Association of dietary oxidative balance score and sleep duration with the risk of mortality: prospective study in a representative US population

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Abstract

Objective: We investigated the association between dietary oxidative balance score (DOBS) and mortality and whether this association can be modified by sleep duration.

Design: We calculated DOBS to estimate the overall oxidative effects of the diet, with higher DOBS reflecting more antioxidant intake and less pro-oxidant intake. Cox proportional hazards models were employed to examine the associations between DOBS and all-cause, CVD and cancer mortality in the general population and people with different sleep durations.

Setting: Prospective analysis was conducted using data from the US National Health and Nutrition Examination Survey (NHANES, 2005–2015).

Participants: A total of 15 991 US adults with complete information on dietary intake, sleep duration and mortality were included.

Results: During a median follow-up of 7.4 years, 1675 deaths were observed. Participants in the highest quartile of DOBS were significantly associated with the lower risk of all-cause mortality (hazard ratio (HR) = 0.75; 95 % CI 0.61, 0.93) compared with those in the lowest. Furthermore, we found statistically significant interactions between DOBS and sleep duration on all-cause mortality ($P_{interaction}$ = 0.021). The inverse association between DOBS and all-cause mortality was significant in short sleepers (HR = 0.66, 95 % CI 0.48, 0.92), but not in normal and long sleepers.

Conclusions: Our study observed that higher DOBS was associated with lower all-cause mortality, and this association appeared to be stronger among short sleepers. This study provides nutritional guidelines for improving health outcomes in adults, especially for short sleepers.

CVD and cancer, as crucial constituents of chronic non-communicable diseases, have accounted for a large proportion of the deaths⁴⁵. It is widely accepted that the generation of free radicals caused by an imbalance between oxidants and antioxidants pushes forward an immense influence on the occurrence and development of these two kinds of diseases⁶⁷.⁸

As an exogenous factor, appropriate daily intake of antioxidants and pro-oxidants is proven to regulate the oxidative balance and affect the oxidative stress level of the body⁹, thus reducing the risk of mortality. Although many studies have investigated the association of dietary antioxidants and pro-oxidants with health outcomes,⁴⁵ these studies focus only on specific nutrients such as dietary fats, vitamin C, vitamin E and main carotenoids. Over the past few decades, human nutrition science has shifted from focusing on specific nutrients to emphasising overall dietary quality. There is compelling evidence that the impact of food on health is influenced not only by individual nutrients but also by their interactions⁹,¹⁰.

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Dietary oxidative balance, sleep and health

Therefore, the relationship between nutrition and health can be more fully assessed in the context of an integrated diet. To estimate the combined pro-/anti-oxidative effects of dietary nutrient exposures, dietary oxidative balance score (DOBS) was constructed, reported, and validated previously(11–13). But evidence regarding the association between DOBS and death was scarce. To our knowledge, only two studies investigated the effect of DOBS on mortality and were limited to specific population subgroups: male smokers(11) and women aged 55–69 years(14). And limited evidence was available on the association of DOBS with all-cause and cause-specific mortality, especially in the general population.

Emerging evidence has pointed out that the length of sleep was related to dietary pro-/anti-oxidants levels. Among people with short sleep duration, lower antioxidant intake and higher pro-oxidant intake are commonly owing to decreased diet quality, altered time of intake and loss of appetite(15–17). Moreover, previous studies found that people with short or long sleep duration might have an increased risk of mortality(18,19). Thus, we hypothesised that sleep duration might modify the association between DOBS and mortality risk.

In order to test this hypothesis, we examined the association of DOBS with the risk of all-cause, CVD, and cancer mortality and additionally evaluated the potential modification effect of sleep duration on this association among US adults using data from the US National Health and Nutrition Examination Survey (NHANES, 2005–2015).

Methods

Study population

NHANES is a project supported by National Center for Health Statistics (NCHS). This continuous survey aims at getting a general picture of nutritional and health status among US residents by collecting and integrating messages from different areas. The survey contents, including interviews, physical examinations and laboratory measurements, are acquired using a multistage and stratified sampling method(20). Other detailed information about NHANES can be found from the website (http://www.cdc.gov/nchs/nhanes.htm). From 2005 to 2010, a total of 31 034 participants were brought into the NHANES database. We selected adults aged 18 years or older (n 18 318) and excluded those who had missing or unavailable information on dietary intake, sleep duration and mortality (n 1568). Pregnant women (n 465) and those with extreme total energy intake (< 500 kcal/d or > 4500 kcal/d, n 299) were excluded as well. At last, 15 991 individuals were included in this study. The detailed procedure was displayed in the flowchart (see online supplementary material, Supplemental Fig. 1).

Assessment of food intake and nutrients

In terms of food intake, all the participants took part in the 24-h dietary recall survey which was conducted twice by seasoned interviewers. For the first time, in the Mobile Examination Center (MEC), the utilisation of images and charts assisted interviewees to quantitatively stating what they have eaten in the past 24 h. Afterwards, the second food investigation was processed by telephone 3–10 d later. Before that, a set of measuring tools, including a booklet, ruler and spoon, were distributed to each person to ensure that their reports were as accurate as possible. Both interviews were carried out using the specific computer software program developed by NHANES. The nutrient intake used for DOBS was the average of two recalls. If people only had one recall, the value of their one recall was used.

Total intake was calculated as diet plus supplement when the supplement was available. For dietary supplements, from 1999 to 2006, only the 30-d dietary supplement use questionnaire recorded whether any dietary supplements had been taken and the total amount of dietary supplements consumed in 30 d, so the 24-h intake of dietary supplements could not be obtained. The 24-h survey for dietary supplements has been used since 2007, so nutrient intake after 2007 was the sum of food and dietary supplements.

Food groups were determined according to the Pyramid Equivalence Database 2.0 (MPED 2·0) for the US Department of Agriculture (USDA). The MyPyramid Food Guidance System contains a total of thirty-seven food groups and subgroups. In this study, some foods of similar species were combined into the same group, and twenty-six major food groups were analysed: total fruits and juices, citrus and melons and berries, other fruits, total vegetables, dark green vegetables, red and orange vegetables, starchy vegetables, other vegetables, total grains, refined grains, whole grains, total meat, cured meat, red meat, poultry, seafood, organ meat, eggs, total dairy, milk, yogurt, cheese, legumes, nuts, soy products, and solid fat.

Main exposure

The DOBS that contained three pro-oxidants (iron, n-6 fatty acids and saturated fats) and nine antioxidants (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein + zeaxanthin, Se, vitamin C, vitamin E and n-3 fatty acids) was developed in the present study. These chosen twelve components are supported by the previous literature which has been published elsewhere(21,22). To begin with, each component was divided into four groups according to sex-specific quartile values of their respective intake and assigned a score of 0, 1, 2, and 3 for pro-oxidants from high to low and for antioxidants from low to high (see online supplementary material, Supplemental Table 1). After that, individual scores were added up to give an overall DOBS ranging from 0 to 36. A higher score indicates an intake of higher antioxidants combined with lower pro-oxidants.
Assessment of sleep duration

Sleep duration was assessed in NHANES using a single question from the Questions on sleep (SLQ): 'How much sleep do you usually get at night on weekdays or workdays (hours)?'. The response categories were integers ranging from 1 to 12, with 12 indicating that the subject slept for 12 h or more. According to the American Academy of Sleep Medicine and Sleep Research Society, 7–8 h of sleep per night was defined as the optimal amount for adults. Therefore, we classified sleep duration into three categories: short (≤ 6 h), normal (7–8 h) and long (≥ 9 h).

Main outcome

NCHS connected data from NHANES to the National Death Index (NDI) by matching the only identification number called respondent sequence number (SEQN) to estimate mortality rates and published information among adults as public-use files. The document covered deaths from the time of participation in the investigation to 31 December 2015. Definitions of specific causes of death were provided by The International Classification of Disease, 10th Revision (ICD-10). The codes for deaths from CVD and cancer were I00–I09 and C00–C97, respectively.

Covariates

Confounding factors comprised age (years), sex (male/female), race/ethnicity (Hispanic/other Hispanic/non-Hispanic Black/non-Hispanic White/other race), education (less than college/college or above), household income (< $20 000/$20 000), smoking status (never/previous/current), alcohol drinking status (never/previous/current), BMI category (underweight, < 18·5 kg/m²; normal weight, 18·5 ≤ 25·0 kg/m²; overweight, 25·0 < 30·0 kg/m²; and obese, ≥ 30·0 kg/m²), physical activity (yes/no), chronic non-communicable diseases (NCD, yes/no, diagnosed with hypertension, diabetes, heart diseases, stroke or cancer), prescription for diabetes (yes/no), prescription for hypertension (yes/no), depression (yes/no), total energy intake (kcal/d), cholesterol intake (mg/d), dietary supplement use (yes/no), red/cured meat intake (oz. eq), non-steroidal anti-inflammatory drugs (yes/no), sleep disorders (yes/no), coffee consumption (gm/d) and tea consumption (gm/d). Physical activity was assessed using several questions from the Questions on physical activity (PAQ)25. Individuals with moderate or vigorous work and recreational activity were considered as physically active. Depression status was measured according to the Patient Health Questionnaire (PHQ–9). Each of the nine questions consisted of a four-point Likert scale (0–3), with an overall score of 0–27. Finally, a score of 10 or more was considered to indicate depressive symptoms. Depression status was treated as a variable which was included in the multivariate Cox proportional hazards regression models, with a score greater than 10 defined as depression and a score less than 10 defined as non-depression.

Statistical analysis

Sample weights, considering the survey design and complex sampling of NHANES, were incorporated to make the analysis nationally representative27. The baseline characteristics of participants were shown as mean (se) and number (percentage) according to the quartiles of DOBS. The differences of these characteristics were compared by using general linear models and χ² test for continuous and categorical variables, respectively. In addition, general linear models were also used to compare the differences in food intake across quartiles 1–4 of DOBS.

Multivariate Cox proportional hazards regression models were applied to elucidate the potential association between DOBS and all-cause, CVD and cancer mortality with the lowest quartile of DOBS as a reference. Multivariable-adjusted hazard ratios (HR) and 95% CI were estimated in three sequential models. Model 1 was adjusted for age, sex and race/ethnicity. Model 2 was additionally adjusted for education, household income, smoking status, alcohol drinking status, BMI category and physical activity. Model 3 was additionally adjusted for NCD, prescription for diabetes, prescription for hypertension, depression, total energy intake, cholesterol intake and dietary supplement use. The medians of the DOBS in each quartile were employed to calculate linear trend tests.

Stratified analysis was carried out to determine whether the association between DOBS and mortality was modified by sleep duration. The interaction test of DOBS and sleep duration category was performed by using the likelihood ratio test comparing models with and without a cross-product term.

To test the robustness and reliability of the results, several sensitivity analyses were conducted in this study. First, considering other potential confounding factors, we adjusted for the additional confounders to minimise their effects of them, including red/cured meat intake and non-steroidal anti-inflammatory drugs; sleep disorders, coffee consumption, and tea consumption. Second, we repeated the analysis by limiting participants to those who were followed up for more than 2 years (n 15 652) and by analysing participants without anxiolytics, sedatives and hypnotics (n 15 414), to assess the extent to which they could explain the findings of the study.

All the above statistical analyses were performed with SPSS for Windows, version 20.0 (SPSS Inc.) and R, version 3.5.3. The P value was two-tailed, and less than 0·05 was considered statistically significant.

Results

Baseline characteristics of participants

In this study, 15 991 individuals meeting the criteria were enrolled, of which 1675 deaths (288 CVD deaths and 375 cancer deaths) were observed during a median follow-up of 7·4 years. Table 1 presents the demographic characteristics of participants by DOBS quartiles. Compared with
those in the lowest quartile, participants in the highest DOBS quartile were more likely to be older, non-smokers and current drinkers, but less likely to be non-Hispanic Black. They tended to have lower BMI and lower prevalence of depression, but with a high prevalence of NCD. Furthermore, they had a higher level of income, education and physical activity, with a higher percentage of dietary supplements use, prescription for hypertension, and intake of energy and cholesterol.

**Associations of DOBS and mortality**

Associations of DOBS with the risk of all-cause, CVD and cancer mortality among US adults are shown in Table 2. After adjusting for model 1, participants in quartile 4 (the highest DOBS) were less likely to die from all-cause (HR 0.56, 95% CI 0.46, 0.67) ($P$ trend $<0.001$), CVD (HR 0.51, 95% CI 0.34, 0.77) ($P$ trend $=0.001$) and cancer (HR 0.64, 95% CI 0.46, 0.89) ($P$ trend $=0.004$) than those in quartile 1 of DOBS. Negative associations were attenuated when controlling for model 2 but remained significant for all-cause mortality. Compared with the lowest quartile of DOBS, the highest quartile was related with lower risk of all-cause mortality (HR 0.77, 95% CI 0.62, 0.95) ($P$ trend $=0.013$). Moreover, after adjustments for model 3, participants in the quartile 4 of DOBS were still significantly associated with a 25% reduction in the risk of all-cause mortality (95% CI 0.61, 0.93, $P$ trend $=0.004$), compared with those in quartile 1 of DOBS.

**Associations of DOBS and mortality stratified by sleep duration**

In the fully adjusted model, we found statistically significant interaction between DOBS and sleep duration on all-cause mortality ($P$ interaction $=0.021$). Participants in the highest quartile of DOBS had lower all-cause mortality risk (HR 0.69, 95% CI 0.49, 0.95) ($P$ trend $=0.021$) than those in the lowest quartile among short sleepers. Simultaneously, a similar tendency was also shown in DOBS and CVD.
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HR, hazard ratio; DOBS, dietary oxidative balance score; NHANES, National Health and Nutrition Examination Survey; NCD, non-communicable disease.

Model 1 was adjusted for age, sex and race/ethnicity.
Model 2 was adjusted for model 1 plus education, household income, smoking status, alcohol drinking status, BMI category and physical activity.
Model 3 was adjusted for model 2 plus NCD, prescription for diabetes, prescription for hypertension, depression, total energy intake, cholesterol intake and dietary supplement use.
mortality in participants with normal sleep (HR 0·54, 95 % CI 0·31, 0·94) (P trend = 0·018). Nevertheless, there was no significant interaction between DOBS and sleep duration on CVD mortality (P interaction = 0·068) and cancer mortality (P interaction = 0·365). In addition, there were no other statistically significant associations between DOBS and mortality stratified by sleep duration (Figs 1–3).

Food intake among DOBS quartiles

A comparison of food intake in terms of DOBS quartiles is presented in online supplementary material, Supplemental Table 2. Compared with those in the lowest quartile, participants with the highest DOBS quartile tended to consume more fruits, vegetables, whole grains, poultry, seafood, eggs, yogurt, nuts and soya products, but fewer
refined grains, red meat, cured meat, cheese and solid fats. There was no obvious difference in the intake of starchy vegetables, organ meat, milk and legumes across quartiles 1–4.

**Sensitivity analyses**
The significantly inverse associations between DOBS and all-cause and cancer mortality among short sleepers did not materially change when further adjusting for red/cured meat intake and non-steroidal anti-inflammatory drugs; sleep disorders, coffee consumption and tea consumption (see online supplementary material, Supplemental Tables 3 and 4). In addition, when the analysis was restricted to those who were followed up for more than 2 years from baseline interview, the results were stable (see online supplementary material, Supplemental Table 5). Furthermore, after excluding participants who have taken anxiolytics, sedatives and hypnotics, the main results did not change (see online supplementary material, Supplemental Table 6).

**Discussion**
In this large-scale prospective cohort study of US adults, we observed that higher DOBS (reflecting more antioxidants intake and fewer pro-oxidants intake) was associated with lower all-cause mortality, independent of traditional dietary and lifestyle factors. We also found that this association was significantly modified by sleep duration, while the decreased risk of all-cause mortality associated with DOBS appeared to be stronger among participants with short sleep duration. And the results remain robust after additionally adjusted for a variety of possible confounding factors.

**Comparison with other studies**
There were three studies before which have focused on the relationship between oxidative balance and mortality, and their results were inconsistent. The first study was conducted among 2814 Belgian smoking men (11). Male smokers with the most pro-oxidant intake had a higher risk of all-cause mortality (RR 1.44, 95% CI 1.13, 1.82) (P < 0.01) and total cancer mortality (RR 1.62, 95% CI 1.07, 2.45) (P < 0.01), compared with those with the most antioxidant intake. No association was observed between DOBS and CVD mortality (RR 1.31, 95% CI 0.86, 2.00) (P = 0.07). In contrast to the above results, an investigation on older White Iowa women aged 55–69 years found that DOBS was not associated with all-cause (RR 0.99, 95% CI 0.94, 1.04) (P = 0.81), CVD (RR 1.02, 95% CI 0.94, 1.11) (P = 0.18) and cancer mortality (RR 0.96, 95% CI 0.87, 1.06) (P = 0.60) (14). Furthermore, in a population-based cohort study from the Reasons for Geographic and Racial Differences in Stroke (REGARDS), a comprehensive oxidative balance score was calculated on the combination of diet and lifestyle for observing the association with mortality (34). People with the greatest balance of antioxidant to pro-oxidant exposures had a lower risk of all-cause mortality (HR 0.70, 95% CI 0.61, 0.81) (P trend < 0.001) and cancer mortality (HR 0.50, 95% CI 0.37, 0.67) (P trend < 0.001). Oxidative balance of diet and lifestyle was not associated with cardiac mortality (RR 0.68, 95% CI 0.40–1.05) (P = 0.07).
sleepers had lower FV consumption (35). Notably, a cohort with those with moderate sleep duration, short and long was a non-linear correlation between sleep duration and vegetable (FV) consumption. It was found that there was a non-linear correlation between sleep duration and FV consumption and sleep duration may be related to polyphenol content (36). The potential mechanism may be that polyphenol content induces a double interexchange of information between the gut microbiota and brain through the gut–brain axis, thereby altering sleep measures, improving sleep and reducing insomnia symptoms (37, 38).

A body of literature have investigated the association between sleep duration and dietary oxidative status. Using the data from 2005 to 2016 of NHANES, Ikonte et al found that short sleep was associated with increased nutrient inadequacy, especially in antioxidant nutrients including vitamins C and E and lycopene (39). Meanwhile, previous studies illustrated that people with abnormal sleep duration tended to have higher fat intake, especially saturated fats, which could promote oxidation and increase the cardio-metabolic diseases risk (40, 41). Furthermore, several studies have investigated the relation of sleep duration and mortality, and most of them found short or long sleep duration affects health status and increases mortality risk (18, 19). And, thus we hypothesised that there were interactive effects between dietary oxidative balance and sleep duration on mortality. Until now, limited evidence was available on the interactive effect of DOBS and sleep duration on mortality. In the present study, we examined the association of DOBS with mortality in people with different sleep durations and indicated that the protective effects of DOBS on all-cause mortality were more significant in short sleepers. Dietary recommendations should be promoted especially among people with short sleep duration.

Potential mechanisms
One crucial finding of our study was that higher DOBS was significantly associated with lower all-cause and CVD mortality. Our study provides evidence for the protective effects of DOBS on health. The underlying mechanism may be due to the following reasons. According to previous research, DOBS was shown to have negative correlations with markers related to inflammation, such as IL-6 and C-reactive protein (13), suggesting that an imbalance of pro-oxidant and antioxidant exposures of diet may have pro-inflammatory effects. Based on two cross-sectional studies, it is suggested that a better oxidative balance was accompanied by lower LDL-cholesterol, total cholesterol, diastolic blood pressure and incidence of abdominal obesity (42, 43). Besides, the association between oxidative balance and the risk of cancer, such as prostate cancer, breast cancer and colorectal cancer, has been supported by a series of case–control and cohort studies (21, 44, 45). This relationship may be partly due to changes in the expression of angiogenesis genes, transcription-related genes, and other genes induced by dietary intake (12, 46). Hence, the above results support the important role of dietary oxidative balance in health promotion.

Another vital finding was that the negative association between DOBS and all-cause and cancer mortality was enhanced among short sleepers. The observed interaction between DOBS and sleep duration on mortality seems reasonable, which may be explained by the following mechanisms. For one thing, according to former research, changes in sleep duration could be associated with the alterations in appetite-related hormones and time of intake (16, 17). It is supposed that short sleep duration may contribute to a decline in dietary quality and a change in diet type (15, 47), as well as an imbalance between dietary pro-oxidants and antioxidants, thereby modifying the association between DOBS and mortality. For another, it is found that sleep function is related to scavenging reactive oxygen species. Animal models and human studies found an augment of reactive oxygen species and markers of oxidative stress (48, 49), and an increased morbidity of CVD in short sleepers (50). A diet with high antioxidants and low pro-oxidants can reduce the harmful effects of free radicals and improve the oxidative balance of the organism (11). Therefore, we speculate that the beneficial health effect of high DOBS in short sleepers may be because a predominant intake of antioxidants over pro-oxidants may offset the oxidative damage attributed to short sleep duration, alleviate adverse health reactions, and thus decrease the risk of mortality.

Strengths and limitations
The strengths of this study are as follows. First, to our knowledge, it is the first study that demonstrates the interactive effect of DOBS and sleep duration on all-cause mortality and cause-specific mortality. Second, participants in this prospective study were from a large, nationally representative population in the USA. Third, we adjusted a series of potential confounding factors and carried out several sensitivity analyses to ensure the robustness of the results. However, several limitations were available. First, sleep duration is self-reported during the interview with no objective measurement, which may introduce information bias, thus contributing to the deviation of results. Second, despite controlling for many covariates, we cannot completely rule out the residual confounding caused by...
other relevant variables. Third, all measurements were conducted only at baseline, but during long-term follow-up, the lifestyle and dietary habits of participants may change over time. These unknowable variations may affect the results.

**Public health implications**
Balanced diet is a critical element to prevent chronic non-communicable diseases and premature death. Nutritional guidelines and intervention strategies should emphasise the importance of oxidative balance in diet. From a public health point of view, individuals, especially those with abnormal sleep duration should focus on the current findings from this study and be aware of the beneficial effects of eating more fruits and vegetables and less red meat and processed meat for reducing the risk of death. Moreover, this study provides ideas for further exploring the association of dietary factors, sleep duration and mortality risk to promote public health.

**Conclusion**
In summary, our study indicates that higher antioxidant combined with lower pro-oxidant intake is associated with a lower risk of mortality, and such association is modified by sleep duration. The association between high DOBS and lower risk of mortality is more significant in short sleepers, namely that this association is more beneficial in short sleepers. Dietary intervention guidelines for eating more fruits and vegetables and less red meat and processed meat should be recommended for adults, especially for short sleepers to prevent premature death. Our findings emphasised the importance of considering lifestyle factors when investigating the relationship between dietary intake and mortality risk, such as sleep behaviours.

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**Supplementary material**
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