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Investigating nutrition and lifestyle factors as determinants of abdominal obesity: An environment-wide study

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ABSTRACT

Background: The increasing global trends in obesity and its associated burden of disease indicate a need to identify modifiable determinants of obesity.

Methods: A total of 182 nutrition and lifestyles factors were investigated in relation to abdominal obesity among 7,403 male and 8,328 female participants of the Third U.S. National Health and Examination Survey (NHANES III). We used the first phase (1988-1991) of the NHANES III to identify factors with a false discovery rate (FDR) of <5%. Of these, we tentatively replicated our findings in the second phase (1992-1994) of the survey. Principal component analysis was performed to identify unobserved factors underlying the association between validated factors and abdominal obesity, defined as waist circumference >88 cm for women and >102 cm for men.

Results: We found 5 tentatively replicated factors showing significant associations with abdominal obesity in men: serum α-carotene, β-carotene, serum β-cryptoxanthin, serum vitamin D, and vigorous physical activity. In women, 7 factors were identified: serum α-carotene, β-carotene, serum β-cryptoxanthin, serum vitamin C, serum vitamin D, vigorous physical activity, and aspartame intake. In contrast to the other factors which showed inverse associations with abdominal obesity, aspartame intake displayed a positive relationship with this outcome (OR: 1.18, 95% CI: 1.10-1.26 for each log increase in aspartame intake in women). Principal component analysis suggested three principal components underlying such associations, each comprising: 1) serum antioxidants; 2) serum vitamin D and vigorous physical activity; and 3) aspartame intake. All three principal components also displayed significant associations with abdominal obesity.

Conclusion: Our observational investigation that systematically investigates multiple modifiable factors simultaneously has enabled the creation of data-driven hypotheses

regarding the possible role of determinants of abdominal obesity and has identified potential avenues for mechanistic investigations to clarify suitable targets of intervention.

Introduction

The obesity pandemic remains a challenging health problem worldwide [1], with approximately 2.1 billion individuals estimated to be overweight or obese in 2013. Although some countries have shown indications of rate stabilisation during the past decade, the prevalence of obesity continues to increase in both developed and developing regions [1–3]. High body mass index (BMI), a widely accepted indicator of obesity, is a well-known risk factor for diseases with serious implications including cardiovascular disease and several cancers [4,5], and accounted for over 33 million disability-adjusted life years (DALYs) lost in 2000 [6]. Moreover, obesity-related diseases confer a large economic burden, with an estimated rise in total medical costs of $48–66 billion/year in the U.S. and £1.9–2 billion/year in the UK by 2030 [7]. Nevertheless, outcomes of public health strategies aimed at reducing obesity rates are unsatisfactory. Although interventions based on reduction in energy intake lead to weight loss, the lengthy period required for an obese individual to reach their normal weight implies limited efficiency [8], which indicates that policies should be directed towards obesity prevention rather than its reversal.

Identification of suitable targets of obesity prevention requires an understanding of at least two key concepts: 1) clinically relevant definition of obesity and 2) factors involved in mechanisms underlying obesity. Although obesity is conventionally defined by high BMI, the Third Report of Adult Treatment Panel (ATP III) of the National Cholesterol Education Program refers to waist circumference as the recommended measurement since abdominal obesity is closely related to metabolic disorders such as abnormal lipid profile, glucose

tolerance, and blood pressure [9]. Nevertheless, heterogeneity in other metabolic features has been reported within the same obesity categories [10], which underlines the importance of taking into account variation in metabolic indicators in assessing determinants of obesity.

Although around 40% of obesity cases are considered heritable [11,12], environmental factors such as energy intake and physical activity remain the major driving forces underlying obesity [13]. Additionally, environmental factors may indirectly contribute to obesity through interaction with susceptibility genes [14]. Many studies have documented correlations between these factors and obesity, but most of them focused on individual associations without their co-existence, which is in contrast with a real life situation. Recently, Patel and colleagues introduced an environment-wide association study approach derived from methods used in genome-wide association studies (GWAS) to investigate the association of multiple nutrition and environmental factors with clinical phenotypes including blood pressure, diabetes, and mortality [15–17]. Here, we utilised this approach to comprehensively assess nutrition and lifestyle factors in relation to abdominal obesity in the Third U.S. Nutrition Health and Examination Survey (NHANES III). Additionally, we took into account other metabolic disorders and unobserved underlying factors while assessing abdominal obesity.

Methods

Study population

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional health survey conducted by the National Center for Health Statistics (NCHS) in representative samples of the non-institutionalized U.S. population [18]. Participants were selected through a multistage stratified, clustered probability sampling. The survey included

an interview conducted at home and an extensive physical examination with a blood sample taken in a mobile examination center (MEC). Institutional Review Board (IRB) approval was obtained for the NHANES and documented consent was obtained from participants. The present study was based on data from the Third NHANES (NHANES III) which was performed in two phases: 1988-1991 and 1991-1994, each of which provides independent unbiased national estimates of health and nutrition characteristics. From this population, we selected a total of 15,721 participants (7,403 men and 8,328 women) aged 20 and older with measurements of waist circumference. The first phase of NHANES III (N = 7,743) was used as a discovery set and findings from this dataset were replicated in the second phase of NHANES III (N = 7,988).

Obesity assessment

Abdominal obesity referred to waist circumference (WC) of >88 cm in women and >92 cm in men as defined by the experts in the Adult Treatment Panel (ATP) under the National Cholesterol Education Program (NCEP) [9]. All body measurements were performed using standardized methods and equipment [18]. WC was measured at the high point of the iliac crest at minimal respiration using a steel measuring tape to the nearest 1 mm [18]. Waist-to-hip ratio was calculated from WC and hip circumference. Body mass index (BMI) was calculated from measured weight and height. Weight was measured with an electronic weight scale in pounds and automatically converted to kilograms. Participants only wore underwear, disposable paper gowns and foam rubber slippers. Standing height was measured with a fixed stadiometer to the nearest 1 mm.

Assessment of exposures and covariates

A total of 182 nutrition and lifestyle factors in NHANES III were assessed (Table S1, Supplementary Data). Data collected ranged from information obtained through the

interview, such as smoking history, as well as physical and laboratory examination, e.g. serum vitamin C concentrations. Examples of markers and categories are presented in Table 1. Excluding reproductive-related factors such as external hormone use, 176 factors were equally assessed in both men and women. These factors were assessed either as continuous or categorical variables. The majority of continuous variables had a right-skewed distribution. We transformed these variables into standardised z-scores by subtracting the mean and dividing by the standard deviation (SD) of the population. For categorical variables, we consistently defined one value as the referent category or the "negative" result, e.g. "never smoker" as the reference for "current smoker". Vigorous physical activity (yes, no) was defined as participating three or more times per week in leisure-time physical activities with metabolic equivalent (MET) ≥6 for those aged 60 and older, and MET ≥7 for those younger than 60 [19]. Secondary exposure to smoking among never smokers (never smoked ≥100 cigarettes) was defined as exposure to smoke at home (≥1 person smoke at home) or at work (≥1 hours smoke exposure at work). All exposure variables were assessed with standard procedures as detailed in the NHANES III documentation [18,20].

The following variables have been suggested to strongly affect environmental factors and obesity and were therefore considered as confounders in our study: age, sex, race/ethnicity, education and socioeconomic status (SES). Race/ethnicity was categorised into Non-Hispanic white, Non-Hispanic black, Mexican-American, and other. We classified educational attainment as less than high school, high school equivalent, and higher than high school. SES was estimated with poverty-to-income ratio (PIR), a ratio of total family income to the official poverty threshold at the family level. A PIR <1 indicated that income was less than the level of poverty. We categorised PIR into <1, 1-<2, 2-<3, and ≥3, indicating lowest to highest SES as previously described [21].

The ATP-III definition of metabolic syndrome apart from waist circumference was used to define presence of other metabolic disorders [9]. Hypertension was defined by blood pressure of ≥130/≥85 mmHg or any use of antihypertensive drugs. Diabetes was defined as fasting glucose ≥110 mg/dL or any use of insulin or glucose-lowering drugs. Any HDL-cholesterol levels <40 mg/dL for men or <50 mg/dL for women were considered as low. Triglyceridaemia was defined as any levels of triglycerides ≥150 mg/dL. Blood pressure was measured with mercury manometer and the average of the second and third blood pressure measurements was taken. Fasting plasma glucose levels were measured by using a modified hexokinase enzymatic method (Roche Diagnostic Systems, Inc., Montclair, NJ). Blood lipids were enzymatically measured using the Hitachi 704 Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) [18].

Statistical analysis

Sampling weights specific to each phase were included in all analyses. Fig 1 summarises the analytical steps in this study which were similar to previously published nutrient- and environment-wide studies [15,16,22,23]. WC was used as an outcome instead of BMI, and was assessed as a dichotomous instead of a continuous outcome given that this definition of abdominal obesity has been widely accepted to be clinically relevant to risk of diabetes and cardiovascular disease [24]. We selected factors corresponding to categories of environmental exposures used in a previous EWAS study as summarised in Table 1 [16]. First, each of the 182 nutrition and lifestyle factors was assessed in relation to abdominal obesity in the discovery set, phase I of the NHANES III. Survey-weighted logistic regression was used to examine the association of continuous and dichotomous nutrition and lifestyle factors in men and women separately. All models were linearly adjusted for age, race/ethnicity, education and PIR by adding each term into the regression model.

Next, we estimated the false discovery rate (FDR) among findings in the discovery set. FDR is the expected proportion of false discoveries, among all significant findings at a given significance level [25]. Using a significance level α of 0.05, we estimated FDR using the Benjamini-Hochberg step down method [26] to select factors with statistically significant association with obesity status and FDR<5% in the discovery set. A sensitivity analysis was performed by selecting all factors with FDR<1%. Because physical activity and smoking status classifications were derived from other variables, we performed a sensitivity analysis including the four categories of physical activity (vigorous, moderate, light physical activity, and sedentary) [19] instead of using it dichotomously. Similarly, for smoking status we repeated our analyses by only including the main smoking categorisation (current, former and never smokers) [27] and continuous levels of serum cotinine, the primary metabolite of nicotine [28], as indicators of smoking exposures.

Replicated analysis of assessed nutrition and lifestyle factors was subsequently performed by re-running similar logistic regression models in the second dataset, namely phase II of NHANES III (Fig 1). Only nutrition or lifestyle factors with both FDR <5% in the first dataset and p value <0.05 in the replication set were considered valid. Furthermore, analysis for replicated factors in the overall study population was repeated in additional multivariable models adjusting for other metabolic disorders (i.e., hypertension, diabetes, low HDL, and hypertriglyceridaemia) and models incorporating all replicated factors. To account for potential effects of body type differences, a sensitivity analysis was performed by a further adjustment for BMI or waist-to-hip ratio as a continuous variable.

Finally, our secondary aim was to understand any underlying factors associated with abdominal obesity based on inter-correlation among tentatively replicated nutrition and lifestyle factors in the overall study population. For this purpose, we performed a principal component analysis with an orthogonal varimax rotation procedure. An eigenvalue of >1 was used to define the number of principal components to be extracted from our data [29]. Proportion of variance in abdominal obesity explained by each principal component was estimated, and 95% confidence intervals of this estimation were obtained using 1000 bootstrap resampling [30]. We further estimated the value of principal components identified and assessed them in relation to abdominal obesity using similar multivariable approach. McFadden R^2 values were computed to estimate variance explained by the model, and the variance explained only by replicated nutrition and lifestyle factors or principal components.

The NHANES III datasets were prepared with Statistical Analysis Software (SAS) release 9.3 (SAS Institute, Cary, NC). All analyses were performed with R version 3.1.2 (R Foundation for Statistical Computing). The *survey* package was used to account for sampling weights and the *psych* package was used to perform principal component analysis.

Results

Characteristics of study participants are shown in Table 2, whereas means, standard deviations and frequencies of investigated nutrition and lifestyle factors are available in Table S1 (Supplementary Information).Using the ATP III definition for abdominal obesity, 55.6% of women and 28.8% of men were abdominally obese. Prevalence of other metabolic disorders was higher in both obese men and women compared to the non-obese counterparts.

We performed a systematic screening of the relationships of the 182 nutrition and lifestyle factors with abdominal obesity in men and women separately. A total of 30 factors with FDR<5% in men and 36 factors in women in the discovery set were examined for significance (P<0.05) in the replication set. In men, this resulted in 5 tentatively replicated factors showing significant inverse associations for serum α-carotene, β-carotene, serum β-cryptoxanthin, serum vitamin D, and vigorous physical activity with abdominal obesity (Table 3). A total of 7 factors were replicated in women: serum vitamin C, serum α-carotene, β-carotene, serum β-cryptoxanthin, serum vitamin D, and vigorous physical activity were inversely correlated with abdominal obesity, whereas aspartame intake was positively associated with abdominal obesity. Replicated findings did not alter when we performed a sensitivity analysis including only factors with FDR<1% in the discovery survey or when we used the alternative categorisation of physical activity and smoking status (results not shown). Fig 2 depicts the distribution of P-values for each investigated factor and effect sizes ("Manhattan plot"). As seen in Fig 2, stronger associations between common factors and abdominal obesity were observed in women compared to men. Detailed results on associations between all factors and abdominal obesity are presented in the Supplementary Information (Table S2 and S3).

Correlation between replicated factors is displayed as a heatmap in Fig S1 (Supplementary Information). To assess any structure underlying replicated nutrition and lifestyle factors, we performed a principal component factor analysis to identify common underlying factors. In men, two principal components (PC) were identified. The first PC mainly consisted of serum antioxidants (antioxidant PC). The second PC comprised serum vitamin D and vigorous physical activity (exercise-related PC). In addition to these two factors, a third factor (food additive PC) was identified in women, which only included aspartame intake. The total

variance of replicated variables explained by these principal components in men was 36% (95% CI: 34-36%) by antioxidant PC and 22% (21-22%) by exercise-related PC. In women, 29% (95% CI: 29-31%) of total variance was explained by antioxidant PC, 16% (15-16%) by exercise-related PC, and 15% (14-15%) by food additive PC).

For the final analysis, we obtained estimates for replicated nutrition and lifestyle factors in relation to abdominal obesity in the overall survey (phase I and phase II) for men and women (Table 4). In addition to these factors, we calculated estimates for antioxidant PC and exercise-related PC from the principal component analysis. We found that these two principal components were inversely associated with abdominal obesity in both sexes (Table 4). In a sensitivity analysis where we adjusted for BMI, only antioxidant PC was consistently associated with abdominal obesity in both men and women (Table S4). Associations remain significant for both principal components when models were adjusted for waist-to-hip ratio (Table S4).

When we adjusted for other metabolic disorders as denoted by presence of one or more components of metabolic syndrome apart from obesity, the results were not altered, and all replicated factors remained significantly associated to abdominal obesity in men and women (Table 4). In a multivariable model incorporating all replicated factors, a lack of statistically significant association with abdominal obesity was observed for serum α-carotene in men and women, and for β-cryptoxanthin in women. Other factors and all principal components identified remained significantly associated with abdominal obesity. The total variance explained by the multivariable model including replicated nutrition and lifestyle factors was 11% and 17% for men and women, respectively. The total variance explained by replicated

factors was 2% and 6% in men and women, respectively. Similar contribution to variance was found for principal components in the multivariable models

Discussion

In a systematic screening of 182 nutrition and lifestyle factors, 5 factors in men and 7 factors in women were found to have statistically significant associations with abdominal obesity after applying the EWAS methodology among a representative sample of the U.S. population. Based on inter-correlation between these factors, three underlying principal components were identified. Lower odds of being abdominally obese were seen with higher quantities of factors representing serum antioxidants and exercise in both men and women, whereas a positive association was observed for aspartame intake in women, but not men.

Circulating levels of common antioxidants including vitamin A metabolites and vitamin C have been reported to be inversely associated with general and abdominal obesity [31–35]. Recently, using repeated measurements, longitudinal associations between levels of these antioxidants and adiposity indicators were observed in postmenopausal women, indicating lower serum β-carotene and higher γ-tocopherol to be associated with higher WC [36]. However, results from clinical studies have failed to demonstrate benefit of antioxidant supplementation in prevention of obesity-related diseases such as cardiovascular disease [37] and breast cancer [38], although confounding by fruit and vegetable consumption may be implicated. Interestingly, a meta-analysis assessing a total of 78 randomised trials showed increased mortality with supplementation of β-carotene or vitamin E, and with higher doses of vitamin A [39]. Our findings support inverse associations between serum antioxidants and abdominal obesity which was robust against variation in BMI and waist-to-hip ratio. The contrasting positive association between vitamin A levels and abdominal obesity, albeit not

seen in the replication set, may underline the discrepancy between absorbed pro-vitamin A and the tightly regulated levels of vitamin A [40,41]. In line with this, different associations between carotenoids and vitamin A levels with respect to other health outcomes such as mortality have also been noted [42]. Our findings also showed disagreement between dietary intake and serum levels of antioxidants in relation to obesity. Nevertheless, it is possible that this discrepancy and the lack of associations for dietary antioxidants that we observed was due to a lack of precision from measurement error and within-individual variation because information was obtained by a single 24-hour dietary recall. Alternatively, such discrepancy may also indicate the implication of physiological regulators of antioxidant metabolism rather than antioxidant intake on obesity and relevant health outcomes. In support of this, experimental evidence indicated that circulating carotenoids reduce adiposity through regulation of adipocyte thermogenesis [43]. These findings may indicate an interplay between antioxidant metabolism and physiological regulation of adiposity warranting further investigations.

The role of physical activity in management of obesity has been well-established [44], as well as their opposing effects on health outcomes such as cardiovascular death [45]. Similarly, decreased levels of serum vitamin D among individuals with general and abdominal obesity have been reported [46–48] but the directionality of this association is unclear. Findings from a meta-analysis of vitamin D supplementation [49] and a Mendelian randomisation study [50] suggested that levels of vitamin D decrease secondary to increasing adiposity. Our study corroborates the inverse relationship between physical activity or serum vitamin D and abdominal obesity in both men and women. Additionally, from the principal component analysis, we observed a high correlation between physical activity and serum vitamin D. Although higher levels of physical activity have been linked to increasing levels of vitamin D

[51,52], their mechanistic association is unclear. Adding to the current evidence, we identified from the principal component analysis that an unobserved PC, which we denoted as 'exercise-related', drove the associations of physical activity and serum vitamin D with abdominal obesity. One possible explanation is that levels of vitamin D may be a proxy for a healthier lifestyle which involves outdoor activities. However, it was suggested that sun exposure and time spent outdoor do not explain the majority of variation in vitamin D levels [53]. On the other hand, the notion that obesity is followed by decreasing vitamin D levels [49,50] may indicate a physical activity-obesity-vitamin D regulation axis. Mechanistic investigations are needed to confirm these plausible pathways.

Aspartame is a methyl ester of a dipeptide and widely used as a synthetic non-nutritive sweetener (NNS) [54]. There is evidence that NNSs may interfere glucose and insulin response by disrupting learned physiological response [55,56]. However, a position statement by the Academy of Nutrition and Dietetics concluded that aspartame consumption was not associated with adverse health effects in humans (Grade I evidence) [57]. In the context of obesity, weight loss and maintenance among obese women have been reported following diet regimens incorporating aspartame-sweetened food and beverages, which are often part of diet-related products such as diet soda, compared to those without [58]. Nevertheless, there is a lack of evidence apart from clinical trials including individuals on weight management programmes. We found that higher aspartame intake correlated to more prevalent abdominal obesity in obese women. Although any obesogenic effect of aspartame intake has not been well-documented, our findings are in line with previous studies suggesting higher risks of subsequent general and abdominal obesity following use of artificially-sweetened beverages [59–61]. On the other hand, the positive association observed between aspartame intake and abdominal obesity in women may also signify certain behavioural patterns secondary to

obesity, such as efforts to moderate energy intake [56] and a 'licensing effect', since artificial sweeteners are often a part of diets based on calorie restriction. The latter refers to disinhibition or 'licensing' of unhealthy behaviours following commitment to a self-perceived healthy behaviour [62,63], a phenomenon increasingly recognised in marketing research and experimental human studies. Further studies are needed to rule out such reverse causality, which is important in clarifying the role of aspartame in obesity and obesity-related outcomes.

One of the strengths of this study is its generalisability following the use of nationally representative data of the U.S. population. We were able to adjust for major confounders such as education and PIR in our analysis. To our knowledge, this is the first study applying GWAS-like analytical approaches in studying determinants of obesity. Robustness of the statistical associations between investigated markers and obesity was ascertained through replication analysis and adjustment for presence of other metabolic factors. The systematic screening was able to eliminate factors with small effects which may be more prone to bias. Additionally, this method overcomes the limitation of selective reporting, which may be an issue with studies focusing on individual exposures. Nevertheless, it is important to address limitations of this study. NHANES III was set up as a cross-sectional study, thus our analysis was unable to identify any causality. Some nutrition and lifestyle factors were only measured in small numbers of the participants and this may have limited statistical power of the analysis. Many of investigated factors, such as smoking status and dietary assessment, were self-reported. For dietary assessment, only one 24-hour dietary recall was used. Such imprecision arising from subjective instruments and potential recall bias [64] may have resulted in the discrepancy between findings from dietary intake and serum levels of antioxidants. Therefore, it is necessary to confirm these results with objective measurements

such as digitalised instruments to monitor energy balance [65]. Nevertheless, these results may also indicate a role of physiological factors involved in oxidative stress response pathways, which may have greater influence than dietary intake of antioxidants in determining their serum levels. Definition of other metabolic disorders was limited by data availability in NHANES III. For instance, diabetes was based on fasting glucose and not all participants were fasting at time of measurements. Although we took into account potential confounders and inter-correlation between replicated factors, residual confounding may have occurred. It should also be noted that there were non-replicated factors in this study such as fat intake and serum cotinine that were individually correlated to abdominal obesity but did not display FDR<5% or significance in the replication set. Similarly, the association between aspartame intake and abdominal obesity may not have been gender-specific given a similar but weaker estimate in men after adjustment for multiple comparisons. These factors may still be associated, albeit weakly, with abdominal obesity. Furthermore, we were unable to exclude the potential role of other relevant factors apart from those assessed in NHANES III. Therefore, obtaining an equivalent definition of 'genome-wide significance' as one would be able to claim in a GWAS analysis may be impractical or otherwise requires more rigorous and thorough assessments of nutrition and lifestyle determinants.

Conclusion

Using a comprehensive screening, our study identified nutrition and lifestyle factors demonstrating robust associations with abdominal obesity. Future mechanistic investigations are necessary in order to draw conclusions which may lead to development of suitable behavioural intervention and public policies aimed to reduce the obesity pandemic.

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Supporting Information