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Serum Soluble CD163 Predicts Risk of Type 2 Diabetes in the General Population

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BACKGROUND: Activation of adipose tissue macrophages with concomitant low-grade inflammation is believed to play a central role in the development of type 2 diabetes. We tested whether a new macrophage-derived biomarker, soluble CD163 (sCD163), identifies at-risk individuals before overt disease has developed.

METHODS: A prospective cohort study of 8849 study participants from the general population, the Copenhagen City Heart Study, was followed for 18 years for incidence of type 2 diabetes. Risk of disease was calculated according to age- and sex-adjusted percentile categories of serum sCD163 concentrations: 0%–33%, 34%–66%, 67%–90%, 91%–95%, and 96%–100%.

RESULTS: A total of 568 participants developed type 2 diabetes. The cumulative incidence increased with increasing baseline sCD163 (trend $P < 0.001$), and sCD163 was strongly associated with known risk factors such as physical inactivity, body mass index, C-reactive protein, and triglycerides (all $P < 0.001$). Multifactorially adjusted hazard ratios for type 2 diabetes were 1.4 (95% CI, 1.0–1.9), 2.4 (1.8–3.2), 3.8 (2.6–5.5), and 5.2 (3.6–7.6) for categories 34%–66%, 67%–90%, 91%–95%, and 96%–100%, respectively, vs the 0%–33% category. In overweight men 50–70 and >70 years of age, serum sCD163 concentrations in the top 5% group predicted an absolute 10-year risk of type 2 diabetes of 29% and 36% vs 7% and 8% in the lowest percentile group. Equivalent values in women were 19% and 24% vs 4% and 5%.

CONCLUSIONS: Increased concentrations of sCD163 predict increased risk of type 2 diabetes in the general population and may be useful for identification of high-risk overweight individuals.

The incidence of obesity and associated diseases such as type 2 diabetes has increased during recent decades and now constitutes a serious health threat globally. The WHO estimates that more than 1.6 billion people are overweight *(1)*, and the number of persons with type 2 diabetes is expected to surpass 350 million in 2030 *(2)*. Long-term prospective clinical trials clearly show that interventions can delay or possibly prevent onset of type 2 diabetes in high-risk individuals *(3, 4)*. Biomarkers that identify at-risk individuals before overt disease has developed will thus be of great clinical value to initiate early prevention and treatment in targeted high-risk groups *(5)*.

Obesity induces a chronic low-grade inflammation in adipose tissue, characterized by infiltration of activated macrophages, increased expression of cytokines, and predisposition to insulin resistance and type 2 diabetes *(6–9)*. The haptoglobin-hemoglobin receptor CD163 is closely related to macrophage activation, since the extracellular part of the molecule, soluble CD163 (sCD163),[7] is shed to blood upon inflammatory activation of the macrophages *(10, 11)*. CD163 is exclusively expressed on macrophages and monocytes and is highly expressed in human adipose tissue *(12, 13)*. Accordingly, increased concentrations of sCD63 have been measured in obese individuals, and sCD163 is a promising biomarker candidate for inflammation in adipose tissues and for the development of type 2 diabetes *(14, 15)*.

We tested the hypothesis that increased concentrations of sCD163 predict risk of type 2 diabetes. For

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[7] Nonstandard abbreviations: sCD163, soluble CD163; BMI, body mass index; ICD, *International Classification of Diseases*; hsCRP, high-sensitivity CRP; TNF-α, tumor necrosis factor-α.

this purpose, we measured serum sCD163 in 8849 participants in the Copenhagen City Heart Study, of whom 568 developed type 2 diabetes during 18 years of follow-up.

Methods

STUDY PARTICIPANTS

We used a population-based prospective study of the Danish general population, the 1991–1994 examination of the Copenhagen City Heart Study *(16, 17)*. Participants age 20 years and older were selected randomly after sex and age stratification into 5-year groups among residents of Copenhagen. Of the 17 180 individuals invited, 10 135 participated, and serum was available for sCD163 determination in 8849 participants. Participants were followed using their unique Central Person Registration number from baseline at the 1991–1994 examination until May 2009. Follow-up was 100% complete. Roughly 99% were whites of Danish descent.

The participants completed a self-administered questionnaire, which was verified by the participant and an investigator on the day of attendance. Participants reported on smoking habits, physical activity, and alcohol consumption. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, and waist/hip ratio as waist circumference divided by hip circumference in centimeters. Blood pressure was measured in the sitting position, on the left upper arm, after 5-min rest by use of a London School of Hygiene sphygmomanometer.

Serum sCD163 was measured a second time in blood samples of 923 participants in the 2001–2003 examination of the Copenhagen City Heart Study. These individuals were free of known diseases at the 1991–1994 and 2001–2003 examinations, allowing correction for regression dilution bias *(18)*.

ENDPOINTS

We collected information on diagnoses of type 2 diabetes [WHO; *International Classification of Diseases* (ICD), 8th edition: code 250; 10th edition: codes E10-E14] from the national Danish Patient Registry and the national Danish Causes of Death Registry. Individuals who had a diabetes diagnosis at entry (n = 153) were excluded, leaving 8696 for prospective analyses.

ETHICS

Studies were approved by institutional review boards and Danish ethics committees (KF V.100.2039/91 and KF 01–144/01, Copenhagen and Frederiksberg committee) and conducted according to the Declaration of Helsinki. Written informed consent was obtained from participants.

LABORATORY ANALYSES

We measured serum concentrations of sCD163 in duplicate in samples that had been frozen for 12–15 years at −80 °C by use of an in-house sandwich ELISA on a BEP-2000 ELISA-analyzer (Dade Behring) essentially as described *(19)*. In each run, we coanalyzed control samples and serum standards with concentrations traceable to purified CD163. The interassay imprecision in the current study (293 runs) was 5.4% (n = 293), 9.1% (n = 45), and 11.4% (n = 293) CV at concentrations of 1.48, 1.82, and 3.86 mg/L, respectively. The limit of detection (lowest calibrator) was 6.25 μg/L. Soluble CD163 is robust to thawing, and stability has been rigorously verified for at least 16 months at −80 °C *(19)*.

We measured nonfasting glucose concentrations in serum using a standard hexokinase/glucose-6-phosphate-dehydrogenase assay *(17)*. High-sensitivity C-reactive protein (hsCRP), fibrinogen, α1-antitrypsin, and orosomucoid were measured by standard clinical laboratory nephelometry or turbidimetry assays. We used colorimetric and turbidimetric assays to measure serum concentrations of total cholesterol, triglycerides, HDL cholesterol (after precipitation of apolipoprotein B–containing lipoproteins), apolipoprotein B, and apolipoprotein AI (all Boehringer Mannheim). We calculated LDL cholesterol according to Friedewald if triglycerides were <354 mg/dL (4 mmol/L) *(20)* but measured it directly at higher triglyceride concentrations (Konelab).

STATISTICAL ANALYSIS

We used STATA version 10 (Stata Corp). Two-sided $P < 0.05$ was considered significant. We stratified serum sCD163 concentrations into percentile categories in sex and 10-year age groups: the 5 percentile categories were 0%–33%, 34%–66%, 67%–90%, 91%–95%, and 96%–100%. The 5 percentile groups were prespecified to evaluate both tertiles in the lower range and extreme phenotypes in the upper range *(21, 22)*. Kruskal–Wallis ANOVA trend test evaluated continuous traits as a function of percentile group. We used Mann–Whitney *U*-test for the comparison of serum concentrations of sCD163 between sexes and Spearman ρ to calculate the correlation between serum sCD163 and various continuous variables.

We plotted cumulative incidence using Kaplan–Meier curves and examined differences between serum sCD163 percentile categories using log-rank tests. We calculated hazard ratios and 95% CIs using Cox regression analysis with age as time scale (left-truncation, which implies that age is automatically accounted for) and adjusted for sex or multifactorially (sex, smoking habits, physical activity, BMI, alcohol consumption, blood pressure). Participants with known diabetes be-

Table 1. Baseline characteristics in relation to sCD163 categories of study participants.[a]

	Serum sCD163					Trend *P*	Spearman ρ
	0%–33%	34%–66%	67%–90%	91%–95%	96%–100%		
All	2944 (33)	2909 (33)	2118 (24)	443 (5)	435 (5)		
Women	1655 (56)	1650 (57)	1195 (56)	250 (56)	245 (56)		
Age, years	61 (48–71)	61 (48–71)	61 (48–71)	61 (49–71)	61 (47–71)		0.22[b]
Smoking[c]	2277 (78)	2110 (73)	1524 (72)	316 (72)	332 (77)	0.003	
Physical inactivity[d]	1870 (64)	1869 (65)	1424 (68)	311 (71)	308 (72)	<0.001	
BMI, kg/m^2	24 (22–27)	25 (22–28)	26 (23–29)	27 (24–31)	26 (23–30)	<0.001	0.29[b]
Waist, cm	84 (75–93)	86 (77–96)	90 (80–100)	94 (84–105)	93 (84–102)	<0.001	0.30[b]
Hip, cm	98 (93–103)	99 (94–105)	101 (96–106)	102 (96–110)	101 (94–107)	<0.001	0.22[b]
Waist/hip ratio	0.86 (0.79–0.92)	0.87 (0.80–0.94)	0.89 (0.82–0.96)	0.91 (0.84–0.98)	0.91 (0.84–0.99)	<0.001	0.24[b]
Alcohol consumption, g/day	10 (3–21)	9 (2–21)	9 (2–22)	9 (0–24)	12 (2–34)	0.95	−0.01
Systolic blood pressure, mmHg	135 (121–150)	136 (122–153)	139 (125–156)	140 (125–157)	140 (125–156)	<0.001	0.22[b]
Diastolic blood pressure, mmHg	83 (75–90)	84 (76–92)	85 (77–94)	86 (78–94)	85 (77–95)	<0.001	0.15[b]

[a] Data are n (%) or median (interquartile range). Statistical comparison across the 5 sCD163 percentile categories used Kruskal–Wallis ANOVA trend test. Spearman ρ was calculated on unadjusted serum sCD163.
[b] $P < 0.001$.
[c] Current or ex-smokers at baseline.
[d] Individuals with <2–4 h per week of light physical activity at baseline.

fore blood sampling were excluded from prospective analyses. We corrected hazard ratios for regression dilution bias using a nonparametric method *(18)*. For this correction, we used serum sCD163 values from 923 individuals attending both the 1991–1994 baseline examination and the 2001–2003 follow-up examination. A regression dilution ratio of 0.86 was computed. We estimated absolute 10-year risk of type 2 diabetes by serum sCD163 percentile categories using the regression coefficients from a Poisson regression model including only the most significant covariates from the Cox regression models: sex, age in 3 groups (<50, 50–70, >70 years), and BMI in 2 groups (≤25, >25 kg/m^2) at the date of blood sampling. Absolute risks are presented as estimated incidence rates (events/10 years) in percentages.

Results

The median serum concentration of sCD163 was 1.71 mg/L (interquartile range, 1.31–2.26 mg/L) in women and 1.76 mg/L (1.37–2.36 mg/L) in men ($P < 0.001$). Serum sCD163 concentrations increased in both sexes with increasing age (*P* for trends, <0.001) (see Supplemental Fig. 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue2). Spearman ρ correlation between serum sCD163 and age was 0.22 ($P < 0.001$) (Table 1). Absolute values of age- and sex-adjusted percentile categories of serum sCD163 concentrations (0%–33%, 34%–66%, 67%–90%, 91%–95%, and 96%–100%) are shown in online Supplemental Table 1.

BASELINE CHARACTERISTICS

Baseline characteristics of participants according to serum sCD163 percentile categories (grouped by 10-year age and sex) are shown in Table 1. Increasing percentile categories were associated with increasing BMI, waist and hip circumferences, waist/hip ratio, systolic blood pressure, and diastolic blood pressure (all *P* for trends, <0.001). Spearman ρ correlations between unadjusted serum sCD163 concentrations and baseline anthropometric factors were strongest for waist circumference, BMI, waist/hip ratio, hip circumference, and systolic blood pressure (Spearman ρ: 0.30, 0.29, 0.24, 0.22, 0.22, respectively; all *P* values <0.001) (Table 1).

sCD163 AND BIOCHEMICAL PARAMETERS AT BASELINE

Increasing serum concentrations of sCD163 by percentile categories were associated with increasing concentrations of glucose (*P* for trend, <0.001); inflammatory markers such as CRP, fibrinogen, α1-antitrypsin, and orosomucoid (all *P* for trends, <0.001); and triglycerides, and inversely with HDL cholesterol and apolipoprotein AI (all *P* for trends, <0.001) (Table 2). Spearman ρ correlations between unadjusted serum sCD163 concentrations and baseline biochemical parameters (Table 2) were strongest for concentrations of

Table 2. Biochemical parameters at baseline in relation to sCD163 categories of study participants.[a]

	Serum sCD163					Trend *P*	Spearman ρ
	0%–33%	34%–66%	67%–90%	91%–95%	96%–100%		
All	2944 (33)	2909 (33)	2118 (24)	443 (5)	435 (5)		
Glucose homeostasis							
Glucose, mmol/L (nonfasting)	5.4 (5.0–5.9)	5.4 (5.0–6.1)	5.5 (5.0–6.2)	5.7 (5.1–6.8)	5.7 (5.1–7.0)	<0.001	0.17[b]
Inflammatory markers							
hsCRP, mg/L	1.54 (1.19–2.45)	1.67 (1.23–2.81)	1.98 (1.36–3.43)	2.46 (1.56–4.78)	2.52 (1.48–5.02)	<0.001	0.25[b]
Fibrinogen, g/L	2.86 (2.39–3.39)	2.99 (2.50–3.58)	3.11 (2.59–3.72)	3.16 (2.63–3.81)	3.14 (2.49–3.88)	<0.001	0.22[b]
α1-Antitrypsin, μmol/L	24.9 (22.3–28.0)	25.0 (22.3–28.1)	25.3 (22.4–28.1)	25.6 (22.8–28.9)	26.7 (23.8–30.2)	<0.001	0.08[b]
Orosomucoid, μmol/L	19.5 (17.0–23.4)	21.4 (18.3–25.3)	22.4 (19.5–26.3)	23.4 (19.5–27.3)	23.4 (18.5–30.2)	<0.001	0.26[b]
Lipid traits							
Total cholesterol, mmol/L	6.0 (5.2–6.9)	6.1 (5.3–7.0)	6.1 (5.3–7.1)	6.0 (5.2–6.9)	5.8 (4.9–6.7)	0.46	0.10[b]
LDL cholesterol, mmol/L	3.6 (3.0–4.4)	3.7 (3.0–4.5)	3.7 (3.0–4.5)	3.6 (2.9–4.4)	3.4 (2.6–4.1)	0.01	0.07
Apolipoprotein B, mg/dL	83 (70–99)	86 (70–101)	87 (71–103)	87 (73–103)	81 (67–99)	0.02	0.12[b]
HDL cholesterol, mmol/L	1.6 (1.3–1.9)	1.5 (1.2–1.9)	1.4 (1.2–1.8)	1.3 (1.0–1.7)	1.4 (1.1–1.7)	<0.001	−0.17[b]
Apolipoprotein AI, mg/dL	143 (124–164)	139 (122–160)	136 (120–155)	130 (113–154)	135 (115–154)	<0.001	−0.11[b]
Triglycerides, mmol/L	1.40 (1.03–1.96)	1.54 (1.08–2.21)	1.64 (1.17–2.44)	1.92 (1.32–2.84)	1.88 (1.20–2.71)	<0.001	0.23[b]

[a] Data are n (%) or median (interquartile range). Statistical comparison across the 5 sCD163 percentile categories used Kruskal–Wallis ANOVA trend test. Spearman ρ was calculated on unadjusted serum sCD163.
[b] $P < 0.001$.

orosomucoid, CRP, triglycerides, and fibrinogen (Spearman ρ: 0.26, 0.25, 0.23, 0.22, respectively; all *P* values <0.001) (Table 2).

sCD163 AND RISK OF TYPE 2 DIABETES

During 18 years of follow-up, 568 of 8696 participants developed type 2 diabetes. The cumulative incidence of type 2 diabetes increased with increasing serum sCD163 percentile categories (log-rank *P* for trend, <0.001) (Fig. 1). At the age of 80 years, 12%, 22%, 39%, and 46%, respectively, of individuals with sCD163 in 34%–66%, 67%–90%, 91%–95%, and 96%–100% categories had type 2 diabetes compared with 8% for the 0%–30% category. At the age of 80 years, 27% of individuals with sCD163 in the combined upper tertile (67%–100%) had diabetes, as shown in online Supplemental Fig. 2.

Multifactorially adjusted (age, sex, smoking, physical inactivity, BMI, alcohol consumption, systolic blood pressure, and diastolic blood pressure) hazard ratios for type 2 diabetes increased as a function of sCD163 percentile group, from 1.4 (95% CI, 1.0–1.9) for serum sCD163 percentile category 34%–66% to 5.2 (3.6–7.6) for 96%–100% vs serum sCD163 percentile category 0%–33% (*P* for trend, <0.001) (Table 3). Hazard ratios were only slightly lower after also adjusting for hsCRP (Table 3) or when not adjusting for regression dilution bias (online Supplemental Table 2).

When expressing sCD163 on a continuous scale, 1 change in 1 U of log CD163 increased the risk of incident type 2 diabetes, with a hazard ratio of 3.0 (95% CI, 2.4–3.8) when corrected for regression di-

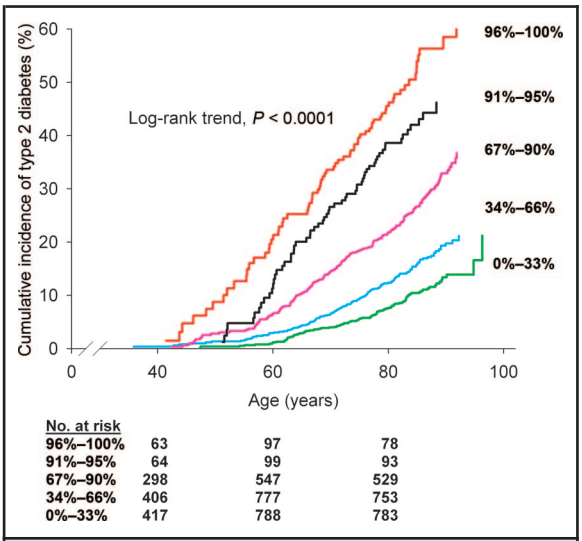

Fig. 1. Cumulative incidence of type 2 diabetes in percentile categories of serum sCD163 concentrations as a function of age in the general population.

Table 3. Risk of type 2 diabetes as a function of serum sCD163 percentile groups in the general population.

	Participants, n (%)[a]	Incidence rate per 10 000 person-years (95% CI)	Age- and sex-adjusted hazard ratio (95% CI)	Trend *P*	Multifactorially adjusted hazard ratio (95% CI)	Trend *P*	Multifactorially and CRP-adjusted hazard ratio (95% CI)	Trend *P*
Serum sCD163				<0.001[b]		<0.001		<0.001
0%–33%	2927	26 (21–32)	1		1		1	
34%–66%	2864	40 (34–47)	1.6 (1.2–2.2)		1.4 (1.0–1.9)		1.3 (1.0–1.8)	
67%–90%	2070	73 (63–84)	3.3 (2.5–4.4)		2.4 (1.8–3.2)		2.2 (1.7–3.0)	
91%–95%	425	118 (90–153)	6.1 (4.2–8.9)		3.8 (2.6–5.5)		3.3 (2.3–4.9)	
96%–100%	410	157 (122–199)	8.7 (6.1–12.5)		5.2 (3.6–7.6)		4.4 (3.0–6.5)	

[a] Nonincident events were excluded, leaving 8696 individuals for Cox regression analysis of type 2 diabetes. In Cox regression models, age was adjusted for by incorporating age in the baseline hazard function (left truncation). In multifactorially adjusted Cox regressions, numbers of participants vary slightly (<4%) according to availability of data. Multifactorial adjustment included age (left truncation), sex, smoking, physical inactivity, BMI, alcohol consumption, systolic blood pressure, and diastolic blood pressure.
[b] *P* for trend over all 5 groups.

lution bias and 2.6 (95% CI, 2.1–3.2) without correction for regression dilution bias.

ABSOLUTE 10-YEAR RISK OF TYPE 2 DIABETES

Absolute 10-year risk of type 2 diabetes increased with increasing sCD163 percentile groups in both women and men and with increasing BMI (≤25 kg/m^2 vs >25 kg/m^2) and age (<50, 50–70, >70) (Fig. 2). In men 50–70 and >70 years of age with BMI >25 kg/m^2, serum sCD163 concentrations predicted an absolute 10-year risk of type 2 diabetes of 29% and 36% in the most extreme percentile group vs 7% and 8% in the lowest percentile group (0%–33%). Equivalent values in women were 19% and 24% vs 4% and 5%.

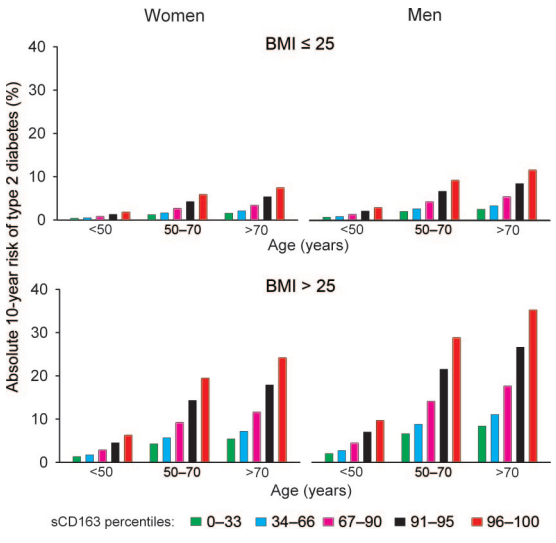

Fig. 2. Absolute 10-year risk of type 2 diabetes by serum sCD163 percentile category, BMI, sex, and age.

Discussion

The principal finding of the present study is that increased serum concentrations of sCD163, independently of age and BMI, predict a highly increased risk of type 2 diabetes. The very high absolute 10-year risk of type 2 diabetes among individuals in the top 5% of sCD163 concentrations implies a role for sCD163 as a marker for detection of high-risk groups, especially among overweight individuals.

It is now widely accepted that obesity and its related diseases are associated with a state of chronic, low-grade inflammation in adipose tissue, with macrophage activation and increased concentrations of several macrophage-derived cytokines such as tumor necrosis factor-α (TNF-α) *(23–26)*. These proinflammatory cytokines are thought to be directly involved in the development of obesity-related disease because they promote insulin resistance, decrease cellular glucose uptake, and increase lipolysis *(6, 25)*; mice lacking TNF-α function are protected from obesity-induced insulin resistance *(27)*.

Macrophages of adipose tissue also strongly express the haptoglobin-hemoglobin receptor CD163 *(12, 28, 29)*. This pathway for removal of hemoglobin is upregulated during inflammation, and inflammatory stimulation of macrophages increases shedding of sCD163 *(11)*. CD163 is shed by proteolytic activity in much the same way that the membrane-bound proform of TNF-α is liberated to the circulation. The function of the circulating sCD163 is largely unknown, and there is no data to suggest a direct role of sCD163 in the

pathogenesis of type 2 diabetes *(30)*. Concentrations of sCD163 have been reported to be increased in various diseases with enhanced loads of activated monocytes/macrophages, such as hemophagocytic syndrome, Gaucher disease, liver diseases, sepsis, and coronary heart disease *(31–36)*.

Previous studies have revealed increased concentrations of sCD163 in obese and diabetic people *(14, 15, 29, 37)*, but the present study reveals that the concentrations of sCD163 predict incident type 2 diabetes and actually discriminate between low- and high-risk type 2 diabetes groups throughout the age spectrum, and importantly, sCD163 can differentiate risk among overweight persons. Only a subset of overweight individuals develops insulin resistance, type 2 diabetes, and other related diseases *(26)*, and consequently, serum markers that identify these particular subsets are of major clinical importance in enabling initiation of preventive strategies or possibly for selection of individuals for medical or surgical treatment *(38)*. We believe that sCD163 in obese patients without an established inflammatory disease reflects the level of adipose tissue inflammation and thus discriminates individuals with low adipose tissue inflammation and low type 2 diabetes risk from those with high adipose tissue inflammation and high type 2 diabetes risk. Soluble CD163 correlates with traditional inflammatory markers such as hsCRP; however, the macrophage-specific nature of CD163 may explain the high risk of diabetes associated with increased sCD163 when adjusting for hsCRP.

Our prospective study involving 8849 individuals from a homogeneous population of whites of Danish ancestry provides high statistical power. However, it limits the transferability of results to other ethnic groups, e.g., Asians, in whom diabetes incidence is high at even lower indices of overweight *(39)*. An important limitation of the present study is the lack of fasting glucose measurements at baseline in this study population, which hinders a direct comparison with sCD163 for diabetes prediction. Also, because this is a register-based study, some individuals may have had unrecognized disease at baseline that might have been identified by fasting glucose concentrations. Furthermore, we were not able to determine to what extent intervention during the study period would modify risk factors and thus the risk of future type 2 diabetes. Finally, sera had been stored for several years, but stability studies of sCD163 have been rigorously performed for only 16 months.

A significant age- and sex-related increase in sCD163 hinders an absolute sCD163 cutpoint value that applies for discrimination between low- and high-risk groups in the entire population. However, the presented use of age- and sex-adjusted percentiles ensures an optimal adjustment of risk estimates and allows for comparison of values across analytical platforms.

In conclusion, in this large prospective study of the general population, we found that increased serum concentrations of sCD163 predict increased risk of type 2 diabetes. Furthermore, the data suggest that sCD163 is a marker for adipose tissue inflammation, and thus for detection of groups at high risk of developing type 2 diabetes, especially among overweight individuals.

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