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The Impact of Vitamin D Deficiency on Modulating of Global DNA Methylation and Obesity-Related Bio-Parameters

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Abstract

Background. Obesity has become a rapidly increasing global epidemic health issue where vitamin D seems to impact both of the disease course and the epigenetic profile of the obese individuals. **Aim.** Given the scarcity of local studies examining the correlation between vitamin D levels, global DNA methylation in the context of obesity, this study was set to explore these potential relationships between VD deficiency and changes in liver enzymes, antioxidant levels, global DNA methylation in addition to body fat distribution as reflected by waist circumference and body mass index in Iraqi obese. **Methods.** The study involved 90 participants, including 60 obese individuals and 30 healthy controls (mean age: 31.5 years, range: 20-50 years). All the obese cases were diagnosed at Al Karkh General Hospital, Baghdad, Iraq and private clinic during the period of the 1st November 2023 to the 30th March 2024 by the consultant medical staff. Demographic data such as sex, age, BMI, WC, PBF% were assessed in addition to obesity-related bio-parameters, including vitamin D3, liver enzymes (ALP, AST, ALT) and total antioxidant capacity (T-AOC) were evaluated. Furthermore, global DNA methylation levels were assessed following DNA extraction using the MethylFlash™ Global DNA Methylation (5 mC%) ELISA Easy Kit. **Results.** The levels of vitamin D3 were significantly lower in obese subjects (22.30 ± 1.61) compared to healthy controls (30.20 ± 1.38) ($P = 0.002$). A positive correlation ($r = 0.3$) was also observed between lower vitamin D3 levels and reduced global DNA methylation. There was a significant increase in ALT enzyme levels in obese individuals (32.12 ± 2.34) compared to healthy controls (24.47 ± 1.13) ($P = 0.02$). This suggests that obesity is associated with elevated ALT levels, which are related to liver function. In addition, obese subjects exhibited significantly lower serum T-AOC levels (0.160 ± 0.004) compared to healthy controls (0.191 ± 0.011) ($P = 0.0024$), indicating a reduction in overall antioxidant defense and a potential increase in oxidative stress. DNA methylation levels were significantly reduced in obese subjects (0.348 ± 0.01) compared to healthy controls (0.559 ± 0.02) ($P < 0.0001$), suggesting that obesity may be associated with changes in DNA methylation. **Conclusion.** Vitamin D deficiency is significantly positively correlated with global DNA hypomethylation (5mC%) and reduced total antioxidant capacity (T-AOC) levels. It is also negatively correlated with obesity-related anthropometric measurements and liver enzymes, suggesting its potential role in the increasing rates of chronic inflammation in obese patients and the pathogenesis of obesity.

Key words: Obesity, Vitamin D3, Global DNA methylation, T-AOC, ALT.
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Introduction

Obesity is a multifactorial disease due to obesogenic environments, psycho-social factors and genetic variants. It has become a global health issue with an escalating prevalence among different age groups. Interestingly, over the past three decades obesity has nearly doubled worldwide (1). Since obesity tend to be highly influenced by different environmental factors, it more likely to be affected by the epimethylome that is prone to be modulated by the activity of epigenetic modifiers including DNMTs

(2). The inverse associations between 25-hydroxyvitamin D (25(OH)D) serum levels with both fat volume and body mass index (BMI) have been confirmed (3).

Additionally, it is believed that vitamin D (VD) deficiency has a key role in both regulation of DNA methylation and obesity development. Both 25 hydroxy vitamin D [25(OH)2D] and its active form, 1,25-dihydroxy vitamin D [1,25(OH)2D] are crucial for maintain normal physiological functions, including decreasing intracellular oxidative stresses and inflammation episodes(4). The VD deficiency potential influence in obesity and its related health complications have been highlighted by several lines of evidence; however, the causal relationship is still unclear. A number of possible explanations about the inverse relationship between increased adiposity, mainly abdominal obesity, and low plasma VD concentrations, have been proposed (5). Additionally, vitamin D influences secretion of insulin, tissue sensitivity to insulin, and systemic infection(6).VD sequestration in fat tissue reduces its bioavailability in obese than in lean individuals (7). In the context of obesity, lipids accumulation has the potential to overexpress genes encoding cytokines, chemokines and adhesion molecules in adipocytes that boost the infiltration of immune cells producing further pro-inflammatory mediators. Accordingly, this dramatic shift in the secretory profile of adipose tissue contributes to the development of obesity-related metabolic and cardiovascular obstacles. Such increased levels of pro-inflammatory adipokines circulating in the blood consequence in enlarged oxidative stress. Considering the key role of VD in regulating of the inflammatory activity of adipocytes, the inflammatory status of adipose tissue could be triggered by the reduced VD levels (8). In addition, previous studies have confirmed a significant role for VD supplementation on liver enzymes or fibrosis level among patients with fatty liver disease (9-12). In metabolic associated fatty liver disease (MAFLD) rat models, active VD treatment reduced liver inflammation and oxidative stress by inhibiting the p53-p21 signalling pathway, thus preventing cell senescence (13). In addition, the activation of VDRs in hepatic macrophages by VD ligands ameliorated liver inflammation, steatosis, and insulin resistance in experimental studies(14). VD has also been shown to modulate gene expression by regulating DNA methylation, contributing to reduced inflammation and oxidative stress (15). DNA methylation alterations, as an epigenetic modification, that have been implicated in the pathogenicity of various health issues including obesity via the regulation the transcription activity of genes connected to key cellular pathways. DNA methylation modification involves the addition of methyl group (CH3) to the cytosine nucleotide procced by guanine in the context known as CpG site. A cluster of CpGs (called CpG island) are usually maps to promoter region and regulate the transcription activity of key cellular genes and their associated pathways. The epigenome’s DNA methylation

landscape (epimethylome) is governed by DNMTase family (including *DNMT1, DNMT3A* and *DNMT3B*). Gene silencing and transcription inactivity could the consequence of promoter DNA hypermethylation. While promoter DNA hypomethylation is linked to with transcription activity of the associated genes (16).

Overall, the present study findings have highlighted the associated between vitamin D deficiency and global DNA hypomethylation in the context of obesity- associated biological alterations. This could provide a better understanding for the contribution of epigenetic modifications (global DNA methylation as it is estimated via the 5mC level) in obesity in relation to VD status. Thus, if such association could be established, new avenues would be opened for the development of treatment or prevention approaches to tackle such devastating health issue.

Materials and Methods

Description of Samples

A total number of 90 subjects were involved the present study including 60 obese (45 females and 15males) and 30 healthy controls. Subjects who smoked, having type 1or type 2 diabetes, stroke, angina or myocardial infarction MI, kidney diseases, blood pressure, eye diseases, and taking lipid-lowering medications were excluded from this study. All the obese cases were diagnosed at Al Karkh General Hospital, Baghdad, Iraq and private clinic during the period of the 1st November 2023 to the 30th march 2024 by the consultant medical staff.

Blood samples (5mL) were collected from all of the participants and used for DNA extraction and serum separation for subsequent experiments. A written consent was obtained before any samples were taken. The age average of the participants was 31.5 years (ranging from 20-50 yrs.).

Blood samples collection

Five mL of peripheral blood specimens were collected from all participants (obese cases and healthy controls). Each blood sample was divided into two parts: the first part of two ml of blood was transferred to an EDTA tube to be used for DNA extraction for 5mC measurement. While the second part contains total 3ml and pushed slowly into disposable serum tubes containing separating gel which was allowed to clot at room temperature for 10-15 min then centrifuged at 3000 rpm for about 10-15 min. This was followed by serum distribution in Eppendorf tubes in equal amounts and stored at -20˚C for later use in serological tests.

Methods

PBF% was measured by in body device 270 manufactured by In Body Co.,Ltd.,South Korean company.

Measuring biochemical parameters

25-Hydroxy vitamin D3(VD3) test:

The levels of serum vitamin D3 were measured using Cobas E411 device (Germany). This device works via electrochemiluminescence immunoassay (ECLIA). This technology uses labelled antibodies that, when they bind to specific analyses in a sample, emit light as a result of an electrochemical reaction. The emitted

light is then measured to determine the concentration of the analyses.

Liver enzymes

The analyses were performed in serum of obese cases and their healthy counterparts for liver enzymes [Alanine aminotransferase (ALT)- Aspartate aminotransferase (AST)- Alkaline phosphatase (ALP)] using (AbbottC4000 device) manufactured by Abbott Diagnostics (USA), a clinical chemistry analyser. It typically uses photometric technology to measure various biochemical parameters in clinical samples.

Serum total antioxidant capacity (T-AOC) levels mmeasurement

The levels of total antioxidants (TAC) in serum of obese cases and healthy controls were estimated using Total Antioxidant Capacity (T-AOC) Assay Kit, Cat No: BC1315, manufactured by Solarbio -China). In this assay, within acidic environment, a colorimetric reaction takes place to reduce Fe3+-TPTZ into blue Fe2+-TPTZ. This color reaction reflects the total antioxidant capacity.

Molecular study

DNA extraction

Genomic DNA was extracted from the whole blood samples for both cases and healthy controls by using gSYNC™ DNA Extraction Kit. Extraction was performed following manufacturer's instructions. The concentration of extracted DNA was estimated using a Quantus Fluorometer (Fluorescence Method) to determine the quality of samples. 200 µl of diluted Quantifluor Dye was mixed with 1 µl of DNA then the mixture was incubated for 5-min at room temperature in a dark environment.

Global DNA methylation assessment (5mC%)

For the evaluation of global DNA methylation levels of the extracted DNA samples in the studied obese and healthy control participants, MethylFlash™ Global DNA Methylation (5mC) ELISA Easy Kit (Catalog # P-1034, Epigentek, USA) was employed. According to the manufacturer's instructions, 100ng of the extracted genomic DNA from each tested sample was diluted in the provided binding solution supplied with an eight-well-assay strips kit. In this assay, the DNA methylation fraction that binds to the well-assay strips monoclonal antibodies is captured to be identified by the consequent analyse steps. These included the addition of wash solution, detection antibody, enhancer solution, developer and stop reaction solution. Eventually, global DNA methylation quantification was deliberate as proportional to the OD intensity read (at 450nm) using micro-plate reader (Thermo Fisher Scientific Inc.). The total DNA methylation percentage (5mC%) was proportionally measured by subtracting the OD of the positive controls, supplied by the kit, from the OD of each tested sample. All of the analysed samples were run in duplicate, along with the use of positive and negative controls provided by the kit to ensure obtaining reliable generated signals.

Statistical analysis

The Statistical Analysis System-SAS (2018) program was utilized to distinguish the effect studied parameters

between the different groups (obese and control). t-Test was used to assess the significant differences between levels' mean. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Sensitivity and Specificity of parameters in patients and control groups in this study. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test the normality distribution of data by graph pad prism version 8. Categorical data were expressed as number and percentage, the parametric data were expressed as mean ± standard error. Student t Test and one-way ANOVA followed by Tukey's test as post hoc were employed for parametric data. Regarding correlation among study variables, Pearson's rank correlation coefficient was performed. The parametric data were expressed as mean ± standard error. The differences were considered significant when P value ≤ 0.05.

Results

The results presented in Table (1) showed the distribution of studied subjects [both obese (n=60) and healthy controls (n=30)] according to sex. Chi-square analysis did not show significant differences (value is 0.03, and the p-value is 0.86) between the number of males and females in the studied obese and healthy controls groups (25.00% *vs,* 26.67% and 75.00% *vs.*73.33%, respectively). However, significant (p ≤ 0.01) differences were observed between males and females within each group.

Table (1) : Distribution of study's subjects according to the sex categories

Factor		Obese (No=60)	HealthyControls (No= 30)	Chi-square value
Sex: No (%)	Male	15 (25.00%)	8 (26.67%)	0.03
	Female	45 (75.00%)	22 (73.33%)	
P-value		0.0001**	0.01**	0.86 NS
** (P≤0.01), NS: Non-Significant.				

Table (2) compared the obese and the healthy controls groups in terms of age, BMI, and waist circumference. The data showed no significant age difference (p>0.05) between the groups (31.53 ±1.24 *vs.* 31.70 ±1.48, respectively). However, there is a highly significant difference (p≤0.01) in both BMI and waist circumference, with the obese group having higher values for both the aforementioned bio-parameters (38.58±1.49 *vs.* 24.34 ±0.13 and 119.81 ±1.54 *vs.* 89.73 ±1.09, respectively).

Table (2): Comparison between obese and healthy controls in age, BMI, and waist circumference

Group	Mean ±SE		
	Age (year)	BMI (kg/m^2)	Waist-Circumference (cm)
Obese	31.53 ±1.24	38.58 ±1.49	119.81 ±1.54
Healthy Controls	31.70 ±1.48	24.34 ±0.13	89.73 ±1.09
P-value	0.935 NS	0.0001**	0.0001**
**** (P≤0.01), NS: Non-Significant.**			

Table (3) provided an insight of waist circumference (WC) (cm) and percent body fat percentage (PBF%) might affected by an individual's sex, within the obese group. The results indicate a statistically significant difference in WC was observed ($p \leq 0.05$), with males having a larger waist circumference (126.45 ± 3.38 *vs.* 117.60 ± 1.61) . Additionally, obese females had a significantly higher mean (P≤0.01) in PBF% (49.67 ± 0.98) compared to males (40.70 ± 1.01) indicating a sex -specific difference in body fat percentage in obesity.

Table (3): Effect of sex in WC and PBF% in obese group.

Parameters	Mean ±SE		P-value
	Male	Female	
Waist-Circumference (cm)	126.45 ±3.38	117.60 ±1.61	0.01*
PBF%	40.70 ±1.01	49.67±0.98	0.0001**
NS: Non-Significant, * (P≤0.05), ** (P≤0.01).			

In Figure (1) obese subjects showed to have significantly lower levels (p≤0.01) of vitamin D3(ng\ml) (22.30±1.61) than that of the control groups (30.20±1.38), suggesting an involvement for vitamin D3 deficiency in the obesity pathogenicity.

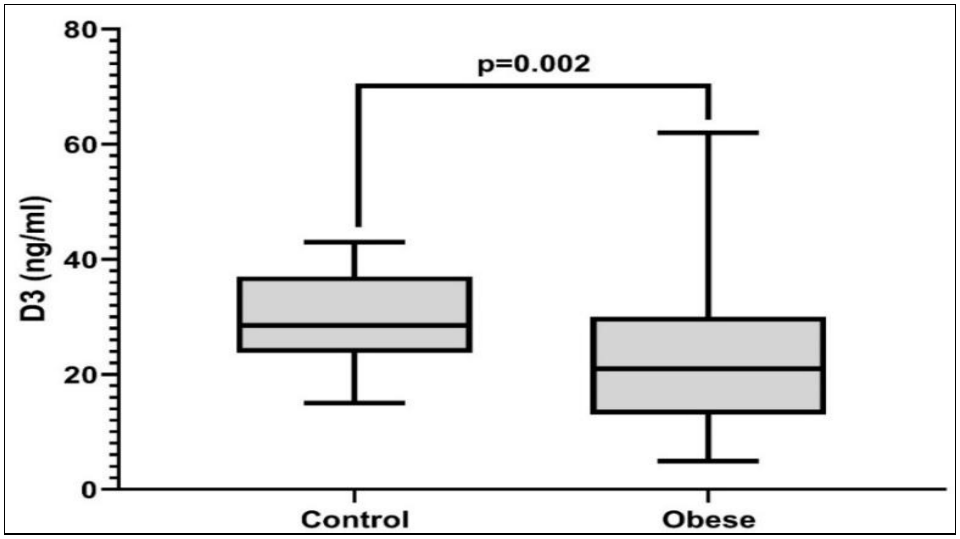

Figure (1): Boxplot of vitamin D3 levels where obese subjects showed to have lower levels than that of the control groups (normal weight).

The serum levels of enzymes related to liver function were assessed in the present study to evaluate their potential associated with obesity. The results of this analysis showed significant elevation of serum ALT levels ($p<0.05$) in obese subjects in comparison to that of the healthy controls (32.12 ±2.34 *vs.* 24.47 ±1.13U/L, respectively). However, this was not the case in respect to the levels of the other investigated liver enzymes ALP and AST, where the results were no significant ($p>0.05$, 76.90 ±2.10 *vs*. 72.27 ±2.37and 22.72 ±1.19 *vs*. 19.40 ±0.55 U/L, respectively) as shown in Table (4).

Obese subjects showed to have significantly lower levels of T-AOC than in control group (0.16 ± 0.004 and 0.19 ± 0.011, respectively, $P \leq 0.01$), Figure (2).

Table (4): Serum levels of liver enzymes of obese and non-obese (control) subjects.

Group	Mean ±SE		
	ALP (U/L)	AST (U/L)	ALT (U/L)
Obese	76.90 ±2.10	22.72 ±1.19	32.12±2.34
Healthy Controls	72.27 ±2.37	19.40 ±0.55	24.47 ±1.13
P-value	0.179 NS	0.06 NS	0.02*
*** (P≤0.05), NS: Non-Significant.**			

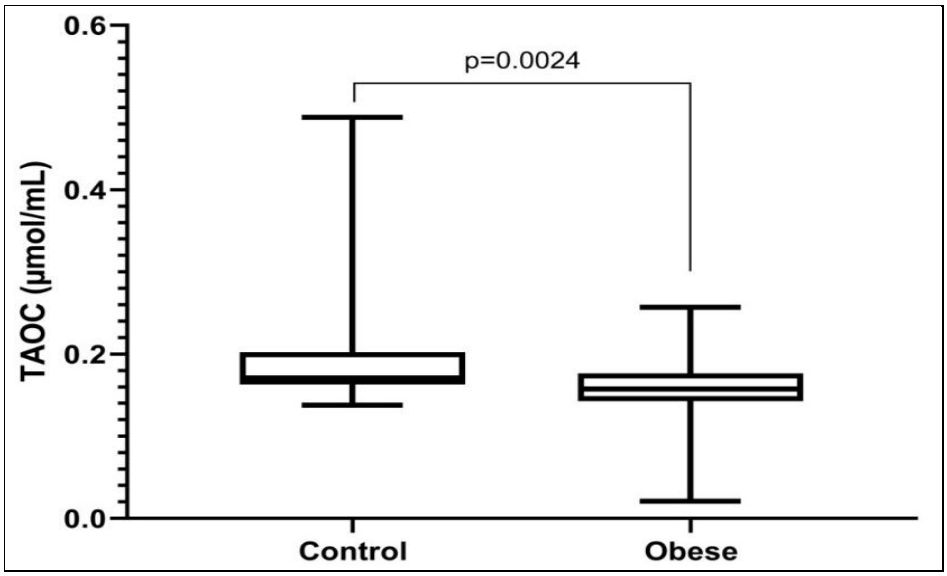

Figure (2): Box plot of the total antioxidant's capacity (T-AOC) levels in obese patients and control groups (normal weight).

The present study findings showed a significant reduction (P≤0.01) in the levels of global DNA methylation (5mC %) of the investigated obese *versus* nonobese healthy subjects. The epimethylome of the investigated obese subjects exhibited to loss DNA methylation by approximately 44.87% than that of non obese (0.348 ±0.01*vs.*0.559 ±0.02, respectively, Figure (3).

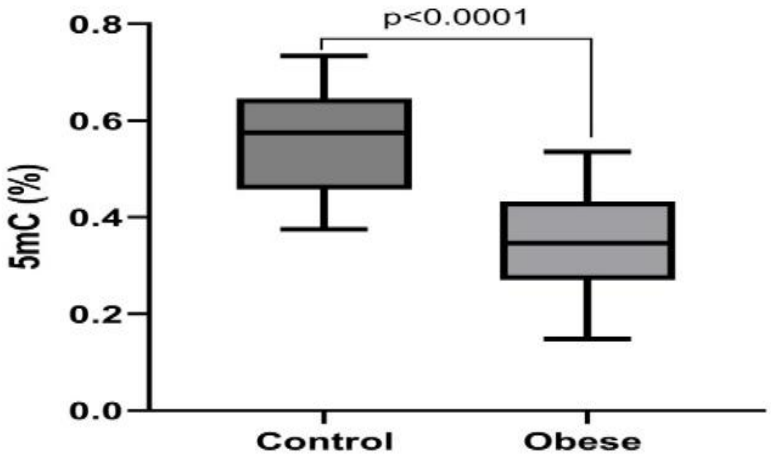

Figure (3): Boxplot of global DNA methylation levels (5mC%) in obese subjects and healthy control groups (normal weight).

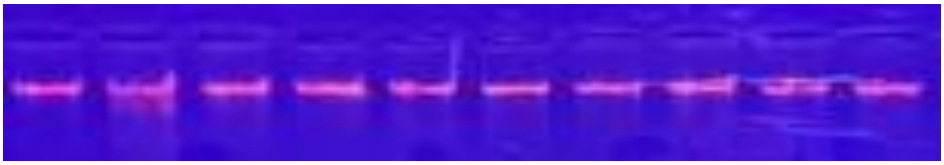

Figure (4): A representative illustration for the extracted DNA samples from the investigated obese and heathy controls subjects.

The current study was also set out to provide an insight about how the epimethylome profile of obese subjects is differ in relation to vitamin D status. The results showed a significant reduction (P= 0.0205) in the levels of global DNA methylation (5mC%) in concomitant with the decreased of vitamin D serum levels in the investigated obese individuals (figure 5). Detailed analysis showed that obese subjects with very lower serum D3 (<19 ng/ml) have significantly lower global DNA methylation levels than those with relatively higher (>19 ng/ml) vitamin D3 levels ($p \leq 0.05$) (0.316 ±0.096 vs. 0.376 ±0.097, respectively, Figure (5). Regarding vitamin D3 deficiency associations, the results of the Figure (6) showed that there was a significant negative correlation with anthropometric parameters (BMI, WC, and PBF) (r=-0.36, -0.32, -0.30, respectively) and liver enzymes [(ALP, r= -0.25), (AST, r=-0.29), (ALT, r= -0.38). These correlations suggest that lower D3 levels are associated with higher body weight and fat accumulation and imply that low Vitamin D3 levels may be associated with elevated ALT levels, which can indicate liver damage or inflammation. Whereas vitamin D3 levels showed to be positively correlated with total antioxidant capacity (T-AOC) (r=0.31), and global DNA hypomethylation (5mC%) (r=0.33). This may reflect the impact of vitamin D3 in reducing levels of antioxidant and suggests that lower D3 levels may be associated with reduced DNA methylation, potentially leading to dysregulation in gene expression.

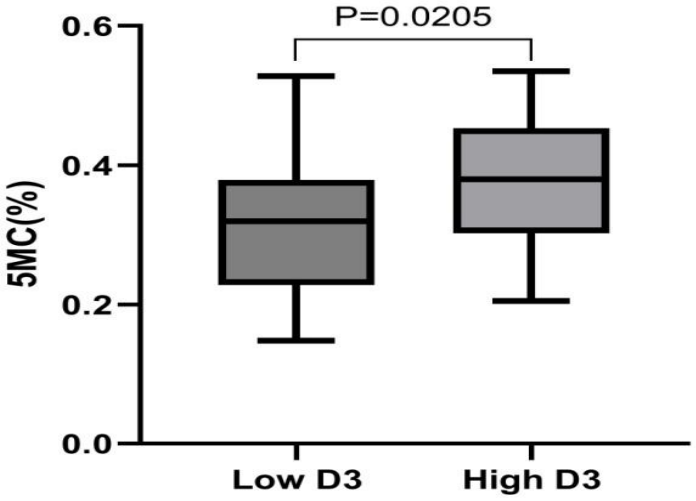

Figure (5): Boxplot of 5mC% levels between low and high vitamin D3 groups in obese patients

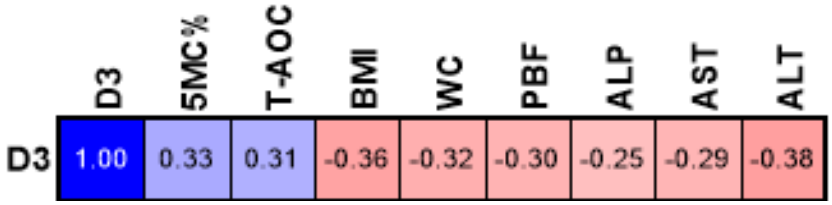

Figure (6): Estimate of correlation coefficient-r between VD3 and 5mC%, T-AOC, BMI, WC, PBF%, and liver enzymes of obese group.

Discussion

Despite significant advances in understanding the pathophysiology and clinical implications of obesity, the role of sex in this context is often overlooked in research, particularly when examining differences between patients and healthy individuals (17). On contrary the biological sex significantly influences disease susceptibility, including obesity. It emphasizes the need to consider sex differences in health, immunity, and gastrointestinal physiology when designing and analysing research studies in both human and animal populations (18). Globally, women are more likely to develop obesity than men. Similarly, the prevalence of obesity in males (14%) was lower than in females (18%) (17, 19, 20). This aligns with the findings of the current study, where the number of obese women exceeds the number of obese men which may be attributed to the hormonal differences between the sexes.

The similarity in average age between obese and control groups ($p=0.935$) in this study can significantly influence the interpretation of findings related to other variables. When age is closely matched, it minimizes confounding factors that could skew results, allowing for a clearer understanding of the impact of specific conditions or treatments. Indeed, a study found that 73% of obese individuals aged 20-50, indicating a higher prevalence in younger populations (21). This is consistent with the results of the current study, where the age groups were between 20-50 years.

The relationship between body mass index (BMI) and obesity is multifaceted, influenced by various demographic factors and health outcomes. Higher BMI is linked to increased risks of multimorbidity, with obese individuals have 1.93 times higher risk compared to those with normal BMI (22). Data presented in our study confirmed that a significant difference ($p\leq0.0001$) in BMI mean values of healthy obese individuals compared to those of normal weight. Of interest, the study found that metabolic health declines with increasing BMI in obese individuals, with only 7.5% of those with a BMI $\geq$ 30 kg/m² being metabolically healthy. This percentage drops to less than 1% at a BMI $\geq$ 36 kg/m² (23). Despite its utility, BMI has limitations, particularly across different ethnicities and genders, which can affect its accuracy in measuring body fat and associated health risk (24) . Waist circumference (WC) is essential for assessing central obesity (25) ,where the results of the present study indicate a clear statistical difference between obese and their healthy controls ($p\leq0.0001$). According to the International Diabetes Federation (IDF), a WC greater than 94 cm for men and 80 cm for women is indicative of increased health risks (26). The prevalence of central obesity in the U.S. rose from 45.2% in 1999-2000 to 56.7% in 2013-2014, with significant increases noted in women (27). These

results underline the critical role of waist circumference as a predictor of obesity-related health risks**.** A significant difference in WC by sex (p = 0.01) suggests that males are generally have higher waist circumference indicating more visceral fat, which is linked to higher triglycerides levels (28). These findings underscore the importance of early intervention and sex -specific strategies for managing central obesity and emphasize the value of WC as a key marker in public health screening and interventions. The study emphasizes that body fat percentage (BFP) is a more accurate obesity evaluation tool than BMI, correlating strongly with physical fitness and health risks associated with obesity(29),and the study defines obesity as 30% body fat for men and 42% for women, emphasizing the importance of direct adiposity measures over BMI for obesity management (30). The present study revealed a significant difference in body fat percentage between obese males and females, with women showing significantly higher levels (p=0.0001). This finding is consistent with previous research and highlights the importance of considering sex as a biological variable in obesity research. Possible mechanisms underlying this sex difference may include hormonal differences, differential fat cell metabolism, and genetic factors. The clinical implications of these findings are significant, as they underscore the need for sex-specific interventions to treat obesity. Future research should explore the biological basis of these sex differences and develop treatment strategies tailored to both men and women. Therefore, in this study WC results showed the differences were also clear and large between the different sexes of obese and control individuals ($p \leq 0.0001$). Higher WC levels are linked to increased infertility rates in women, with a 2% rise in infertility incidence for each cm increase in WC and the inflection point for this relationship was identified at 116.6 cm, emphasizing the need for targeted management of WC (31). The data of the present study demonstrated a significant reduction (p=0.002) in vitamin D3 levels among obese than their control group. A research indicated that Vitamin D deficiency and obesity are a worldwide health issue,and ,obese people have low vitamin D levels for several reasons including larger volume of distribution, where vitamin D tightly bound in fatty tissues, reduced absorption, and diets with low vitamin D (32). The results of vitamin D3 levels supports the well-established association between obesity and vitamin D deficiency and align with current scientific understanding and highlight the importance of addressing vitamin D deficiency in obese individuals (33). The findings also showed that vitamin D3 levels were relatively lower in obese men (18.78±2.24) compared to obese women (23.47±1.99). In addition, the average of methylation levels was lower in obese males (0.307±0.02) than in obese females (0.361±0.01). The lower 25(OH)D levels in women can be explained by the fact that generally women have more fat than men (34). While it consistent with other research, where the greater amount of body fat in women results in an ability to store more vitamin D from skin synthesis than in men, which also explains the fact that vitamin D levels are more variable over the year in men (35). The present study findings for ALT (P=0.02) are in line with previous studies,were serum ALT is considered a precise marker for hepatic dysfunction. Approximately 58% of participants in the general obesity group and 55% of the participants in the abdominal obesity group had at least one

or more elevated hepatic enzymes. Furthermore, serum levels of ALT showed a significant association with only general obesity in the regression models. Also, individuals with both general and abdominal obesity showed an increased risk of developing fatty liver compared to obese persons with no abdominal obesity (36). Additionally, studies showed that ALT levels are significantly higher in obese individuals compared to healthy controls, indicating a correlation with body mass index (BMI) and potential liver health issues (37). Obesity is also characterized by chronic oxidative stress, which affects mitochondrial function and promotes level of chronic inflammation through increased adipocyte size (38). The present study finding also shown that obese individuals exhibit a reduced levels of total antioxidant capacity (T-AOC) compared to non-obese controls ($p=0.0024$). In response to heightened oxidative stress, the body may upregulate its antioxidant defences to neutralize ROS. However , As obesity is commonly associated with increased oxidative stress the reducing T-AOC levels in obese individuals could reflect a compensatory response to the excess reactive oxygen species (ROS) generated during obesity-related metabolic dysfunction. Overall, these findings emphasize the dynamic nature of antioxidant defences in obesity and underscore the need for further research to explore the role of oxidative stress and antioxidant capacity in the pathophysiology of obesity. As obesity has a significant adverse impact on different aspects of human health. As far as we aware, no pervious local study has addressed the association between global DNA methylation and obesity. Accordingly, the present study was set out to provide an insight about how the epimethylome profile of obese subject is differ in comparison to that of their healthy counterparts. The present study results showed a significant reduction ($P< 0.0001$) in the levels of global DNA methylation, as it is assessed by the 5mC%, in the obese individuals than the control group. Several lines of evidence support the involvement of epigenetic alterations in the obesity pathogenic. Of these the fat cell epigenetic signature in post-obese women is characterized by global hypo methylation and differential DNA methylation of adipogenesis genes (39). Furthermore, 35 differentially methylated positions (DMPs) showed hypomethylation in the overall obese group (40). Moreover, studies have identified an inverse association between global DNA methylation levels and weight loss depending on individual genetic variants for different genes(41). On the contrary DNA hypermethylation in obesity onset could be attributed to either altered DNMT activity or expression (42). Interestingly, another experimental study proven that high-fat diet induced obesity alters *DNMT1-DNMT3A* levels and global DNA methylation patterns in mouse ovary and testis (43, 44). The present study results showed a significant reduction ($P= 0.0205$) in the levels of global DNA methylation (5mC%) in concomitant with the decreased of vitamin D serum levels in the investigated obese individuals. This global DNA hypomethylation demonstrated by the obese subjects seems to be consistence with the findings of previous studies that reported genome hypomethylated signature of the assessed obese in comparison to those who are not (45-47). Furthermore, higher degrees of VD deficiency are linked to lower DNA methylation levels and increased expression of inflammatory adipokines such as IL12A and CXCL8 in adipose tissues (48) .This seems quite interesting

finding and has the potential to link reduce vitamin D level with the loss of global DNA methylation. (41).These results underscore the importance of maintaining optimal Vitamin D3 levels in obese individuals, not only for metabolic health but also for preserving epigenetic stability. Globally, the prevalence of obesity has increased markedly especially among children and young adults due to the tendency of consumption of greasy, high calories of fast and processed food that suit the rhythm of modern life (49-51). Thus, understanding the underlying obesity-associated biological alterations could help with the management of this devastating health issue. Research concluded that plasma 25(OH)D is a useful marker to indicate the risk of clinical deficiency and insufficiency (52). A study reported a negative correlation between vitamin D levels and BMI, with lower vitamin D levels observed in obese individuals compared to healthy controls (53). Research indicates that vitamin D levels negatively correlate with visceral adipose tissue (VAT) and waist circumference, suggesting that higher abdominal fat is linked to lower vitamin D availability(54). A national survey demonstrated that lower serum vitamin D levels were associated with increased fat mass in various body regions, particularly in individuals with obesity (55).This is consistent with the negative relationship between VD3 deficiency and anthropometric measurement in this study results. Obesity is known to suppress the expression of CYP2R1, the enzyme responsible for converting vitamin D to its active form, leading to lower vitamin D levels and potentially higher ALT due to liver dysfunction (56). The present study results also highlight a clear association between vitamin D3 deficiency, obesity, and elevated liver enzyme levels. This suggests that vitamin D3 may play a role in reducing inflammation and improving liver function in individuals with obesity. The negative correlation between D3 and ALT is particularly significant (r = -0.38), as ALT is a key marker of liver health, these findings are consistent with recent research linking vitamin D3 deficiency to increased risks of obesity and chronic conditions such as non-alcoholic fatty liver disease (NAFLD)(57-59). The study found that vitamin D3 deficiency in obese individuals is associated with significantly lower total antioxidant capacity (TAC), indicating a potential link between low vitamin D levels and increased oxidative stress, which may negatively impact overall health (60). The association of T-AOC with VD3 deficiency(r=0.31) in the current study was consistent with what was stated in the previous studies above.

Epigenetic modifications, including DNA methylation, are considered as the genome guardian and regulator where the transcription activity and gene silencing of key cellular processes/ pathways are tightly controlled by the epigenetic marks (61, 62). Global methylation patterns associated with intake of vitamin D supplements combined with other lifestyle habits, such as physical activity (63). Furthermore, the positive correlation of VD3 with DNA methylation (5mC%) (r = 0.33) indicates that vitamin D3 might influence epigenetic regulation, potentially affecting gene expression as stated by Mirza *et al*, (46, 64). On the other hand, a study found that obesity is associated with decreased vitamin D metabolites and altered methylation of genes like CYP27A1, CYP2R1, and CYP27B1, which are crucial for vitamin D metabolism(65). The inverse relationship between vitamin D3 and

obesity markers suggests that maintaining adequate Vitamin D3 levels might be crucial in managing weight and metabolic health, especially for the epigenetic landscape that governs the genome transcription activity *via* DNA methylation and histone modifications.

Ethics approval

This study was approved by the Ethical Committee, Department of Biology, College of Science, University of Bagdad and the Iraqi Ministry of Health, Baghdad, Iraq under the reference number CSEC/1023/0086 in 29th of October 2023.

Conclusion

Maintaining adequate vitamin D3 levels in individuals with obesity could prevent increases in liver enzyme levels and mitigate the negative impacts of obesity on overall health. Large-scale studies are recommended to validate the relationship between vitamin D deficiency, global hypomethylation and obesity related-biomarkers, specifically inflammation markers.

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