanic participants from two ongoing population-based studies (the San Antonio Heart Study and the San Luis Valley Diabetes Study). The IRAS protocol was approved by local institutional review committees and all participants provided written informed consent.

A total of 1,625 individuals participated in the baseline IRAS examination (56% women; age range 40–69 years), which occurred between October 1992 and April 1994. After an average of 5.2 years (range 4.5–6.6 years), follow-up examinations were conducted using the baseline protocol. Participants who returned for the follow-up visit (response rate 81%) were eligible for analysis if they were non-diabetic at the baseline examination (n=1,065). We excluded participants with no data on incident diabetes (death, n=22; no OGTT data at the follow-up visit, n=153) or white cell count (n=24). Therefore, the present report includes information on 866 participants (222 African-American, 300 Hispanic and 344 non-Hispanic white). These participants were similar to those who were excluded in terms of demographics, baseline metabolic variables and lymphocyte count (all comparisons, p>0.05), but were different with regard to smoking status and total white cell, neutrophil and monocyte counts (Electronic Supplementary Material [ESM] Table 1). Smoking explained the differences in total white cell and neutrophil counts, but not in monocyte count (ESM Table 2).

Clinical measurements and procedures

The IRAS protocol required two visits, 1 week apart, of approximately 4 h each. Protocols were identical at the baseline and 5 year follow-up examinations. Individuals were asked prior to each visit to fast for 12 h, abstain from heavy exercise and alcohol for 24 h, and refrain from smoking on the morning of the examination. Data on age, sex, race/ethnicity, family history of diabetes, cigarette smoking and medications were gathered by trained personnel. Duplicate measures of anthropometry were made following a standardised protocol, and averages were used in the analyses.

During the first baseline and follow-up visits, a 75g OGTT was administered to assess glucose tolerance status. During the second baseline visit, insulin sensitivity and insulin secretion were determined using the FSIVGTT [25]. Some modifications were made to the original FSIVGTT. An injection of regular insulin rather than tolbutamide was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance. Glucose in the form of 50% solution (0.3 g/kg) and regular human insulin (0.03 U/kg) were injected through an i.v. line at 0 and 20 min, respectively. Blood was collected at −5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100 and 180 min for measurement of plasma glucose and insulin. S_I was calculated using mathematical modelling methods (MINMOD program version 3.0 [1994] developed at the laboratory R. Bergman, Department of Physiology and Biophysics, University of Southern California Medical School, Los Angeles, CA, USA). There was a strong degree of agreement between S_I estimated by minimal model analysis of the insulin-modified FSIVGTT and insulin sensitivity obtained with the euglycaemic–hyperinsulinaemic clamp [26]. AIR was calculated as the mean of 2 and 4 min insulin concentrations after glucose administration.

The same laboratory carried out analytical procedures for all samples except for complete blood cell counts. Complete blood cell counts were performed with standard techniques (analytical CV <9% for total white cell count and differential) [27] at each centre in accredited laboratories [25]. There were no significant differences between instruments (Coulter T540, Beckman-Coulter, Fullerton, CA, USA; Minos STX, Roche Diagnostics,

Basel, Switzerland; and H-1 Analyzer, Technicon, Tarrytown, NY, USA) except for lymphocyte count (lower lymphocyte counts using the H-1 Analyzer). Plasma glucose and insulin levels were measured at the central IRAS laboratory at the University of Southern California (Los Angeles, CA, USA). Glucose concentration was determined by the glucose oxidase method (Yellow Springs Equipment Co., Yellow Springs, OH, USA) and insulin concentration by the dextran–charcoal radioimmunoassay (CV 19%). This assay had a high cross-reactivity with proinsulin [25]. Intact proinsulin concentration was measured at the laboratory of the Department of Clinical Biochemistry at Addenbrooke's Hospital (Cambridge, UK) (CV 14%) [28]. To determine hsCRP, we used an in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA, USA) (CV 8.9%) [12].

Obesity was defined as BMI ≥30 kg/m^2. We used the 2003 ADA criteria to define diabetes (fasting glucose ≥7.0 mmol/l and/or 2 h glucose ≥11.1 mmol/l) and impaired glucose tolerance (2 h glucose ≥7.8 and <11.1 mmol/l) [29]. Individuals who reported current treatment with glucose-lowering medications were considered to have diabetes. HOMA-IR was calculated according to Matthew's formula [30]. We grouped participants as never smokers (<100 cigarettes during the lifetime), former smokers (>100 cigarettes during the lifetime but not actively smoking) and current smokers (actively smoking). The proinsulin:insulin ratio was used as a measure of disordered processing of insulin. The neutrophil:lymphocyte ratio was defined as the $\log_e$ neutrophil count / $\log_e$ lymphocyte count.

Statistical analyses

Analyses were carried out using the SAS (version 9.2, SAS Institute, Cary, NC, USA) and R Project statistical software packages (version 2.9.2, The R Foundation for Statistical Computing, Vienna, Austria). We assessed baseline differences in anthropometric variables, selected known risk factors for diabetes and white cell count (including major types of white cells) by diabetic status at follow-up using one-way ANCOVA (for continuous variables) and logistic regression analysis (for dichotomous variables). The strength of the relationship between metabolic risk factors and white cell count (total and individual cell types) and neutrophil:lymphocyte ratio was quantified using Pearson's correlation coefficients and linear regression. The effect of confounders on the relationship between cell counts and incident diabetes was assessed by multiple logistic regression analysis. In separate models, appropriate interaction terms were introduced to assess the impact of sex, race/ethnicity, clinic, family history of diabetes, obesity, smoking and glucose tolerance on the relationship between cell count and incident diabetes. We fitted a different logistic regression model to the data to model incident diabetes with a restricted cubic polynomial spline for total white cell count to estimate the varying effects of total white cell count (or subfractions) over its full range [31]. We used $\log_e$-transformed values of total and differential white cell counts, AIR, proinsulin:insulin ratio, HOMA-IR and hsCRP in all analyses to minimise the influence of extreme observations. We also used the $\log_e$ transformation of (S_I + 1) and (number of cigarettes + 1), given that some participants had S_I=0 or did not smoke. We considered p<0.05 to be statistically significant.

Results

The range for white cell count among the 866 non-diabetic participants was 2.1–15.4×10^9 cells/l. The numbers of individuals who were non-smokers, former smokers and active smokers were 405, 336 and 125, respectively. Progression to diabetes was associated with older age and current smoking, but not with sex or race/ethnicity (Table 1). Among smokers, the number of cigarettes smoked per day was not related to incident diabetes. After adjusting

for age, sex, race/ethnicity and clinic, progression to diabetes was associated with lower S_I and AIR, as well as higher adiposity, HOMA-IR, proinsulin:insulin ratio and levels of plasma glucose, fasting insulin and hsCRP. In addition, baseline white cell and lymphocyte counts were higher in individuals who developed diabetes compared with those who had no diabetes at follow-up. However, no significant differences were demonstrated for neutrophil and monocyte counts, neutrophil:lymphocyte ratio, and white blood cell differential measured as percentage of each type of white blood cell.

After controlling for age, sex, race/ethnicity, clinic and smoking, neutrophils were the white cell type with the strongest correlation to total white cell count (Table 2). Direct correlations were demonstrated between subfractions, but were relatively weak. Neutrophil:lymphocyte ratio had a strong relationship with neutrophil and lymphocyte counts (direct and inverse, respectively). White cell counts and subfractions tended to have weak correlations with measures of adiposity, insulin resistance/sensitivity and subclinical inflammation. Proinsulin:insulin ratio was not related to any white cell type. The weak relationship between AIR and total white cell and lymphocyte counts was partially explained by S_I. None of the metabolic markers except hsCRP had a significant relationship with neutrophil:lymphocyte ratio. Similar results were obtained without controlling for the effect of any covariate (ESM Table 3).

Additional analyses were carried out to further assess the relationship between established risk factors for diabetes (plasma glucose, fasting insulin, S_I, AIR and hsCRP) and total white cell count and subfractions (Table 3). Fasting insulin rather than S_I had a consistent and independent relationship with total white cell count and subfractions except for neutrophils. In addition, hsCRP had a consistent and independent relationship with total white cell count and subfractions except for lymphocytes. Two additional independent relationships were demonstrated: BMI with lymphocyte count and 2 h glucose with total white cell and neutrophil counts. The only independent correlate of neutrophil:lymphocyte ratio was hsCRP.

A total of 138 participants (15.9%) developed diabetes. The diagnosis was made in 126 (91.3%) of these participants by OGTT criteria. The other 12 participants were already receiving glucose-lowering medications. Figure 1 presents the relationship between incident diabetes and total white cell count and subfractions modelled by a smooth function. Results were adjusted for age, sex, race/ethnicity and clinic. The relationship was linear for all cell types (Wald test for linearity, $p>0.3$). It was statistically significant for total white cell count (OR×1 SD unit increase, 1.40 [95% CI 1.09, 1.80], $p=0.028$) and lymphocyte count (OR 1.59 [1.20, 2.09], $p=0.004$), but not for neutrophil count (OR 1.21 [0.99, 1.60], $p=0.161$) or monocyte count (OR 1.12 [0.88, 1.43], $p=0.600$). None of the white cell types expressed as percentage of total white cell count was associated with incident diabetes (data not shown).

We also examined ORs (95% CIs) of incident diabetes comparing middle and upper tertiles with the lower tertile of total white cell, absolute neutrophil, lymphocyte and monocyte counts, and neutrophil:lymphocyte ratio by multiple logistic regression analyses (Table 4). Associations for white cell and neutrophil counts were largely explained by the effect of family history of diabetes, fasting glucose and smoking. ORs for lymphocyte count increased in a stepwise fashion. The lymphocyte association was partially explained by insulin sensitivity/resistance (S_I, fasting insulin or HOMA-IR) or 2 h glucose, but not by fasting glucose, smoking, BMI, AIR or hsCRP. Monocyte count and neutrophil:lymphocyte ratio were not associated with incident diabetes. A more comprehensive analysis is presented in ESM Table 4.

In separate models, we examined the effect of sex, race/ethnicity, clinic, smoking, obesity and glucose tolerance status on the relationship between white cell and lymphocyte counts and incident diabetes (Fig. 2). None of the interaction terms was significant for either total white cell count models or lymphocyte count models. Even though interaction terms smoking × lymphocyte count and smoking × white cell count were not statistically significant, neither white cell count nor lymphocyte count was associated with progression to diabetes in current smokers. In addition, none of the interaction terms was statistically significant in models that examined the relationship between neutrophil count and incident diabetes (data not shown).

Discussion

This study has several novel findings. In non-diabetic individuals, lymphocyte count was associated with insulin sensitivity; neutrophil and monocyte counts with subclinical inflammation, as measured by hsCRP; and total white cell count with both insulin sensitivity and subclinical inflammation. The lymphocyte was the white cell type with the strongest relationship to incident diabetes, some of which is explained by insulin resistance rather than low-grade inflammation.

Many studies have reported a significant relationship between white cell count and type 2 diabetes [10, 14–19], but a concern of publication bias has been raised by Gkrania-Klotsas et al. in a systematic review and meta-analysis [16]. In addition, the confounding effect of established risk factors for type 2 diabetes other than family history of diabetes, adiposity and/or fasting glucose has not been examined [14–19], except for in the study by Vozarova et al [10]. Among 272 Pima Indians with normal glucose tolerance at baseline, the study by Vozarova et al. demonstrated an independent relationship between white cell count and incident diabetes after controlling for adiposity, insulin action (measured by the hyperinsulinaemic clamp) and AIR [10]. Despite the evidence relating white cell count to incident diabetes, attempts to include white cell count in risk-prediction models have produced mixed results [19–21]. Our data link white cell count to the development of diabetes, but side with studies reporting a limited ability of white cell count to reclassify individuals according to their risk of diabetes [20, 21]. In the IRAS, white cell count was not associated with incident diabetes beyond the effect of family history of diabetes, smoking and fasting glucose.

Few studies have examined the relationship between differential white cell count and incidence of diabetes. Among 12,330 middle-aged participants in the Atherosclerosis Risk in Communities study, neutrophil and lymphocyte counts were associated with incident type 2 diabetes [15]. Similar results were obtained among the 15,550 participants in the EPIC-Norfolk study [16]. Both of these studies adjusted their results for smoking, family history of diabetes, physical activity and adiposity (and fasting glucose in the Atherosclerosis Risk in Communities study), but neither study examined the impact of insulin resistance, glucose tolerance, insulin secretion or subclinical inflammation. In contrast to these reports, Vozarova et al. described no significant relationship between neutrophil and lymphocyte counts and incident diabetes in 154 Pima Indians with normal glucose tolerance at baseline [10]. In our study, the lymphocyte appears to be the major white cell type that is associated with incident diabetes. The results were consistent across varying categories of sex, race/ethnicity, adiposity and glucose tolerance. This association remained significant after controlling for factors that influence white cell count and/or diabetes risk (e.g. smoking, family history of diabetes, fasting glucose and BMI). It is partially explained by insulin sensitivity rather than subclinical inflammation. On the other hand, the neutrophil association is largely explained by family history of diabetes, fasting glucose and smoking.

White cell count is one of the markers of subclinical inflammation linked to the metabolic syndrome [11–13]. Several studies have described a relationship between white cell count and insulin action measured by the hyperinsulinaemic clamp [10, 13, 32]. The association with insulin secretion is less well established: raised white cell count has been related to lower insulinogenic index [11], but not to lower AIR [10]. In the latter study, elevated white cell count was also associated with a longitudinal decline in insulin action, but not with worsening AIR [10]. In addition, the relationships of white cell count to adiposity [15, 21, 32] and fasting insulin levels [32–34] are well described. White cell count has been independently related to glucose tolerance and subclinical inflammation (fibrinogen) in multiple regression analyses [34]. Our results suggest that white cell count has an independent relationship with markers of insulin resistance, glucose tolerance and subclinical inflammation. The association with fasting insulin concentration is not fully explained by S_I, a direct measure of insulin resistance. In the IRAS, white cell count was not independently associated with adiposity and measures of insulin secretion such as AIR and proinsulin:insulin ratio, a marker of beta cell stress.

Our cross-sectional analysis revealed a distinctive metabolic pattern of relationships for each white cell subfraction: neutrophil and monocyte counts with subclinical inflammation (measured by hsCRP) and glucose tolerance; and lymphocyte count with insulin sensitivity/resistance and adiposity. Although neutrophil count has tended to be associated with subclinical inflammation [22, 34], the relationship of neutrophil count to insulin resistance [22], and that of lymphocyte count to subclinical inflammation [22] and insulin resistance and BMI [34], has been less consistent. However, these studies have generally had relatively small sample sizes with which to carry out a comprehensive analysis of the effect of major confounders. In the IRAS, post-load plasma glucose and subclinical inflammation appeared to account for the relationship between neutrophil count and insulin resistance, whereas insulin resistance accounted for the relationship of lymphocyte count to plasma glucose and hsCRP.

A notable number of studies have favoured the use of the neutrophil:lymphocyte ratio as a marker of inflammation, for example, to predict survival in patients with myocardial infarction [35] and progression to steatohepatitis in those with non-alcoholic fatty liver disease [36]. In apparently healthy non-diabetic individuals, a raised neutrophil:lymphocyte ratio was been associated with the metabolic syndrome and elevated hsCRP [24]. In our study, the neutrophil:lymphocyte ratio linked only to subclinical inflammation as measured by hsCRP, and was not associated with progression to diabetes. The absence of a direct relationship between neutrophil:lymphocyte ratio and insulin resistance (and adiposity) may reflect concurrent recruitment of cells of myeloid and lymphoid lineage in the adipose tissue with weight gain [37]. Thus, abnormal neutrophil:lymphocyte ratios should be explained by processes other than adiposity and insulin resistance.

Increased numbers of macrophages, neutrophils, T cells, B cells and mast cells infiltrate adipose tissue with weight gain, and appear to play an essential role in insulin resistance [37]. Macrophages become activated and secrete proinflammatory cytokines such as IL-6, TNF-α and IL-1β, which can contribute to insulin resistance [38]. The adaptive immune system also has an important role in metabolic regulation and type 2 diabetes [37]. T cell infiltration has been shown to precede the recruitment of macrophages in an experimental model of obesity and to correlate with waist circumference in individuals with type 2 diabetes [39]. The production of TNF-α, IFN-γ and IL-6 by activated T cells, both T helper type 1 cells and cytotoxic CD8+ T cells, contributes to metabolic dysfunction [37]. In addition, Foxp3+ regulatory T cells (Tregs), which inhibit autoimmunity and protect against tissue injury, are decreased in the peripheral blood both in experimental models of obesity and individuals with type 2 diabetes [37, 40]. The generation of Tregs is reciprocally

interconnected to that of proinflammatory IL-17-producing T helper (Th17) cells [41]. It is not known whether T helper cell subset polarisation occurs prior to the development of type 2 diabetes. Nevertheless, IL-6, a key cytokine in the generation of Th17 cells [41], is upregulated in adipose tissue in individuals with obesity [37, 42].

Our study has several strengths. The IRAS has a well-characterised sample population and uses validated measures of both insulin sensitivity and insulin secretion. In contrast to earlier reports, our regression models assessed the effects of major correlates of white cell count and established risk factors for type 2 diabetes (smoking, insulin sensitivity, insulin secretion, glucose tolerance, adiposity and chronic subclinical inflammation). The results were consistent across categories of sex, race/ethnicity, clinic, glucose tolerance and adiposity. The study also has limitations. The assay to measure insulin concentration has a significant interassay CV. It is plausible that a more specific assay for insulin concentration could have resulted in a more precise assessment of the relationship between white cell count (or subfractions) and measures of insulin sensitivity and secretion. However, our method of measuring insulin sensitivity was validated against the euglycaemic–hyperinsulinaemic clamp [26]. A single determination of white cell count and differential is common practice in most epidemiological studies, but carries significant intraindividual variation: 11% for total white cell count, 7–16% for neutrophil count, 10–12% for lymphocyte count and 11–18% for monocyte count measurement [43]. Finally, white cell counts and subfractions were measured in each clinic centre. This might have contributed to ascertainment error of diabetic risk. Nevertheless, this type of error would tend to bias results towards the null hypothesis. Furthermore, the results were consistent across centres.

In summary, elevated total white cell, neutrophil and lymphocyte counts may be detected in individuals who are at increased risk of diabetes. The lymphocyte association was the strongest among the white cell types. This was partially explained by insulin sensitivity rather than low-grade inflammation, as measured by hsCRP. Future studies need to examine the dynamic interactions between white cell subfractions, macrophages and adipocytes in relation to weight gain and declining glucose tolerance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AIR	Acute insulin response
CRP	C-reactive protein
FSIVGTT	Frequently sampled IVGTT
hsCRP	High-sensitivity C-reactive protein
IRAS	Insulin Resistance Atherosclerosis Study
S_I	Insulin sensitivity index

Th17	IL-17-producing T helper
Treg	Foxp3+ regulatory T cell

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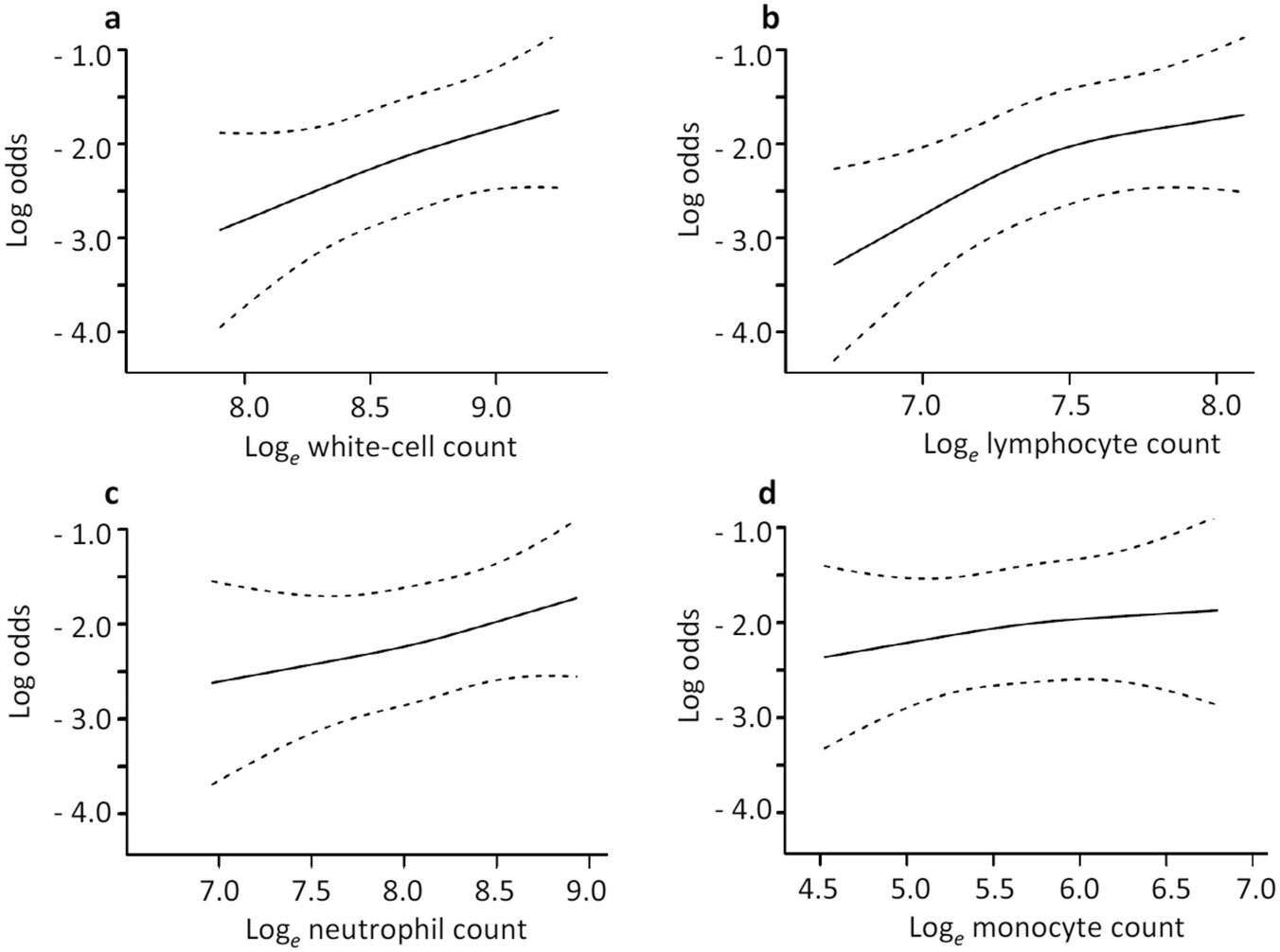

Figure 1.

Relationship between 5 year risk of type 2 diabetes and total white cell count and subfractions modelled by a smooth function. The relationship was linear for all cell types (Wald for linearity, $p>0.3$) and statistically significant for (**a**) white cell count (p value of the Wald $\chi^2=0.028$) and (**b**) lymphocyte count ($p=0.004$). (**c**) Neutrophil count ($p=0.161$) and (**d**) monocyte count ($p=0.600$) were not associated with incident diabetes. Results were adjusted for age, sex, race/ethnicity and clinic

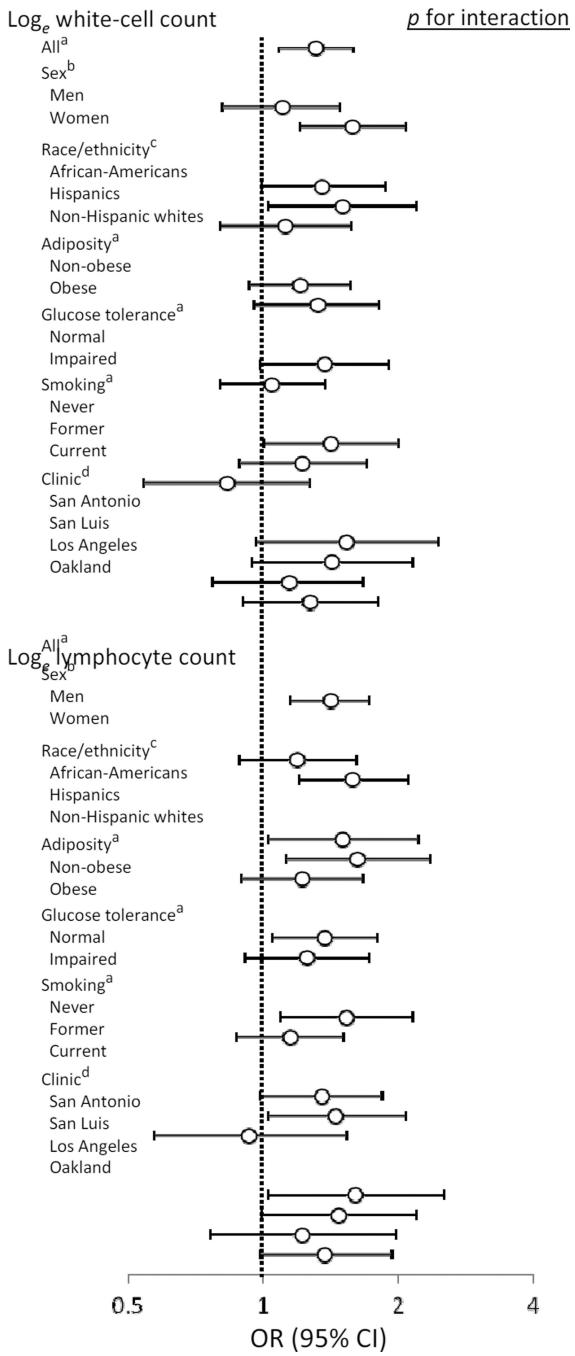

Figure 2.

Heterogeneity analyses for the relationship of white cell and lymphocyte counts to the 5 year incidence of diabetes. Results adjusted for [a]age, sex, race/ethnicity and clinic; [b]age, race/ethnicity and clinic; [c]age, sex and clinic; and [d]age, sex and race/ethnicity

Table 1

Baseline characteristics by diabetes status at follow-up

Characteristic	No diabetes	Diabetes	*p* value
n	728	138	–
Age (years)[a]	54.3±0.3	56.2±0.7	0.007
Female[a]	56.5 (52.8, 60.0)	60.1 (51.8, 68.0)	0.422
Ethnicity[a]			
African-American	25.7 (22.6, 29.0)	25.4 (18.8, 33.3)	0.936
Hispanic	34.1 (30.7, 37.6)	37.7 (30.0, 46.0)	0.413
Non-Hispanic white	40.2 (36.7, 43.9)	37.0 (29.3, 45.3)	0.469
Cigarette smoking[a]			
Never smokers	48.4 (44.7, 50.2)	38.4 (30.7, 46.8)	0.032
Former smokers	39.3 (35.8, 42.9)	36.2 (28.7, 44.6)	0.500
Current smokers	12.4 (10.2, 15.0)	25.4 (18.8, 33.3)	<0.001
Cigarettes per day among current smokers[a]	15.2±1.5	14.7±2.4	0.860
BMI (kg/m^2)	27.9±0.2	31.0±0.5	<0.001
Waist circumference (cm)	89.1±0.4	95.6±1.0	<0.001
Fasting glucose (mmol/l)	5.37±0.02	5.87±0.04	<0.001
2 h glucose (mmol/l)	6.59±0.07	8.38±0.14	<0.001
Fasting insulin (pmol/l) [b]	71.4±1.2	105.6±5.4	<0.001
HOMA-IR [b]	2.83±0.06	4.57±0.28	<0.001
S_I ($\times10^{-5}$ min^{-1} pmol^{-1} l^{-1})[b]	3.21±0.10	1.75±0.18	<0.001
AIR (pmol/l)[b]	318.0±9.6	235.8±16.8	<0.001
AIR adjusted for S_I (pmol/l)[b]	327.6±10.2	198.6±12.0	<0.001
Proinsulin:insulin ratio (×100)[b]	6.08±0.12	6.93±0.36	0.029
hsCRP (mg/l)[bc]	1.67±0.07	2.61±0.25	<0.001
White cell count ($\times10^9$/l)[b]	5.39±0.06	5.78±0.14	0.007
Neutrophils (%)	59.8±0.3	59.7±0.3	0.993
Lymphocytes (%)	32.0±0.3	32.3±0.7	0.670
Monocytes (%)	6.06±0.10	5.76±0.22	0.197
Neutrophil count ($\times10^9$/l)[b]	3.11±0.05	3.33±0.11	0.064
Lymphocyte count ($\times10^9$/l)[b]	1.65±0.02	1.81±0.05	<0.001
Monocyte count ($\times10^9$/l)[b]	0.30±0.01	0.31±0.01	0.318
Neutrophil:lymphocyte ratio[b]	1.087±0.002	1.082±0.005	0.349

Data are *n*, mean ± SEM or per cent (95% CI)
Results are adjusted for age, sex, race/ethnicity and clinic
Neutrophil:lymphocyte ratio = log$_e$-transformed neutrophil count / log$_e$-transformed lymphocyte count

[a] Non-adjusted values

[b] Log$_e$-transformed values then back-transformed for presentation

[c] To convert hsCRP to nM/l, multiply by 9.524

Table 2

Pearson partial correlation coefficients between total white cell counts and subfractions and selected metabolic risk factors at baseline

Variable	Log_e white cell count	Log_e neutrophil count	Log_e lymphocyte count	Log_e monocyte count	Neutrophil: lymphocyte ratio
Log_e neutrophil count	0.92***	–	–	–	–
Log_e lymphocyte count	0.54***	0.22***	–	–	–
Log_e monocyte count	0.46***	0.35***	0.21***	–	–
Neutrophil: lymphocyte ratio	0.39***	0.69***	−0.55***	0.15***	–
Fasting glucose	0.11**	0.08*	0.12***	0.02	−0.02
2 h glucose	0.20***	0.18***	0.15***	0.08*	0.04
BMI	0.19***	0.15***	0.20***	0.07*	−0.02
Waist circumference	0.19***	0.14***	0.20***	0.06	−0.03
Log_e fasting insulin	0.25***	0.17***	0.25***	0.12***	−0.04
Log_e HOMA-IR	0.25***	0.17***	0.25***	0.12***	−0.04
Log_e S_I	−0.22***	−0.18***	−0.22***	−0.08*	0.01
Log_e AIR	0.09*	0.07	0.09*	0.07	0.00
Log_e AIR[a]	0.02	0.00	0.01	0.05	0.00
Log_e proinsulin: insulin ratio	−0.05	−0.02	−0.06	−0.03	0.03
Log_e hsCRP	0.28***	0.27***	0.13***	0.13***	0.13***

Pearson partial correlation coefficients controlling for age, sex, race/ethnicity, clinic and log_e-cigarettes currently smoked per day

*$p<0.05$;

**$p<0.01$;

***$p<0.001$

[a]Pearson partial correlation coefficients also controlling for log_e S_I

Table 3

Relationship between selected metabolic variables and total and individual white cell type counts (dependent variable) by multiple linear regression analyses

Variable	Log_e white cell count β (95% CI)	Log_e neutrophil count β (95% CI)	Log_e lymphocyte count β (95% CI)	Log_e monocyte count β (95% CI)	Neutrophil: lymphocyte count β (95% CI)
Adjustment model 1					
BMI	0.005±0.011	0.006±0.016	0.025±0.012*	−0.008±0.019	−0.003±0.003
Fasting glucose	0.003±0.010	0.003±0.015	−0.005±0.011	−0.005±0.018	0.001±0.002
Log_e fasting insulin	0.047±0.011***	0.043±0.015**	0.057±0.012***	0.049±0.018**	−0.003±0.002
Log_e hsCRP	0.059±0.010***	0.079±0.015***	0.014±0.011	0.045±0.018*	0.009±0.002***
Adjustment model 2					
BMI	0.007±0.011	0.000±0.016	0.027±0.012*	−0.002±0.019	−0.004±0.003
2 h glucose	0.031±0.011**	0.049±0.016**	0.014±0.013	0.039±0.020*	0.005±0.003
Log_e S_I	−0.018±0.013	−0.011±0.018	−0.036±0.014*	0.005±0.022	0.004±0.003
Log_e hsCRP	0.061±0.011***	0.087±0.015***	0.011±0.012	0.048±0.018**	0.010±0.002***
Log_e AIR	0.013±0.011	0.018±0.015	0.011±0.012	0.034±0.019	0.001±0.002
Adjustment model 3					
BMI	−0.001±0.011	−0.006±0.016	0.017±0.013	−0.011±0.020	−0.003±0.003
2 h glucose	0.028±0.011**	0.043±0.015**	0.011±0.012	0.029±0.018	0.004±0.002
Log_e fasting insulin	0.042±0.012***	0.033±0.017	0.050±0.013***	0.056±0.021**	−0.003±0.003
Log_e S_I	−0.004±0.013	−0.003±0.019	−0.017±0.015	0.017±0.023	0.002±0.003
Log_e hsCRP	0.058±0.011***	0.085±0.015***	0.007±0.012	0.045±0.018*	0.010±0.002***

Adjustment model 1: age, sex, race/ethnicity, clinic, family history of diabetes, log_e-cigarette smoking, cardiovascular disease, emphysema, treatment with medications, BMI, fasting glucose, fasting insulin and log_e hsCRP were all included as independent variables in all five regression models

Adjustment model 2: all variables of adjustment model 1, but 2 h glucose, log_e S_I and log_e AIR were substituted for fasting glucose and insulin

Adjustment model 3: all variables of adjustment model 1, but 2 h glucose and log_e S_I were substituted for fasting glucose

Variable estimates expressed per 1 SD unit increase;

* $p<0.05$;

** $p<0.01$;

*** $p<0.001$

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Table 4

Risk of developing diabetes associated with total white cell count and subfractions

Variable	Adjustment model	Lower tertile	Middle tertile	Upper tertile	*p* for trend
White cell count					
Model 1	Demographics (age, sex, ethnicity and clinic)	1.00	1.70 (1.06, 2.74)	1.80 (1.10, 2.92)	0.020
Model 2	Demographics + family history + fasting glucose	1.00	1.61 (0.96, 2.70)	1.52 (0.89, 2.59)	0.141
Model 3	Demographics + family history + fasting glucose + smoking	1.00	1.44 (0.86, 2.44)	1.15 (0.66, 2.02)	0.683
Neutrophil count					
Model 1	Demographics (age, sex, ethnicity and clinic)	1.00	1.42 (0.87, 2.30)	1.67 (1.04, 2.71)	0.037
Model 2	Demographics + family history + fasting glucose	1.00	1.30 (0.76, 2.21)	1.51 (0.89, 2.56)	0.127
Model 3	Demographics + family history + fasting glucose + smoking	1.00	1.18 (0.69, 2.02)	1.17 (0.67, 2.03)	0.607
Lymphocyte count					
Model 1	Demographics (age, sex, ethnicity and clinic)	1.00	1.73 (1.06, 2.83)	2.30 (1.41, 3.76)	<0.001
Model 2	Demographics + family history + fasting glucose	1.00	1.71 (1.00, 2.93)	2.20 (1.29, 3.76)	0.004
Model 3	Demographics + family history + fasting glucose + smoking	1.00	1.54 (0.89, 2.66)	1.96 (1.13, 3.37)	0.017
Model 4	Model 3 + BMI	1.00	1.59 (0.92, 2.75)	1.88 (1.08, 3.25)	0.028
Model 5	Model 3 + 2 h glucose	1.00	1.44 (0.81, 2.56)	1.64 (0.92, 2.91)	0.098
Model 6	Model 3 + $\log_e$ fasting insulin	1.00	1.54 (0.88, 2.67)	1.69 (0.97, 2.96)	0.074
Model 7	Model 3 + $\log_e$ HOMA-IR	1.00	1.54 (0.88, 2.67)	1.69 (0.97, 2.96)	0.074
Model 8	Model 3 + $\log_e S_I$	1.00	1.38 (0.78, 2.45)	1.44 (0.80, 2.58)	0.242
Model 9	Model 3 + $\log_e$ AIR	1.00	1.53 (0.88, 2.69)	2.02 (1.15, 3.56)	0.014
Model 10	Model 3 + $\log_e S_I$ + $\log_e$ AIR	1.00	1.31 (0.73, 2.35)	1.47 (0.81, 2.67)	0.214
Model 11	Model 3 + $\log_e$ hsCRP	1.00	1.62 (0.93, 2.82)	1.89 (1.08, 3.30)	0.028
Monocyte count					
Model 1	Demographics (age, sex, ethnicity and clinic)	1.00	1.26 (0.79, 2.01)	1.18 (0.73, 1.90)	0.506
Model 2	Demographics + family history + fasting glucose	1.00	1.34 (0.80, 2.23)	1.27 (0.76, 2.12)	0.382
Model 3	Demographics + family history + fasting glucose + smoking	1.00	1.32 (0.79, 2.22)	1.13 (0.67, 1.92)	0.671
Neutrophil:lymphocyte ratio					
Model 1	Demographics (age, sex, ethnicity and clinic)	1.00	1.29 (0.81, 2.03)	0.89 (0.55, 1.45)	0.618
Model 2	Demographics + family history + fasting glucose	1.00	1.21 (0.74, 1.98)	0.85 (0.50, 1.45)	0.540

Variable	Adjustment model	Lower tertile	Middle tertile	Upper tertile	*p* for trend
Model 3	Demographics + family history + fasting glucose + smoking	1.00	1.15 (0.70, 1.89)	0.74 (0.43, 1.27)	0.257

Values are OR (95% CI) expressed per 1 SD unit increase

Range for total and differential white cell count tertiles ($\times 10^9$/l): white cell count: lower 2.1–4.9, middle 5–6.2, upper 6.3–15.4; neutrophil count: lower 0.41–2.82, middle 2.83–3.80, upper 3.81–11.5; lymphocyte count: lower 0.55–1.47, middle 1.48–1.92, upper 1.93–4.18; monocyte count: lower 0–0.26, middle 0.27–0.36, upper 0.37–1.13

Range for neutrophil:lymphocyte ratio tertiles: lower 0.78–1.06, middle 1.07–1.11, upper 1.12–1.42

Family history indicates family history of diabetes; smoking was expressed as $\log_e$-cigarettes currently smoked per day