**Effect of selenium supplementation on changes in glycated haemoglobin (HbA1c):
Results from a multiple-dose, randomized controlled trial**

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**Effect of selenium supplementation on changes in glycated haemoglobin (HbA_{1c}):
Results from a multiple-dose, randomized controlled trial**

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ABSTRACT

Aims: Long-term selenium supplementation may have adverse effects on glucose metabolism and risk of type-2 diabetes in selenium-replete populations, such as the US. There is limited trial evidence on the effect of selenium supplementation on glucose metabolism in European populations whose selenium status is much lower than that of the US. We investigated the effect of selenium supplementation at different dose levels on changes in HbA_{1c} after 6 months and 2 years in a population of relatively low selenium status.

Materials and methods: The Denmark PRECISE study was a single-centre, randomized, double-blinded, placebo-controlled, multi-arm, parallel clinical trial with four groups. In total, 491 volunteers aged 60-74 years were randomly assigned to treatment with 100, 200, or 300 µg selenium/d as selenium-enriched-yeast or placebo-yeast. HbA_{1c} measurements were available for 489 participants at baseline, 435 at 6 months, and 369 after 2 years of selenium supplementation. Analyses were performed by intention to treat.

Results: The mean (SD) age, plasma-selenium concentration, and blood HbA_{1c} at baseline were 66.1 (4.1) years, 86.5 (16.3) ng/g, and 36.6 (7.0) mmol/mol, respectively. During the initial 6-month intervention period, mean HbA_{1c} (95% CIs) decreased by 1.5 (-2.8 to -0.2) mmol/mol for 100 µg/d of selenium supplementation and by 0.7 (-2.0 to 0.6) mmol/mol for the 200 and 300 µg/d groups compared with placebo ($P = 0.16$ for homogeneity of changes across the four groups). After 2 years of selenium supplementation, HbA_{1c} had decreased significantly in all treatment groups, with no difference between active treatment and placebo.

Conclusions: Selenium supplementation in an elderly European population of relatively low selenium status did not significantly affect HbA_{1c} levels after 2 years. Our findings corroborate a possible U-shaped response of selenium supplementation on glucose metabolism.

INTRODUCTION

Selenium (Se) is a key component of a number of selenoproteins involved in essential functions such as redox homeostasis, endocrine and metabolic activities (1). Because of the potential of selenoproteins to protect against oxidative stress, Se was hypothesized to prevent the development of several chronic diseases including cancer, cardiovascular disease (CVD), and type 2 diabetes. However, findings from observational studies and randomized clinical trials have raised concerns that high Se exposure may lead to adverse cardio-metabolic effects, including an increased risk of type 2 diabetes in Se-replete populations such as the US (2-4).

Indeed, in a *post-hoc* analysis of the Nutritional Prevention of Cancer (NPC) trial in the eastern US, we found Se supplementation (200 µg/day), compared to placebo, increased the risk of type 2 diabetes, particularly among individuals with high baseline plasma Se (5). In addition, a number of selenoproteins, primarily selenoprotein P (SEPP1) and glutathione peroxidase (GPx1) have been linked with diabetogenic effects (6-9).

There are gaps in the current available evidence. First, many of the observational studies are cross-sectional surveys or prospective investigations based on a single analysis of Se status at one time point only, and are therefore subject to reverse causation and incidence/prevalence biases (10-13). Moreover, there have been disappointing findings from several costly clinical trials of antioxidant supplements showing not only an absence of health benefits but also potential harm (5, 14-18). Lastly, most previous research in this area has been conducted in North American populations where baseline Se status is substantially higher than in Europe. Dietary intakes of Se vary considerably between countries and regions largely due to the variability of the Se content of plant foods (and hence of animal forage) from one part of the world to another (1, 19).

We determined the effect of different doses of Se supplementation (100, 200, and 300 µg/d or placebo) on HbA_{1c} levels after 6 months and 2 years in a double-blind, randomized, controlled trial in Denmark, a country with lower Se status than that of the US (18, 20). Double-blind, placebo controlled randomized clinical trials are less prone to many types of biases than observational studies, and provide the strongest level of evidence on the effect of dietary interventions on health outcomes.

MATERIALS AND METHODS

Study design and sample size

The Denmark PRECISE (PREvention of Cancer by Intervention with SElenium) pilot study (ClinicalTrials.gov ID: NCT01819649) was a single-center, randomized, double-blinded, placebo-controlled, multi-arm, parallel clinical trial with four groups (allocation ratio 1:1:1:1) run from Odense University Hospital, Denmark (18, 21-22). Denmark PRECISE was one of two pilot studies for the proposed international PRECISE trial of Se in cancer prevention; the other was carried out in the UK (23-24). No formal power calculations were performed *a priori*; the sample size was set at 500 participants, which was considered sufficient to assess whether recruitment, adherence, and retention during follow-up would be sufficient for a large trial. From November 1998 to June 1999, 2897 potential participants, males and females aged 60–74 years from the County of Funen, Denmark, were invited to take part in the trial; 630 accepted the invitation to be screened for inclusion at Odense University Hospital. Exclusion criteria were: A Southwest Oncology Group performance-status score > 1, indicating impairment in general well-being and activities of daily life; active liver or kidney disease; previous diagnosis of cancer (excluding non-melanoma skin cancer); diagnosed HIV infection; receiving immunosuppressive

therapy; being unable to understand written/spoken information; and receiving $\geq 50 \mu\text{g/d}$ of Se supplements in the previous 6 months.

Randomization was computer-generated, blocked and non-stratified (18, 21-22). Participating couples living at the same address were allocated to the same intervention. Participants, research staff and investigators were blinded to treatment assignment (18, 21-22). Participants deemed suitable for inclusion provided blood samples and were given yeast tablets for an open-label 4-week placebo run-in phase. Those ($n=491$) who met the inclusion criteria, displayed good adherence in the placebo run-in phase, and gave written, informed consent, were randomised to 0 (placebo-yeast), 100, 200, or 300 $\mu\text{g/d}$ of Se as Se-enriched yeast (SelenoPrecise[®] Pharma Nord, Vejle, Denmark) (**Figure 1**). Participant evaluation was carried out at Odense University Hospital at baseline, 6, 12, 18 months, 2, 3 and 5 years, as previously described in detail (18, 21-22). The intervention was delivered for 5 years and participants were followed up, post-treatment, for an additional 10 years for mortality ascertainment.

The regional Data Protection Agency and Scientific Ethics Committees of Vejle and Funen counties approved the study prior to data collection (Journal nr. 19980186).

Baseline measurements

Demographic characteristics, smoking status, height, weight, and supplement use were collected at baseline. Medications used were obtained from medical records. Morbidity data were obtained from the Danish National Patient Registry which has records of major and secondary diagnoses for all in-patient discharges since 1977 and all emergency and outpatient contacts since 1995 (25). The Charlson comorbidity index was computed by adding 19 comorbid conditions diagnosed prior to randomization (26).

Selenium measurement

As previously described in detail (18, 21-22), total Se was measured gravimetrically (ng/g) in lithium-heparin plasma at LGC Limited, Teddington, United Kingdom, by inductively coupled-plasma mass spectrometry at baseline, 6-months and 5-years. Analysis of a matrix-certified reference material indicated good accuracy of the method. The intra-assay coefficients of variation (CVs) ranged from 0.5% for samples of high-Se concentration to 3% for samples of low-Se concentration. The inter-assay CV was 3.4%.

HbA_{1c} measurement

HbA_{1c} at baseline and at the 6-month and 2-year visits were measured in stored red blood cells at the Department of Clinical Biochemistry, King's College Hospital, United Kingdom, between June and August 2015. Measurements were performed using a Premier Hb9210 System (A Menarini Diagnostics Ltd, Winnersh-Wokingham, Berkshire, United Kingdom), employing the principles of boronate affinity and high-performance liquid chromatography. Samples were diluted 1:300 using the Hamilton Diluter. The intra-assay CVs ranged from 0.9% for samples of high HbA_{1c} concentration to 1.2% for samples of low HbA_{1c} concentration. The inter-assay CV was 2.8%. HbA_{1c} concentrations were reported in mmol/mol.

Power calculations for intention-to-treat analysis

The two study endpoints were the differences in HbA_{1c} concentration in each intervention group compared with placebo at 6 months and 2 years of follow-up. For the average sample size of 117 participants retained in each treatment group after 6 months of follow-up and a two-sided significance level of 0.05, the power to detect underlying differences of 1, 1.5, and 2 mmol/mol in mean HbA_{1c} changes after 6 months of follow-up comparing any active treatment group with

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3 placebo were 35.6, 66.4, and 88.9%, respectively. Given the smaller average number of 105
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5 participants remaining in each group after 2 years of follow-up, the power to detect the same
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7 differences in mean HbA_{1c} changes after 2 years was 20.4, 39.5, and 61.8%, respectively.
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11 **Statistical analysis**
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14 For the analysis of randomized groups, all trial participants were assigned to their randomized
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16 treatment group, irrespective of compliance (intention-to-treat analysis). The effect of 100, 200,
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18 and 300 µg/d of Se supplementation on changes in HbA_{1c} after 6 months and 2 years was
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20 estimated by using a linear mixed model (22-23, 27), with fixed effects for treatment groups,
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22 follow-up times, and treatment-by-time interactions, and random between-subject variations in
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24 baseline HbA_{1c} levels (intercepts) and HbA_{1c} changes over follow-up (time slopes). The model is
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26 specified in detail in the Statistical Appendix. Treatment effects were estimated as the
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28 differences in mean HbA_{1c} changes from baseline to 6 months and 2 years comparing the three
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30 active treatment groups with placebo. In sensitivity analyses, we excluded visits after participants
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32 received diabetes medications at baseline or during the intervention period.
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37 We also evaluated treatment-effect modifications across pre-specified subgroups defined
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39 by baseline age (< or ≥ 65 years), sex, smoking status (non-current or current), alcohol drinking
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41 (≤ or > 2 drinks/week), body mass index (< or ≥ 25 kg/m²), baseline plasma Se (< or ≥ 80 ng/g),
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43 and baseline HbA_{1c} (< or ≥ 42 mmol/mol) by including all main terms and interactions between
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45 treatment groups, time, and the corresponding covariate as fixed effects in the above mixed
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47 model.
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51 In addition to the intention-to-treat analysis, we evaluated the cross-sectional association of
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53 plasma Se and HbA_{1c} levels at baseline and the longitudinal association between changes in
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55 plasma Se and HbA_{1c} levels after 6 months (plasma Se measurements were not available at 2
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years). As described in the Statistical Appendix, we used a linear mixed model (22-23, 28) with random intercepts, random time slopes, and fixed slopes for baseline Se levels and Se changes at 6 months to estimate the mean difference in baseline HbA_{1c} levels per 50-ng/g increase in baseline Se levels (cross-sectional association), as well as the difference in mean HbA_{1c} changes from baseline to 6 months for each 50-ng/g change in Se levels (longitudinal association). We also categorized baseline Se levels and Se changes into quartiles in the above mixed model and compared mean baseline HbA_{1c} levels across quartiles of baseline Se and mean HbA_{1c} changes after 6 months across quartiles of Se change. Cross-sectional and longitudinal associations were adjusted for baseline age (continuous), sex, smoking status (never, former, or current), alcohol drinking (≤ 2 , 3–10, or > 10 drinks/week), body mass index (continuous), and use of diabetes medications.

All reported *P* values were two-sided and the significance level was set at 0.05. Statistical analyses were performed with Stata, version 14 (StataCorp).

RESULTS

Participants

Of the 491 randomized participants, 23 withdrew from treatment before 6 months of follow-up, 48 dropped out between 6 months and 2 years, and the remaining 420 participants completed the 2-year follow-up period (**Figure 1**). The 71 drop-outs before the end of the study period were equally distributed across treatment groups ($P = 0.36$). HbA_{1c} measurements were available for 489 participants at baseline, 435 at 6 months, and 369 at 2 years (**Figure 1**). Participants with and without available HbA_{1c} measurements at 6 months and 2 years did not differ in baseline HbA_{1c} levels or other baseline characteristics (data not shown). Non-protocol use of over-the-counter Se was deemed to be rare since only one of the 108 participants allocated to placebo

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3 (0.9%) had plasma Se concentrations more than two standard deviations above the mean at 6
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5 months. Six participants were receiving diabetes medications at baseline, six at 6 months, and 14
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7 at 2 years, with no significant differences between treatment groups at any time ($P = 0.70, 0.73,$
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9 and 0.12 ; respectively).
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14 **Analysis of randomized groups**
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16 The mean (SD) age, plasma Se concentration, and HbA_{1c} at baseline were 66.1 (4.1) years, 86.5
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18 (16.3) ng/g, and 36.6 (7.0) mmol/mol, respectively. There were no significant differences
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20 between treatment groups at baseline in plasma Se concentrations, HbA_{1c}, or other participant
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22 characteristics (**Table 1**). Mean plasma Se concentrations (95% confidence intervals [CIs])
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24 increased significantly by 64.8 (57.7 to 71.9) ng/g, 120.9 (113.7 to 128.0) ng/g, and 169.8 (162.4
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26 to 177.1) ng/g after 6 months of Se supplementation at 100, 200, and 300 µg/d, respectively, but
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28 were virtually unchanged in the placebo group ($P < 0.001$ for homogeneity of changes across the
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30 four groups).
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34 However, small changes in HbA_{1c} were observed in the treatment groups after 6 months
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36 of intervention (**Table 2**). Compared with placebo, mean HbA_{1c} differences (95% CIs) at 6
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38 months were -1.5 (-2.8 to -0.2) mmol/mol for 100 µg/d and -0.7 (-2.0 to 0.6) mmol/mol for 200
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40 and 300 µg/d of Se supplementation ($P = 0.16$ for homogeneity of changes across the four
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42 groups). The decrease in HbA_{1c} levels between the 100 µg/d Se and placebo groups remained
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44 significant after excluding participants who received diabetes medications during the initial 6-
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46 month intervention period (change -1.6 mmol/mol, 95% CI -2.9 to -0.3 mmol/mol). In subgroup
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48 analyses, there were no significant treatment-effect modifications at 6 months by participant
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50 characteristics (**Figure 2**), although 300 µg/d of Se supplementation showed larger HbA_{1c}
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decreases in participants with lower plasma Se concentrations and pre-diabetes HbA_{1c} levels at baseline.

After 2 years, HbA_{1c} decreased significantly in the four treatment groups but there were no significant differences between active treatment groups and placebo (**Table 2**). Compared with placebo, mean HbA_{1c} levels (95% CIs) changed by -0.9 (-2.7 to 0.9), 0.1 (-1.7 to 2.0), and -1.0 (-2.8 to 0.8) mmol/mol for 100, 200, and 300 µg/d of Se supplementation, respectively ($P = 0.52$ for homogeneity of changes across the four groups). The exclusion of visits after participants received diabetes medications did not materially alter the results at 2 years (data not shown).

The decrease in HbA_{1c} levels after two years of intervention in the placebo group was not explained by selective drop-outs (mean baseline HbA_{1c} levels of 35.7 and 36.1 mmol/mol among 109 retained participants and 17 drop-outs from the placebo group, respectively, $P = 0.77$) or diabetes treatment initiation (mean HbA_{1c} decrease of 2.0 mmol/mol, 95% CI 0.8 to 3.2 mmol/mol, after excluding 2 participants in the placebo group who received diabetes medications during follow-up). Hawthorne or placebo effects might account for part of the observed decline in HbA_{1c} levels over time in this elderly population. Anyway, the estimated treatment effects were corrected for changes in the placebo group since they were subtracted from the corresponding longitudinal HbA_{1c} changes in the three active treatment groups.

Association between plasma selenium and HbA_{1c} levels

In cross-sectional analyses at baseline, plasma Se concentrations were not associated with HbA_{1c} levels (**Table 3**). In longitudinal analyses, there was a U-shaped association between changes in plasma Se and HbA_{1c} levels after 6 months (Figure 3). Compared with the first quartile of Se change, mean (95% CI) HbA_{1c} levels changed by -1.5 (-2.6 to -0.3), -0.4 (-1.5 to 0.8) and 0.3 (-

0.8 to 1.5) mmol/mol from baseline to 6 months in the second, third, and fourth quartiles, respectively (**Table 4**).

Adverse events

Eight participants died during the 2-year follow-up period and 21 participants discontinued the study due to non-fatal adverse events (**Figure 1**), with no significant differences between treatment groups ($P = 0.75$ and 0.25 ; respectively). Eighteen participants withdrew due to adverse reactions to treatment (**Figure 1**), which were mainly hair loss, skin reactions, and grooved nails. These reactions were equally distributed across placebo and the three active treatment groups ($P = 0.63$).

DISCUSSION

Overall, findings from the Denmark PRECISE trial do not support a beneficial or harmful effect of Se supplements on glucose metabolism. Specifically, in this European population of elderly individuals of relatively low Se status, supplementation at different dose levels of Se did not significantly affect HbA_{1c} levels after 2 years of supplementation. However, compared to placebo, small beneficial changes in HbA_{1c} were observed in the treatment groups after 6 months of intervention, particularly at the lowest dose level of Se supplementation (100 µg/d).

The latter finding seems to corroborate the notion of a U-shaped association of selenium status and supplementation with glucose metabolism, and possibly other health outcomes (1-4, 29).

Evidence from *in vivo* and *in vitro* studies suggests that Se could enhance insulin sensitivity by mediating insulin-like actions (30). However, results from human studies on Se and glucose metabolism or diabetes risk are conflicting. Findings from observational studies and randomised clinical trials from the US, a Se-replete population, indicate that high Se status or Se

supplementation may be associated with an increased risk of type 2 diabetes (5, 10-11).

Specifically, in a *post-hoc* analysis of the Nutritional Prevention of Cancer (NPC) trial, in the eastern US, we found that supplementation with Se (200 µg/day as high-Se yeast), compared to placebo, increased the risk of type 2 diabetes (5), particularly among participants with high baseline plasma Se (hazard ratio of 2.70 in the highest tertile of plasma Se, i.e. >121.6 µg/l).

Moreover, results from the large Se and Vitamin E Cancer Prevention Trial (SELECT) in 35,533 North American men aged ≥ 50 y (15), showed a small, though non-significant, increase in the number of cases of adult-onset diabetes in subjects supplemented with Se alone (200 µg/day as selenomethionine). However, after a further 33 months of follow-up in the SELECT trial, the risk of diabetes had diminished: RR 1.04; 99% CI 0.91 to 1.18 (16).

The overall trial evidence on the effect of Se supplementation on diabetes risk was pooled together by our group in a Cochrane review (29). We found a small increased risk of type-2 diabetes with Se supplementation, which did not reach statistical significance (RR 1.06, 95% CI 0.97 to 1.15). However, that review included only the limited number of randomized clinical trials that administered Se alone, all carried out in US populations (29).

In European populations, where Se status is generally lower than in the US, the evidence linking Se to glucose metabolism is conflicting. For example, in an early analysis of the EVA (Epidemiology of Vascular Ageing) study in France, plasma Se concentrations were positively, though non-significantly, associated with baseline glucose levels in women and with prevalent diabetes in men (12). However, a later report from the same study showed that high plasma Se (1.19-1.97 µmol/L) was associated with a marginally significant reduced risk of hyperglycaemia (impaired fasting glucose or diabetes) in men over the 9-year follow-up (31). Conversely, in a prospective study on a large sample of women from Northern Italy, an increased risk of type-2

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diabetes was associated with higher dietary Se intake; however it has to be admitted that dietary assessments of Se intake are unreliable so not much weight can be attached to this result (13).

With regard to trial evidence, in the French SU.VI.MAX study, no effect of combined supplementation with antioxidants including Se (100 µg/day as high-Se yeast), was observed on fasting plasma glucose after 7.5 y of follow-up despite a positive association between glucose and Se concentrations at baseline in the whole population (32). More recently, in the UK PRECISE Trial among 501 elderly volunteers treated for 6 months with 100, 200 or 300 µg Se/d as high-Se yeast or placebo yeast, we found no effect of Se supplementation on plasma adiponectin, a surrogate marker of type-2 diabetes risk (24).

Findings from the Denmark PRECISE trial seem to suggest that Se supplementation is unlikely to impair glucose metabolism and increase diabetes risk in populations of relatively low Se status. Moreover, small beneficial changes in HbA_{1c} were observed in the treatment groups after 6 months of intervention, particularly at the lowest dose level of Se supplementation (100 µg/d), which is in line with a possible U-shaped relationship between selenium and glucose metabolism (1-4, 29).

In general, the degree to which populations are adequately supplied with a nutrient is more variable for Se than for many nutrients. Because of wide differences in geology, soil and climatic factors, foods and fodder from different parts of the world vary greatly in their ability to provide Se for dietary intake (4). Thus intake ranges from deficient to excessive in China, is generally low in Europe, adequate in North America and Japan and is high in Venezuela (4). Se status – often measured as serum, plasma, or toenail Se – varies accordingly, reflecting the difference in intake in these locations (4). Therefore, the geographic variations in Se intake and status across countries and populations are likely to represent one plausible explanation for the discrepant results in the published literature.

In terms of potential mechanisms to justify a role of Se in glucose metabolism, *in vivo* and *in vitro* studies have shown anti-diabetic and insulin mimetic effects of Se (generally as selenate) (30); however, doses have been used that would be toxic to humans calling into question the relevance of these findings (7, 33).

Type-2 diabetes is associated with oxidative stress attributable to the production of excess levels of reactive oxygen species in hyperglycemia (34). As pancreatic β cells are poorly protected by intrinsic enzymatic antioxidants and are very susceptible to oxidative injury, it might be thought that the antioxidant selenoenzyme, GPx, could protect against that stress (34). Indeed overexpression of GPx in islets provided enhanced protection against oxidative stress (34), while Se, as selenite or selenate at physiological levels, was able to stimulate pancreatic β -cell gene expression and enhance islet function (35). Whether as cause or consequence, plasma GPx3 concentration was found to be decreased in newly diagnosed, drug-naïve diabetic patients compared to subjects with normal or impaired glucose tolerance and was lower in *db/db* than in normal mice (36). Another selenoprotein, SELENOP, appears to protect the β cell against endoplasmic reticulum (ER) stress and oxidative stress and may protect against β -cell apoptosis (37). Binding of insulin to its receptor initiates the insulin-signalling cascade, which is accompanied by a burst of hydrogen peroxide that acts as a second messenger (7). High activity of (cytosolic) GPx1 can interfere with insulin signalling by removing hydrogen peroxide (38). Thus, transgenic mice overexpressing GPx1 developed insulin resistance, hyperglycaemia, hyperinsulinaemia and obesity (38-39), while knockout of GPx1 improved insulin-induced glucose uptake and insulin resistance (40).

SELENOP has also been implicated in insulin resistance (6). *In vitro*, in the presence of insulin, SELENOP modulated insulin signalling to upregulate mRNA expression of

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gluconeogenic enzymes, resulting in 30% increase in glucose release. Treatment of mice with purified SELENOP impaired insulin signalling in liver and skeletal muscle and induced glucose intolerance (6). Conversely, knockdown of the gene for SELENOP in liver improved glucose tolerance and reduced insulin resistance in mice with type-2 diabetes while mice deficient in SELENOP showed improved glucose tolerance and enhanced insulin signalling (6). It is thought that the effect of SELENOP is at least partly through the inactivation of AMP-activated protein kinase, a positive regulator of insulin synthesis and secretion in pancreatic β -cells (6).

Our study has limitations: changes in HbA_{1c} were not the primary endpoint of the Denmark PRECISE Trial, hence the need for cautious interpretation of our findings, as they result from post-hoc analyses; it was conducted in apparently healthy elderly, so the results may not extend to other age groups or participants with different co-morbidities; 71 participants dropped out before completing the 2-year intervention period, though they were equally distributed between treatment groups and captured in the intention-to-treat mortality analysis. Follow-up was only for six months and 2 years, which may not have been long enough to see an effect. Importantly, the role of chance in our findings cannot be ruled out as the sample size was small and the study was not designed to assess the effect of Se supplementation on HbA_{1c}.

In conclusion, our findings add to the limited trial evidence on the effect of Se, as a single agent at different dose levels, on diabetes risk, in a non-Se-replete population, as most of the previous evidence was based on randomized clinical trials conducted among US populations (5, 15-16, 29). Overall, our results are reassuring as they do not support potential harmful effects of Se supplements on glucose metabolism in populations of relatively low Se status. Further research is needed to identify the optimal range of Se intake and status in order to minimize potential adverse effects on glucose metabolism while optimising type-2 diabetes prevention (4).

Author Contributions: SS, MPR and SC designed research; KHW, SC and RPB conducted research and analysed data; RPB performed statistical analysis; SS, MPR, and RPB wrote the paper with help from KHW and EG; SS (guarantor) and MPR had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1. Baseline characteristics of trial participants overall and by treatment group*

Characteristic	Overall	Placebo	Selenium dose (µg/d)			P value†
			100	200	300	
No. of participants	491	126	124	122	119	
Age (years)	66.1 (4.1)	65.4 (3.8)	66.4 (4.2)	66.3 (4.4)	66.5 (4.1)	0.13
Sex						0.49
Men	255 (51.9)	60 (47.6)	70 (56.5)	66 (54.1)	59 (49.6)	
Women	236 (48.1)	66 (52.4)	54 (43.5)	56 (45.9)	60 (50.4)	
Smoking status						0.49
Never	160 (32.6)	35 (27.8)	42 (33.9)	40 (32.8)	43 (36.2)	
Former	185 (37.7)	48 (38.1)	47 (37.9)	52 (42.6)	38 (31.9)	
Current	146 (29.7)	43 (34.1)	35 (28.2)	30 (24.6)	38 (31.9)	
Alcohol drinking (drinks/week)						0.76
≤ 2	170 (34.6)	47 (37.3)	41 (33.1)	40 (32.8)	42 (35.3)	
3–10	209 (42.6)	48 (38.1)	51 (41.1)	55 (45.1)	55 (46.2)	
> 10	112 (22.8)	31 (24.6)	32 (25.8)	27 (22.1)	22 (18.5)	
Body mass index (kg/m ²)	26.8 (4.1)	26.5 (4.0)	27.1 (4.0)	27.2 (4.3)	26.5 (4.0)	0.40
Plasma selenium						0.17
ng/g	86.5 (16.3)	86.0 (15.2)	87.5 (16.4)	88.3 (16.2)	83.9 (17.1)	
µg/L	88.6 (16.7)	88.2 (15.6)	89.7 (16.8)	90.5 (16.6)	86.0 (17.5)	
HbA _{1c}						0.34
mmol/mol	36.6 (7.0)	35.7 (5.7)	37.4 (7.4)	36.6 (7.9)	36.7 (7.0)	
%	5.5 (0.6)	5.4 (0.5)	5.6 (0.7)	5.5 (0.7)	5.5 (0.6)	
Use of diabetes medication	6 (1.2)	1 (0.8)	3 (2.4)	1 (0.8)	1 (0.8)	0.70

* Data are means (SDs) or numbers (%).

† P value for homogeneity of means or proportions across the four treatment groups.

HbA_{1c}, glycated hemoglobin.

Table 2. Effect of selenium supplementation on changes in HbA_{1c} and plasma selenium concentrations after 6 months and 2 years*

Variable	Placebo	Selenium dose (µg/d)			P value†
		100	200	300	
HbA_{1c} (mmol/mol)					
Mean (SD) at baseline	35.7 (5.7)	37.4 (7.4)	36.6 (7.9)	36.7 (7.0)	
Mean (SD) at 6 mo	36.4 (5.2)	37.1 (8.3)	36.9 (7.3)	36.5 (4.8)	
Change from baseline to 6 mo (95% CI)	0.7 (-0.2 to 1.6)	-0.8 (-1.7 to 0.1)	0.1 (-0.9 to 1.0)	0.0 (-0.9 to 0.9)	
Difference in change (95% CI)	0 (Reference)	-1.5 (-2.8 to -0.2)	-0.7 (-2.0 to 0.6)	-0.7 (-2.0 to 0.6)	0.16
P value		0.02	0.32	0.30	
Mean (SD) at 2 yr	34.0 (7.2)	34.8 (7.2)	34.9 (7.7)	33.8 (7.8)	
Change from baseline to 2 yr (95% CI)	-1.8 (-3.1 to -0.6)	-2.7 (-4.0 to -1.4)	-1.7 (-3.0 to -0.3)	-2.8 (-4.1 to -1.5)	
Difference in change (95% CI)	0 (Reference)	-0.9 (-2.7 to 0.9)	0.1 (-1.7 to 2.0)	-1.0 (-2.8 to 0.8)	0.52
P value		0.35	0.88	0.30	
Plasma selenium‡ (ng/g)					
Mean (SD) at baseline	86.0 (15.2)	87.5 (16.4)	88.3 (16.2)	83.9 (17.1)	
Mean (SD) at 6 mo	85.3 (14.2)	152.4 (23.7)	209.1 (41.5)	253.7 (54.1)	
Change from baseline to 6 mo (95% CI)	-0.8 (-8.0 to 6.4)	64.8 (57.7 to 71.9)	120.9 (113.7 to 128.0)	169.8 (162.4 to 177.1)	
Difference in change (95% CI)	0 (Reference)	65.6 (55.6 to 75.7)	121.7 (111.6 to 131.9)	170.6 (160.3 to 180.9)	< 0.001
P value		< 0.001	< 0.001	< 0.001	

* Results were obtained from linear mixed models with fixed treatment-by-time interactions and random between-subject variations in both baseline HbA_{1c} levels and HbA_{1c} changes over time.
† Overall P value comparing the three active treatment groups with placebo.
‡ Plasma selenium measurements were not available at 2 years of follow-up.
HbA_{1c}, glycated hemoglobin.

Table 3. Cross-sectional association between plasma selenium and HbA_{1c} levels at baseline*

Variable	50-ng/g increase in baseline selenium	Quartile of baseline selenium (ng/g)				<i>P</i> value for trend†
		First (43 to 76)	Second (77 to 85)	Third (86 to 95)	Fourth (96 to 159)	
Median baseline selenium (ng/g)	85	70	80	89	104	
No. of participants	488	126	129	114	119	
HbA_{1c} (mmol/mol)						
Mean (SD) at baseline	36.6 (7.0)	37.4 (8.0)	36.5 (6.7)	36.0 (5.5)	36.6 (7.6)	
Adjusted mean difference (95% CI)	-1.1 (-2.6 to 0.5)	0 (Reference)	-0.3 (-1.7 to 1.1)	-1.2 (-2.7 to 0.2)	-0.8 (-2.3 to 0.6)	0.18

* Results were obtained from linear mixed models with random between-subject variations in baseline HbA_{1c} levels and adjusted for baseline age (continuous), sex, smoking status (never, former, or current), alcohol drinking (≤ 2 , 3–10, or > 10 drinks/week), body mass index (continuous), and use of diabetes medications.

† *P* value for linear trend using an ordinal variable with the median baseline selenium level in each quartile.

HbA_{1c}, glycated hemoglobin.

Table 4. Longitudinal association between changes in plasma selenium and HbA_{1c} levels after 6 months*

Variable	50-ng/g increase in selenium over time	Quartile of selenium change (ng/g)				P value†
		First (-48 to 24)	Second (25 to 81)	Third (82 to 138)	Fourth (139 to 355)	
Median selenium change (ng/g)	81	-2	58	107	172	
No. of participants	432	108	108	110	106	
HbA_{1c} (mmol/mol)						
Mean (SD) change at 6 mo	-0.1 (4.8)	0.5 (4.1)	-1.2 (4.6)	-0.5 (6.2)	0.9 (3.8)	
Adjusted difference in change (95% CI)	0.1 (-0.1 to 0.4)	0 (Reference)	-1.5 (-2.6 to -0.3)	-0.4 (-1.5 to 0.8)	0.3 (-0.8 to 1.5)	0.02

* Results were obtained from linear mixed models with random between-subject variations in both baseline HbA_{1c} levels and HbA_{1c} changes over time and adjusted for baseline selenium concentration (continuous), age (continuous), sex, smoking status (never, former, or current), alcohol drinking (≤ 2, 3–10, or > 10 drinks/week), body mass index (continuous), and use of diabetes medications.

† Overall P value comparing the three highest quartiles of selenium change with the lowest quartile.

HbA_{1c}, glycated hemoglobin.

FIGURE LEGENDS

Figure 1. Study flow diagram.

HbA_{1c}, glycated hemoglobin.

Figure 2. Differences in mean HbA_{1c} changes after 6 months comparing the three active treatment groups with placebo by pre-specified baseline subgroup.

Subgroup-specific differences in mean HbA_{1c} changes (squares with area inversely proportional to the variance) and their 95% CIs (horizontal lines) were obtained from linear mixed models with fixed interactions between treatment group, time, and the corresponding baseline covariate and random between-subject variations in both baseline HbA_{1c} levels and HbA_{1c} changes over time. HbA_{1c}, glycated hemoglobin; Se, selenium.

Figure 3. Changes in HbA_{1c} levels after 6 months by change in plasma selenium concentration.

Curve represents mean changes in HbA_{1c} levels from baseline to 6 months (solid line) and their 95% CIs (shaded region) based on restricted quadratic splines for changes in plasma selenium concentrations with knots at the 5th, 50th, and 95th percentiles (-11, 81, and 217 ng/g, respectively). Results were obtained from a linear mixed model with random between-subject variations in both baseline HbA_{1c} levels and HbA_{1c} changes over time and adjusted for baseline selenium levels (continuous), age (continuous), sex, smoking status (never, former, or current), alcohol drinking (≤ 2 , 3–10, or > 10 drinks/week), body mass index (continuous), and use of diabetes medications.

Scatterplot represents changes in plasma selenium and HbA_{1c} levels after 6 months for participants randomized to placebo (green dots), 100 $\mu\text{g/d}$ (blue dots), 200 $\mu\text{g/d}$ (orange dots), and 300 $\mu\text{g/d}$ (red dots) of selenium supplementation. HbA_{1c}, glycated hemoglobin.

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STATISTICAL APPENDIX

For the analysis of randomized groups, the linear mixed model for HbA_{1c} levels Y_{ij} at baseline $j = 0$, 6-month $j = 1$, and 2-year visits $j = 2$ for participant i was

$$\begin{aligned} Y_{ij} = & (\beta_{00} + b_{i0}) + \beta_{01}T_{i1} + \beta_{02}T_{i2} + \beta_{03}T_{i3} \\ & + (\beta_{10} + b_{i1})t_1 + \beta_{11}T_{i1}t_1 + \beta_{12}T_{i2}t_1 + \beta_{13}T_{i3}t_1 \\ & + (\beta_{20} + b_{i2})t_2 + \beta_{21}T_{i1}t_2 + \beta_{22}T_{i2}t_2 + \beta_{23}T_{i3}t_2 + \varepsilon_{ij}, \end{aligned}$$

where T_{i1} , T_{i2} , and T_{i3} were treatment indicators for the three active treatment groups of 100, 200, and 300 µg/d of selenium supplementation, respectively; t_1 and t_2 were time indicators for 6-month and 2-year follow-up visits, respectively; and the within-subject error ε_{ij} and the between-subject variations b_{i0} , b_{i1} , and b_{i2} were assumed to be independent and normally distributed with mean 0 and constant variances. In this model, the fixed effect β_{00} represented the mean baseline HbA_{1c} level for the placebo group; the fixed effects β_{01} , β_{02} , and β_{03} represented the differences in mean baseline HbA_{1c} levels for the three active treatment groups compared with placebo; and the random effect b_{i0} represented the unexplained between-subject variation in baseline HbA_{1c} levels. Also, the fixed effect β_{10} represented the mean HbA_{1c} change from baseline to 6 months for the placebo group; the fixed effects β_{11} , β_{12} , and β_{13} represented the differences in mean HbA_{1c} changes from baseline to 6 months for the three active treatment groups compared with placebo (treatment effect at 6 months); and the random effect b_{i1} represented the unexplained between-subject variation in HbA_{1c} changes from baseline to 6 months. Similarly, the fixed effect β_{20} represented the mean HbA_{1c} change from baseline to 2 years for the placebo group; the fixed effects β_{21} , β_{22} , and β_{23} represented the differences in mean HbA_{1c} changes from baseline to 2 years for the three active treatment groups compared with placebo (treatment effect at 2 years); and the random effect b_{i2} represented the unexplained between-subject variation in HbA_{1c} changes from baseline to 2 years.

To assess the cross-sectional and longitudinal associations between plasma selenium and HbA_{1c} concentrations, the linear mixed model for HbA_{1c} levels Y_{ij} at baseline $j = 0$ and 6 months $j = 1$ for participant i was

$$Y_{ij} = (\beta_{00} + b_{i0}) + \beta_{01}x_{i0} + \beta_{02}z_{i0} + (\beta_{10} + b_{i1})t_1 + \beta_{11}(x_{ij} - x_{i0}) + \varepsilon_{ij},$$

where x_{ij} was the plasma selenium level at visit j and z_{i0} were other covariates measured at baseline, including age, sex, smoking status, alcohol drinking, body mass index, and use of diabetes medications. The fixed effect β_{00} represented the mean baseline HbA_{1c} level at 0 values of selenium and other baseline covariates; the fixed effect β_{01} represented the covariate-adjusted mean difference in baseline HbA_{1c} levels per unit increase in baseline selenium levels (cross-sectional association at baseline); the fixed effects β_{02} represented the adjusted coefficients for the other baseline covariates; and the random effect b_{i0} was the unexplained between-subject variation in baseline HbA_{1c} levels. In addition, the fixed effect β_{10} represented the mean HbA_{1c} change from baseline to 6 months when selenium remained unchanged; the fixed effect β_{11} represented the difference in mean HbA_{1c} changes from baseline to 6 months per unit change in selenium levels adjusted for baseline covariates (longitudinal association at 6 months); and the random effect b_{i1} was the unexplained between-subject variation in HbA_{1c} changes over time.