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**Myeloperoxidase, superoxide dismutase-3, cardiometabolic risk factors and distal sensorimotor polyneuropathy: KORA F4/FF4 study**

Christian Herder1,2, Julia M. Kannenberg1,2, Cornelia Huth2,3, Maren Carstensen-Kirberg1,2, Wolfgang Rathmann2,4, Wolfgang Koenig5,6, Alexander Strom1,2, Gidon J. Bönhof1, Margit Heier3, Barbara Thorand2,3, Annette Peters2,3, Michael Roden1,2,7, Christa Meisinger2,3,8, Dan Ziegler1,2,7

1Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany;

2German Center for Diabetes Research (DZD), München-Neuherberg, Germany;

3Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany;

4Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany;

5Deutsches Herzzentrum München, Technische Universität München, München, Germany;

6German Center for Cardiovascular Research (DZHK), Partner site Munich Heart Alliance, München, Germany;

7Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany.

8Chair of Epidemiology, Ludwig-Maximilians-Universität München, UNIKA-T Augsburg, Germany;

Correspondence to:

Prof. Dr. Christian Herder, Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Auf’m Hennekamp 65, 40225 Düsseldorf, Germany. Phone: +49 211 3382 647. Fax: +49 211 3382 603. E-mail: christian.herder@ddz.uni-duesseldorf.de

**Authors’ email addresses**

christian.herder@ddz.uni-duesseldorf.de (Christian Herder), julia.kannenberg@ddz.uni-duesseldorf.de (Julia M. Kannenberg), huth@helmholtz-muenchen.de (Cornelia Huth), maren.carstensen@ddz.uni-duesseldorf.de (Maren Carstensen-Kirberg), rathmann@ddz.uni-duesseldorf.de (Wolfgang Rathmann), koenig@dhm.mhn.de (Wolfgang Koenig), alexander.strom@ddz.uni-duesseldorf.de (Alexander Strom), gidon.boenhof@ddz.uni-duesseldorf.de (Gidon J. Bönhof), heier@helmholtz-muenchen.de (Margit Heier), thorand@helmholtz-muenchen.de (Barbara Thorand), peters@helmholtz-muenchen.de (Annette Peters), michael.roden@ddz.uni-dueseldorf.de (Michael Roden), christa.meisinger@helmholtz-muenchen.de (Christa Meisinger), dan.ziegler@ddz.uni-duesseldorf.de (Dan Ziegler)

**Abstract**

**Aims:**Oxidative stress has been proposed as an important pathomechanism of cardiometabolic diseases and distal sensorimotor polyneuropathy (DSPN), a common comorbidity of type 2 diabetes and peripheral ischaemia. However, the relevance of biomarkers of oxidative stress has not been investigated in this context. Therefore, this study aimed to assess the associations of the prooxidant myeloperoxidase (MPO) and the antioxidant extracellular superoxide dismutase (SOD3) with cardiometabolic risk factors and with prevalence and incidence of DSPN.

**Methods:** Cross-sectional analyses comprised 1069 participants aged 62-81 years of the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006-2008), 181 of whom had DSPN at baseline. Prospective analyses included 524 individuals without DSPN at baseline who also participated in the KORA FF4 study (2013-2014), 132 of whom developed DSPN during the 6.5-year follow-up. Serum MPO and SOD3 were measured by ELISA, and their associations with cardiometabolic risk factors and DSPN were estimated using linear and logistic regression analysis.

**Results:**Higher MPO and SOD levels showed multiple positive associations with cardiometabolic risk factors including age, indices of obesity, insulin resistance, blood pressure, serum lipids, renal dysfunction and biomarkers of inflammation. Higher MPO levels were associated with prevalent DSPN (age and sex-adjusted OR 1.36 (95% CI 1.11; 1.67) per doubling of MPO) even after adjustment for cardiometabolic risk factors. Higher baseline SOD3 levels were related to incident DSPN (age and sex-adjusted OR 2.14 (1.02; 4.48) per doubling of SOD3), which was partially explained by cardiometabolic risk factors.

**Conclusions:** Both oxidative stress and enhanced antioxidative defence may be involved in cardiometabolic risk and the development of DSPN, suggesting that these processes may be common pathogenetic mechanisms determining the susceptibility to these conditions.

**Keywords:** oxidative stress, myeloperoxidase, superoxide dismutase, cardiovascular risk factors, polyneuropathy, neuropathy

**Background**

Oxidative stress has been implicated in the development of type 2 diabetes and its complications [Baynes 1991, Giugliano 1996, Brownlee 2005, Karimi Galougahi 2015, Shah 2016]. One of the most frequent complications of type 2 diabetes is distal sensorimotor polyneuropathy (DSPN) [Ziegler 2014, Pop-Busui 2017], which also represents a common comorbidity of cardiovascular diseases (CVD) [Papanas 2015]. DSPN is characterised by a substantial adverse impact on quality of life and is associated with higher risk of mortality [Tesfaye 2010, Ziegler 2014, Pop-Busui 2017], but our incomplete understanding of its aetiology limits current preventive and therapeutic options.

Hyperglycaemia, age, obesity, hypertension, dyslipidaemia, low physical activity and smoking emerged as risk factors of DSPN in epidemiological studies, which therefore largely overlap with those identified for type 2 diabetes and CVD [Papanas 2015, Callaghan 2016]. On the other hand, DSPN has been shown to predict CVD in patients with type 2 diabetes [Brownrigg 2014, Brownrigg 2016]. Thus, in order to characterise novel risk factors that specifically influence the risk of DSPN, studies need to take into account multiple cardiometabolic risk factors.

Associations between biomarkers of oxidative stress and DSPN have mainly been assessed in small cross-sectional studies. Increased systemic generation of the prooxidative biomarker superoxide anion, higher levels of of the protein carbonylation marker methylglyoxal as well as lower levels of the antioxidant biomarkers reduced glutathione and glutathione peroxidase in peripheral blood mononuclear cells or serum/plasma have been linked to the presence of DSPN pointing to an imbalance between prooxidative substances and antioxidant capacity in peripheral blood [Ziegler 2004, Kasznicki 2012, Bierhaus 2012, Mendez 2015, Almogbel 2017]. However, other studies failed to confirm some of these findings [Hansen 2015]. Importantly, higher plasma superoxide generation preceded a larger decline in nerve conduction velocity over six years in a prospective study [Ziegler Acta Diab 2015], but we are not aware of further data from large cohort studies in this context.

Although analytical issues such as the relative instability of many biomarkers of oxidative stress represent major challenges [Karimi Galougahi 2015], the measurement of enzymes involved in the generation or dismutation of reactive oxygen species allows an estimate of the oxidative stress burden in the circulation. Myeloperoxidase (MPO) catalyses the conversion of H2O2 to reactive oxygen species (ROS) and has been found associated with multiple cardiometabolic risk factors and the incidence of cardiovascular events [Baldus 2003, Tang 2011, Karakas 2012, Olza 2012]. Extracellular superoxide dismutase (SOD3) is the major antioxidant enzyme in the circulation catalysing the dismutation of superoxide radicals (O2-). Lower systemic SOD3 levels were observed in people with type 2 diabetes and DSPN, and gene variants associated with lower SOD3 levels were related to higher cardiovascular risk [Mohammedi 2015, Strom 2017].

We hypothesised that higher serum levels of MPO and lower serum levels of SOD3 are associated with the development of DSPN independently of cardiometabolic risk factors. Therefore, we aimed assess whether both biomarkers of oxidative stress are associated with prevalent and incident DSPN in a large population-based cohort. We also aimed to characterise the relationship of these two proteins to established cardiometabolic risk factors and to investigate to what extent these risk factors might explain any association between MPO, SOD3 and DSPN.

**Study participants and Methods**

**Study population**

The Cooperative Health Research in the Region of Augsburg (KORA) F4 (2006-2008) and FF4 studies (2013-2014) are follow-up examinations of the population-based KORA S4 study (1999-2001), which were conducted in Augsburg (Germany) and two surrounding counties. The study design has been described previously in detail [Rathmann 2003, Rathmann 2009, Herder DC2017]. The studies were carried out in accordance with the Declaration of Helsinki, including written informed consent from all participants, and were approved by the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany).

For the cross-sectional analyses this study is based on data from all study participants aged between 62 and 81 years in KORA F4 (*n*=1161). After exclusion of individuals with unclear glucose tolerance status due to missing values for fasting and/or 2-hour glucose, type 1 diabetes, drug-induced diabetes or other missing covariables (Figure S1), data from 1069 study participants were available for analysis.

For the prospective analyses on incident DSPN individuals had to be excluded if no follow-up data from KORA FF4 were available or if they had DSPN in KORA F4, which resulted in a sample size of 524 individuals (Figure S1). Follow-up time was 6.46 ± 0.23 years (mean ± SD).

**Assessment of anthropometric, metabolic, lifestyle and immunological variables**

The standardised assessment of height, body weight, waist circumference, systolic and diastolic blood has been described before [Rathmann 2003, Rathmann 2009, Herder DC2017]. Body mass index (BMI) was calculated as body weight (kg)/[height (m)]². Actual hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive medication given that the study participants were aware of being hypertensive.

The assessment of glucose tolerance status was based on standard 75-g oral glucose tolerance test (OGTT) for all individuals without known type 2 diabetes using the criteria of the American Diabetes Association for fasting and 2-hour glucose [TheExpertCommittee ADA 2003]. Known type 2 diabetes was defined if study participants reported a previous diagnosis of type 2 diabetes that could be validated by the responsible physician, or as current use of glucose-lowering medication. Measurement methods of glucose, insulin levels and HbA1c and calculation of the homeostasis model assessment of insulin resistance (HOMA-IR) and whole-body insulin sensitivity index (ISI[composite]) were also reported before [Herder EJE2015, Herder CVDB2017, Herder EJE2017, Carstensen-Kirberg 2017].

The measurement of serum lipid levels and the assessment of kidney function from the estimated glomerular filtration rate (eGFR) using the chronic kidney disease epidemiology (CKD-EPI) creatinine equation were done as described [Herder EJE2015, Carstensen-Kirberg 2017].

Information on medical history, physical activity, smoking, alcohol consumption and use of medication were obtained by medical interviewers [Rathmann 2009]. Individuals were considered physically active if they reported more than one hour of physical activity per week during leisure time in either summer or winter. Smoking status was classified as never, former or current smoking, and alcohol consumption was classified as none, moderate or high based on alcohol intakes of 0, 0.1 to 39.9 or 40 g/d and above for men and 0, 0.1 to 19.9 or 20 g/d and above for women.

Plasma concentrations of high-sensitivity C-reactive protein (hsCRP) and interleukin-18 (IL-18) and serum concentrations of interleukin (IL)-6, tumour-necrosis factor-α (TNFα), IL-1 receptor antagonist (IL-1RA), soluble intercellular adhesion molecule-1 (sICAM-1), adiponectin and omentin were quantified using a high-sensitivity latex-enhanced nephelometric assay (for hsCRP) or using ELISAs (for all other analytes) [Herder 2013, Herder DME2015, Herder DC2015].

**Assessment of DSPN**

The examination part of the Michigan Neuropathy Screening Instrument (MNSI) was used to define presence and incidence of DSPN [Feldman 1994]. The MNSI contains the following items: appearance of feet (normal or deformities, dry skin, callus, infection, fissure or other irregularities), foot ulceration, ankle reflexes and vibration perception threshold (VPT) at the great toes. The VPT becomes impaired with increasing age, so that age-dependent limits of normal VPT were used as described [Martina 1998]. The neuropathy assessment was extended by the bilateral examination of sensory perception using a 10-g monofilament (Neuropen) [Paisley 2012], so that the total MNSI score ranged from 0 (all aspects normal) to a maximum of 10 points [Herder DC 2017]. DSPN was defined using a cut-off at >3 points in accordance with our previous analysis on biomarkers and incident DSPN in this cohort [Herder DC2017], thus satisfying the minimal diagnostic criteria for possible DSPN [Tesfaye 2010].

**Measurement of MPO and SOD3**

Fasting serum samples were stored at -80°C between blood sampling and analysis of MPO and SOD3 concentrations in 2017. MPO concentrations were measured using the Human Myeloperoxidase Quantikine ELISA (R&D Systems, Wiesbaden, Germany). SOD3 concentrations were measured using the ELISA for Superoxide Dismutase 3, Extracellular (SOD3) from Cloud-Clone Corp. (Houston, TX, USA). Limits of detection (LOD) were 0.16 ng/ml for MPO and 125 pg/ml for SOD3. All sera yielded levels above the LOD for both analytes. Coefficients of variation (CV) were estimated using three control sera that were measured in duplicates on 16 plates. Mean intra- and inter-assay CV were 3.2% and 5.6%, respectively, for MPO and 4.5% and 7.1%, respectively, for SOD3.

**Statistical analysis**

Characteristics of the study population are given stratified by quarters of serum MPO or SOD3 (with *P* values from linear regression analysis for associations between both log2-transformed serum levels of both proteins and these characteristics) and stratified by presence of DSPN at baseline (with *P* values from logistic regression analysis, i.e. likelihood ratio tests comparing models with the respective variable, age and sex as independent variables to models with age and sex only).

Cross-sectional associations between log2(MPO) or log2(SOD3) and presence of DSPN at baseline (KORA F4) were assessed by multivariable linear regression analysis using the following potential confounders in line with previous KORA analyses [Herder DC2017]:

Model 1: adjusted for age, sex.

Model 2: model 1 + physical activity, smoking, alcohol consumption.

Model 3: model 2 + waist circumference, height.

Model 4: model 3 + HDL cholesterol, LDL cholesterol, triglycerides, lipid-lowering medication, hypertension, use of non-steroidal anti-inflammatory drugs, history of myocardial infarction, estimated glomerular filtration rate, neurological conditions that might cause nerve damage.

Model 5: model 4 + IL-6, TNFα.

Age, alcohol consumption, BMI, HDL cholesterol, LDL cholesterol, triglycerides, eGFR, IL-6 (log2-transformed) and TNFα (log2-transformed) were used as continuous variables in these models.

Prospective associations between log2(MPO) or log2(SOD3) and incidence of DSPN between baseline (KORA F4) were assessed by fitting logistic regression models using the aforementioned covariables.

Statistical analyses were carried out with R version 3.3.3 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) and Python version 3.6.1 (Python Software Foundation, https://www.python.org/). Statistical significance was inferred at a 2-tailed *P* value <0.05.

**Results**

**Associations of MPO and SOD3 with cardiometabolic risk factors**

The cross-sectional analysis including 1069 participants of the KORA F4 study revealed positive associations of serum MPO and SOD3 with multiple cardiometabolic risk factors after adjusting for age and sex. Higher levels of both proteins were associated with higher age, male sex, higher fasting insulin and insulin resistance, higher systolic and diastolic blood pressure, lower eGFR and higher levels of the majority of the biomarkers of inflammation studied (Table 1, Table 2). Both proteins also positively correlated with each other. In addition, higher MPO levels were associated with lower HbA1c and a higher proportion of smokers and ex-smokers (Table 1), whereas higher SOD3 levels were associated with higher BMI and waist circumference, higher 2-hour glucose and insulin, higher proportion of impaired glucose regulation and hypertension, lower HDL cholesterol, higher triglycerides and lower physical activity (Table 2).

Most of the aforementioned associations remained statistically significant after further adjustment for waist circumference. This adjustment slightly attenuated the association between MPO and age (Table 1) as well as the associations between SOD3 and 2-hour glucose, 2-hour insulin, glucose tolerance status and serum lipids, but strengthened the inverse association between MPO and fasting glucose and the positive association between SOD3 and adiponectin (Table 2).

**MPO, SOD3 and prevalence of DSPN**

At baseline, individuals with DSPN (*n*=181) were older, more frequently male, had a less favourable cardiometabolic risk profile and higher MPO serum levels than individuals without DSPN (*n*=888), whereas SOD3 serum levels did not differ between both groups (Table 3). The difference in MPO levels between both groups translated into an age and sex-adjusted OR for prevalent DSPN of 1.36 (95% CI 1.11; 1.67, *P*=0.003) per doubling of MPO levels (Table 4, model 1). Further adjustment for multiple cardiometabolic risk factors (e.g. lifestyle factors, waist circumference, HbA1c, serum lipids), medication use, history of myocardial infarction, kidney function and other neurological conditions had almost no impact on this association (fully adjusted model 5: 1.35 (95% CI 1.08; 1.68, *P*=0.007). Despite a similar OR, SOD3 levels were not associated with prevalent DSPN in any model due to a considerably wider 95% CI (Table 4).

**MPO, SOD3 and incidence of DSPN**

Table S1 shows the baseline characteristics of the KORA F4 participants stratified by DSPN incidence. The description of an almost identical study sample has been published in a previous report on biomarkers of inflammation and incident DSPN [Herder DC2017]. Briefly, individuals with incident DSPN (*n*=132) were older and had an overall less favourable cardiometabolic risk profile including higher levels of most biomarkers of inflammation than individuals without incident DSPN (*n*=392), and adiponectin levels were lower in incident cases (Table S1). Serum MPO levels did not differ significantly between cases and controls (median (25th; 75 pecentiles) 149 (97; 221) vs. 132 (88; 198) ng/ml, respectively; age and sex-adjusted *P*=0.095), whereas cases had significantly higher SOD3 levels than non-cases (129 (97; 221) vs. 132 (88; 198) ng/ml; age and sex-adjusted *P*=0.043).

Age and sex-adjusted OR (95% CI) for incident DSPN were 1.23 (0.96; 1.58, *P*=0.098) for MPO and 2.14 (1.02; 4.48, *P*=0.044) for SOD3 (Table 5). However, further adjustment for cardiometabolic risk factors and other confounders in models 2-5 attenuated these associations (all *P*>0.1).

**Discussion**

This study has three main findings: (i) Higher levels of serum MPO and SOD3 were associated with multiple cardiometabolic risk factors independent of age, sex and waist circumference. (ii) Only MPO showed a robust association with prevalent DSPN that was independent of cardiometabolic risk factors, whereas no such association was found for SOD3. (iii) Higher SOD3 levels were significantly associated with higher risk of DSPN, but about half of this association was explained by cardiometabolic risk factors, while no prospective association with incident DSPN was observed for MPO in any model.

**MPO, cardiometabolic risk factors and DSPN**

MPO, a prooxidant enzyme from leukocytes, is an important source of ROS which lead to oxidative damage of multiple lipid species and proteins. This study represents the most comprehensive population-based analysis of associations between serum MPO and cardiometabolic risk factors so far. Our findings of multiple positive associations with cardiometabolic risk factors including age, HOMA-IR, blood pressure, impaired kidney function, smoking and subclinical inflammation are in line with the physiological function of MPO and with previous reports linking higher MPO levels with cardiometabolic risk factors and subsequent higher risk of cardiovascular events in cohorts with preexisting CVD [Brennan 2003, Baldus 2003, Tang 2011] or in samples from the general population [Meuwese 2007, Rana 2011, Karakas 2012]. We extend the current literature by showing positive associations between MPO, fasting insulin and HOMA-IR, but not with the OGTT-based variables 2-hour insulin and ISI(composite). These novel data suggest that higher MPO levels are primarily linked with hepatic insulin resistance, but less so with peripheral or whole-body insulin resistance.

Associations between circulating MPO levels and prevalent or incident DSPN have not been investigated before. We show that higher MPO levels are robustly and independently associated with the prevalence of DSPN. With respect to incident DSPN, we observed a positive trend, but adjustment for cardiometabolic risk factors attenuated this association. The weaker association in the prospective analysis may be attributable to the smaller sample size compared to the cross-sectional analysis. Alternatively, the cross-sectional association may be overestimated because of residual confounding, i.e. confounding by unknown parameters that were not assessed, or reverse causality as often seen in cardiovascular research [Sattar 2017]. Thus, MPO may be involved in the pathogenesis of DSPN, but possibly restricted by the available sample size, we cannot provide evidence of its utility as biomarker for incident DSPN in contrast to the proinflammatory cytokines IL-6 and TNFα, which independently predicted incident DSPN in this study sample [Herder DC 2017].

**SOD3, cardiometabolic risk factors and DSPN**

SOD3 represents the major antioxidant enzyme in the circulation and the most important scavenger of superoxide in the extracellular compartment. It is produced in response to ROS and proinflammatory cytokines such as TNFα and IFNγ and has neuroprotective effects *in vitro* [Kemp 2010]. In contrast to MPO, which has been investigated before in the context of cardiometabolic risk factors, comparable studies for SOD3 are lacking. Thus, our findings showing associations of higher serum SOD3 levels with higher age, BMI, glycaemia, insulin resistance, blood pressure and subclinical inflammation and with lower kidney function and less physical activity are novel and extend the current literature considerably. This study also contains the first observation between higher SOD3 levels and higher risk of developing DSPN, which is partly explained by cardiometabolic risk factors.

Based on two previous observations we had expected associations in the opposite direction. One study found that SOD3 levels were lower in individuals with recent-onset type 2 diabetes compared to non-diabetic individuals and in diabetic individuals with DSPN compared to DSPN-free controls [Strom 2017]. Another study reported that gene variants that were linked to higher SOD3 levels had a protective effect on the risk for cardiovascular events in patients with diabetes [Mohammedi 2015]. Thus, lower antioxidant capacity should be linked with higher cardiometabolic risk.

However, our study provides consistent evidence that both the presence of cardiometabolic risk factors including all aspects of the metabolic syndrome and subclinical inflammation as well as the increased risk of developing DSPN is associated with *higher* SOD3 levels. Overall, these data are directly comparable to findings for interleukin-1 receptor antagonist (IL-1RA), a major anti-inflammatory regulator [Herder TEM 2015]. IL-1RA levels are positively associated with multiple cardiometabolic risk factors, and increases in systemic IL-1RA levels are observed even 5-15 years before the onset of type 2 diabetes [Carstensen 2010, Herder DOM 2013] or the manifestation of cardiovascular events [Herder ATVB 2017] and in people at risk for progression of DSPN [Herder DC 2017]. The increases in systemic levels in SOD3 and IL-1RA most likely reflect an upregulation to chronic subclinical inflammation, oxidative stress and metabolic stimuli, which is, however, not sufficient to protect against the development of cardiometabolic diseases and DSPN. Interestingly, a similar association of enhanced mitochondrial SOD2 expression with longer diabetes duration and sympathovagal dysbalance has been observed [Ziegler Diabetologia 2015].

Importantly, one meta-analysis also showed that genetic upregulation of IL-1RA is associated with lower inflammation and higher insulin sensitivity [Herder Diabetes 2014], which mirrors the aforementioned observation of lower cardiovascular risk in individuals with genetically upregulated SOD3 levels [Mohammedi 2015]. Collectively, these findings point towards the necessity to differentiate between genetic and environmental mechanisms to counteract inflammation and oxidative stress.

**Clinical implications for the prevention and treatment of DSPN**

Despite the biological plausibility that oxidative stress is involved in the development of DSPN, prospective studies on biomarkers of oxidative stress and risk of DSPN are very scarce. Our previous study showed an association between higher plasma superoxide production and subsequent decline in nerve conduction and cardiac autonomic function over 6 years in patients with diabetes [Ziegler Acta Diab 2015], and the present study adds the observation that higher SOD3 levels were associated with incident DSPN in the general population. Thus, studies analysing further biomarkers of oxidative stress are necessary to better understand the role of different prooxidative processes in the development of DSPN. Self-evident analytes are ROS or reactive nitrogen species, but technical issues related to their instability may be limiting factors. In addition, further sources of ROS, biomarkers modified by interaction with ROS (e.g. biomarkers of lipid peroxidation, tyrosine nitration and protein carbonylation) and biomarkers produced in response to oxidative stress (e.g. other antioxidant enzymes) are promising candidates [Karimi Galougahi 2015].

The impact of approaches targeting oxidative stress in the prevention and treatment of DSPN has been addressed in few studies so far. The best evidence for beneficial effects on the prevention of pregression of DSPN comes from studies using α-lipoic acid [Ziegler 2011, Papanas 2014], but further prevention and intervention trials are necessary to explore this promising avenue of pathogenesis-derived treatment.

**Strengths and limitations**

Strenghts of the study included the large sample size, the detailed phenotyping, the population-based design and the accuracy of the MPO and SOD3 assays, which allowed a valid and comprehensive analysis of the association between circulating levels of both proteins and cardiometabolic risk factors. This study provides the first data for links between MPO, SOD3 and DSPN based on cross-sectional and prospective associations taking into account multiple cardiovascular risk factors as potential confounders.

This study also has limitations that warrant attention. First, we were not able to measure ROS or RNS with direct cellular effects because of their short half-life and instability in frozen samples, so that further biomarkers need to be investigated in order to assess links between systemic oxidative stress, cardiometabolic risk and DSPN in more detail. Despite the comparatively large cohort for studies of DSPN, we had a smaller sample size for the prospective compared to the cross-sectional analysis, which reduced our statistical power. Moreover, the study examined older individuals of German descent which limits the generalisability of our findings to younger populations and people with different ethnic background.

**Conclusion**

Serum levels of MPO and SOD3 showed positive associations with multiple cardiometabolic risk factors. Higher MPO levels were independently associated with prevalent DSPN. We also observed an association between higher SOD3 levels and incident DSPN, but about half of the excess risk was explained by cardiometabolic risk factors. Collectively, our data indicate that oxidative stress and an antioxidative counterregulation may be linked to cardiometabolic risk and the development of DSPN.

**Additional file**

**Table S1**. Baseline characteristics of the KORA F4 study population stratified by incidence of DSPN.

**Figure S1.** Description of the study design.

**Authors’ contributions**

CHe and DZ designed the study. BT contributed to the study design. CHe, CHu, MCK, WR, WK, AS, GJB, MH, BT, AP, MR and CM contributed data. CHe and JMK drafted the statistical analysis plan. JMK performed the statistical analysis. CHe wrote the manuscript. All authors contributed to, critically revised and approved the final version of the manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The data are subject to national data protection laws and restrictions were imposed by the Ethics Committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with KORA via the online portal KORA.passt (<https://epi.helmholtz-muenchen.de/>). Please contact the corresponding author Christian Herder in case of further questions.

**Ethics and consent to participate**

The investigations were carried out in accordance with the Declaration of Helsinki, including written informed consent from all participants. The study was approved by the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany).

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**Table 1** **Description of the KORA F4 study population stratified by quarters of serum concentrations of MPO**

| **Variable** | **Quarter 1** | **Quarter 2** | **Quarter 3** | **Quarter 4** | ***P* a** | ***P* b** |
| --- | --- | --- | --- | --- | --- | --- |
| *n* | 268 | 267 | 267 | 267 |  |  |
| MPO (ng/ml) | 68.7 (53.8; 83.9) | 118.7 (105.9; 130.8) | 177.0 (159.9; 191.8) | 271.3 (233.4; 327.7) |  |  |
| Age (years) | 69.8 ± 5.3 | 69.9 ± 5.3 | 70.4 ± 5.5 | 70.5 ± 5.5 | **0.042** | 0.055 |
| Sex (% male) | 48.1 | 47.9 | 47.6 | 61.0 | **<0.001** | **0.007** |
| BMI (kg/m²) | 28.1 ± 3.9 | 29.0 ± 4.2 | 29.1 ± 4.7 | 28.6 ± 4.8 | 0.266 | 0.434 |
| Waist circumference (cm) | 96.5 ± 12.1 | 98.6 ± 12.2 | 98.7 ± 12.1 | 99.3 ± 12.2 | 0.106 | 0.106 |
| Fasting glucose (mmol/l) c | 5.5 ± 0.7 | 5.5 ± 0.9 | 5.4 ± 0.7 | 5.5 ± 0.6 | 0.088 | **0.029** |
| 2-h glucose (mmol/l) c | 7.2 ± 2.4 | 7.1 ± 2.5 | 7.0 ± 2.1 | 7.1 ± 2.2 | 0.935 | 0.670 |
| HbA1c (%) c | 5.65 ± 0.39 | 5.65 ± 0.46 | 5.60 ± 0.36 | 5.59 ± 0.37 | **0.022** | **0.011** |
| HbA1c (mmol/mol) c | 38 ± 4 | 38 ± 5 | 38 ± 4 | 38 ± 4  | **0.022** | **0.011** |
| Fasting insulin (µU/ml) c,d | 4.6 (3.3; 7.8) | 5.3 (3.5; 9.7) | 4.8 (3.3; 7.8) | 5.2 (3.3; 9.2) | **0.002** | **0.007** |
| 2-h insulin (µU/ml) c,d | 54.3 (29.9; 93.6) | 56.6 (34.5; 88.6) | 51.4 (30.2; 83.0) | 53.4 (28.3; 89.4) | 0.315 | 0.597 |
| HOMA-IR c,d | 1.1 (0.8; 2.0) | 1.3 (0.8; 2.5) | 1.2 (0.7; 1.9) | 1.3 (0.8; 2.4) | **0.005** | **0.008** |
| ISI (composite) (1/((mmol/l)x(pmol/l))) c,d | 17.8 (9.8; 29.1) | 16.3 (9.2; 26.3) | 17.4 (10.4; 28.3) | 16.4 (9.6; 28.1) | 0.335 | 0.540 |
| Glucose tolerance status: NGT / IFG / IGT / IFG&IGT / ndT2D / T2D (%)  | 34.3/23.9/10.8/10.4/7.1/13.4 | 37.8/18.7/10.5/12.4/5.6/15.0 | 44.6/12.4/9.7/11.6/6.0/15.7 | 40.1/22.1/9.0/9.7/6.4/12.7 | 0.629 | 0.417 |
| Systolic blood pressure (mmHg) e | 121.3 ± 13.6 | 119.4 ± 14.3 | 120.1 ± 13.8 | 117.7 ± 13.2 | **0.023** | **0.022** |
| Diastolic blood pressure (mmHg) e | 73.2 ± 8.0 | 72.4 ± 8.3 | 71.7 ± 8.1 | 69.7 ± 8.7 | **0.001** | **<0.001** |
| Hypertension (%) | 61.9 | 59.5 | 62.9 | 64.4 | 0.635 | 0.922 |
| Total cholesterol (mmol/l) f | 5.94 ± 1.05 | 5.98 ± 1.05 | 5.89 ± 0.97 | 5.88 ± 0.96 | 0.930 | 0.888 |
| LDL cholesterol (mmol/l) f | 3.80 ± 0.91 | 3.85 ± 0.92 | 3.74 ± 0.86 | 3.79 ± 0.90 | 0.959 | 0.998 |
| HDL cholesterol (mmol/l) f | 1.49 ± 0.40 | 1.45 ± 0.38 | 1.47 ± 0.38 | 1.39 ± 0.33 | 0.161 | 0.464 |
| Triglycerides (mmol/l) d,f | 1.25 (0.89; 1.75) | 1.29 (0.96; 1.82) | 1.20 (0.90; 1.69) | 1.33 (1.01; 1.82) | 0.351 | 0.725 |
| Use of lipid-lowering drugs (%) | 22.4 | 24.0 | 26.2 | 26.6 | 0.336 | 0.327 |
| eGFR (ml/min per 1.73m²)  | 78.7 ± 13.5 | 77.3 ± 13.7 | 75.3 ± 15.7 | 75.1 ± 16.2 | **<0.001** | **<0.001** |
| Smoking (never/former/current) (%) | 57.8/38.1/4.1 | 50.9/42.7/6.4 | 54.3/38.6/7.1 | 40.8/46.4/12.7 | **<0.001** | **<0.001** |
| Physically active (%) | 53.0 | 52.8 | 46.8 | 48.7 | 0.064 | 0.108 |
| Alcohol consumption (none/moderate/high) (%) | 32.8/54.9/12.3 | 31.1/49.1/19.9 | 32.6/52.4/15.0 | 30.3/49.1/20.6 | 0.151 | 0.201 |
| hs C-reactive protein (mg/l) d | 1.14 (0.64; 2.24) | 1.56 (0.80; 2.72) | 1.52 (0.78; 3.26) | 2.25 (1.03; 5.51) | **<0.001** | **<0.001** |
| IL-6 (pg/ml) d | 1.38 (0.94; 2.12) | 1.58 (1.11; 2.17) | 1.59 (1.24; 2.43) | 2.01 (1.27; 3.13) | **<0.001** | **<0.001** |
| IL-18 (pg/ml) d | 296 (247; 378) | 312 (253; 412) | 332 (255; 423) | 330 (252; 440) | **0.001** | **0.002** |
| TNFα (pg/ml) d | 1.91 (1.38; 2.86) | 2.05 (1.48; 3.01) | 1.95 (1.45; 2.74) | 2.15 (1.51; 3.11) | **0.016** | **0.025** |
| IL-1 receptor antagonist (pg/ml) d | 243 (194; 327) | 292 (238; 374) | 325 (259; 415) | 369 (298; 480) | **<0.001** | **<0.001** |
| sICAM-1 (ng/ml) d | 222 (194; 250) | 228.0 (198; 256) | 227 (198; 262) | 242(213; 274) | **<0.001** | **<0.001** |
| Adiponectin (µg/ml) | 10.03 (6.29; 15.05) | 10.28 (6.83; 15.66) | 10.31 (6.73; 16.11) | 9.76 (6.92; 14.19) | 0.699 | 0.412 |
| Omentin (ng/ml) | 506 ± 156 | 512 ± 165 | 508 ± 197 | 507 ± 163 | 0.422 | 0.350 |
| SOD3 (ng/ml) | 126 ± 26 | 127 ± 23 | 131 ± 31 | 135 ± 36 | **<0.001** | **<0.001** |

Data are given as mean ± SD, median and 25th; 75th percentiles or percentages, unless indicated otherwise.

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; hs, high-sensitivity; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL, interleukin; LDL, low-density lipoprotein; MPO, myeloperoxidase; ndT2D, newly diagnosed type 2 diabetes; NGT, normal glucose tolerance; sICAM-1, soluble intercellular adhesion molecule-1; SOD3, extracellular superoxide dismutase; TNF, tumour necrosis factor; T2D, type 2 diabetes.

a *P* values are adjusted for age and sex using linear regression analysis. The analysis for age is adjusted for sex only, the analysis for sex is adjusted for age only.

b *P* values are additionally adjusted for waist circumference.

c Individuals with known type 2 diabetes (*n*=152) excluded.

d Variables were log2-transformed for the linear regression analysis.

e Individuals with anti-hypertensive medication (*n*=665) excluded.

f Individuals with lipid-lowering medication (*n*=265) excluded.

**Table 2** **Description of the KORA F4 study population stratified by quarters of serum concentrations of SOD3**

| **Variable** | **Quarter 1** | **Quarter 2** | **Quarter 3** | **Quarter 4** | ***P* a** | ***P* b** |
| --- | --- | --- | --- | --- | --- | --- |
| *n* | 268 | 267 | 267 | 267 |  |  |
| SOD3 (ng/ml) | 99 ± 9 | 119 ± 4 | 134 ± 5 | 167 ± 30 |  |  |
| Age (years) | 69.7 ± 5.1 | 70.1 ± 5.5 | 70.0 ± 5.4 | 70.8 ± 5.5 | **0.003** | **0.006** |
| Sex (% male) | 43.3 | 50.6 | 55.8 | 55.1 | **0.003** | 0.263 |
| BMI (kg/m²) | 28.4 ± 4.4 | 28.4 ± 4.3 | 28.6 ± 4.5 | 29.3 ± 4.5 | **0.003** | 0.132 |
| Waist circumference (cm) | 96.5 ± 12.5 | 97.7 ± 11.7 | 98.0 ± 11.6 | 100.8 ± 12.6 | **<0.001** | **<0.001** |
| Fasting glucose (mmol/l) c | 5.4 ± 0.6 | 5.5 ± 0.7 | 5.5 ± 0.6 | 5.6 ± 0.9 | 0.206 | 0.798 |
| 2-h glucose (mmol/l) c | 6.9 ± 2.0 | 7.0 ± 2.2 | 7.0 ± 2.1 | 7.5 ± 2.8 | **0.038** | 0.210 |
| HbA1c (%) c | 5.59 ± 0.33 | 5.65 ± 0.39 | 5.57 ± 0.33 | 5.68 ± 0.52 | 0.561 | 0.994 |
| HbA1c (mmol/mol) c | 38 ± 4 | 38 ± 4 | 38 ± 4 | 39 ± 6 | 0.561 | 0.994 |
| Fasting insulin (µU/ml) c,d | 4.4 (3.2; 6.9) | 5.1 (3.4; 8.1) | 4.9 (3.3; 8.7) | 5.9 (3.8; 11.2) | **<0.001** | **0.009** |
| 2-h insulin (µU/ml) c,d | 49.3 (25.4; 75.3) | 53.6 (32.1; 94.0) | 50.1 (29.2; 89.2) | 63.0 (37.3; 98.0) | **0.015** | 0.238 |
| HOMA-IR c,d | 1.1 (0.7; 1.7) | 1.2 (0.8; 2.1) | 1.2 (0.8; 2.2) | 1.4 (1.0; 2.8) | **<0.001** | **<0.001** |
| ISI (composite) (1/((mmol/l)x(pmol/l))) c,d | 18.8 (11.2; 30.9) | 15.5 (10.2; 27.5) | 18.2 (9.3; 29.7) | 14.2 (8.1; 22.4) | 0.434 | 0.834 |
| Glucose tolerance status: NGT / IFG / IGT / IFG&IGT / ndT2D / T2D (%)  | 44.4/19.4/10.4/10.1/ 4.5/11.2 | 40.1/20.2/10.5/11.6/ 5.6/12.0 | 39.0/21.0/10.9/10.5/ 4.5/14.2 | 33.3/16.5/8.2/12.0/10.5/19.5 | **0.030** | 0.261 |
| Systolic blood pressure (mmHg) e | 120.1 ± 12.8 | 120.5 ± 11.9 | 119.1 ± 15.2 | 118.5 ± 15.5 | **0.026** | **0.021** |
| Diastolic blood pressure (mmHg) e | 72.5 ± 7.8 | 72.3 ± 7.7 | 71.2 ± 8.5 | 71.1 ± 9.8 | **0.025** | **0.019** |
| Hypertension (%) | 53.7 | 62.5 | 60.7 | 71.9 | **<0.001** | **0.004** |
| Total cholesterol (mmol/l) f | 5.89 ± 1.05 | 6.03 ± 0.93 | 5.91 ± 1.08 | 5.85 ± 0.98 | 0.540 | 0.837 |
| LDL cholesterol (mmol/l) f | 3.78 ± 0.94 | 3.85 ± 0.84 | 3.77 ± 0.95 | 3.78 ± 0.86 | 0.739 | 0.803 |
| HDL cholesterol (mmol/l) f | 1.50 ± 0.40 | 1.48 ± 0.37 | 1.43 ± 0.36 | 1.40 ± 0.36 | **0.006** | 0.114 |
| Triglycerides (mmol/l) d,f | 1.22 (0.84; 1.73) | 1.27 (0.96; 1.69) | 1.27 (0.95; 1.74) | 1.29 (0.97; 1.90) | **0.035** | 0.276 |
| Use of lipid-lowering drugs (%) | 29.1 | 16.9 | 25.5 | 27.7 | 0.534 | 0.503 |
| eGFR (ml/min per 1.73m²)  | 79.3 ± 12.8 | 78.7 ± 13.0 | 76.7 ± 14.6 | 71.6 ± 17.5 | **<0.001** | **<0.001** |
| Smoking (never/former/current) (%) | 56.7/39.2/4.1 | 47.9/42.3/9.7 | 50.9/42.7/6.4 | 48.3/41.6/10.1 | 0.139 | 0.121 |
| Physically active (%) | 57.8 | 53.2 | 49.8 | 40.5 | **<0.001** | **0.003** |
| Alcohol consumption (none/moderate/high) (%) | 33.6/48.5/17.9 | 32.2/51.3/16.5 | 27.7/55.8/16.5 | 33.3/49.8/16.9 | 0.654 | 0.503 |
| hs C-reactive protein (mg/l) d | 1.34 (0.74; 2.78) | 1.34 (0.64; 3.00) | 1.52 (0.90; 2.98) | 2.04 (1.01; 3.96) | **<0.001** | **<0.001** |
| IL-6 (pg/ml) d | 1.52 (1.08; 2.40) | 1.48 (0.96; 2.16) | 1.53 (1.13; 2.29) | 1.84 (1.37; 2.09) | **<0.001** | **<0.001** |
| IL-18 (pg/ml) d | 303 (239; 388) | 306 (253; 413) | 325 (252; 418) | 339 (264; 436) | **<0.001** | **<0.001** |
| TNFα (pg/ml) d | 1.90 (1.40; 2.69) | 1.98 (1.40; 2.87) | 1.94 (1.43; 2.79) | 2.33 (1.59; 3.48) | **<0.001** | **0.001** |
| IL-1 receptor antagonist (pg/ml) d | 286 (222; 378) | 295 (230; 376) | 320 (246; 413) | 344 (258; 461) | **<0.001** | **<0.001** |
| sICAM-1 (ng/ml) d | 223 (197; 252) | 224 (197; 258) | 225 (200; 256) | 242 (205; 287) | **<0.001** | **<0.001** |
| Adiponectin (µg/ml) | 10.02 (6.25; 15.34) | 10.28 (6.55; 16.36) | 10.19 (6.92; 14.09) | 9.88 (6.88; 14.62) | 0.358 | **0.044** |
| Omentin (ng/ml) | 500 ± 166 | 499 ± 181 | 517 ± 169 | 517 ± 168 | **0.022** | **0.008** |
| MPO (ng/ml) | 137 (90; 195) | 138 (86; 193) | 148 (102; 220) | 166 (100; 230) | **<0.001** | **<0.001** |

Data are given as mean ± SD, median and 25th; 75th percentiles or percentages, unless indicated otherwise. *P1* values are adjusted for age and sex using linear regression analysis.

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; hs, high-sensitivity; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL, interleukin; LDL, low-density lipoprotein; MPO, myeloperoxidase; ndT2D, newly diagnosed type 2 diabetes; NGT, normal glucose tolerance; sICAM-1, soluble intercellular adhesion molecule-1; SOD3, extracellular superoxide dismutase; TNF, tumour necrosis factor; T2D, type 2 diabetes.

a *P* values are adjusted for age and sex using linear regression analysis. The analysis for age is adjusted for sex only, the analysis for sex is adjusted for age only.

b *P* values are additionally adjusted for waist circumference.

c Individuals with known type 2 diabetes (*n*=152) excluded.

d Variables were log2-transformed for the linear regression analysis.

e Individuals with anti-hypertensive medication (*n*=665) excluded.

f Individuals with lipid-lowering medication (*n*=265) excluded.

**Table 3 Baseline characteristics of the KORA F4 study population stratified by presence of DSPN**

| **Variable** | **No DSPN** | **DSPN** | ***P*** |
| --- | --- | --- | --- |
| *n* | 888 | 181 |  |
| Age (years) | 69.7 ± 5.3 | 72.5 ± 5.2 | **<0.001** |
| Sex (% male / female) | 49.3 / 50.7 | 60.2 / 39.8 | **0.009** |
| BMI (kg/m2) | 28.4 ± 4.2 | 30.1 ± 5.2 | **<0.001** |
| Waist circumference (cm) | 97.1 ± 11.8 | 103.7 ± 12.9 | **<0.001** |
| Height (cm) | 165 ± 9 | 168 ± 10 | **<0.001** |
| HbA1c (%) | 5.75 ± 0.63 | 5.96 ± 0.83 | **0.004** |
| HbA1c (mmol/mol) | 39 ± 7 | 42 ± 9 | **0.004** |
| Glucose tolerance status (NGT / IFG / IGT / IFG&IGT / ndT2D / kT2D) (%) | 40.2 / 19.9 / 10.4 /10.9 / 6.5 / 12.1 | 34.2 / 16.0 / 8.3 / 11.6 / 5.0 / 24.9 | **0.010** |
| Hypertension (%) a | 61.8 | 64.1 | 0.355 |
| Total cholesterol (mmol/l) b | 5.98 ± 1.02 | 5.63 ± 0.90 | **0.002** |
| LDL cholesterol (mmol/l) b | 3.84 ± 0.91 | 3.57 ± 0.81 | **0.005** |
| HDL cholesterol (mmol/l) b | 1.46 ± 0.38 | 1.40 ± 0.33 | 0.235 |
| Fasting triglycerides (mmol/l) b | 1.28 (0.96; 1.76) | 1.17 (0.85; 1.81) | 0.335 |
| Use of lipid-lowering drugs (%) | 24.2 | 27.6 | 0.620 |
| eGFR (ml/min per 1.73m2) | 77.4 ± 14.5 | 72.5 ± 16.1 | 0.104 |
| Smoking (never / former / current) (%) | 52.1 / 40.2 / 7.7 | 45.3 / 47.5 / 7.2 | 0.555 |
| Alcohol intake (none / moderate / high (%) | 31.2 / 52.8 / 16.0 | 34.2 / 44.2 / 21.6 | **0.008** |
| Physically active (%) | 52.0 | 42.0 | 0.189 |
| Myocardial infarction (%) | 5.4 | 8.8 | 0.650 |
| Neurological conditions that might cause nerve damage (%) | 15.8 | 30.9 | **<0.001** |
| Use of nonsteroidal anti-inflammatory drugs (%) c | 3.6 | 7.2 | 0.209 |
| MNSI | 2.0 (1.0; 2.0) | 4.0 (3.5; 4.5) | **<0.001** |
| MPO (ng/ml) | 140 (92; 206) | 172 (115; 237) | **0.002** |
| SOD3 (ng/ml) | 126 (111; 142) | 129 (114; 150) | 0.234 |
| hsCRP (mg/l) | 1.53 (0.78; 3.20) | 1.50 (0.78; 2.84) | 0.615 |
| IL-6 (pg/ml)  | 1.55 (1.08; 2.32) | 1.85 (1.33; 2.89) | 0.909 |
| IL-18 (pg/ml) | 314 (251; 413) | 332 (259; 425) | 0.399 |
| TNFα (pg/ml) | 1.99 (1.45; 2.90) | 2.14 (1.56; 3.00) | 0.864 |
| IL-1RA (pg/ml) | 341 ± 180 | 385 ± 216 | **0.005** |
| sICAM-1 (ng/ml) | 235 ± 56 | 246 ± 63 | 0.102 |
| Adiponectin (µg/ml) | 10.11 (6.56; 15.19) | 10.18 (6.77; 15.65) | 0.690 |
| Omentin (ng/ml) | 505 ± 170 | 523 ± 177 | 0.486 |

Data are given as mean ± SD, median and 25th/75th percentiles or percentages. The *P* values are derived from logistic regression analysis (likelihood ratio tests comparing models with the respective variable, age and sex as independent variables to models with age and sex only). All analyses were adjusted for age and sex except associations with age (sex-adjusted only) or sex (age-adjusted only). Biomarkers of oxidative stress and subclinical inflammation were log2-transformed prior to logistic regression.

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL, interleukin; IL-1RA, IL-1 receptor antagonist; LDL, low-density lipoprotein; MPO, myeloperoxidase; ndT2D, newly diagnosed type 2 diabetes; NGT, normal glucose tolerance; sICAM-1, soluble intercellular adhesion molecule-1; SOD3, extracellular superoxide dismutase; TNF, tumour necrosis factor; T2D, type 2 diabetes.

a Blood pressure of 140/90 mmHg or higher, or antihypertensive medication given that the subjects were aware of being hypertensive.

 b Individuals using lipid-lowering drugs excluded (*n*=265).

 c Nonsteroidal anti-inflammatory drugs except acetylsalicylic acid used as platelet aggregation inhibitor.

**Table 4**  **Associations of serum concentrations of MPO and SOD3 with prevalent DSPN**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Model** | **MPO** |  | **SOD3** |  |
|  | **OR (95% CI)** | ***P*** | **OR (95% CI)** | ***P*** |
| 1 | **1.36 (1.11; 1.67)** | **0.003** | 1.39 (0.81; 2.38) | 0.233 |
| 2 | **1.33 (1.08; 1.63)** | **0.007** | 1.36 (0.79; 2.35) | 0.267 |
| 3 | **1.36 (1.10; 1.68)** | **0.005** | 1.17 (0.67; 2.05) | 0.589 |
| 4 | **1.36 (1.09; 1.69)** | **0.007** | 1.20 (0.66; 2.17) | 0.552 |
| 5 | **1.38 (1.10; 1.72)** | **0.005** | 1.23 (0.68; 2.23) | 0.501 |

MPO and SOD3 levels were log2-transformed for the logistic regression analysis, so that OR (95% CI) refer to a doubling in biomarker levels levels.

Model 1: adjusted for age, sex.

Model 2: model 1 + physical activity, smoking, alcohol consumption.

Model 3: model 2 + waist circumference, height.

Model 4: model 3 + HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, lipid-lowering medication, hypertension, use of non-steroidal anti-inflammatory drugs, history of myocardial infarction, estimated glomerular filtration rate, neurological conditions that might cause nerve damage.

Model 5: model 4 + IL-6, TNFα.

**Table 5**  **Associations of serum concentrations of MPO and SOD3 with incident DSPN**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Model** | **MPO** |  | **SOD3** |  |
|  | **OR (95% CI)** | ***P*** | **OR (95% CI)** | ***P*** |
| 1 | 1.23 (0.96; 1.58) | 0.098 | **2.14 (1.02; 4.48)** | **0.044** |
| 2 | 1.21 (0.94; 1.57) | 0.135 | 1.77 (0.83; 3.81) | 0.141 |
| 3 | 1.21 (0.94; 1.57) | 0.142 | 1.72 (0.78; 3.76) | 0.177 |
| 4 | 1.20 (0.92; 1.56) | 0.180 | 1.66 (0.74; 3.73) | 0.217 |
| 5 | 1.17 (0.90; 1.53) | 0.243 | 1.61 (0.71; 3.66) | 0.254 |

MPO and SOD3 levels were log2-transformed for the logistic regression analysis, so that OR (95% CI) refer to a doubling in biomarker levels levels.

Model 1: adjusted for age, sex.

Model 2: model 1 + physical activity, smoking, alcohol consumption.

Model 3: model 2 + waist circumference, height.

Model 4: model 3 + HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, lipid-lowering medication, hypertension, use of non-steroidal anti-inflammatory drugs, history of myocardial infarction, estimated glomerular filtration rate, neurological conditions that might cause nerve damage.

Model 5: model 4 + IL-6, TNFα.

**Table S1 - Baseline characteristics of the KORA F4 study population stratified by incidence of DSPN**

| **Variable** | **No DSPN** | **Incident DSPN** | ***P*** |
| --- | --- | --- | --- |
| *n* | 392 | 132 |  |
| Age (years) | 67.9 ± 4.6 | 70.2 ± 5.0 | **<0.001** |
| Sex (% male / female) | 49.5 / 50.5 | 56.1 / 43.9 | 0.176 |
| BMI (kg/m2) | 27.6 ± 3.8 | 29.1 ± 4.0 | **<0.001** |
| Waist circumference (cm) | 94.8 ± 11.3 | 99.9 ± 11.4 | **<0.001** |
| Height (cm) | 166 ± 9 | 167 ± 9 | **0.018** |
| HbA1c (%) | 5.66 ± 0.48 | 5.83 ± 0.68 | **0.013** |
| HbA1c (mmol/mol) | 38 ± 5 | 40 ± 7 | **0.013** |
| Glucose tolerance status (NGT / IFG / IGT / IFG&IGT / ndT2D / kT2D) (%) | 45.7 / 21.4 / 9.2 /9.7 / 5.6 / 8.4 | 37.9 / 20.4 / 9.8 / 11.4 / 5.3 / 15.2 | 0.518 |
| Hypertension (%) a | 56.1 | 65.9 | 0.330 |
| Total cholesterol (mmol/l) b | 6.05 ± 1.00 | 5.84 ± 1.12 | 0.115 |
| LDL cholesterol (mmol/l) b | 3.87 ± 0.92 | 3.79 ± 0.94 | 0.610 |
| HDL cholesterol (mmol/l) b | 1.49 ± 0.38 | 1.37 ± 0.32 | **0.004** |
| Fasting triglycerides (mmol/l) b | 1.27 (0.97; 1.74) | 1.29 (1.03; 1.66) | 0.699 |
| Use of lipid-lowering drugs (%) | 22.7 | 25.8 | 0.728 |
| eGFR (ml/min per 1.73m2) | 80.3 ± 13.0 | 77.0 ± 14.1 | 0.470 |
| Smoking (never / former / current) (%) | 51.5 / 42.6 / 5.9 | 55.3 / 33.3 / 11.4 | **0.003** |
| Alcohol intake (none / moderate / high (%) | 30.9 / 52.5 / 16.6 | 29.6 / 52.3 / 18.2 | 0.586 |
| Physically active (%) | 62.0 | 42.4 | **0.001** |
| Myocardial infarction (%) | 5.1 | 6.8 | 0.888 |
| Neurological conditions that might cause nerve damage (%) | 14.9 | 20.4 | 0.064 |
| Use of nonsteroidal anti-inflammatory drugs (%) c | 1.0 | 2.3 | 0.388 |
| MNSI | 2.0 (0.5; 2.0) | 2.0 (2.0; 2.5) | **<0.001** |
| MPO (ng/ml) | 132 (88; 198) | 149 (97; 221) | 0.095 |
| SOD3 (ng/ml) | 123 (110; 136) | 129 (112; 148) | **0.043** |
| hsCRP (mg/l) | 1.32 (0.70; 2.52) | 1.60 (0.76; 3.42) | **0.027** |
| IL-6 (pg/ml)  | 1.33 (0.94; 1.96) | 1.71 (1.23; 2.50) | **0.005** |
| IL-18 (pg/ml) | 307 (251; 402) | 325 (255; 416) | 0.970 |
| TNFα (pg/ml) | 1.92 (1.39; 2.74) | 2.11 (1.60; 3.52) | **0.013** |
| IL-1RA (pg/ml) | 306 ± 128 | 349 ± 175 | **0.015** |
| sICAM-1 (ng/ml) | 224 ± 47 | 240 ± 58 | **0.008** |
| Adiponectin (µg/ml) | 10.15 (6.57; 15.44) | 9.44 (5.41; 13.58) | **0.046** |
| Omentin (ng/ml) | 496 ± 150 | 506 ± 155 | 0.706 |

Data are given as mean ± SD, median and 25th/75th percentiles or percentages. The *P* values are derived from logistic regression analysis (likelihood ratio tests comparing models with the respective variable, age and sex as independent variables to models with age and sex only). All analyses were adjusted for age and sex except associations with age (sex-adjusted only) or sex (age-adjusted only). Biomarkers of oxidative stress and subclinical inflammation were log2-transformed prior to logistic regression.

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL, interleukin; IL-1RA, IL-1 receptor antagonist; LDL, low-density lipoprotein; MPO, myeloperoxidase; ndT2D, newly diagnosed type 2 diabetes; NGT, normal glucose tolerance; sICAM-1, soluble intercellular adhesion molecule-1; SOD3, extracellular superoxide dismutase; TNF, tumour necrosis factor; T2D, type 2 diabetes.

a Blood pressure of 140/90 mmHg or higher, or antihypertensive medication given that the subjects were aware of being hypertensive.

 b Individuals using lipid-lowering drugs excluded (*n*=123).

 c Nonsteroidal anti-inflammatory drugs except acetylsalicylic acid used as platelet aggregation inhibitor.

Please note that a description of an almost identical study sample has been published in a previous report on biomarkers of inflammation and incident DSPN [Herder DC2017].

**Figure S1 - Description of the study design.**