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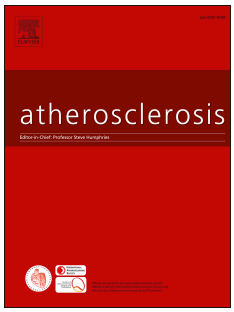

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Association between plasma CD36 levels and incident risk of coronary heart disease among Danish men and women

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ABSTRACT

Background and aims: CD36 is a cholesterol receptor involved in the uptake of oxidized low-density lipoprotein cholesterol and development of atherosclerotic plaques. Cross-sectional studies have shown correlations between plasma CD36 and atherosclerosis but no prospective study has examined the association yet. We prospectively examined the association between plasma CD36 levels and risk of incident coronary heart disease (CHD) in a Danish population.

Methods: Plasma CD36 levels were measured in a case-cohort study nested within the Danish population-based cohort, the Diet, Cancer and Health Study. A total of 1,963 incident CHD events occurred between baseline (1993-1997) and 2008, and a sub-cohort of 1,759 participants were randomly selected as reference. Cox proportional hazard regression models were used to compute the hazard ratio (HR) and corresponding 95% confidence interval (CI).

Results: After adjusting for CHD risk factors, including history of hypercholesterolemia and diabetes, elevated plasma CD36 levels were not associated with higher CHD risk in the total population, and the HR comparing the highest *versus* lowest tertile of CD36 levels was 1.02 (95% CI 0.84-1.23). High CD36 levels were only found to be associated with risk of CHD in combination with prevalent diabetes (HR=2.83, 95% CI: 1.08-7.45) *vs*. the joint reference group of lowest CD36 tertile and no diabetes.

Conclusions: Plasma CD36 levels were not predictive of CHD risk in the general population.

Keywords: case-cohort study, coronary heart disease, plasma CD36, prospective study

INTRODUCTION

Coronary heart disease (CHD) arising from atherosclerosis is a leading cause of death and morbidity worldwide [1]. Atherosclerosis is considered a chronic inflammatory disease consists of plaque initiation, progression and thrombosis [2]. The transmembrane glycoprotein CD36 is an important multi-ligand class B scavenger receptor in monocytes and macrophages that internalizes oxidized low-density lipoprotein (ox-LDL) cholesterol in the subendothelial spaces of arteries and subsequently differentiates the macrophages into foam cells, which is the hallmark of early atherosclerotic lesions [3, 4]. In addition, CD36 is also a fatty acid transporter in metabolically active tissues (muscle, liver and adipocytes) that is implicated in the development of insulin resistance [5-7], which is another important risk factor for developing atherosclerosis [8]. The importance of CD36 in the pathogenesis of atherosclerosis has been shown in animal studies where double apoE/CD36 knockout mice who develop significantly smaller atherosclerotic lesions compared to the wild-type controls [9], have a doubling in lesion area when CD36 is reintroduced [10]. In addition, several human genome-wide linkage studies have shown that the location of the *CD36* gene locus on chromosome 7q is associated with myocardial infarction and stroke [11]. In addition, in comparison to individials with asyumptomatic carotid plaques, CD36 gene expression has been found to be up-regulated in patients with symptomatic carotid plaques [12]. This suggests that CD36 could be a useful biomarker in the early development of cardiovascular disease.

The expression of CD36 is increased in macrophages, smooth muscle cells, and endothelial cells in atherosclerosis plaques [13]. However, previous studies of membrane CD36 in monocytes and macrophages require fresh blood samples for measurement [14-17], and thus were not well suited for large population-based epidemiological studies. To tackle this issue, Handberg et al. [18] developed an assay to analyze the stored plasma samples, and

identified a circulating form of CD36 in human plasma. Plasma CD36 was hypothesized to be released into the circulation as part of the low-grade inflammatory state in insulin resistance and atherosclerosis [18], and the levels have been found to be moderately correlated with membrane CD36 expression in liver tissue [19]. While two cross-sectional studies have reported correlations between plasma CD36 and carotid atherosclerosis among both healthy populations and patients with high-grade internal carotid stenosis [20, 21], one study did not find such correlation among patients with early coronary artery disease [22]. Thus far, no prospective studies have been conducted to evaluate the association between plasma CD36 and risk of CHD.

Therefore, we conducted a case-cohort study in a large population-based cohort among Danish men and women to examine the association between plasma CD36 and risk of CHD. We also investigated whether this association is modified by important cardiovascular risk factors including obesity, smoking status, as well as history of hypercholesterolemia and diabetes.

MATERIALS AND METHODS

Study population

The Danish Diet, Cancer and Health study is an ongoing prospective study established between 1993 and 1997, and recruited 57,053 cancer-free participants aged between 50 and 65 years who lived in the urban areas of Copenhagen and Aarhus [23]. At baseline, participants filled out self-administered lifestyle questionnaires, and the questions included self-reported type 2 diabetes and hypercholesterolemia. In addition, technicians obtained anthropometric measurements and collected non-fasting blood samples at the study clinic. Blood specimens were separated into plasma, serum, lymphocytes, and erythrocytes and frozen at −150° C within two hours of collection. The detailed design of the study has

described previously [23]. The study protocol complied with the Helsinki declaration and was approved by the National Committee on Health Research Ethics and the Danish Data Protection Agency (KF 01-116/96). Informed consent was completed and obtained from all participants at the baseline interview.

CHD cases were identified via the National Diabetes Registry using the personal identification number assigned to all Danish citizens in the Danish Civil Registration System. Cases were identified when participants registered with a first-time discharge diagnosis of myocardial infarction (International Classification of Diseases [ICD], 8th revision codes 410 to 410.99; and ICD 10th revision codes I21.0-I21.9) [24, 25]. Medical records were retrieved from hospitals, reviewed in accordance with current guidelines [26], and myocardial infarctions diagnoses in the National Diabetes Registry are recorded with a high degree of validity [27]. Furthermore, we included participants with a sudden cardiac death diagnosis in the Cause of Death Register (ICD 8: 427.27 or ICD 10: I46.0-I46.9) if the cardiac arrest after validation was believed to be caused by a myocardial infarction.

We investigated the association between plasma CD36 and risk of CHD (non-fatal myocardial infarction and fatal CHD) in a case-cohort study nested within the Danish Diet, Cancer and Health study. For the current analysis, all confirmed incident cases between study entry and May 2008 (n=1,977) were included along with a randomly chosen sub-cohort of participants drawn from the entire study population at baseline (n=1,824). After additional exclusion of participants with missing covariate values, the case-cohort included 1,963 incident CHD cases (58 within the reference sub-cohort) and 1,701 non-cases (sub-cohort total n=1,759).

Biochemical measurements

For the measurement of CD36 concentrations, plasma samples from the baseline exam were sent to Aarhus University hospital and Handberg's *in-house* ELISA assay was

used [18]. While phosphate-buffered saline was served as background, a pool of ethylenediaminetetraacetic acid (EDTA) plasma was applied in increasing dilutions and used to produce a standard concentration curve. Absorptions were calculated relative to the standard EDTA plasma pool and expressed as relative units. Internal controls consisting of an EDTA plasma pool and recombinant CD36 (generously donated by Randox, Laboratories [Antrim, United Kingdom]) were run in duplicates and in four concentrations on each plate. Analytical runs were accepted if one of the internal controls was within mean ± 1 standard deviation (SD) and the other control was within ± 2 SD. The intra-assay coefficient of variation (CV) was 11% (plasma pool, mean 0.14 arbitrary units), and total day-to-day assay CV was 25% (plasma pool) and 19% (recombinant CD36). The relatively high CVs (≥15%) suggested the existence of moderate variability between batches. To account for batch variability, we performed recalibration by regressing CD36 levels on batch and other variables associated with CD36 levels including age, sex, smoking status, alcohol intake, and education [28], that might have been unevenly distributed across batches by chance.

Statistical methods

We evaluated the baseline characteristics of participants who developed CHD during follow-up and the random sub-cohort members separately with medians and 5^{th}/95^{th} percentiles. The difference of plasma CD36 levels between gender, smoking status, adiposity level, as well as history of diabetes and hypercholesterolemia were examined by two-tailed t tests in sub-cohort population with adjustment for age and sex. Plasma levels of CD36 were categorized into tertiles based on the distribution of CD36 in sub-cohort participants. Cox proportional hazard regression using age as the underlying time-scale with standard inverse probability weights and robust variation to account for the case-cohort design was used to estimate the hazard ratio (HR) and corresponding 95% confidence interval (CI) of CHD comparing the highest *versus* lowest tertile of plasma CD36 levels. Person-years were

calculated from the study entry to diagnosis of CHD, death, emigration, or end of follow-up in 2008, whichever came first. Multivariable model was adjusted for potential confounders including age (continuous), sex (men, women), smoking (never; former; current <15, 15-24, ≥25 grams of tobacco/day), length of school education (short < 8; medium 8-10; long >10 years), BMI (continuous), alcohol intake (nondrinker; drinker, <5, 5-9, 10-19, 20-39, ≥40 grams of alcohol/day), as well as self-reported hypercholesterolemia and diabetes (yes, no). In addition, we also examined the possible non-linear relation between plasma CD36 and CHD risk using restricted cubic spline regression with 3 knots at 25^{th}, 50^{th} and 75^{th} percentiles of plasma CD36 concentrations. If no deviation from linearity was detected, we also calculated the CHD risk associated with per SD increment of plasma CD36. Moreover, age- and sex-adjusted means of plasma CD36 levels were compared between subgroups of sex (men, women), smoking status (current smokers, non-smokers), history of diabetes (yes, no), body mass index (<25 kg/m^2, 25-<30 kg/m^2, ≥30 kg/m^2) and history of hypercholesterolemia (yes, no). Furthermore, we evaluated the joint effect between tertiles of plasma CD36 and different cardiovascular risk factors, using the lowest tertile of plasma CD36 and the low-risk category of each risk factor as the reference. This corresponds to the evaluation of biological interaction on the additive scale, as per Rothman. From these results, we can qualitatively judge whether the combined exposure to high CD36 and a risk factor, such as diabetes, is greater than expected based on the independent “effect” of each. To get a *P* for interaction, we used the multiplicative model where plasma CD36 was modeled continuously and included also the risk factor and interaction term between them. Data were analyzed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina). Two-sided *p* values of <0.05 were considered to be statistically significant.

RESULTS

Population characteristics

The characteristics of the case-cohort participants are shown in **Table 1**. The median age at baseline was 58 years for the participants that developed CHD and 55 years for the randomly selected sub-cohort individuals. In comparison to the sub-cohort participants, those who developed CHD were more likely to be male, current smokers, had higher BMI, lower education, and history of diagnosed hypercholesterolemia or diabetes. In addition, cases had higher concentrations of plasma CD36 compared with sub-cohort participants. Within the sub-cohort, the mean value of plasma CD36 levels was substantially higher among men compared to women (p=0.04; **Table 2**), and among obese participants compared to subjects with normal weight (p=0.02; **Table 2**).

Associations of plasma CD36 and CHD risk

After adjustment for age, sex and lifestyle factors, elevated plasma CD36 levels were not associated with higher risk of CHD; the HR comparing the highest *versus* lowest tertile of plasma CD36 levels was 1.02 (95% CI 0.84- 1.23; **Table 3**). Restricted cubic spline regression analysis did not suggest a non-linear relationship between plasma CD36 and CHD risk (p=0.99 for nonlinearity; **Supplementary Figure 1**). When modelling plasma CD36 as a continuous variable, the HR (95% CI) for CHD with per-1 SD increment in plasma CD36 was 1.01 (0.93-1.07) in the multivariable model. The results were materially unchanged after further adjustment for postmenopausal status and hormone therapy. In sensitivity analyses, we repeated the analysis after trimming CD36 levels by 5^{th} and 95^{th} percentile, and found a similar association between plasma CD36 and CHD risk (HR per SD:1.05; 95% CI 0.92-1.19).

Joint analysis with cardiovascular risk factors

In joint models, a higher CD36 level did not add to the risk of CHD beyond sex,

198 smoking, obesity, and hypercholesterolemia (**Table 4)**. However, elevated plasma CD36
199 levels were associated with higher CHD-risk among people with diabetes. Compared with
200 participants who were in the lowest plasma CD36 tertile and free of diabetes, higher CD36
201 levels were not associated with higher CHD-risk among non-diabetic individuals (HR
202 comparing the highest *versus* lowest tertile of plasma CD36 levels was 1.00 (95% CI 0.83-
203 1.21), whereas the HR among participants was 2.83 (95% CI: 1.08, 7.45) for diabetic
204 participants with the highest CD36 level. Nevertheless, no significant interactions were
205 observed between CD36 with all these cardiovascular risk factors (all *P*-interaction >0.05).
206

207 **DISCUSSION**

208 In this large, prospective case-cohort study among Danish men and women, elevated
209 plasma CD36 levels were not associated with higher CHD-risk in the overall population.
210 However, a suggestive positive association between elevated plasma CD36 levels and higher
211 CHD-risk was observed among participants with prevalent diabetes.

212 Thus far, only cross-sectional studies have been conducted to explore the relationship
213 between plasma CD36 and presence of atherosclerosis [20-22]. Among 62 Norwegian
214 patients with high-grade internal carotid stenosis, Handberg et al. [21] found that patients
215 with echolucent carotid plaques had higher plasma CD36 than those with
216 echogenic/heterogeneous plaques, and suggested that CD36 may play a critical role in plaque
217 instability and symptomatic carotid atherosclerosis. Furthermore, a study of 1029 healthy
218 individuals from 14 European countries found a weak correlation (r=0.10; p<0.01) between
219 plasma CD36 and carotid atherosclerosis as reflected by intima-media thickness [20].
220 However, in contrast, a recent study from Poland did not find any significant correlations
221 between plasma CD36 concentrations and atherosclerosis (using a comprehensive set of
222 radiological parameters) among 70 patients with early-onset coronary artery disease [22].

Reverse causality is a concern in these reports since the temporal relations cannot be determined from cross-sectional studies. To the best of our best knowledge, the current study is the first prospective population-based study to investigate the association between plasma CD36 and CHD-risk. During the 14-year follow-up, we did not observe a positive association between plasma CD36 levels and CHD risk in the general population, but we found a moderate positive association among participants with self-reported diabetes. However, given the multiple statistical tests and small number of diagnosed diabetes cases, our observed association between plasma CD36 and CHD among participants with prevalent diabetes could also be due to chance, and should be interpreted with caution.

Although the underlying mechanism is not clear yet, plasma CD36 was previously hypothesized to be released into the circulation as part of the low-grade inflammatory state in insulin resistance and atherosclerosis in a previous study [18]. Plasma CD36 levels moderately correlated with membrane CD36 expression in liver tissue (correlation=0.37; p=0.07) [19], and elevated plasma CD36 levels were observed in obese people and patients with type 2 diabetes, in accordance with raised tissue CD36 expression reported by others [5, 15, 16, 29, 30].

Several lines of experimental evidence also suggests that membrane CD36 is implicated in the pathophysiology of developing insulin resistance and atherosclerosis [31-33]. In the presence of high glucose levels or insulin resistance, membrane CD36 transcription and expression is upregulated and could lead to an almost 10-fold increase in CD36 mediated ox-LDL uptake [15, 29, 34], and thus may provide a mechanism for accelerated atherosclerosis in diabetic patients [29]. In addition, ox-LDL uptake by CD36 has shown to be dependent on the fatty acid that simultaneously binds to the same receptor [35]. Interestingly, recent studies have demonstrated that medications (peroxisome proliferator-activated receptor-gamma agonist and metformin), exercise and food extracts

(green tea polyphenols and cinnamon) could decrease CD36 expression in animal models and plasma levels in humans [34, 36-39]. Future studies should investigate whether such interventions might lower plasma CD36 among people with type 2 diabetes, and if lowering the plasma CD36 level translates into decreasing CHD-risk

Given the previous associations particularly with echolucent plaques, we propose that it would be worthwhile to expand the endpoint from pure CHD to all cardiovascular disease (such as including stroke events). Our current study only included CHD events and we cannot exclude that associations with stroke might be stronger.

Our study has the strength of assessing the association between plasma CD36 and CHD risk, as well as exploring potential interactions with other CVD risk factors. In addition, the present study is a prospective design with large sample size; hence the recall bias in the exposure data prior to CHD diagnosis does not exist. However, some limitations merit consideration. First, we were only able to investigate the risk of CHD as we did not have plasma samples from stroke cases. Moreover, type 2 diabetes cases were self-reported in the current study and not identified by standardized blood testing, thus, underestimation of the type 2 diabetes may exist. In addition, we included relatively small number of diabetes cases and thus may have limited statistical power for the stratified analyses, however, our direction of association pointed towards the same direction compared to previous observations [18, 40, 41]. Furthermore, we observed moderate batch variability, which was accounted for by batch-recalibration. However, even though this methodology can break any potential association between CD36 and potential confounders in the final Cox models, a smaller CV for the CD36 measurement would provide greater statistical power and precision in our analysis. As such, we cannot exclude that the largely null result in our study could be partly explained by our measurement error. Additionally, the present study was conducted among a Caucasian population living in Northern Europe, and the results may not be applicable to

other ethnic groups.

Conclusions

In conclusion, we have observed that elevated plasma CD36 levels not associated with higher CHD risk in a general population. A tendency for a higher risk was observed among participants with diabetes. Plasma (or circulating) CD36 concentration could be an interesting new marker that may link diabetes and atherosclerosis but future longitudinal studies are needed to examine the role of CD36 and risk of cardiovascular disease, particularly stroke, and to validate our findings in other ethnic groups.

Conflict of interest

Dr. Handberg and the Ideas Clinic at Aalborg University Hospital hold two patents for the measurement of CD36 in plasma: "Method of evaluation of the relative risk of developing atherosclerosis in patients" 2006, WO2005/116644 and ”A method for diagnosing atherosclerotic plaques by measurement of CD36”, 2008,WO2008/ 095492.

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Author contributions

MKJ conceived the study, interpreted the data, and critically revised the reports. JZ analyzed and interpreted the data, and drafted the reports. YW drafted and critically revised the reports. AH measured the plasma samples of CD36 and critical revised the reports. KO, AT and EBR critically revised the reports.

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REFERENCES

[1] C. Weber, H. Noels, Atherosclerosis: current pathogenesis and therapeutic options, Nat. Med. 17 (2011) 1410-1422.

[2] S. Gautam, M. Banerjee, The macrophage Ox-LDL receptor, CD36 and its association with type II diabetes mellitus, Mol. Genet. Metab. 102 (2011) 389-398.

[3] Y. Iwashima, M. Eto, A. Hata, et al., Advanced glycation end products-induced gene expression of scavenger receptors in cultured human monocyte-derived macrophages, Biochem. Biophys. Res. Commun. 277 (2000) 368-380.

[4] J.M. Zingg, R. Ricciarelli, E. Andorno, et al., Novel 5' exon of scavenger receptor CD36 is expressed in cultured human vascular smooth muscle cells and atherosclerotic plaques, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 412-417.

[5] D.P. Koonen, R.L. Jacobs, M. Febbraio, et al., Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity, Diabetes. 56 (2007) 2863-2871.

[6] R.W. Schwenk, G.P. Holloway, J.J. Luiken, et al., Fatty acid transport across the cell membrane: regulation by fatty acid transporters, Prostaglandins. Leukot. Essent. Fatty Acids. 82 (2010) 149-154.

[7] X. Su, N.A. Abumrad, Cellular fatty acid uptake: a pathway under construction, Trends. Endocrinol. Metab. 20 (2009) 72-77.

[8] J. Nigro, N. Osman, A.M. Dart, et al., Insulin resistance and atherosclerosis, Endocr. Rev. 27 (2006) 242-259.

[9] M. Febbraio, E.A. Podrez, J.D. Smith, et al., Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice, J. Clin. Invest. 105 (2000) 1049-1056.

[10] M. Febbraio, E. Guy,R.L. Silverstein, Stem cell transplantation reveals that absence of macrophage CD36 is protective against atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 2333-2338.

[11] R. Sherva, M.B. Miller, J.S. Pankow, et al., A whole-genome scan for stroke or myocardial infarction in family blood pressure program families, Stroke. 39 (2008) 1115-1120.

[12] T.B. Dahl, A. Yndestad, M. Skjelland, et al., Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization, Circulation. 115 (2007) 972-980.

[13] J.M. Zingg, R. Ricciarelli, A. Azzi, Scavenger receptor regulation and atherosclerosis, Biofactors. 11 (2000) 189-200.

[14] M. Piechota, A. Banaszewska, J. Dudziak, et al., Highly upregulated expression of CD36 and MSR1 in circulating monocytes of patients with acute coronary syndromes, Protein. J. 31 (2012) 511-518.

[15] M.J. Sampson, I.R. Davies, S. Braschi, et al., Increased expression of a scavenger receptor (CD36) in monocytes from subjects with Type 2 diabetes, Atherosclerosis. 167 (2003) 129-134.

[16] A. Bonen, M.L. Parolin, G.R. Steinberg, et al., Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36, Faseb. J. 18 (2004) 1144-1146.

[17] A. Bonen, N.N. Tandon, J.F. Glatz, et al., The fatty acid transporter FAT/CD36 is upregulated in subcutaneous and visceral adipose tissues in human obesity and type 2 diabetes, Int. J. Obes. 30 (2006) 877-883.

[18] A. Handberg, K. Levin, K. Hojlund, et al., Identification of the oxidized low-density lipoprotein scavenger receptor CD36 in plasma: a novel marker of insulin resistance, Circulation. 114 (2006) 1169-1176.

[19] S. Heeboll, M.K. Poulsen, M.J. Ornstrup, et al., Circulating sCD36 levels in patients with non-alcoholic fatty liver disease and controls, Int. J. Obes. 41 (2017) 262-267.

[20] A. Handberg, K. Hojlund, A. Gastaldelli, et al., Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population, J. Intern. Med. 271 (2012) 294-304.

[21] A. Handberg, M. Skjelland, A.E. Michelsen, et al., Soluble CD36 in plasma is increased in patients with symptomatic atherosclerotic carotid plaques and is related to plaque instability, Stroke. 39 (2008) 3092-3095.

[22] M. Rac, A. Krzystolik, M. Rac, et al., Is plasma-soluble CD36 associated with density of atheromatous plaque and ankle-brachial index in early-onset coronary artery disease patients?, Kardiol. Pol. 74 (2016) 570-575.

[23] A. Tjonneland, A. Olsen, K. Boll, et al., Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: a population-based prospective cohort study of 57,053 men and women in Denmark, Scand. J. Public Health. 35 (2007) 432-441.

[24] T.F. Andersen, M. Madsen, J. Jorgensen, et al., The Danish National Hospital Register. A valuable source of data for modern health sciences, Dan. Med. Bull. 46 (1999) 263-268.

[25] C.B. Pedersen, H. Gotzsche, J.O. Moller, et al., The Danish Civil Registration System. A cohort of eight million persons, Dan. Med. Bull. 53 (2006) 441-449.

[26] R.V. Luepker, F.S. Apple, R.H. Christenson, et al., Case definitions for acute coronary heart disease in epidemiology and clinical research studies: a statement from the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on Epidemiology and Prevention; the European Society of Cardiology Working Group on Epidemiology and Prevention; Centers for Disease Control and Prevention; and the National Heart, Lung, and Blood Institute, Circulation. 108 (2003) 2543-2549.

376 [27] A.M. Joensen, M.K. Jensen, K. Overvad, et al., Predictive values of acute coronary
377 syndrome discharge diagnoses differed in the Danish National Patient Registry, J. Clin.
378 Epidemiol. 62 (2009) 188-194.
379 [28] B. Rosner, N. Cook, R. Portman, et al., Determination of blood pressure percentiles in
380 normal-weight children: some methodological issues, Am. J. Epidemiol. 167 (2008) 653-666.
381 [29] E. Griffin, A. Re, N. Hamel, et al., A link between diabetes and atherosclerosis: Glucose
382 regulates expression of CD36 at the level of translation, Nat. Med. 7 (2001) 840-846.
383 [30] C. Aguer, J. Mercier, C.Y. Man, et al., Intramyocellular lipid accumulation is associated
384 with permanent relocation ex vivo and in vitro of fatty acid translocase (FAT)/CD36 in obese
385 patients, Diabetologia. 53 (2010) 1151-1163.
386 [31] J.F. Glatz, J.J. Luiken,A. Bonen, Membrane fatty acid transporters as regulators of lipid
387 metabolism: implications for metabolic disease, Physiol. Rev. 90 (2010) 367-417.
388 [32] D.P. Koonen, M.K. Jensen,A. Handberg, Soluble CD36- a marker of the
389 (pathophysiological) role of CD36 in the metabolic syndrome?, Arch. Physiol. Biochem. 117
390 (2011) 57-63.
391 [33] M. Snel, J.T. Jonker, J. Schoones, et al., Ectopic fat and insulin resistance:
392 pathophysiology and effect of diet and lifestyle interventions, Int. J. Endocrinol. 2012 (2012)
393 983814.
394 [34] C.P. Liang, S. Han, H. Okamoto, et al., Increased CD36 protein as a response to defective
395 insulin signaling in macrophages, J. Clin. Invest. 113 (2004) 764-773.
396 [35] A.G. Jay, A.N. Chen, M.A. Paz, et al., CD36 binds oxidized low density lipoprotein
397 (LDL) in a mechanism dependent upon fatty acid binding, J. Biol. Chem. 290 (2015)
398 4590-4603.

399 [36] D. Glintborg, K. Hojlund, M. Andersen, et al., Soluble CD36 and risk markers of insulin
400 resistance and atherosclerosis are elevated in polycystic ovary syndrome and significantly
401 reduced during pioglitazone treatment, Diabetes Care. 31 (2008) 328-334.
402 [37] A.C. Smith, K.L. Mullen, K.A. Junkin, et al., Metformin and exercise reduce muscle
403 FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced
404 hyperglycemia, Am. J. Physiol. Endocrinol. Metab. 293 (2007) E172-181.
405 [38] B. Qin, M.M. Polansky, D. Harry, et al., Green tea polyphenols improve cardiac muscle
406 mRNA and protein levels of signal pathways related to insulin and lipid metabolism and
407 inflammation in insulin-resistant rats, Mol. Nutr. Food. Res. 54 Suppl 1 (2010) S14-23.
408 [39] B. Qin, M.M. Polansky, R.A. Anderson, Cinnamon extract regulates plasma levels of
409 adipose-derived factors and expression of multiple genes related to carbohydrate metabolism
410 and lipogenesis in adipose tissue of fructose-fed rats, Horm. Metab. Res. 42 (2010) 187-193.
411 [40] A. Handberg, A. Lopez-Bermejo, J. Bassols, et al., Circulating soluble CD36 is associated
412 with glucose metabolism and interleukin-6 in glucose-intolerant men, Diab. Vasc. Dis. Res. 6
413 (2009) 15-20.
414 [41] R. Liani, B. Halvorsen, S. Sestili, et al., Plasma levels of soluble CD36, platelet activation,
415 inflammation, and oxidative stress are increased in type 2 diabetic patients, Free. Radic. Biol.
416 Med. 52 (2012) 1318-1324.
417

Table 1. Characteristics of participants who developed CHD during follow-up and sub-cohort members in the Diet, Cancer and Health study

Variable	Sub-cohort	CHD cases
N	1759	1963
Age (yrs)	55.0 (50.0, 64.0)	58.0 (51.0, 64.0)
Women	46.5%	27.2%
Postmenopausal (women)[a]	58.3%	71.0%
Current estrogen use[a]	31.7%	29.0%
Education < 8 yrs, % (N)	33.5%	45.0%
Current smoker, % (N)	37.8%	54.8%
Alcohol, g/d	13.6 (0.7, 65.7)	13.6 (0.2, 69.2)
Physical activity, METs/wk	60.00 (19.5, 155.0)	58.5 (16.5, 163.8)
BMI (kg/m^2)	25.6 (20.5, 33.0)	26.7 (21.1, 34.7)
Diabetes[b]	1.8%	5.5%
Hypertension[b]	18.4%	31.3%
Hypercholesterolemia[b]	8.0%	13.0%
Plasma CD36, arbitrary units	0.62 (0.08, 2.67)	0.65 (0.08, 2.57)

Median (5th and 95th percentiles) or %.

[a]Among women.

[b]Self-reported physician-diagnoses of diabetes, hypertension, and hypercholesterolemia.

Table 2. Least-squares means of plasma CD36 level in randomly selected participants from the Danish Diet, Cancer and Health Study.

	Mean CD36 level (95% CI)	***p***[a]
Men	0.91 (0.85, 0.96)	
Women	0.82 (0.76, 0.88)	0.04
Non smokers	0.90 (0.84, 0.95)	
Current smokers	0.81 (0.75, 0.88)	0.07
Non-diabetes	0.87 (0.83, 0.91)	
Diabetes	0.71 (0.40, 1.03)	0.33
Normal weight	0.82 (0.75, 0.88)	
Overweight	0.88 (0.82, 0.94)	0.18
Obesity	0.97 (0.86, 1.08)	0.02
Non hypercholesterolemia	0.86 (0.81, 0.90)	
Hypercholesterolemia	0.97 (0.82, 1.12)	0.16

Data were means (95% CI), adjusted for age and sex (where appropriate).

[a]*p* values for test of difference in means of CD36.

Table 3. Hazard Ratios (HRs) and 95% confidence intervals (95% CI) for coronary heart disease risk according to plasma CD36 level.

	Tertiles of plasma CD36			**Continuous**	
	T1	**T2**	**T3**	**Per SD (0.87 unit)**	***p***
N cases/N at sub-cohort	595/586	676/588	692/585		
Median (interquartile range)	0.24 (0.12-0.33)	0.62 (0.52-0.73)	1.32 (1.03-2.04)		
Age and sex adjusted HR (95% CI)	1 (ref)	1.03 (0.87, 1.23)	1.03 (0.87, 1.22)	1.00 (0.93, 1.07)	0.89
Multivariable model HR[a] (95% CI)	1 (ref)	1.00 (0.83, 1.21)	1.02 (0.84, 1.23)	1.01 (0.94, 1.09)	0.71

HRs were obtained from Cox proportional hazard regression models stratified by sex.

Tertiles created based on the distribution in the random sub-cohort. *p*-values were calculated using the continuous CD36 variables.

[a]Multivariable model: adjusted for age, sex, BMI, smoking, alcohol, physical activity, education, self-reported hypercholesterolemia, and diabetes.

Table 4. Hazard ratios (HRs) and 95% confidence intervals for risk of coronary heart disease by joint categorization of CD36 tertiles and cardiovascular disease risk factors and continuous CD36 within strata.

	Tertiles of plasma CD36			Continuous	
	T1	**T2**	**T3**	**Per SD (0.87 unit)**	***p***
Gender					
Female (N=1,336/N cases=534)	1 (ref)	1.08 (0.81, 1.43)	1.02 (0.76, 1.36)	0.98 (0.86, 1.13)	0.8
Male (N=2,328/N cases=1,429)	2.76 (2.10, 3.64)	2.68 (2.07, 3.48)	2.80 (2.16, 3.62)	1.03 (0.94, 1.13)	0.5
Current smoking status					
No smoking (N=1,961/N cases=888)	1 (ref)	1.01 (0.79, 1.28)	0.96 (0.76, 1.22)	0.99 (0.90, 1.09)	0.8
Current smoking (N=1,703/N cases=1,075)	2.01 (1.54, 2.63)	2.02 (1.57, 2.60)	2.16 (1.66, 2.80)	1.04 (0.93, 1.16)	0.5
Normal/Overweight/Obesity					
BMI <25 kg/m^2 (N=1,362/N cases=603)	1 (ref)	1.15 (0.86, 1.55)	1.08 (0.80, 1.47)	1.03 (0.90, 1.18)	0.6
BMI 25-30 kg/m^2(N=1,638/N cases=923)	1.51 (1.13, 2.02)	1.47 (1.11, 1.95)	1.59 (1.19, 2.11)	1.01 (0.91, 1.11)	0.9
BMI ≥30 kg/m^2 (N=664/N cases=437)	2.71 (1.71, 4.30)	2.09 (1.45, 3.00)	1.97 (1.38, 2.82)	0.96 (0.79, 1.18)	0.7
Diabetes					
No diabetes (N=3,530/N cases=1,855)	1 (ref)	1.00 (0.83, 1.21)	1.00 (0.83, 1.21)	1.01 (0.93, 1.08)	0.9
Diabetes (N=134/N cases=108)	2.11 (0.87, 5.13)	1.99 (0.99, 3.99)	2.83 (1.08, 7.45)	1.37 (0.92, 2.04)	0.1
Hypercholesterolemia					
No hypercholesterolemia (N=3,281 /N cases=1,707)	1 (ref)	1.06 (0.87, 1.30)	1.09 (0.89, 1.33)	1.01 (0.94, 1.09)	0.8
Hypercholesterolemia (N=383/N cases=256)	2.41 (1.45, 3.98)	1.48 (0.96, 2.27)	1.49 (0.98, 2.26)	1.06 (0.80, 1.42)	0.7

Models were adjusted for age, alcohol, physical activity, education; and for sex, smoking, BMI, self-reported hypercholesterolemia, and diabetes where appropriate.

- CD36 was not associated risk of coronary heart disease in the total population.
- There was a suggestion of higher risk of coronary heart disease among participants with both high CD36 levels and existing diabetes.