

## CLINICAL STUDY

# Body iron stores and the risk of type 2 diabetes in middle-aged men

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## Abstract

**Objective:** We investigated the risk of type 2 diabetes mellitus (T2DM) over a wide range of body iron stores.

**Methods:** Prospective cohort of 1613 men in the Kuopio Ischemic Heart Disease Risk Factor study, aged 42–60 years, free of T2DM and hereditary hemochromatosis at baseline in 1984–1989. Baseline serum ferritin (sF) and serum-soluble transferrin receptor (sTfR) concentrations were used to predict incident T2DM. T2DM was assessed by questionnaires, blood glucose measurements, and medication reimbursement register.

**Results:** There were 331 cases of incident T2DM during the mean follow-up of 16.8 years (27 098 person-years). At baseline, subjects who later developed T2DM had average sF concentrations of 191 µg/l (s.d. 155) vs 151 µg/l (s.d. 119) among those who remained healthy,  $P < 0.001$ . In a multivariate-adjusted logistic regression, each 100 µg/l increase in sF corresponded to an average of 14% increased (odds ratio = 1.14, 95% CI 1.03–1.26,  $P = 0.009$ ) risk of developing T2DM. In a Cox regression, a markedly increased risk of developing T2DM was observed from the fourth sF quintile (185 µg/l, the median) upward (hazard ratio (HR) first vs fifth quintile = 1.5, 95% CI 1.0–2.2,  $P$ -trend = 0.05). In a corresponding Cox model in sTfR, the subjects in the third quintile (1840 µg/l, the median) had the least risk (HR = 0.63, 95% CI 0.42–0.97,  $P = 0.04$ ).

**Conclusions:** Body iron within the sF reference range is not an important determinant of T2DM risk, whereas high normal and above is associated with markedly increased risk. Iron depletion toward iron deficiency as assessed by sTfR is not protective against T2DM. A rule of thumb safe range could be 30–200 µg/l of sF.

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## Introduction

Impaired glucose homeostasis, mainly due to  $\beta$ -cell dysfunction, and the later progression to type 2 diabetes mellitus (T2DM) in the iron accumulation disorder hereditary hemochromatosis are well documented (1). Several etiological pathways from iron-induced oxidative damage (2, 3) to impaired insulin sensitivity and increased insulin resistance (4, 5, 6, 7, 8), have been suggested in iron-T2DM hypothesis. Despite the fact that ferritin is an acute-phase reactant that may be elevated in the presence of liver disease, inflammation, and neoplasm, it has been predominantly used as a marker of body iron stores especially in epidemiological studies (9, 10). Serum-soluble transferrin receptor (sTfR) is the soluble fragment of the transferrin receptor domain (a membrane glycoprotein) and it is elevated in expanded or inefficient erythropoiesis such as in iron deficiency (11).

Several observational studies (10, 12, 13, 14, 15, 16), a lifestyle intervention study (17) and a placebo arm of a randomized controlled trial (18) have

examined the association between mildly elevated body iron stores and T2DM with no clear-cut range within which the body iron stores should be maintained. A recent meta-analysis of relevant studies addressing this study question further provided supportive evidence base for mildly elevated body iron stores as a risk factor of T2DM but also did not report the ideal range (19).

Thus, we decided to investigate the risk of T2DM over a wide range of body iron stores, as assessed by serum ferritin (sF), and whether iron depletion toward mild iron deficiency, as assessed by sTfR, offers protection against T2DM.

## Subjects and methods

### Study population

The Kuopio Ischemic Heart Disease Risk Factor (KIHD) study is an ongoing prospective population-based study designed to investigate the risk factors for

cardiovascular disease, atherosclerosis, and other chronic diseases in middle-aged men from Kuopio and the surrounding communities in eastern Finland (20). The baseline examinations were carried out in 1984–1989, with a random sample of men living in the city of Kuopio and its environs. A total of 2682 men (83% of the eligible) in the four age strata, 42, 48, 54, or 60 years old at baseline, were recruited in two cohorts. The first cohort included 1166 men who were aged 54 years as at 1984–1986, and the second cohort included 1516 men aged 42, 48, 54, or 60 years during 1986–1989. This was followed by a 4-year examination round (1991–1993) in which 1038 men from the second cohort (88% of the eligible) participated. At the 11-year examination round (1998–2001), all men from the second cohort were invited and 854 men (95% of the eligible) participated. During the 20-year examination round, all eligible participants from the first and second cohort were invited to the study site. A total of 1241 men (80% of the eligible) participated. We excluded participants with prevalent T2DM at baseline (162), who had not participated in at least one of the re-examination rounds (881), who had no values for sF (23), and who had high ferritin hereditary hemochromatosis (3), leaving a total of 1613 for the current analysis (Supplementary Figure 1, see section on supplementary data given at the end of this article). The cases of high ferritin hereditary hemochromatosis in our data sets were identified based on Cys282Tyr HFE genotyping. There were 553 subjects for whom there was no value for sTfR. The Research Ethics Committee of Kuopio University has approved the KIHd study and the participants gave their written informed consent to participate.

### Data collection

Details of data collection on anthropometry measurements (20), physical activity, which was assessed using the KIHd 12-Month Leisure-Time Physical Activity Questionnaire (21), blood pressure (22), hypertension medication (22), dyslipidemia medication (22), smoking status, number of cigarettes smoked, duration of smoking in years (23), alcohol consumption (23), and dietary intake of foods and nutrients including dietary iron, using a 4-day food recording (24), have been described earlier. The family history of diabetes was defined as positive if a first-degree relative of the subject had a history of diabetes. T2DM was defined as a self-reported physician-set diagnosis of T2DM and/or fasting plasma glucose  $\geq 7.0$  mmol/l (blood glucose  $\geq 6.25$  mmol/l) or 2-h oral glucose tolerance test plasma glucose  $\geq 11.1$  mmol/l (blood glucose  $\geq 9.9$  mmol/l) at any of the study visits and by record linkage to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for T2DM for the entire study period at the end of the study.

### Biomarkers and other measurements

Subjects visited the study site to give blood samples in the morning hours between 0800 and 1000 h, after an overnight fast and abstinence from alcohol use for 3 days and tobacco smoking for 12 h before giving the sample. The baseline sF measurement was assayed from stored serum samples by RIA based on a double-antibody technique (RIA Amersham International, Amersham, UK), and sTfR concentration was assayed from stored samples by the IDEa Transferrin Receptor Immunoassay (ORION Diagnostica, Espoo, Finland). Detailed descriptions of these markers of body iron stores (23), serum LDL (22), serum HDL (22), serum triglyceride (22), serum C-reactive protein (25), and serum gamma-glutamyl transpeptidase (GGT) (26) have been published earlier. The blood glucose measurements were assessed by the glucose dehydrogenase method (27).

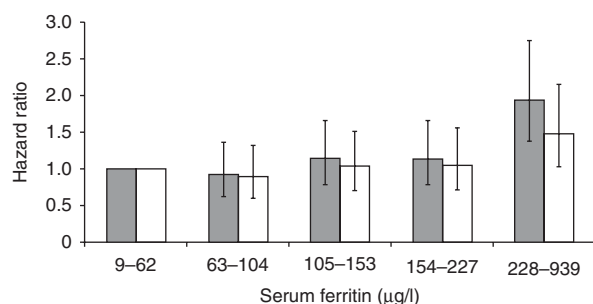
### Statistical analyses

Test of linear trend across quintiles of baseline sF was carried out for relevant characteristics of the study population. Mann–Whitney *U* test was used to compare the sF concentration of those who later developed T2DM and those who remained free of T2DM. Multivariable-adjusted logistic regression model was used to analyze the association between baseline sF and incident T2DM (Table 1). Multivariable-adjusted Cox proportional hazards regression model was used to analyze the association between baseline sF and incident T2DM (Fig. 1) and also the association between baseline sTfR and incident T2DM (Fig. 2).

The potential confounders in our data that were tested for included age (years); examination date (years); BMI ( $\text{kg}/\text{m}^2$ ); serum C-reactive protein concentration ( $\text{mg}/\text{l}$ , high sensitivity method); physical activity (conditioning leisure-time sessions per week); smoking (pack years); systolic and diastolic blood pressure

**Table 1** Multivariate-adjusted odds ratio for incident type 2 diabetes.

Characteristics	Odds ratio	95% CI (low–high)	P value
Age (years)	0.99	0.96–1.01	0.32
Examination date (years)	0.94	0.86–1.02	0.15
Ferritin (per 100 $\mu\text{g}/\text{l}$ )	1.14	1.03–1.26	0.009
BMI ( $\text{kg}/\text{m}^2$ )	1.08	1.01–1.16	0.03
Systolic BP (mmHg)	1.01	1.00–1.02	0.18
Diabetes in family (yes)	1.85	1.41–2.43	<0.001
Drug for hypertension (yes)	1.46	1.05–2.02	0.02
Drug for dyslipidemia (yes)	3.71	1.07–12.89	0.04
Serum GGT (U/l)	1.01	1.00–1.10	0.02
Serum triglyceride (mmol/l)	1.13	0.97–1.33	0.13
Alcohol intake (g/week)	1.01	1.00–1.03	0.07
Waist circumference (cm)	1.02	1.00–1.05	0.08
Smoking (pack years)	1.01	1.00–1.02	0.17
Total energy intake (kcal/day)	1.01	1.00–1.01	0.14



**Figure 1** Risk of type 2 diabetes with increasing serum ferritin quintiles. Gray bars represent the age and examination year-adjusted risk. The white bars represent the fully adjusted risk. The lines indicate the 95% CI.

(mmHg, mean of six measurements); medication for hypertension (yes, %); medication for dyslipidemia (yes, %); family history of diabetes (yes, %); serum LDL, HDL, and triglyceride concentrations (mmol/l); serum GGT (U/l); alcohol intake (grams of absolute ethanol per week); mean waist circumference (cm); and total daily energy intake (kcal, derived from a 4-day food diary).

The relative risk of incident T2DM per 100 µg/l increase in sF concentration, adjusting for covariates, was computed as odds ratio (OR) by logistic regression. The final covariate set was defined by the backward stepwise method,  $P \leq 0.2$ , for removal. The missing values in the continuous covariates were  $< 5\%$  and were replaced by the cohort mean.

Tests of linear trend were conducted by assigning the median values for each category of exposure variable and treating those as a single continuous variable. For the tests of quadratic (non-linear) trend, the variable was squared after centering it at median sTfR value. The statistical analyses were performed using SPSS 19 for Windows, and test for statistical significance (at  $P < 0.05$ ) was two sided.

## Results

The mean age of the subjects was 52.5 years (s.d. 5.7, range 42.0–61.3 years). The mean sF concentration was 159 µg/l (s.d. 128, range 9–939 µg/l) in the whole population. Those with higher sF within the population were more likely to have higher BMI, higher waist circumference, higher blood pressure, higher dyslipidemia, higher intake of alcohol, lower total daily energy intake, and fewer sessions of leisure-time physical activity per week (Table 2). The subjects in the highest sF quintile had the highest blood glucose concentration (4.66 mmol/l (s.d. 0.48),  $P$ -trend  $< 0.001$ ) and the highest serum insulin concentration (13.28 mU/l (s.d. 7.19),  $P$ -trend  $< 0.001$ ) (Table 2).

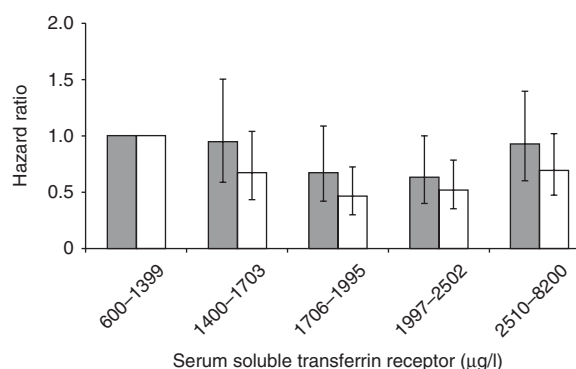
During the average follow-up time of 16.8 years (27 098 person-years), there were 331 cases of incident T2DM among the 1613 men. In the baseline sF quintiles, lowest to highest, we observed 16.0% (51 in

319), 15.8% (52 in 330), 21.2% (68 in 320), 20.3% (66 in 324), and 29.3% (94 in 320) cumulative T2DM cases. At baseline, subjects who later developed T2DM had higher average sF concentration, 191 µg/l (s.d. 155) vs 151 µg/l (s.d. 119), than in subjects that remained free of T2DM ( $P < 0.001$ ). In the backward conditional logistic regression analysis with all the potential confounders in our data set, an increase of 100 µg/l in sF corresponded to an average of 14% increase (OR = 1.14, 95% CI 1.03–1.26,  $P = 0.009$ ) (Table 1) in the risk of developing T2DM.

In the multivariate-adjusted Cox regression model, the subjects in the highest sF quintile had a statistically significant 50% increased (hazards ratio (HR) = 1.5, 95% CI 1.0–2.2,  $P$ -trend = 0.05) risk to develop T2DM during the follow-up, compared with the subjects in the lowest quintile (Fig. 1). Other statistically significant predictors of incident T2DM were BMI, family history of diabetes, hypertension medication, dyslipidemia medication, serum GGT, and alcohol intake (Table 1). A linear increase in the risk of developing T2DM over the normal sF range (30–300 µg/l for male) was not observed; rather, variation in the risk was observed with marked increase in the risk of T2DM between the fourth and fifth sF quintiles (Fig. 1).

In the analysis in which sTfR was used in the multivariable-adjusted Cox regression model, the subjects in the third quintile had the least risk (HR = 0.63, 95% CI 0.42–0.97,  $P = 0.04$ ) of developing T2DM when compared with subjects in the lowest quintile (Fig. 2). In the *post hoc* analysis, we performed a nonlinear trend test that showed a borderline nonlinear trend ( $P = 0.057$ ), suggesting a U-shaped type of association between sTfR and T2DM.

In a subgroup of subjects ( $n = 1060$ ) for whom data were available to compute sTfR to sF ratio (sTfR:sF), those subjects in the lowest quintile had statistically significant highest risk (HR = 1.75, 95% CI 1.06–2.88,  $P$ -trend = 0.17) to develop T2DM in the multivariate-adjusted Cox regression model.



**Figure 2** Risk of type 2 diabetes with increasing serum-soluble transferrin receptor quintiles. Gray bars represent the age and examination year-adjusted risk. The white bars represent the fully adjusted risk. The lines indicate the 95% CI.

**Table 2** Baseline characteristics of the study population of 1613 eastern Finnish men in the Kuopio Ischemic Heart Disease Risk Factor study, 1984–1989.

Characteristics	Serum ferritin quintiles (µg/l)					P-trend
	1 (9–62)	2 (63–104)	3 (105–153)	4 (154–227)	5 (228–939)	
Blood glucose (mmol/l)	4.50±0.45	4.48±0.44	4.56±0.44	4.58±0.47	4.66±0.48	<0.001
Serum insulin (mU/l)	9.70±4.70	9.44±4.32	10.46±6.86	11.03±5.59	13.28±7.19	<0.001
Age (years)	53±5.5	53±5.6	53±5.6	52±6.0	51±5.7	<0.001
BMI (kg/m <sup>2</sup> )	25.7±3.0	26.1±3.0	26.6±3.2	26.9±3.0	27.9±3.4	<0.001
Smoking (pack years)	7.0±15.1	6.4±14.3	7.1±14.2	5.6±13.3	5.4±12.3	0.11
Systolic BP (mmHg)	130±15	131±16	132±16	132±16	135±15	<0.001
Diastolic BP (mmHg)	86±10	87±10	87±9	88±10	90±10	<0.001
Drug for high BP (yes %)	12.9	19.7	25	18.5	19.4	0.09
Drug for dyslipidemia (yes %)	1.6	0.9	0.9	0.3	0	0.01
Serum C-reactive protein (mg/l)	1.9±3.0	1.7±2.4	2.5±5.8	2.3±3.5	2.2±4.6	0.09
Serum GGT (U/l)	23.2±26.8	21.6±13.4	24.4±17	27.4±19.1	35.3±31	<0.001
Serum LDL-cholesterol (mmol/l)	3.86±1.00	4.02±0.97	3.97±1.01	3.92±0.97	3.96±0.89	0.54
Serum HDL-cholesterol (mmol/l)	1.34±0.31	1.34±0.32	1.30±0.28	1.27±0.29	1.27±0.31	<0.001
Serum triglyceride (mmol/l)	1.15±0.59	1.23±0.63	1.24±0.67	1.33±0.91	1.55±1.03	<0.001
Alcohol intake (g/week)	41.4±70.3	56.4±92.3	64.6±102.9	70.7±99.3	106.0±164.6	<0.001
Total energy intake (kcal/day)	2433±624	2447±566	2416±684	2336±609	2285±588	<0.001
Waist circumference (cm)	87.9±8.3	89.0±8.2	89.9±8.7	90.6±8.3	93.3±8.9	<0.001
Leisure-time physical activity (sessions/week)	143±151	131±132	146±145	117±125	119±127	0.009
<sup>a</sup> sTfR (µg/l)	1914±606	1911±723	1891±633	1952±708	2023±712	0.07
<sup>a</sup> sTfR/sF	48.4±19.5	23.6±9.9	15.0±5.3	10.6±4.0	6.3±2.6	<0.001

<sup>a</sup>Study population, *n*=1060.

## Discussion

In this prospective cohort study, higher body iron stores, as assessed by the sF concentration, was associated with increased risk of T2DM. However, iron depletion toward mild iron deficiency, as assessed by the sTfR concentration, was not protective against T2DM. When observed over the wide sF range, there appeared to be little variation in the risk of T2DM, with marked increase in the risk only between the fourth and fifth sF quintiles (median values of 185 and 310 µg/l respectively) (Fig. 1). After adjusting for confounders, we observed that there seemed to be a U-shaped association between sTfR concentration and the risk of T2DM, with the lowest risk at the middle quintile.

In overt iron overload such as in hereditary hemochromatosis, iron accumulation deteriorates glucose homeostasis and results in T2DM (28). Also, in non-iron overload disorders, accumulating epidemiological studies (10, 12, 13, 14, 15, 16) have found supportive evidence for iron-T2DM hypothesis. In a recent meta-analysis of six epidemiological studies that investigated the association between body iron stores and the risk of T2DM, a pooled relative risk for T2DM in an individual with the highest vs the lowest sF concentrations was 1.70 (19).

Still, none of these studies suggested any range within which the body iron stores should be maintained, and most of them concluded on mild-to-moderate elevated body iron stores as a risk factor for T2DM. In line with these studies, we also found that for every 100 µg/l

increase in sF, there was an average 14% increase in the risk of developing T2DM.

The normal range of sF could vary between laboratories, but in males, 30–300 µg/l is the common reference range. We observed from our data that the risk of developing T2DM begins to be markedly increased at around 185 µg/l (the median of the fourth quintile of sF) (Fig. 1). Our data further suggested that the risk of T2DM is the least at the third quintile of sTfR (Fig. 2). On the basis of findings recorded in Figs 1 and 2, a rule of thumb safe range for body iron with regard to T2DM risk could be 30–200 µg/l of sF. Indirect supportive evidence was observed in a cross-sectional study conducted by Fernandez-Real *et al.* (29) in Spain with 181 middle-aged healthy men, in which they investigated the relationship between iron stores and insulin sensitivity between blood donors and non-donors. They showed that frequent blood donors, who had the mean sF concentration maintained at 101.5 µg/l, had increased insulin sensitivity and decreased insulin secretion compared with non-donors who had the mean sF concentration of 162 µg/l. An interventional study by Facchini (30) assessed the effect of lowering of body iron stores by phlebotomy on plasma glucose and insulin concentration. He found that lowering sF from 75 to 38 µg/l led to 37 and 19% reduction in 2-h plasma insulin and glucose concentrations respectively in ten healthy individuals. Also, a few controlled interventional studies have demonstrated the positive effect of iron depletion on insulin resistance (31, 32), which could be more pronounced in subjects with



elevated baseline sF than in those with relatively normal baseline sF (31). These studies (29, 30) suggest that maintaining the sF below high normal could possibly prove useful in reducing the prevalence of T2DM.

In the analysis in our data using sTfR, we observed that the subjects in the third, fourth, and fifth quintiles of sTfR had 37, 33, and 6% respectively reduction in the risk of developing T2DM when compared with subjects in the lowest quintile. It was speculated that if iron excess could increase the risk of T2DM, then iron depletion toward iron deficiency should induce the reverse phenomenon of protection (30). This of course partially holds within sF reference range as demonstrated in our study, but further depletion of body iron stores nearing iron deficiency, using sTfR as the indicator, appeared not to offer additional protection against T2DM. Although sTfR assay is of valuable clinical importance because the concentration does not increase in anemia of chronic inflammation but rather in iron-deficient erythropoiesis and iron deficiency anemia, its concentration may also increase in conditions or processes that stimulate erythropoiesis, such as hemolytic anemia, erythropoietin therapy, and erythroid dysplasia or hyperplasia (11).

Our finding is supported by the results of a prospective study among 54 non-diabetic premenopausal women that investigated the effect of iron deficiency on insulin resistance (33). After treatment with oral iron preparation, a statistically significant decrease in fasting insulin concentrations following the anemia treatment was observed in younger women (<40 years) and in women with low BMI (<27 kg/m<sup>2</sup>). Also, Ford *et al.* (34) found in a cross-sectional study of 8296 adults who participated in the National Health and Nutrition Examination Survey (NHANES) that iron deficiency tends to increase HbA1c level rather than decreasing it. In addition, Shakoury-Elizeh *et al.* (35) showed that iron deficiency leads to impairment in glucose metabolism by altering glucose storage.

It is not far-fetched that iron-deficient cells will exhibit depletion of iron enzymes and thus affect processes in which iron serves as a cofactor. Every cell in the body, including the hepatocytes, needs a minimum level of iron, excess of which on the other hand is toxic to the cell. Thus, the optimal range needs to be clearly established.

The mechanism behind the association between body iron stores and T2DM is still not known in detail, but oxidative damage by excess iron stores in the liver, pancreas, and the muscle has been reported (2, 3), with subsequent insulin resistance (4, 5, 8). Also, the oxidative damage of the lipid cell membrane may be a predisposing factor for decreased cellular glucose uptake (36). Others include iron-regulated adiponectin and iron-mediated adipocyte insulin resistance (37, 38).

The prospective design and the long follow-up are the strengths of our study. A limitation in this study is the data on biomarkers of body iron stores that were from a

single blood sample, which may have changed during the follow-up and may not thus accurately reflect the exposure level during the relevant time period. Because of the large proportion of missing data in the sTfR concentration ( $n_m = 553$ ) in the studied population, we could not compute for all study participants the sTfR/sF, which has been proposed as the preferred measure of body iron stores by some authors (39). Over 40% of the subjects who later developed T2DM were lost in the subgroup analysis, reducing the power to find a significant trend in the multivariate-adjusted Cox regression model. It is known that elevated sF can be found in other conditions that are risk factors of T2DM, such as obesity, metabolic syndrome, inflammation, and liver disease. Thus, we extensively adjusted our results for these conditions by controlling for BMI, waist circumference, serum GGT, serum C-reactive protein, alcohol consumption, and medications for hypertension and dyslipidemia. However, we cannot completely exclude the effect of residual confounding. The relatively few incident cases of T2DM within the normal sF range did not allow for subgroup analyses strictly within this range.

In conclusion, accumulating epidemiological findings suggest elevated body iron stores as a risk factor for developing T2DM, but the safety range is less known. Our study has provided additional knowledge about the likely threshold of sF at which the risk of T2DM becomes marked, the sTfR range within which the risk of T2DM is expected to be the least, and a rule of thumb safe range of sF with regard to T2DM risk. However, these findings would require more observational studies reporting the likely thresholds in their data as well.

## Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-13-0145>.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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## Author contribution statement

A Aregbesola, S Voutilainen, J K Virtanen, and T-P Tuomainen were involved in the conception and design of the study including quality assurance and control. A Aregbesola, T-P Tuomainen, S Voutilainen, and J K Virtanen were involved in the acquisition of data. A Aregbesola, S Voutilainen, J K Virtanen, J Mursu, and T-P Tuomainen were involved in literature search, material and methods, and study analytical concepts. A Aregbesola, T-P Tuomainen, J K Virtanen, S Voutilainen, and J Mursu were involved in analysis and interpretation of data. A Aregbesola, S Voutilainen,

J K Virtanen, J Mursu, and T-P Tuomainen were involved in discussion section. A Aregbesola, S Voutilainen, J K Virtanen, J Mursu, and T-P Tuomainen were involved in final review and approval of the version to be published.

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