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Association of DNA damage, vitamin D and heavy metals in hair of obese women

Running title: DNA damage associated with vitamin D and chromium

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Abstract

Background: Obesity has been linked to DNA damage. The modifiable risk factors may modulate the impact of obesity on DNA damage. **Objective**: This study aimed to assess DNA damage and its association with dietary nutrient, serum 25-hydroxyvitamin D (25(OH)D) and concentration of hair heavy metals of obese and non-obese women. **Method:** A case-control study was conducted involving 134 women aged between 20 to 50 years. Serum 25(OH)D, fasting glucose, and lipid profile were assessed. Indicators of DNA damage such as percentage of tail DNA, tail moment, tail olive moment, tail intensity and tail length were measured using an alkaline-Comet assay. Concentrations of hair heavy metals were quantified using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Participants' daily energy, macro, and micronutrient intake were collected using the Food Frequency Questionnaire (FFQ). **Results:** Mean values of 25(OH)D was 31.8 ± 0.9 nmol/L. Majority of participants (96.3%) were 25(OH)D (<50 nmol/L) deficient. The mean BMI was 26.3 ± 0.5 kg/m^2. Half of the participants (50.7%) have a high frequency of DNA strand breaks. Mean concentration of hair heavy metals (mg/kg) were 0.1 ± 0.03 (arsenic), 1.0 ± 0.4 (mercury), 2.8 ± 0.8 (lead), 0.2 ± 0.1 (cadmium) and 6.2 ± 0.4 (chromium). There was no significant difference for the mean of 25(OH)D, indicators of DNA damage, concentrations of hair heavy metals and dietary nutrients between obese and non-obese groups ($p > 0.05$). Obese women with 25(OH)D level of ≥ 31nmol/L had a significantly lower tail moment ($p=0.029$) and tail olive moment ($p=0.031$); thus, indicating less DNA damage. Additionally, obese women with hair chromium concentration of ≥5.88 mg/kg had a significantly higher tail moment ($p=0.047$), indicating more DNA damage. **Conclusion:** DNA damage among obese women correlated with serum 25(OH)D and hair chromium.

Abstract word count: 285 words

Keywords: DNA damage; Heavy metals; Nutrients; Obese; Vitamin D

Introduction

Age, gender, diet and lifestyle factors significantly influence the extent of DNA damage through individual or synergistic effects[1]. Endogenous factors are non-modifiable, but diet is a modifiable factor that resonates with a healthy lifestyle[2]. Studies in the past decade reported that diet of middle-aged and older adults might influence the level of biological aging[3].

Common micronutrient deficiencies are likely to damage DNA using similar mechanisms as radiation and xenobiotics[4]. The human body requires a minuscule amount of these micronutrients to produce enzymes, hormones and other substances essential for cellular metabolism, maintain optimum tissue function, growth and development[5]. Vitamin D3 is a potent antioxidant with immunosuppression, anti-inflammatory, and anti-proliferation properties[6]. It induces the expression of antioxidant defence system including hepatic glucose 6-phosphate dehydrogenase (G6PD)vity[7], CuZn-superoxide dismutase (CuZn-SOD)[8]; and suppresses markers oxidative stress such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[9]. Suboptimal levels of vitamin D levels have been associated with oxidative stress and DNA damage[10]. Besides, Vitamin D maintains DNA integrity and regulates cell growth rate, preventing propagation of damaged DNA in DDR pathway regulation[11].

DNA single-strand breaks (SSBs) lesions are regarded as an indicator of early damage that is repairable and reversible[12]. However, these lesions may convert to lethal double-strand breaks (DSBs) during DNA replication which pose a significant threat to genomic integrity[13]. Accumulation of DNA damage has been linked to the onset of age-related diseases, which involved

dysregulation of metabolic homeostasis[14]. A study revealed high DNA SSBs in obese individual[15]. Obese women have approximately double DNA damages than non-obese women[16], resulting in higher susceptibility to cancer and precocious aging[15].

The worldwide increase of overweight and obese populations in this recent decade is alarming and results in many adverse health consequences, including infertility, cancer, inflammation, and accelerated aging[17]. Micronutrients have been associated with various underlying metabolic processes responsible for the development of obesity[18]. Micronutrient deficiencies can potentially impair glucose metabolism and cause resistance to insulin[19]. High prevalence of micronutrient deficiencies among obese women has been reported[20] Most micronutrient deficiencies cause DNA damage such as breaking of single- or double-strands and oxidative lesions; these mechanisms are similar to effects of radiation and xenobiotics[4]. This suboptimal nutrient intake has been linked to genomic instability and is likely to be a significant cause of cancer [3,21].

Heavy metals are a heterogeneous group of highly reactive substances affecting various aspects of metabolism, such as the substitution of essential micronutrients and vital metals as well as induction of oxidative stress[22]. Accumulation of heavy metals in the human body due to dietary sources is an emerging public health concern[23]. Additionally, diet is a primary source of heavy metal to human[24] . Cumulative exposure to heavy metals has been associated with underlying mechanisms responsible for obesity-associated pathologies[25], particularly among women. It leads to oxidative stress, production of cytokines and increases markers of systemic inflammation[26]. When oxidative stress overwhelms cells' intrinsic antioxidant, it causes DNA strand breaks[27].

Micronutrients can modify the body's response to heavy metals by altering its metabolism and transport[28]. These nutritional factors are capable of modifying the adverse effects of toxicants and their elimination from the body. Insufficient micronutrients increase one's susceptibility to heavy metal toxicity and play a vital role in obesity. Given the context that obese women experience more serious DNA damage, the incidence of micronutrient deficiency coupled with dietary intake of heavy metals synergistically lead to more significant DNA damage. Varying mechanisms causing genome instability have been reported among those occupationally exposed to heavy [29]. However, data regarding heavy metal exposure and its health consequences are not well established in non-occupational exposed groups. The impact of modifiable and preventable dietary-driven risk factors on DNA damage is often overlooked. Thus, this study aimed to assess DNA damage and its association with dietary nutrient intakes, serum 25(OH)D and hair heavy metals concentrations in obese and non-obese women.

Materials and Methods (Word count: 1003 words)

Participants recruitment

Women aged between 20 and 50 were recruited upon obtaining their informed consent. Women who were pregnant, lactating, having chronic or malignant diseases, or were occupationally exposed to heavy metals were excluded in this study.

Blood collection and biochemical tests

Whole blood was collected using the venepuncture method according to standard protocol. Blood fasting lipid (LDL-cholesterol, triglycerides, HDL-cholesterol, and total cholesterol), fasting glucose, and serum 25(OH)D were measured. According to the NCEP-ATPIII (2001)[30] guideline for lipid profile, total cholesterol levels were defined as desirable, borderline, and high risk; LDL-cholesterol levels were defined as normal, near-optimal, borderline, high risk and very high risk for; HDL-cholesterol levels were defined as normal or not normal, triglycerides levels were defined as normal, borderline, and high risk. Fasting glucose levels were defined as normal, pre-diabetes, and diabetes[31,32] . Serum 25(OH)D level less than 12.5 nmol/L were defined as severe deficiency, less than 25nmol/L were defined as moderate deficiency, less than 50 nmol/L were defined as mild deficiency [33,34], and 50-74 nmol/L as vitamin D insufficiency[35].

Power and sample size calculation

The sample size was computed using the formula $n = \frac{r+1}{r} \frac{SD^2(Z_\beta + Z_{1-\alpha/2})2.}{d^2}$ [36]. Z1-α/2 is the value from the standard normal distribution holding 1- α/2 below it, which is 1.96, and Zβ is the standard normal variate for a power of 80%, which is 0.84. SD is the standard deviation of the percentage of DNA comet tail (%) in the elderly population, which was previously reported to be 1.16 [37]. d is the expected mean difference between the low and high nutrient intake groups, which is 0.5%. r is the ratio of the two groups, which is 1. Thus, a minimum of $n = \frac{1+1}{1} \frac{1.16^2(0.84+1.96)2.}{0.5^2}$, =84 participants were needed for this study.

Quantification of hair heavy metals

Clean stainless-steel scissors were used to cut 1.5 to 2 cm of hair from the occipital region. The 0.5 grams of hair sample was digested with 1 ml of hydrogen peroxide (30%) and 5 ml of nitric acid (65%). Then, samples were radiated in a microwave digester for 15 minutes [38]. Samples were diluted upon cooling with distilled water to a volume of 50 ml.

Concentration of mercury, arsenic, lead, chromium, and cadmium was quantified using inductively coupled plasma mass spectrometry (ICP-MS) in duplicates. Method validation for accuracy was carried out using certified reference material (GBW 07601a, GSH-1a human hair). Serial dilutions of stock solutions were performed to generate seven points calibration curve at 1, 2.5, 5, 10, 25, 50, and 100. Rhodium was added into every sample as an internal standard. Any carry-overs or cross-contamination of heavy metals were determined by quantifying blank samples for every ten samples. Method sensitivity was determined as method limits of detection (MLOD), which was calculated as three times the standard deviation for digestion blanks (n = 5). The MLODs for arsenic, cadmium, lead, mercury, and chromium were all 0.001 mg/kg.

Food frequency data collection and analysis – leave for normina

All participants completed the food frequency questionnaire (FFQ), which was adapted from previous publication[39]. Analysis of food data was performed using the computerized dietary analysis software program (Nutritics, 2019). The intakes of macronutrients were expressed as a percentage of total energy (TE) consumed, whereas micronutrients were expressed as energy

adjusted to unit/TE (kcal)x1000kcal. To estimate under-reporting in the study population, a cut-off of total energy intake/basal metabolic rate (TE: BMR) <1.2 was used to indicate under-reporting [40-42]. TE: BMR ≥2.4 was used to identify over-reporting[42]. In the current study, none of the participants was found to be under or over-reporting. Therefore, all the participants were included in the analysis.

Alkaline Comet Assay – leave for farah

Two hundred microliters of whole blood were used to measure DNA SSBs by alkaline comet assay (also known as single-cell gel electrophoresis)[43]. At a pH 12.1–12.4, this assay facilitates the detection of single and double-strand breaks, incomplete excision repair sites and cross-links; whereas at a pH greater than 12.6, alkali labile sites [44] (e.g., apurinic sites) is transformed to strand breaks that expressed as SSB[45,46]. The assay was performed under alkaline (pH> 13) as described[43]. Cells were microscopically observed at 20x magnification using a fluorescence microscope (Carl Zeiss Axiovert.A1, Germany) with an emission wavelength of 522 nm and an excitation wavelength of 498 nm. Nuclei, with/without DNA damage, were captured with Axiocam MR using Zen2012 imaging software. At least 70 cells were randomly selected and scored with online software (TriTek CometScore 2.0). The damaged DNA migrated out of the cell under electrophoresis, creating a "comet tail", while the undamaged DNA remained within the cell membrane, creating the "comet head[47]. The DNA damage level was expressed in % tail DNA, tail moment, tail olive moment, tail intensity and tail length. Based on the tail moment and % tail DNA, the cells were classified to without DNA damage (tail moment < 5, % tail DNA < 10) and cells with DNA damage (tail moment > 5, % tail DNA > 10)[48]. The intensity of the tail increases as the damage is greater. Tail

length only can be used at low levels of DNA damage. The tail moment combines tail length and tail intensity in one single value, that make the most useful and frequently used parameter[49].

Statistical analysis

SPSS version 22 was used for statistical analysis. Data were presented as number (percentage) or mean ± standard error (SE). Log transformation was carried out to transform non-normally distributed data into normally distributed ones. BMI was categorized into two groups, non-obese (BMI < 27.5kg/m^2 n= 87) and obese (BMI≥27.5kg/m^2 n= 47), for analysis [50]. One way ANCOVA with Bonferroni post hoc test was carried out to ascertain the differences in the mean values of general characteristics, serum 25(OH)D, DNA damage parameters, hair heavy metals levels, and dietary parameters between the non-obese and obese groups. The multivariate general linear model (GLM) was performed to assess interactions between BMI groups and dietary nutrients, serum 25(OH)D and hair heavy metals on DNA damage parameters. A statistical probability level of $p<0.05$ (two-sided) was considered significant.

Results

Demographics of the study participants were reported in Table 1. Most participants (96.3%) population had a vitamin D deficiency (<50 nmol/L). Of all 134 participants, none of them had a sufficient level of 25(OH)D. The mean values (±SE) for fasting blood sugar was 4.78 ± 0.07 mmol/L, and for total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride were 5.20 ± 0.68, 3.20 ± 0.06, 1.55 ± 0.25 and 1.12 ± 0.34 mmol/L, respectively. Some participants had a high risk for

lipid profile, including total cholesterol (11.2%), LDL-cholesterol (8.9%), HDL-cholesterol (14.9%), and triglyceride (2.2%).

Table 1 Demographics of participants

Demographics	Frequency (%)
Age	
20-29	81 (60.4%)
30-39	41 (30.6%)
40-50	12 (8.9%)
Smoking History	
Yes	4 (3%)
No	130 (97%)
Second-hand smoking	
no exposure	51 (38.1%)
everyday	27 (20.1%)
weekday	16 (11.9%)
weekend	40 (29.9%)
Serum Vitamin D	
Insufficiency 50-74 nmol/L	5 (3.7%)
Mild deficiency 25-50 nmol/L	92 (68.7%)
Moderate deficiency 24- 12.4 nmol/L	35 (26.1%)
Severe deficiency <12.5 nmol/L	2 (1.5%)
Lipid profile	
Total cholesterol	
Desirable < 5.2 mmol/L	63 (47%)
Borderline 5.2-6.2 mmol/L	56 (41.8%)
High risk >6.2 mmol/L	15 (11.2%)
LDL-Cholesterol	
Normal <2.58 mmol/L	26 (19.4%)
Near-optimal 2.58-3.34 mmol/L	51 (38.1%)
Borderline 3.35-4.11 mmol/L	45 (33.6%)
High 4.12-4.89 mmol/L	9 (6.7%)
Very high >4.9 mmol/L	3 (2.2%)
HDL-cholesterol	
Low <1.3 mmol/L	20 (14.9%)
Normal ≥1.3 mmol/L	114 (85.1%)
Triglyceride	
Normal < 1.7 mmol/L	121 (90.3%)

Borderline	1.7 -2.25 mmol/L	10 (7.5%)
High	2.26-5.64 mmol/L	3 (2.2%)
Fasting blood glucose		
Normal	< 6.1	130 (97 %)
High fasting glucose	≥ 6.1	4 (3 %)

Table 2 showed differences in mean values of age, anthropometric parameters, serum 25(OH)D, DNA damage parameters, hair heavy metals levels and dietary parameters between the non-obese and obese groups. Results revealed significant differences for height, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR) between the non-obese and obese groups ($p>0.05$).

Percentage of tail DNA values were distributed between 5 and 35, of which 50.7% of participants had a high level of DNA damage. Tail moment values ranged from 0 to 9, only 11.2% marked with DNA damage. The range of the tail olive moment, tail intensity and tail length were between 0 to 8, 10377 to 167469 % and 7.081 ± 4.63 µm, respectively. The mean for percent energy from carbohydrate, protein, and fat were 49.6 ± 0.9 %, 19.4 ± 0.4 %, and 31.0 ± 0.6 %, respectively.

Table 2: Differences in the mean values (±SE) of anthropometric, blood biochemical, DNA damage, heavy metals and dietary parameters between the non-obese and obese groups

Variables	Mean ± SE **(n=134)**	**Non-obese (n=87)**	**Obese (n=47)**	**p-value**
Age (y)	29.96 ± 0.63	28.66 ± 0.7	30.81 ± 0.9	0.064
Anthropometric parameters				
[1]Height (cm)	157.39 ± 0.58	156.1 ± 0.8	159.30 ± 0.74	**0.013***
[1]Weight (kg)	67.2 7 ± 1.75	56.50 ± 1.1	83.84 ± 2.26	**<0.001***
[1]BMI (kg/m^2)	26.3 ± 0.5	23.10 ± 0.36	32.79 ± 0.73	**<0.001***
[1]WC (cm)	84.57 ± 1.31	77.53 ± 1.12	95.41 ± 1.78	**<0.001***
[1]HC (cm)	104.7 ± 1.18	98.28 ± 0.98	114.57 ± 1.63	**<0.001***
[1]WHR	0.81 ± 0.0	1.16 ± 0.0	1.36 ± 0.0	**0.001***
Blood biochemical parameter				

[2]Serum 25(OH)D (nmol/L)	31.8 ± 0.9	31.91 ± 1.2	31.51 ± 1.4	0.767
DNA damage parameters				
[2]Tail moment	1.7 ± 0.2	1.64 ± 0.2	1.67 ± 0.3	0.813
[2] % DNA in comet tail (%)	11.5 ± 0.5	11.20 ± 0.6	11.98 ± 0.9	0.731
[2]Tail olive moment	2.8 ± 0.2	2.77 ± 0.2	2.85 ± 0.3	0.988
[2]Tail intensity (%)	53438 ± 2582.4	53229 ± 3268.1	53825 ± 4243.2	0.654
[2]Tail length (μm)	7.09 ± 4.6	7.09 ± 0.5	7.07 ± 0.7	0.696
Scalp hair heavy metals levels				
[2]Arsenic (mg/kg)	0.1 ± 0.03	0.12 ± 0.03	0.15 ± 0.05	0.959
[2]Lead (mg/kg)	2.8 ± 0.8	2.36 ± 0.4	3.72 ± 2.1	0.229
[2]Cadmium (mg/kg)	0.2 ± 0.1	0.16 ± 0.05	0.27 ± 1.3	0.176
[2]Chromium (mg/kg)	6.2 ± 0.4	6.85 ± 0.5	4.85 ± 0.5	0.973
[2]Mercury (mg/kg)	1.0 ± 0.4	1.37 ± 0.5	0.27 ± 0.1	0.611
Dietary parameters				
[2]Total energy intake (kcal)	2402.4 ± 84.3	2239.9 ± 116	2652.5 ±108.2	0.377
[3]Carbohydrate intake (g)	297.7 ± 11.6	282.2 ±16.3	321.6 ± 26.3	0.354
[3]Protein intake (g)	114.8 ± 4.5	106.3 ± 5.4	127.9 ± 7.4	0.150
[3]Fat intake (g)	83.5 ± 3.6	76.2 ± 4.7	94.9 ± 5.2	0.759
[3]Percent energy from carbohydrate (% of TE)	49.9 ± 2.7	50.0 ± 1.1	48.8 ± 1.2	0.825
[3]Percent energy from protein (% of TE)	19.4 ± 0.4	19.4 ± 0.5	19.3 ± 0.7	0.523
[3]Percent energy from fat (% of TE)	31.0 ± 0.6	30.6 ± 0.8	31.9 ± 0.8	0.880

There was no significant difference in serum 25(OH)D, DNA damage parameters, hair heavy metal levels and dietary parameters between the non-obese and obese groups ($p > 0.05$). However, some associations were observed among the obese group as listed in the following subheadings.

Serum 25(OH)D level and DNA damage in the obese group

Serum 25(OH)D and chromium levels were dichotomized into two groups based on their respective median value of 31 nmol/L and 5.88 mg/kg, respectively (for all participants) (Figures

1-3). Multivariate general linear model analysis revealed that individuals with serum 25(OH)D level of ≥31nmol/L had a significantly lower tail moment (1.06 ± 0.22 nmol/L versus 2.37 ± 0.60 nmol/L; p=0.029; Figure 1), and tail olive moment (2.36 ± 0.24 nmol/L versus 3.41 ± 0.46 nmol/L; p=0.031; Figure 2), compared to those with lower serum 25(OH)D level, in the obese group, after adjustment for covariates age, height, weight, smoking status, exposure to second-hand smoking, physical activity level, total energy intake, and concentration of hair heavy metal including lead, mercury, cadmium, chromium and arsenic (p interaction=0.045 and 0.050, respectively). However, such associations were not found in the non-obese group (p>0.05).

Figure 1

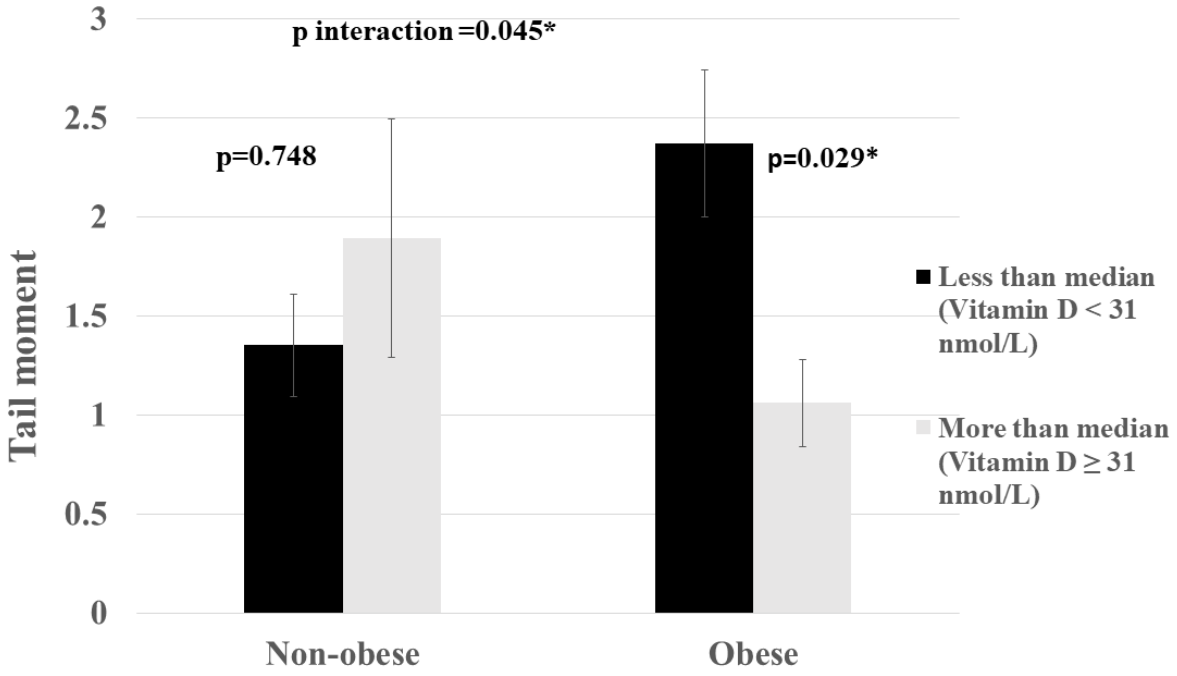
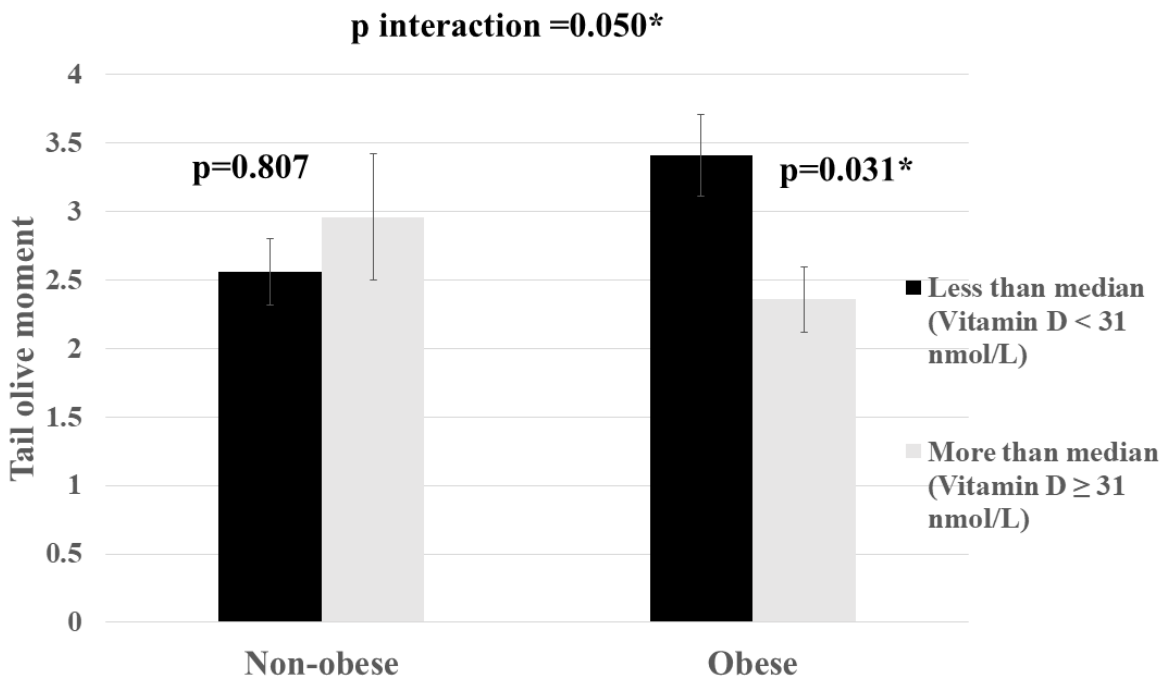


Figure 2



Chromium level and DNA damage in the obese group

The multivariate general linear model analysis also revealed that individuals with chromium level of ≥5.88 had a significantly higher tail moment (2.33 ± 0.75 versus 1.29 ± 0.24; p=0.047) (Figure 3), compared to those with lower chromium level, in the obese group, after adjusting for covariates age, height, weight, smoking status, exposure to second-hand smoking, physical activity level, total energy intake, serum 25(OH)D and concentration of lead, mercury, cadmium and arsenic in hair (p interaction= 0.011). However, such association was not found in the non-obese group (p>0.05).

Figure 3

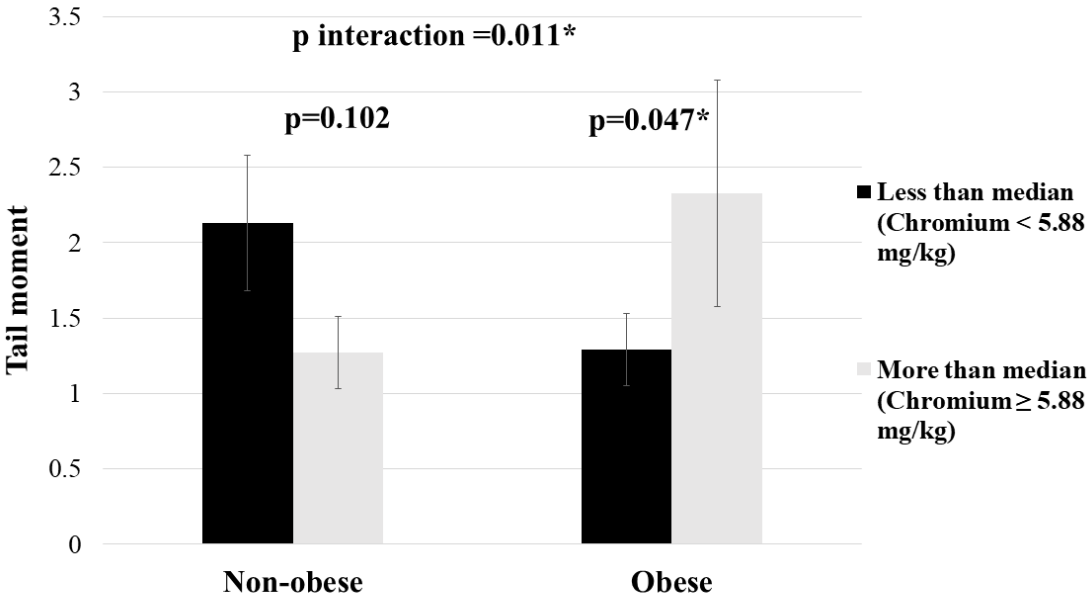
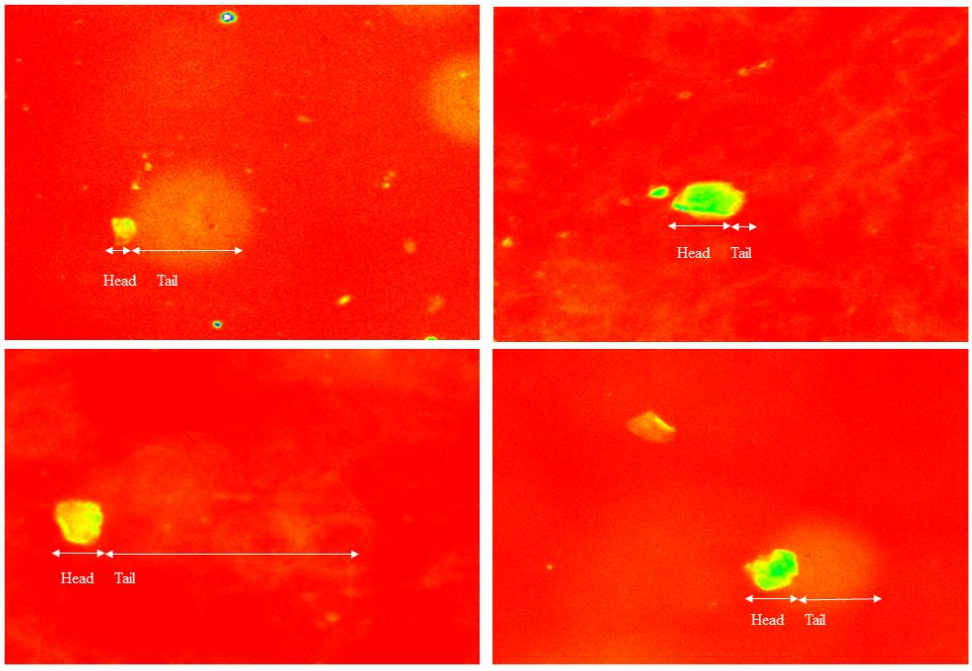


Figure 4



Discussion

Dietary habits and environmental exposure to toxicants significantly affect human's health [51]. It is known that the mutation rate may increase if one is deficient in nutrient involved in DNA metabolism [52].

This study revealed that obese participants with serum 25(OH)D of ≥ 31nmol/L had significantly lower DNA damage. In concordance with this result, several studies had reported lower DNA damage given an adequate level of vitamin D [53-56]. Vitamin D supplementation showed a positive influence on glucose metabolic enzymes and reduced DNA damage where comet tail length showed an average of ~20 μm compared to ~30 μm in non-supplemented diabetic mice [56]. In a human study, vitamin D supplementation increased genomic and chromosomal stability in overweight and obese population [57]. On the contrary, deficiency of vitamin D had been reported to cause greater DNA damage with elevated total damage score ~~was reported~~ in vitamin D deficient subjects (vitamin D < 30 ng/ml) [58]. These data supported the observation in this study which reported lower DNA damage in participants with higher serum 25(OH)D. The amount and choice of food and supplements have a robust impact on micronutrients' cellular concentration through mechanisms that reflect their role in DNA synthesis and repair [1]. Hence, having adequate levels of micronutrients are vital for the optimal function of DNA, particularly in women.

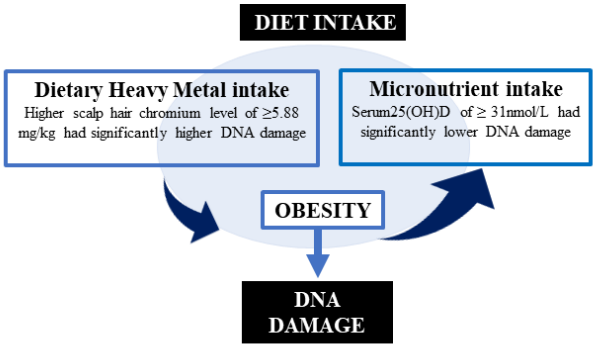
Diet is a primary source of heavy metal to humans contributing to more than 90% of exposure[24,59]. Considering that ubiquitous exposure to heavy metal exposure is a significant threat to women health,[60,61] bio-monitoring of hair heavy metal analysis have been proposed for routine

clinical screening and diagnosis[62]. Hair heavy metals is an indicator reflecting elements from diet than environmental exposure. This study found that a higher hair chromium concentration of ≥5.88 mg/kg resulted in significantly greater DNA damage among obese women. Food is a principal source of exposure to chromium[63] which trivalent chromium Cr(III) has been considered an essential trace element. However, research has shown controversial results for Cr(III) as an essential or toxic micronutrient[64]. Compared to Cr(III), hexavalent chromium Cr(VI) has long been known as a human respiratory carcinogen[64]. Overdosing of chromium may lead to various cytotoxic and genotoxic reactions[63]. Both Cr(III) and Cr(VI) have shown to damage DNA and break chromosomes, impacting genomic integrity leading to cancer[64] . Thus, hair heavy metals reflected heavy metals contamination in diet of which concentration of hair chromium was associated with DNA damage; all these are further complicated by obesity

There was no significant difference for serum 25(OH)D, DNA damage parameters, hair heavy metal levels, and dietary parameters between the non-obese and obese groups. This is likely due to the homogeneity of lifestyle among the study participants. The impact of homogeneous lifestyle on DNA oxidation and DNA damage had also been reported in other studies [65,66]. Besides, the integrative review suggested that psychological distress, educational attainment, physical activity, and sleep duration play a significant part in affecting DNA integrity[67]. Other than dietary intake, other lifestyle factors (e.g. exercise, alcohol, smoking and recreational drugs) profoundly affect DNA damage[1]. DNA SSBs damage lesions can be easily and rapidly repaired[12] if there is a proper diet with modification of lifestyle. A randomized double-blind placebo-controlled intervention study among non-smoking postmenopausal women reported that dietary supplementation protects against DNA damage[68]. In all, the contribution of modifiable and

preventable dietary-driven risk factors like nutrient deficiency and dietary heavy metal exposure in causing DNA damage should not be overlooked. A sufficient level of micronutrient intake plays a paramount role in modifying the pre-existing DNA damage in the state of obesity as well as the heavy metal related DNA damage (figure 5). In this study, DNA damage of non-obese participants was not associated with any studied factors, which is likely because of their healthier lifestyle choices. Reports have supported the impact of a healthy lifestyle on reducing DNA damage [66,69].

Figure 5



Conclusion

There was no significant difference in serum 25(OH)D, DNA damage parameters, concentrations of hair heavy metals, and dietary parameters between the non-obese and obese groups in this study. However, some associations were observed among the obese group. Higher serum 25(OH)D (> 31nmol/L) was associated with lower DNA damage in obese women. A higher concentration of hair chromium increased DNA damage in obese women. As both obesity and DNA damage had been linked to a higher risk of non-communicable diseases, especially cancer, distinct

attention should be given to control the heavy metals in food. Supplementation of vitamin D may modulate the impact of obesity on DNA damage.

There are some limitations in this study. The causal inference of association between DNA damage and diet, vitamin D and heavy metal could not be established in this study. It will be essential to measure the level of micronutrients in the blood as they have been identified as facilitators of the absorption or accumulation of heavy metals in human organs. This study serves as a preliminary study and calls for large-scale studies to confirm its findings.

Total word count: 2882 words

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Authors' contribution

Ng Chiat Yin, Farahnaz Amini, Normina Ahmad Bustami and Eugenie Tan Sin Sing contributed to the research's conception and design. Ng Chiat Yin contributed to the acquisition, analysis, or interpretation of the data. Ng Chiat Yin, Farahnaz Amini, Pui Yee Tan and Soma Roy contributed

analysis and interpretation of the data. Ng Chiat Yin, Pui Yee Tan, Farahnaz Amini drafted the manuscript. All authors critically revised the manuscript, agreed to be fully accountable for ensuring the work's integrity and accuracy, and read and approved the final manuscript.

Conflict-of-interest statement

All authors declare that they have no conflict of interest.

Ethical approval

Ethics approval was obtained from the Medical Research and Ethics Committee (MREC), Malaysia (NMRR-17-224-34092). Informed consent was collected prior to recruitment and sample collection. The research methodology was conducted in accordance with the principles outlined in the Declaration of Helsinki.

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Table Footnote

Table 2:
One-way ANCOVA was carried out to ascertain differences in the mean values of arthropometrics, serum 25(OH)D, concentration of hair heavy metals, DNA damage parameters, and dietary parameters between the non-obese and obese groups, after adjusting for covariates in different model [1]age, physical activity status, smoking status and exposure to second-hand smoking; [2] model[1] + BMI; [3] model[2] +total energy intake.
Obesity is defined as BMI≥27.5kg/m^2.
**p<0.05 was considered significant.*
Body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), total energy intake (TE)

Figure Legends (Word count: 243 words)

Figure 1: Association of serum 25(OH)D level and the tail moment in obese and non-obese groups

The effect was evaluated by using a multivariate general linear model after adjusting for covariates age, height, weight, smoking status, exposure to second-hand smoking, physical activity level, total energy intake, and hair heavy metals (lead, mercury, cadmium, chromium and arsenic) . Serum 25(OH)D *was dichotomized into two groups based on the median value of 31nmol/L (for all participants) for analysis.*
**p<0.05 was considered significant.*

Figure 2: Association of serum 25(OH)D **and DNA damage (tail olive moment) in the obese and non-obese groups**

The effect was evaluated using a multivariate general linear model after adjusting for covariates age, height, weight, smoking status, exposure to second-hand smoking, physical activity level, total energy intake, and hair heavy metal (lead, mercury, cadmium, chromium and arsenic). Serum 25(OH)D *was dichotomized into two groups based on the median value of 31nmol/L (for all participants) for analysis. Obesity is defined as BMI≥27.5kg/m^2.*
**p<0.05 was considered significant.*

Figure 3: Association of chromium level and tail moment

The effect was evaluated by using a multivariate general linear model after adjusting for covariates age, height, weight, smoking status, exposure to second-hand smoking, physical activity level, total energy intake, serum 25(OH)D *and concentration of lead, mercury, cadmium and arsenic. Chromium concentration was dichotomized into two groups based on the median value of 5.88 mg/kg (for all participants) for analysis.*
**p<0.05 was considered significant.*

Figure 4: An example of comet assay results

The comet assay illustrates the comet cells of a participant (27 years old) with low serum 25(OH)D (30 nnmol/L), % tail DNA, and tail moment indicated DNA damage in this participant. The software estimates the DNA damage parameters including the length of the comet tail, the percentage of the tailed DNA, the tail moment (TM) and the Olive tail moment (OTM). The tail moments are calculated by the formulas as follow: $$\mathrm{TM} = \frac{\text{Tail length} \times \text{Tail\%DNA}}{100}$$

Figure 5: The potential correlation of micronutrient intake, dietary heavy metals exposure, obesity and DNA damage