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Soluble CD14 and *CD14* Variants, Other Inflammatory Markers, and Glucose Dysregulation in Older Adults: The Cardiovascular Health Study

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OBJECTIVE

Experimental studies have implicated soluble (s)CD14, an effector of lipopolysaccharide-induced inflammation, in insulin resistance, but its role in human metabolic endotoxemia has not been studied. We evaluated sCD14 in relation to dysglycemia in older adults and how this compares to other markers of inflammation.

RESEARCH DESIGN AND METHODS

We investigated associations of sCD14, interleukin-6 (IL-6), CRP, and white blood cell (WBC) count with insulin resistance (quantitative insulin-sensitivity check index and HOMA 2 of insulin resistance) and incident type 2 diabetes in a population-based cohort of older adults. We also assessed the causal role of sCD14 in insulin resistance using an instrumental variable approach by Mendelian randomization.

RESULTS

After adjustment for conventional risk factors, each of the four biomarkers showed positive cross-sectional associations with both insulin resistance measures. These associations persisted after mutual adjustment for all markers except sCD14. Over a median follow-up of 11.6 years, 466 cases of diabetes occurred. All biomarkers except sCD14 were positively associated with diabetes, although only WBC count remained associated (hazard ratio 1.43 per doubling [95% CI 1.07, 1.90]) after mutual adjustment. Instrumental variable analysis did not support a causal role for sCD14 in insulin resistance.

CONCLUSIONS

Among older adults, sCD14 was associated with insulin resistance, but this disappeared after adjustment for other biomarkers, showed no evidence of a causal basis, and was not accompanied by a similar association with diabetes. IL-6, CRP, and WBC count were each associated with insulin resistance and diabetes, WBC count most robustly. These findings do not support a central role for sCD14, but they highlight the preeminence of WBC count as an inflammatory measure of diabetes risk in this population.

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The role of chronic low-grade inflammation in insulin resistance and subsequent development of diabetes has been well documented experimentally (1). Consistent with such experimental findings, epidemiologic studies have linked various inflammatory biomarkers, including CRP and interleukin-6 (IL-6), to risk of incident diabetes in women (2), middle-aged European cohorts (3), and middle-aged to older multiethnic cohorts (4). This also includes previous work from the Cardiovascular Health Study (CHS), which found a significant association in older adults between CRP and development of diabetes 3 to 4 years later (5). Additionally, a meta-analysis in 2010 combined data from 20 studies to conclude that an increased total white blood cell (WBC) count is associated with a higher risk of diabetes (6). The pathways implicated in such chronic inflammation and consequent dysregulation of glucose metabolism remain incompletely understood.

CD14, a pattern-recognition receptor that has specificity for lipopolysaccharides (LPSs) and other bacterial wall–derived components, is expressed primarily by myeloid cells but also by a range of nonmyeloid tissues (7). The receptor is anchored to the plasma membrane by a glycosylphosphatidylinositol moiety but is also detectable in plasma as a soluble form lacking this membrane-anchoring component (7). Such soluble (s)CD14 mainly arises from monocytes/macrophages following cleavage of the membrane receptor by proteases or from direct secretion of the anchor-free form by hepatocytes and adipocytes (8).

Experimental data suggest that the composition of the gut microbiome and translocation of bacterial components, namely LPSs, contribute importantly to obesity-related inflammation, so-called metabolic endotoxemia (9,10). As a key factor in signal transduction of LPS-related proinflammatory cascades by Toll-like receptors, CD14 has been shown to play a pivotal role in this process (11,12). sCD14 is an acute-phase reactant, but it can have differing effects on LPS-related inflammatory signaling (7). On one hand, sCD14 can act to potentiate LPS-related inflammation in cells both harboring and lacking membrane-bound CD14 (13). On the other hand, high levels of sCD14 can buffer LPS by promoting its transfer to lipoprotein particles, preventing binding of surface CD14 on monocytes/macrophages (14). Previous work by our group in CHS has shown that elevated sCD14 is associated with incident cardiovascular disease and mortality in older adults (15). The link between sCD14 level and insulin resistance has been examined in small clinical studies, which have suggested an inverse association (16) or one that differs by obesity status (17), but findings have been inconclusive. The relationship between sCD14 level and incident diabetes has not been examined to date. In this study, we sought to determine the associations of sCD14 with abnormal glucose metabolism in a large cohort of older adults, both alone and in the context of other inflammatory biomarkers not previously evaluated in relation to long-term incidence of type 2 diabetes in this age-group. We also undertook an instrumental variable analysis using Mendelian randomization to investigate whether observed associations of sCD14 with insulin resistance may have a causal basis.

RESEARCH DESIGN AND METHODS

Study Population
The design and rationale of CHS have been described before (18). Briefly, CHS is a prospective population-based cohort study of older adult men and women recruited from four U.S. field centers: Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA. Candidates were randomly sampled from Medicare eligibility lists, and those who were institutionalized, wheelchair-bound, in hospice, receiving treatment for cancer, not expected to remain in the area for the next 3 years, or unable to give informed consent were excluded. A total of 5,201 participants (original cohort) were recruited in 1988–1989 from random samples of Medicare eligibility lists. An additional 687 primarily black participants were recruited in 1992–1993 (supplemental cohort) for a total cohort of 5,888 individuals. During in-person visits, participants underwent evaluation for demographic and lifestyle factors, physical examination, and fasting blood collection for laboratory measurement and storage using standardized protocols, as previously described (18,19). After excluding individuals with treated diabetes (type 2, in this age range) at baseline, we had a data set of 5,380 participants. Of those,

5,296 individuals had measures of both fasting glucose and fasting insulin at baseline, who were included in analyses of insulin resistance measures. For these analyses, we had baseline measures of sCD14 in 4,999 participants and of IL-6, CRP, and WBC count in 4,927, 5,302, and 5,305 participants, respectively. After further exclusion of 515 individuals with prevalent type 2 diabetes at baseline by fasting glucose ($\geq$126 mg/dL), random glucose ($\geq$200 mg/dL), or anti-hyperglycemic medication, our study sample comprised 4,865 participants for assessment of the association with incident type 2 diabetes (hereafter referred to as diabetes). For this set of analyses, we had baseline measures of sCD14 in 4,558 participants and of IL-6, CRP, and WBC count in 4,502, 4,836, and 4,850 participants, respectively. For instrumental variable analyses of sCD14 in relation to insulin resistance measures using Mendelian randomization, which were stratified by race, genotypic data were available in 3,387 white and 422 African American participants.

Inflammatory Measures
sCD14 was measured in baseline plasma specimens using a commercial ELISA (R&D Systems, Minneapolis, MN) with an interassay coefficient of variation of 5.3–12.4% (20). CRP was measured using an ultra-sensitive ELISA developed at the CHS Central Laboratory at the University of Vermont with an interassay coefficient of variation of 5.5% (20). IL-6 was measured using commercial ELISA kits (Quantikine IL-6; R&D Systems, Minneapolis, MN) with an interassay coefficient of variation of 6.3%. Complete blood counts (including WBC counts) were measured at each of the participating clinical centers using the following instruments: Coulter Stack S cell counter (Coulter, Inc., Hialeah, FL) (University of Pittsburgh, Pittsburgh, PA, and University of California, Davis, Davis, CA) and the Sysmex NE8000 counter (Toa Electronics, Inc., Chicago, IL) (Wake Forest University, Winston-Salem, NC, and Johns Hopkins University, Baltimore, MD).

Genotyping
Genotyping done in this cohort has been described in detail (15). Fine-mapping of the *CD14* locus was performed in 3,950 European American (3,660 with sCD14 measured) and 792 African American

(683 with sCD14 measured) participants who were genotyped using the custom gene-centric IBCv2 genotyping array that contains high single nucleotide polymorphism (SNP) marker density and linkage disequilibrium coverage for various cardiovascular, metabolic, and inflammation-related genes, including *CD14* (21). IBCv2 array used a "cosmopolitan" tagging approach to capture the genetic diversity across candidate genes in the multiple ethnic populations represented in the HapMap, including both Europeans and West Africans. Tag SNPs were selected to capture known variation with minor allele frequency >0.02 and an r^2 of at least 0.8 in HapMap populations. The top SNP in whites was identified as rs5744455, while that in African Americans was identified as rs5744451.

Insulin Resistance and Diabetes

Fasting glucose and insulin values were analyzed using standard enzymatic methods at the University of Vermont Central Laboratory. Measurements were performed in serum at baseline, as well as in all but one of the follow-up examinations, which used EDTA plasma. Specimens were frozen at −70°C <1 h after collection (22). Values were harmonized across examinations, as described previously (23). Two measures of insulin resistance were selected: *1*) quantitative insulin-sensitivity check index (QUICKI), which is derived from a formula using fasting insulin and fasting glucose and correlates well with glucose clamp studies (24,25), and *2*) HOMA 2 index of insulin resistance (HOMA2IR), which improves upon the original HOMA index by using a computer program (26,27).

Participants were followed up through year 2011 for incident diabetes, defined as a fasting glucose ≥126 mg/dL, random glucose ≥200 mg/dL, or use of antihyperglycemic medication. As previously described, after the 1989–1990 examination, glucose measurements were obtained in 1992–1993, 1994–1995, 1996–1997, 1998–1999, and, in a subset, 2005–2006 (23). All were on fasting samples, with the exception of 1994–1995, which were random.

Covariates

Hypertension was defined by systolic and diastolic cutoffs of 140 and 90 mmHg, respectively, or by self-report and antihypertensive therapy. Trained personnel performed anthropometric measurements in standardized fashion. Measurement of physical activity in kilocalories has been previously reported (23). Heavy alcohol use was defined as consumption of >14 drinks/week in men or >7 drinks/week in women. Prevalent atherosclerotic cardiovascular disease (ASCVD) consisted of coronary heart disease, stroke or transient ischemic attack, and peripheral arterial disease. Along with heart failure (HF), prevalent ASCVD components were ascertained at the 1989–1990 and 1992–1993 examinations and in the intervening period through a combination of CHS questionnaires, medical record review, and physician confirmation (19,28). Additionally, fasting baseline samples were used to measure creatinine and lipids (22). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology equation (29).

Statistical Methods

Baseline characteristics are presented as median (interquartile range) for continuous variables and count (percent) for categorical variables, both in the cohort overall and by quartiles of sCD14 level. We tested for trend across increasing quartiles of sCD14 by using contrasts in generalized linear models for continuous variables and the Cochran-Armitage test for categorical variables. The four biomarkers showed positive skew and were converted to base-2 logarithm to normalize their distributions and to evaluate their associations with outcomes uniformly per doubling of their levels. Pearson correlation coefficients were calculated between log-transformed inflammatory biomarkers. Using minimally and fully adjusted linear regression models, we tested the associations between the biomarkers and QUICKI and HOMA2IR. To avoid disproportionate influence of extreme upper values for HOMA2IR, this measure was winsorized at the 99th percentile. The initial model adjusted for age, sex, and race, while the full model additionally adjusted for BMI, systolic blood pressure, use of antihypertensive medication, smoking status, heavy alcohol use, physical activity, estrogen use, prevalent ASCVD, prevalent HF, and eGFR. A subsequent model examined whether additional adjustment for LDL cholesterol influenced the findings; as triglycerides and HDL cholesterol are influenced by insulin resistance, they were considered part of the outcome and therefore not included for adjustment. Additional sensitivity analyses evaluated the impact of further adjustment for use of statins, oral corticosteroids, and nonsteroidal anti-inflammatory drugs. In the second part of the analyses, participants with prevalent diabetes at baseline based on fasting glucose, random glucose, and medication use were excluded. Using Cox proportional hazards models with the same covariates for the initial and full models, we tested for association between the four biomarkers and incident diabetes. In both linear regression and Cox regression analyses, we assessed the functional form of the relationship between inflammatory markers and outcome measures using generalized additive model (GAM) plots. For analyses of insulin resistance and incident diabetes, we tested for interaction by age, sex, race, BMI, prevalent ASCVD, and prevalent HF by including appropriate cross-product terms in the full models. Last, we also tested for associations of the biomarkers with QUICKI, HOMA2IR, and incident diabetes independent of each other by including the four biomarkers simultaneously in the full model.

For the Mendelian randomization analysis, we used the two-stage least-squares method using sCD14 as the endogenous regressor; SNPs rs5744455, rs778584, and rs4914 in whites and rs5744451 in African Americans as instrumental variables; and QUICKI and HOMA2IR as the continuous outcomes.

We used SAS 9.4 (SAS Institute, Cary, NC) and Stata 15 (StataCorp, College Station, TX) for all analyses. A two-tailed *P* value <0.05 was considered statistically significant.

RESULTS

Table 1 describes the baseline characteristics of the cohort both overall and divided by quartiles of sCD14 level. There were decreasing proportions of men, blacks, estrogen users (among women), and former smokers, along with declining levels of BMI, physical activity, and eGFR, with increasing quartiles of sCD14. By contrast, age, LDL cholesterol, and triglycerides tended to be higher at increasing quartiles of sCD14, as were proportions of heavy alcohol users,

Table 1—Baseline characteristics by quartile of sCD14

	Full cohort (n = 5,380)	Quartile 1 (n = 1,250)	Quartile 2 (n = 1,249)	Quartile 3 (n = 1,250)	Quartile 4 (n = 1,250)	P for trend
sCD14, ng/mL	1,593 (1,399, 1,823)	1,270 (1,187, 1,339)	1,497 (1,450, 1,546)	1,696 (1,649, 1,757)	2,000 (1,888, 2,196)	<0.001
Age, years	72 (68, 76)	71 (68, 75)	71 (68, 75)	72 (68, 76)	73 (69, 78)	<0.001
Men, n (%)	2,245 (41.7)	659 (52.7)	578 (46.3)	451 (36.1)	396 (31.7)	<0.001
Black, n (%)	769 (14.3)	301 (24.1)	154 (12.3)	121 (9.7)	108 (8.6)	<0.001
BMI, kg/m^2	26.0 (23.4, 28.9)	26.3 (24.0, 29.3)	26.1 (23.6, 29.1)	25.8 (23.3, 28.8)	25.3 (22.7, 28.2)	<0.001
Systolic blood pressure, mmHg	134 (121, 149)	133 (120, 147)	134 (121, 150)	134 (121, 150)	134 (121, 151)	0.025
Antihypertensive medication, n (%)	2,451 (45.6)	511 (40.9)	546 (43.8)	588 (47.1)	632 (50.6)	<0.001
Smoking, n (%)						
Never	2,505 (46.6)	584 (46.9)	576 (46.2)	558 (44.6)	601 (48.1)	0.741
Past	2,221 (41.3)	545 (43.7)	532 (42.6)	521 (41.7)	464 (37.1)	<0.001
Current	648 (12.1)	117 (9.4)	139 (11.2)	171 (13.7)	185 (14.8)	<0.001
Heavy alcohol use, n (%)	509 (9.5)	89 (7.1)	118 (9.5)	143 (11.5)	126 (10.1)	0.003
Physical activity, kilocalories/week	1,081 (395, 2,356)	1,134 (442, 2,416)	1,179 (444, 2,446)	1,115 (428, 2,401)	901 (271, 2,116)	<0.001
Estrogen use, n (%)*	389 (12.4)	138 (23.4)	86 (12.8)	80 (10.0)	61 (7.2)	<0.001
LDL cholesterol, mg/dL	129 (107, 152)	125 (104, 147)	129 (109, 151)	131 (109, 154)	130 (106, 158)	<0.001
HDL cholesterol, mg/dL	52 (44, 64)	52 (43, 64)	52 (44, 63)	53 (44, 64)	52 (44, 65)	0.525
Triglycerides, mg/dL	121 (93, 164)	117 (91, 164)	125 (96, 163)	120 (94, 163)	122 (91, 169)	0.045
Statin, n (%)	111 (2.1)	30 (2.4)	36 (2.9)	22 (1.8)	20 (1.6)	0.056
Oral corticosteroid, n (%)	114 (2.1)	18 (1.4)	21 (1.7)	28 (2.2)	37 (3.0)	0.005
NSAID, n (%)	686 (12.8)	159 (12.7)	129 (10.3)	173 (13.9)	167 (13.4)	0.194
Prevalent ASCVD, n (%)	1,189 (22.1)	248 (19.8)	261 (20.9)	266 (21.3)	313 (25.0)	0.002
Prevalent HF, n (%)	222 (4.1)	32 (2.6)	49 (3.9)	42 (3.4)	74 (5.9)	<0.001
eGFR, mL/min/1.73 m^2	64.4 (53.5, 75.8)	67.4 (57.4, 76.9)	66.1 (56.1, 76.3)	63.4 (52.9, 75.2)	59.6 (47.3, 73.7)	<0.001
CRP, mg/L	2.5 (1.2, 4.5)	1.9 (0.9, 3.5)	2.2 (1.2, 3.9)	2.6 (1.4, 4.5)	3.2 (1.6, 7.7)	<0.001
IL-6, pg/mL	1.7 (1.1, 2.5)	1.5 (1.0, 2.2)	1.6 (1.1, 2.3)	1.7 (1.2, 2.6)	1.9 (1.3, 3.1)	<0.001
WBC count, ×1,000/mm^3	6.0 (5.0, 7.1)	5.7 (4.9, 6.8)	6.0 (5.1, 7.1)	6.0 (5.0, 7.1)	6.2 (5.2, 7.6)	<0.001
Baseline glucose, mg/dL	100 (94, 109)	99 (93, 108)	101 (94, 109)	100 (94, 108)	99 (93, 109)	0.019
Baseline insulin, IU/mL	12 (9, 17)	12 (9, 17)	12 (9, 17)	12 (9, 16)	12 (9, 17)	0.125
QUICKI	0.32 (0.31, 0.34)	0.32 (0.29, 0.34)	0.32 (0.31, 0.34)	0.32 (0.31, 0.34)	0.32 (0.31, 0.34)	0.255
HOMA2IR	1.62 (1.21, 2.24)	1.61 (1.20, 2.24)	1.62 (1.22, 2.28)	1.61 (1.23, 2.18)	1.62 (1.21, 2.25)	0.161

Data are median (interquartile range) for continuous variables unless otherwise indicated; 25th, 50th, and 75th percentiles of sCD14 are 1,399, 1,593, and 1,823, respectively. NSAID, nonsteroidal anti-inflammatory drug. *Only among women.

participants using oral corticosteroids, and those with hypertension, prevalent ASCVD, and prevalent HF. Levels of QUICKI and HOMA2IR did not differ across sCD14 quartiles, but those for IL-6, CRP, and WBC count tended to increase with increasing sCD14 quartile categories.

sCD14 was positively correlated with IL-6, CRP, and WBC count, with Pearson ρ values of 0.17, 0.24, and 0.11, respectively (all P < 0.001). IL-6 was also positively correlated with CRP and WBC count, with Pearson ρ values being 0.50 and 0.30, respectively (all P < 0.001). Meanwhile, CRP was positively correlated with WBC count, with a Pearson ρ of 0.27 (P < 0.001).

Table 2 describes the associations of sCD14 and the other inflammatory biomarkers in minimally and fully adjusted linear regression models in relation to QUICKI and HOMA2IR, as well as Cox proportional hazards models in relation to incident diabetes. All four biomarkers were significantly inversely related with QUICKI (decreasing QUICKI values reflect higher insulin resistance) after adjustment for demographic variables. These inverse associations were attenuated but remained significant after full adjustment. The magnitude of these associations was comparable for sCD14, IL-6, and CRP, but not for WBC count, which showed a numerically stronger relationship with QUICKI. In the case of HOMA2IR, significant positive associations were again observed for all biomarkers. These associations were attenuated after full adjustment for all biomarkers except for sCD14; all of the relationships remained significant. Once again, the numerically strongest association was observed for WBC count, followed by numerically similar associations for sCD14 and IL-6, and a numerically weaker association for CRP. Additional adjustment for LDL cholesterol did not meaningfully alter the observed associations. GAM plots showed that associations of inflammatory biomarkers with QUICKI and HOMA2IR were consistent with linear relationships (data not shown).

To test for the association of the four biomarkers with QUICKI and HOMA2IR independent of each other, we next tested the full model with the four biomarkers included together. As shown in

Table 2—Relation of inflammatory biomarkers to insulin resistance and incident diabetes

Biomarker	Model	QUICKI			HOMA2IR			Incident diabetes		
		N	β (95% CI)	P value	N	β (95% CI)	P value	Events/N	HR (95% CI)	P value
Log₂sCD14	Model 1	4,964	−0.0027 (−0.0051, −0.0004)	0.024	4,917	0.0899 (0.0001, 0.1797)	0.050	427/4,448	0.92 (0.67, 1.29)	0.641
	Model 2	4,894	−0.0023 (−0.0045, −0.0001)	0.031	4,847	0.0909 (0.0089, 0.1728)	0.029	421/4,383	0.99 (0.70, 1.42)	0.993
Log₂IL-6	Model 1	4,896	−0.0066 (−0.0074, −0.0058)	<0.001	4,853	0.2516 (0.2220, 0.2812)	<0.001	431/4,401	1.33 (1.19, 1.47)	<0.001
	Model 2	4,828	−0.0031 (−0.0039, −0.0023)	<0.001	4,785	0.1236 (0.0956, 0.1516)	<0.001	425/4,338	1.15 (1.03, 1.29)	0.018
Log₂CRP	Model 1	5,265	−0.0038 (−0.0042, −0.0034)	<0.001	5,217	0.1407 (0.1236, 0.1577)	<0.001	464/4,724	1.20 (1.12, 1.27)	<0.001
	Model 2	5,191	−0.0016 (−0.0020, −0.0012)	<0.001	5,143	0.0616 (0.0455, 0.0776)	<0.001	458/4,655	1.09 (1.02, 1.16)	0.017
Log₂WBC	Model 1	5,279	−0.0133 (−0.0149, −0.0117)	<0.001	5,232	0.4578 (0.3929, 0.5226)	<0.001	464/4,737	1.67 (1.33, 2.11)	<0.001
	Model 2	5,205	−0.0098 (−0.0114, −0.0082)	<0.001	5,205	0.3242 (0.2652, 0.3832)	<0.001	458/4,668	1.50 (1.17, 1.92)	0.002

Model 1 adjusts for age, sex, and race; model 2 adjusts for model 1 adjustments and BMI, systolic blood pressure, antihypertensive medication, smoking status, heavy alcohol use, physical activity, estrogen use, prevalent atherosclerotic cardiovascular disease, prevalent HF, and eGFR. HR, hazard ratio.

Fig. 1A and B, after such adjustment, sCD14 ceased to have a statistically significant association with QUICKI and HOMA2IR, while the remaining three biomarkers continued to have a significant association. CRP showed attenuated associations, while WBC count seemed to retain the strongest numerical association with either outcome.

Over a median follow-up of 11.6 years, we documented 466 cases of incident diabetes. As shown in Table 2, sCD14 was not significantly associated with new-onset diabetes in minimally or fully adjusted models. By contrast, IL-6, CRP, and WBC count were significantly positively associated with incident diabetes after adjustment for demographic factors. These associations were attenuated after full adjustment for potential confounding variables but remained statistically significant. GAM plots were consistent with linear relationships. Once again, the strongest numerical association noted was for WBC count. Further adjustment for LDL cholesterol did not materially alter the findings. When all biomarkers were additionally adjusted for each other, the relationship of WBC count with diabetes did not change substantively, whereas the associations for IL-6 and CRP became nonsignificant (Fig. 1C).

In the sensitivity analysis undertaking adjustment for use of statins, oral steroids, or nonsteroidal anti-inflammatory drugs in addition to covariates in the main model (model 2), there were no meaningful changes in risk estimates for insulin resistance or incident diabetes observed for the four inflammatory markers (Supplementary Tables 1 and 2). Assessment for interaction by age, sex, race, BMI, prevalent ASCVD, and prevalent HF did not reveal consistent evidence of effect modification across outcomes for any of the four inflammatory biomarkers (data not shown).

Table 3 shows the results of instrumental variable analysis using Mendelian randomization of sCD14 in relation to QUICKI and HOMA2IR, stratified by race. In the two-stage least-squares approach, F values for all gene variants in whites and African Americans were >10, attesting to the SNPs serving as good instruments. Second-stage P values for sCD14 for whites with SNPs rs5744455, rs778584, and rs4914 were nonsignificant for both QUICKI and HOMA2IR. Similarly, for African Americans, SNP

rs5744451 exhibited second-stage P values that were nonsignificant for QUICKI and HOMA2IR.

CONCLUSIONS

In this population-based cohort of older adults, we evaluated the association of plasma sCD14, alongside the three inflammatory markers, IL-6, CRP, and WBC count, with insulin resistance and incident diabetes. Like IL-6, CRP, and WBC count, sCD14 was cross-sectionally associated with insulin resistance as determined by QUICKI and HOMA2IR after adjustment for potential confounders. However, Mendelian randomization analysis did not support a causal basis for the observed association between sCD14 and QUICKI or HOMA2IR. Upon adjustment for one another, IL-6, CRP, and WBC count remained associated with these two insulin resistance measures, but sCD14 did not. sCD14 was also not significantly associated with incident diabetes, as opposed to IL-6, CRP, and WBC count, all of which showed significant associations with this outcome. When all four biomarkers were entered simultaneously in the model, only WBC count remained significantly associated with incident diabetes.

The concept that insulin resistance and diabetes have an inflammatory basis is supported by laboratory and, increasingly, clinical studies. Expansion of the adipose compartment from caloric excess subjects adipocytes to metabolic and ischemic stress, resulting in macrophage infiltration, inflammation, and adipocyte insulin resistance (30). The resultant lipid excess promotes ectopic fat deposition, inflammation, and insulin resistance in liver and skeletal muscle, further impairing glucose disposal (31). Compensatory hypersecretion of insulin by the pancreas maintains glucose homeostasis until β-cell failure ultimately supervenes, sometimes fostered by pancreatic islet inflammation of its own (32), eventuating in hyperglycemia.

However, obesity has also been linked to perturbations in the gut microbiome (dysbiosis) and intestinal barrier function (33). Attendant low-grade increases in circulating LPSs have been shown in animal models of obesity to promote adipose tissue inflammation and glucose dysregulation (9,10). Such so-called metabolic endotoxemia has been supported by some, but not all, clinical studies (34).
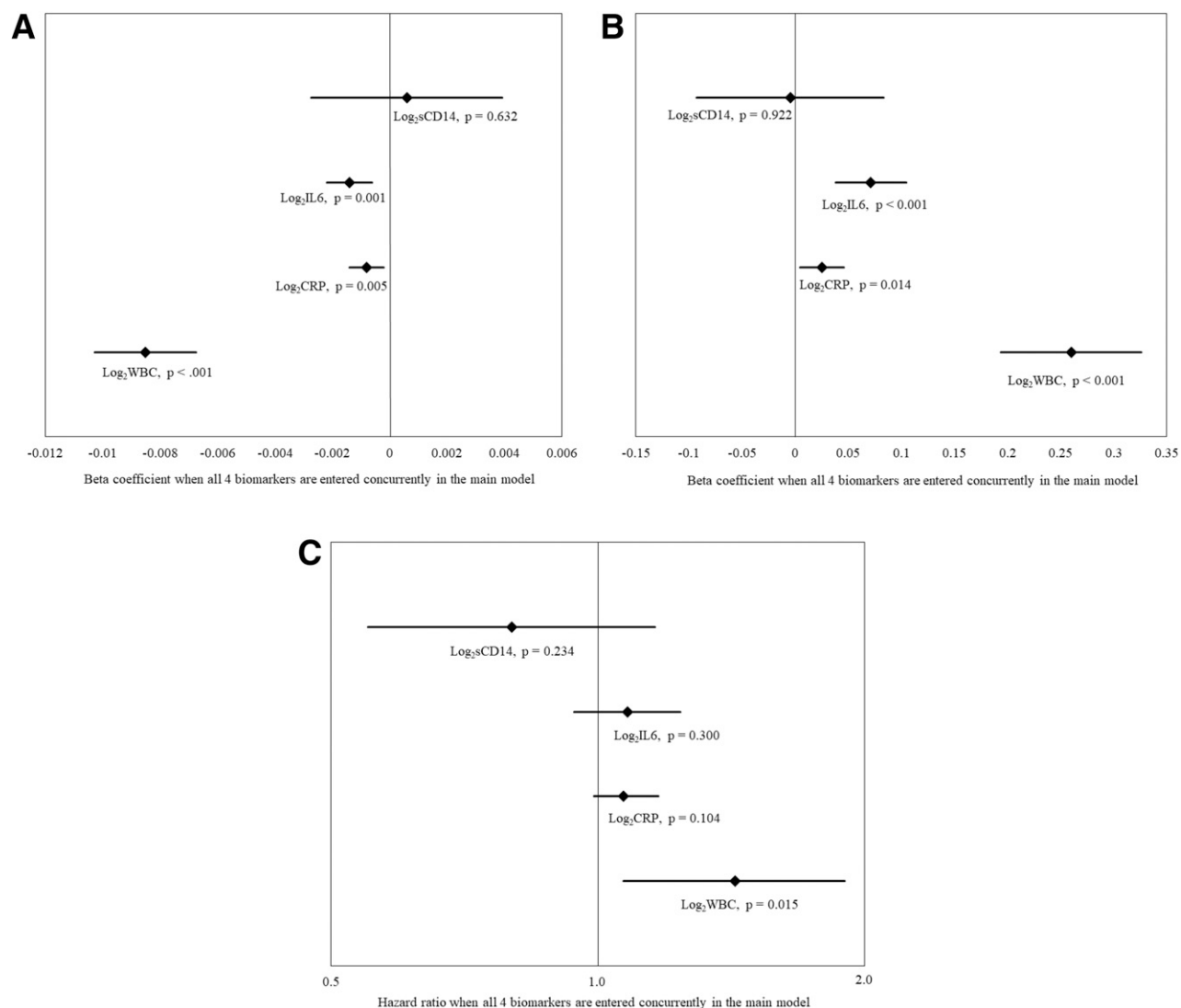
Figure 1—*A* and *B*: β estimates and *P* values per doubling of sCD14, IL-6, CRP, and WBC count after all four were simultaneously included in the main model—comprising age, sex, race, BMI, systolic blood pressure, antihypertensive medication, smoking status, heavy alcohol use, physical activity, estrogen use, prevalent atherosclerotic cardiovascular disease, prevalent HF, and eGFR—to test for association with QUICKI (*A*) and HOMA2IR (*B*). *C*: Hazard ratios and 95% CIs per doubling of sCD14, IL-6, CRP, and WBC count after all four were simultaneously included in the main model—comprising the same covariates as in *A* and *B*—to test for association with incident diabetes.

In this context, the multifunctional pattern-recognition receptor CD14, which mediates LPS's potent proinflammatory actions, has drawn interest, demonstrating a role in adipose tissue inflammation and insulin resistance in experimental studies (35). Clinical studies have in turn reported an inverse association between sCD14 and insulin resistance in men only after adjustment for triglycerides (16) or disparate associations of sCD14 with insulin resistance in nonobese (positive) and morbidly obese (inverse) individuals (17), but these studies have been modest in size. Hence, whether CD14 is a central player in the intestinal–adipose tissue–dysmetabolic axis in humans has remained unclear.

In this study, we found sCD14 to be associated with insulin resistance independent of multiple covariates, including postmenopausal estrogen replacement, with which it was inversely associated, but we did not detect a similar association with incident diabetes. Moreover, the observed relationship with insulin resistance was lost upon concurrent adjustment for other inflammatory markers, and there was no support for a causal association from instrumental variable analysis using Mendelian randomization. Our findings therefore would not appear to support a preeminent role for CD14 as a

lynchpin of dysbiosis, inflammation, and glucose dysregulation. This may owe to the fact that sCD14 is an acute-phase reactant (36), reflecting varied inflammatory stimuli that may not set it apart from other inflammatory markers, or not itself acting as a proximate driver of key pathogenic pathways, as relates to insulin resistance. The lack of a predominant role seen for sCD14 could also be attributable to sCD14's duality of effects. sCD14 may serve to buffer circulating LPS's proinflammatory consequences by promoting its sequestration to triglyceride-rich lipoproteins (14), but can also act to induce proinflammatory responses in cells lacking (37), and even expressing

Table 3—Instrumental variable analysis for causal association of sCD14 with measures of insulin resistance

| | | Mendelian randomization results for sCD14 as endogenous regressor | | | | | |
| | | | | | First stage | | Second stage |
Race	Outcome	Instrumental variables	N	F	P value	F	P value
White	QUICKI	rs5744455 (top SNP in whites)	3,387	101.3	<0.001	0.00	0.960
White	HOMA2IR	rs5744455 (top SNP in whites)	3,356	101.82	<0.001	0.32	0.574
White	QUICKI	rs778584	3,387	59.87	<0.001	2.4	0.120
White	HOMA2IR	rs778584	3,356	59.26	<0.001	1.15	0.283
White	QUICKI	rs4914	3,385	20.57	<0.001	1.1	0.286
White	HOMA2IR	rs4914	3,354	20.40	<0.001	1.36	0.244
Black	QUICKI	rs5744451 (top SNP in blacks)	422	27.39	<0.001	1.8	0.187
Black	HOMA2IR	rs5744451 (top SNP in blacks)	414	25.93	<0.001	2.39	0.123

(13), membrane-bound CD14. The extent to which sCD14's protective and harmful functions are determined by tissue provenance, cellular and molecular milieu, stoichiometry of sCD14 and LPS levels, and other factors is not well defined, but such offsetting influences complicate assessment of the molecule's pathophysiologic contributions using epidemiologic approaches. Thus, the present findings cannot exclude an important pathophysiologic role for LPS-CD14 in metabolic dysregulation not discernible from the evaluation of circulating sCD14 levels.

The other notable finding of the current study is the emergence of WBC count as the most robust inflammatory measure associated with glucose dysregulation, showing the strongest association with insulin resistance, and remaining the single marker related to long-term incidence of diabetes, after accounting for all others. Previous studies have shown prospective associations between various inflammatory markers and diabetes, including CRP, IL-6, and WBC count (2–4). Of note, in a previous analysis of CHS assessing new-onset diabetes in the middle term, CRP but not WBC count showed a significant relationship with this outcome, but the number of events was small (15). To our knowledge, ours is the first study to document the stronger and independent association of WBC count with incidence of diabetes after simultaneous adjustment for other commonly studied markers. That WBC count superseded CRP, and IL-6, with which it is related, supports its role as an integrative measure of systemic inflammation in ubiquitious use in clinical practice. Apart from confirming the association between inflammation and glucose dysregulation among older adults, the segment of the population at highest risk of diabetes (38), this finding suggests that WBC count could potentially have a role in stratification of diabetes risk. As anti-inflammatory therapies are tested for prevention of diabetes or its complications (39), the question of whether WBC count may be useful in identifying individuals for such interventions will merit scrutiny. Likewise, while the current investigation is not focused on prediction, whether addition of WBC count could enhance prediction of diabetes risk in elders will warrant separate study.

The current study has several strengths, including its large and well-characterized population-based sample of older adults with long-term follow-up and regular measures of glycemia, its concurrent assessment of multiple inflammatory markers, and availability of *CD14* sequencing for Mendelian randomization. Various weaknesses must also be acknowledged. Neither hemoglobin A_{1c} nor postload glucose was regularly available in CHS, which may have led to misclassification of baseline or follow-up diabetes status. Glucose was mostly measured in serum and not in sodium fluoride or citrated tubes. Specimens were frozen soon after collection, however, which should have minimized glucose consumption by blood-cell glycolysis and attendant nondifferential bias. The hyperinsulinemic-euglycemic clamp method, the gold standard to measure insulin sensitivity, was not obtained in CHS, yet QUICKI and HOMA2IR have been shown to yield good estimates of insulin resistance for use in large-scale epidemiologic studies (24–27). Concurrent measurement of LPS was not available, which would have permitted more direct assessment of the contribution of microbial translocation to circulating sCD14 and other inflammatory markers, although accurate measurement of LPS poses challenges (34). WBC differential was not performed in CHS, and we could not evaluate the specific relationships of WBC subtypes with study outcomes, including neutrophils, for which the role in the pathogenesis of dysglycemia is being increasingly recognized (40). Information on recent infection or antibiotic use was not obtained at the baseline examination, which may have biased the associations under study toward the null hypothesis. Last, our findings come from a biracial, but predominantly European ancestry, population and are not necessarily generalizable to other ethnic groups.

In conclusion, in this sample of community-dwelling older adults, sCD14 was significantly associated with insulin resistance, but not with incident diabetes. The association of sCD14 with insulin resistance was not independent of other inflammatory markers, nor did it appear to have a causal basis on instrumental variable analysis using Mendelian randomization. By contrast, CRP, IL-6, and WBC count were significantly associated with incident diabetes, but this association persisted only for WBC count upon mutual adjustment, such that WBC count emerged as the strongest inflammatory marker of future diabetes risk. These findings do not support a key role for sCD14 in diabetes risk in elders, although complex actions of sCD14 preclude direct assessment as to the role of metabolic endotoxemia in this population. The foremost association for WBC count provides impetus for further study of this marker's potential role in diabetes risk stratification and prediction.

References

1. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–867
2. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327–334
3. Spranger J, Kroke A, Möhlig M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003;52:812–817
4. Bertoni AG, Burke GL, Owusu JA, et al. Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care 2010;33:804–810
5. Barzilay JI, Abraham L, Heckbert SR, et al. The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. Diabetes 2001;50:2384–2389
6. Gkrania-Klotsas E, Ye Z, Cooper AJ, et al. Differential white blood cell count and type 2 diabetes: systematic review and meta-analysis of cross-sectional and prospective studies. PLoS One 2010;5:e13405
7. Zanoni I, Granucci F. Role of CD14 in host protection against infections and in metabolism regulation. Front Cell Infect Microbiol 2013;3:32
8. Pan Z, Zhou L, Hetherington CJ, Zhang DE. Hepatocytes contribute to soluble CD14 production, and CD14 expression is differentially regulated in hepatocytes and monocytes. J Biol Chem 2000;275:36430–36435
9. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772
10. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–1481
11. Roncon-Albuquerque R Jr., Moreira-Rodrigues M, Faria B, et al. Attenuation of the cardiovascular and metabolic complications of obesity in CD14 knockout mice. Life Sci 2008;83:502–510
12. Luche E, Cousin B, Garidou L, et al. Metabolic endotoxemia directly increases the proliferation of adipocyte precursors at the onset of metabolic diseases through a CD14-dependent mechanism. Mol Metab 2013;2:281–291
13. Hailman E, Vasselon T, Kelley M, et al. Stimulation of macrophages and neutrophils by complexes of lipopolysaccharide and soluble CD14. J Immunol 1996;156:4384–4390
14. Kitchens RL, Thompson PA, Viriyakosol S, O'Keefe GE, Munford RS. Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. J Clin Invest 2001;108:485–493
15. Reiner AP, Lange EM, Jenny NS, et al. Soluble CD14: genomewide association analysis and relationship to cardiovascular risk and mortality in older adults. Arterioscler Thromb Vasc Biol 2013;33:158–164
16. Fernández-Real JM, Broch M, Richart C, Vendrell J, López-Bermejo A, Ricart W. CD14 monocyte receptor, involved in the inflammatory cascade, and insulin sensitivity. J Clin Endocrinol Metab 2003;88:1780–1784
17. de Courten B, Moreno-Navarrete JM, Lyons J, et al. Contrasting association of circulating sCD14 with insulin sensitivity in non-obese and morbidly obese subjects. Mol Nutr Food Res 2016;60:103–109
18. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1991;1:263–276
19. Psaty BM, Kuller LH, Bild D, et al. Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. Ann Epidemiol 1995;5:270–277
20. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52–58
21. Keating BJ, Tischfield S, Murray SS, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One 2008;3:e3583
22. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. Clin Chem 1995;41:264–270
23. Kizer JR, Arnold AM, Benkeser D, et al. Total and high-molecular-weight adiponectin and risk of incident diabetes in older people. Diabetes Care 2012;35:415–423
24. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402–2410
25. Chen H, Sullivan G, Quon MJ. Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. Diabetes 2005;54:1914–1925
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419
27. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191–2192
28. Ives DG, Fitzpatrick AL, Bild DE, et al. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. Ann Epidemiol 1995;5:278–285
29. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612
30. Crewe C, An YA, Scherer PE. The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. J Clin Invest 2017;127:74–82
31. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest 2016;126:12–22
32. Eguchi K, Nagai R. Islet inflammation in type 2 diabetes and physiology. J Clin Invest 2017;127:14–23
33. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest 2011;121:2126–2132
34. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: is it real and is it relevant? Biochimie 2016;124:11–20
35. Fernández-Real JM, Pérez del Pulgar S, Luche E, et al. CD14 modulates inflammation-driven insulin resistance. Diabetes 2011;60:2179–2186
36. Bas S, Gauthier BR, Spenato U, Stingelin S, Gabay C. CD14 is an acute-phase protein. J Immunol 2004;172:4470–4479
37. Pugin J, Schürer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci U S A 1993;90:2744–2748
38. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and trends in diabetes among adults in the United States, 1988-2012. JAMA 2015;314:1021–1029
39. Goldfine AB, Shoelson SE. Therapeutic approaches targeting inflammation for diabetes and associated cardiovascular risk. J Clin Invest 2017;127:83–93
40. Fadini GP, Menegazzo L, Scattolini V, Gintoli M, Albiero M, Avogaro A. A perspective on NETosis in diabetes and cardiometabolic disorders. Nutr Metab Cardiovasc Dis 2016;26:1–8