Med. J. Cairo Univ., Vol. 91, No. 4, December: 1607-1617, 2023
www.medicaljournalofcairouniversity.com

Study of Vitamin D Status and Non Alcoholic Fatty Liver Disease in Obese and Normal Weight Subjects with Different Metabolic Phenotypes: A Case Control Study

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Abstract

Background: Obesity is a common metabolic disorder and is usually associated with non-alcoholic fatty liver disease (NAFLD).

Aim of Study: This study aimed to determine vitamin D level and presence of NAFLD in obese and normal weight subjects with different metabolic phenotypes.

Patients and Methods: Twenty-five obese and twenty-five non obese healthy adult males were included. Anthropometric measurements, serum vitamin D level, diagnosis of NAFLD by ultrasonography and fatty liver index (FLI) were performed. Metabolic health profile was determined in all subjects with classification of each group into metabolic healthy and unhealthy subgroups. ANOVA was used for comparing the four studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. And Person coefficient was used to correlate between normally distributed quantitative variables.

Results: Significant lower serum vitamin D level and disturbed metabolic profile were observed in metabolically unhealthy obese (MUO) subgroup than the other studied subgroups (p<0.001). Both metabolically unhealthy normal weight (MUNW) and MUO subgroups showed lower levels as compared to their corresponding healthy ones (p<0.001). 90% of metabolically healthy obese (MHO) and 93.3% MUO had fatty liver. While only 7.7% of metabolically healthy normal weight (MHNW) and 33.3% of MUNW had fatty liver. Significantly negative correlations were noticed between serum vitamin D levels and body mass index (BMI), serum triglycerides (TG), low density lipoprotein (LDL), homeostasis model assessment for insulin resistance (HOMA-IR), high sensitive C reactive protein (hs-CRP), and fatty liver index (FLI) in MHO, MUNW and MUO subgroups.

Conclusion: Disturbed metabolic health could be related to low vitamin D and NAFLD in obese and normal weight subjects.

Key Words: *Vitamin D – Non alcoholic fatty liver disease – Obesity – Metabolic phenotypes.*

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Introduction

OBESITY is a common metabolic disorder with excessive fat accumulation that may affect health and constitutes a public health problem **[1]**. Obesity is linked with the presence of chronic low-grade inflammation, elevated risk of cardio-metabolic disorders, certain types of cancer and non-alcoholic fatty liver disease (NAFLD) **[2]**.

The difference between obese subjects in their risk for having metabolic dysfunctions and related-

List of Abbreviations:

NAFLD	: Non-alcoholic fatty liver disease.
MUO	: Metabolically unhealthy obese.
MUNW	: Metabolically unhealthy normal weight.
MHO	: Metabolically healthy obese.
MHNW	: Metabolically healthy normal weight.
NASH	: Non-alcoholic steatohepatitis.
IR	: Insulin resistance.
BMI	: Body mass index.
FFA	: Free fatty acids.
TG	: Triglycerides.
NHANESIII	: Third National Health and Nutrition Examination Survey.
VDR	: Vitamin D receptor.
AT	: Adipose tissue.
TNF α	: Tumor necrosis factor α.
NFKβ	: Nuclear factor kappa–light chain-enhancer of activated B cells.
P PAR	: Peroxisome proliferator-activated receptor.
FLI	: Fatty liver index
Fib-4	: Fibrosis 4.

problems has been noticed. Several attempts have been developed to subdivide subjects according to their metabolic health profile and grade of obesity resulting in different obese and non-obese phenotypes. A subgroup of obese subjects has been described to be free from metabolic dysfunction and its associated complications known as metabolic healthy obese (MHO). Conversely, a subgroup of normal weight subjects may present metabolic abnormalities usually found in the obese, this is known as the metabolic unhealthy normal weight (MUNW). Notably, the metabolically unhealthy obese (MUO) persons are characterized by more macrophage infiltration and higher degree of inflammation in the visceral adipose tissue as compared to the MHO persons [2]. Therefore, investigating metabolic health parameters that differentiate individuals into different metabolic phenotypes may produce a newinsight into mechanisms linked to consequences of obesity.

NAFLD is characterized by increased liver fat content >5%, without any secondary causes of steatosis as alcohol abuse, and viral hepatitis [3]. NAFLD is classified as nonalcoholic fatty liver (NAFL), showing histological evidence of hepatic steatosis only, and nonalcoholic steatohepatitis (NASH), having steatosis with lobular inflammation and hepatocyte ballooning with or without perisinusoidal fibrosis [4]. NASH may predispose to cirrhosis and hepatocellular carcinoma. However the associated cardiometabolic complications may lead to morbidity and mortality in those patients [5].

Vitamin D has been connected to several disorders including autoimmune, cardiovascular, inflammatory, and liver diseases [6]. The link of vitamin D level and NAFLD has been lately recognized [7]. Wang et al., have reported that vitamin D deficiency is common among patients with NAFLD. Never the less this relation is still contradictory [8].

The anti-fibrotic and anti-inflammatory effect of vitamin D on hepatic cells was studied by Abramovitch et al., [9]. They confirmed inhibition of hepatic stellate cells proliferation by vitamin D in animal model. It was also reported that Vitamin D decreases insulin resistance (IR) caused by free fatty acids in peripheral tissues and hepatocytes indicating that low vitamin D level may induce intrahepatic fatincrease responsible for NAFLD [10].

Karampela et al., [11] suggested that obesity and low vitamin D are associated and may present increased prevalence worldwide. The volumetric dilution of vitamin D into the largeramount of fat tissue, serum, liver, and muscle could be responsible for the inverse relation between vitamin D serum level and BMI, but the presence of anexact cause is still ambivalent [12].

Given that changes of vitamin D status could be related to NAFLD and obesity as previously studied, it remains unclear whether these relations are affected by changes in metabolic health status. Therefore the aim of this study is to determine the relationship between vitamin D status and NAFLD in obese and normal weight subjects with different metabolic phenotypes.

Patients and Methods

Study design:

Twenty-five apparently healthy obese adult males (BMI ≥30kg/m^2, WC >94cm) and twenty-five apparently healthy non obese adult males (BMI <25kg/m^2, WC ≤94) were selected from the outpatient clinic in Medical Research Institute (MRI), Alexandria university, Egypt.

All methods were carried out in accordance with relevant guidelines and regulations.

History taking and clinical examination were performed. All participants were non-smokers, non- alcoholics, free from diabetes mellitus, hypertension and other chronic diseases. They were not receiving any medications at the time of the study.

Anthropometric measurements:

BMI calculation was performed according to anthropometric measurements of weight (kg) / [height (m^2)]. Waist circumference (WC) measurement was carried out with the patient standing. WC is midway between lower rib margin and superior anterior iliac spine. Measurement was done at the end normal expiration [13,14].

Diagnosis of NAFLD:

B-mode abdominal ultrasonography was performed using Siemens sonograph equipped with 3.5mHz sector transducer scanner to assess the presence of fatty liver. Ultra Sound (US) features consist of liver brightness, contrast between the liver and the kidney, US appearance of the intrahepatic vessels, liver parenchyma and diaphragm. Fatty liver was diagnosed based on ultrasonography findings, negative diagnosis of other liver diseases as viral hepatitis, autoimmune hepatitis and metabolic liver diseases. All patients had negative history for alcohol consumption [15].

• Calculation of fatty liver index (FLI) [16]:

$$FLI = (e(0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745)) / (1 + e(0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745)) \times 100.$$

Where TG denotes triglycerides (mg/dL), GGT is γ- glutamyl transferase (U/L), and WC is the waist circumference (cm). Fatty liver disease is ruled out by a FLI <30 and confirmed with a FLI ≥60.

• Calculation of FIB-4 score for detection of degree of liver fibrosis if present **[16]**:

FIB-4 = (age x AST [IU/L]) / PLT [10^9/L] x √((ALT[IU/L])).

Where ALT; alanine aminotransferase; AST; Aspartate aminotransferase, PLT; Platelets, √; Square root.

A FIB-4 score of ≥2.67, had an 80% positive predictive value and a FIB-4 score of ≤1.30 had a 90% negative predictive value for advanced fibrosis. While a FIB-4 cut-off of ≥1.43 detect stage 1 fibrosis or higher **[17]**.

Biochemical analysis:

Blood samples were obtained from participants by venipuncture following a 12h fasting period (5ml). Samples were left for 30 minutesto clot, then were centrifuged at 3000 rpm for 5 minutes and stored at –20°C until assay. Laboratory tests were carried out to evaluate serum glucose and liver functions; albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) in addition to lipid profile [total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-c) using enzymatic colorimetric method. Determination of hs-CRP using turbidimetry by Kit from Linear chemicals, Montgat, Barcelona, Spain. Basal insulin was quantified by ELISA kits purchased from Millipore Corporation, Billerica, USA.

Low density lipoprotein (LDL-C) fraction was calculated in accordance with the Friedewald'formula LDL-c (mg/dL) = TC (mg/dL) – HDL-c (mg/dL) – TG (mg/dL)/5 **[18]**.

Insulin resistance (IR) was recognized by HOMA-IR index attained from the following calculation: HOMA-IR = Fasting serum /plasma insulin (μU/L) × fasting blood glucose (mmol/L/22.5) **[19]**.

Determination of serum vitamin D:

Vitamin D analysis was measured by Abcam ab213966 25(OH) Vitamin D ELISA kit Cambridge, UK.The results were compared to the normal threshold points established by Endocrine Society clinical practice guidelines. Thus, levels of serum concentration of 25(OH) vitamin D were categorized into deficient (<20ng/ml), insufficient (20–29.9ng/ml), and sufficient (≥30ng/ml and <100ng/ml) **[20]**.

Determination of metabolic health status:

The Karelis criterion was used to define metabolic risk factors (MRF) **[21]**.

Having ≥2 metabolic factors (HOMA-IR ≥2.7, TG ≥1.7mmol/L or use of lipid-lowering drugs, HDL-C ≤1.0/1.3mmol/L for men/women, LDL-C ≥2.6mmol/L, or hsCRP ≥3.0mg/L) is considered metabolically unhealthy. Individuals with less than two MRFs are considered metabolically healthy. According to these criteria, study subjects were categorized into four subgroups:

• Metabolically healthy normal weight (MHNW).

• Metabolically unhealthy normal weight (MUNW).

• Metabolically healthy obese (MHO).

• Metabolically unhealthy obese (MUO).

Statistical analysis:

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). For continuous data, they were tested for normality by the Kolmogorov- Smirnov. Distributed data were expressed as mean and Standard Error of Mean. ANOVA was used for comparing the four studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. And Pearson coefficient was used to correlate between normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

Table (1) illustrates the number, percentage, age, anthropometric data and biochemical characteristics of the four studied subgroups. As shown in table, the number & percentage of the MHNW, MUNW, MHO and MUO subgroups are [(13,52 %), (12,48%), (10,40%) and (15,60%)] respectively. ANOVA test shows significant differences between the 4 subgroups in all parameters except FBS. The MUO subgroup demonstrates significant higher serum TG, Total cholesterol, LDL, hs-CRP and HOMA-IR (p<0.001) than all the other subgroups. Whereas HDL level in the MUO was significantly lower than MHNW and MHO. (p<0.05). The MUNW had significantly higher TG, Total cholesterol and LDL serum levels and a significantly lower HDL. (p<0.05) than MHNW.

Table (1): Anthropometric and biochemical characteristics of all subjects included in the four studied subgroups.

	Normal weight (n = 25)		Obese (n = 25)		F	*p*
	MHNW (n = 13) 52%	MUNW (n=12) 48%	MHO (n=10) 40%	MUO (n=15) 60%		
Age (years)	34.54±2.36	30.58±2.05	38.60**b**±1.61	39.33**b**±1.40	4.553*	0.007*
Waist circumference (cm)	96.15±1.0	100.3±1.61	116.90**ab**±3.11	121.0**ab**±2.68	31.058*	<0.001*
BMI (Kg/m^2)	23.47±0.31	24.19±0.13	31.59**ab**±0.64	33.35**ab**±0.86	72.937*	<0.001*
TG (mg/dl)	132.85±3.91	157.08**a**±3.23	152.20±7.15	186.67abc±5.97	20.195*	<0.001*
Total Cholesterol (mg/dl)	179.0±2.11	199.75**a**±3.48	194.0±1.64	231.60**abc**±6.66	26.787*	<0.001*
HDL-c (mg/dl)	41.23±0.75	35.25**a**±0.33	39.10**ab**±0.41	35.73**ac**±0.45	29.797*	<0.001*
LDL-c (mg/dl)	111.20±2.52	133.0**a**±3.09	125.78±1.52	158.53**abc**±5.52	28.482*	<0.001*
FBS (mg/dl)	85.69±3.31	95.25±3.05	88.40±2.28	90.0±1.78	2.246	0.096
Insulin (μIU/ml)	5.92±0.68	8.56±0.82	8.36±0.81	17.64**abc**±2.26	13.403*	<0.001*
HOMA-IR	1.25±0.16	2.06±0.24	1.85±0.20	3.99**abc**±0.55	11.433*	<0.001*
hs-CRP (mg/L)	2.07±0.42	2.07±0.32	2.47±0.67	5.44**abc**±0.71	9.193*	<0.001*

Data was stated by using Mean ± SE.
SE: Standard Error of Mean
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey).
p: *p*-value for comparing between the studied groups.
a: Significant with MHNW
b: Significant with MUNW
c: Significant with MHO
*: Statistically significant at $p \leq 0.05$.

Metabolically healthy normal weight (MHNW), metabolically unhealthy normal weight (MUNW), metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO). Body mass index (BMI), Triglycerides (TG), High density lipoprotein –cholesterol (HDL-c), Low density lipoprotein–cholesterol (LDL-c), Fasting blood sugar (FBS), homeostasis model assessment-estimated insulin resistance (HOMA-IR), high-sensitivity C-reactive protein (hs-CRP).

Fig. (1) illustrates comparison of serum 25 (OH) vitamin D level (ng/ml) in the four studied subgroups. It is clear that serum vitamin D is significantly decreased in MUO subgroup than the other studied subgroups (p<0.001). As regards the comparison according to the metabolic health status, the unhealthy subgroups MUNW and MUO showed lower levels as compared to their corresponding healthy ones (p<0.001).

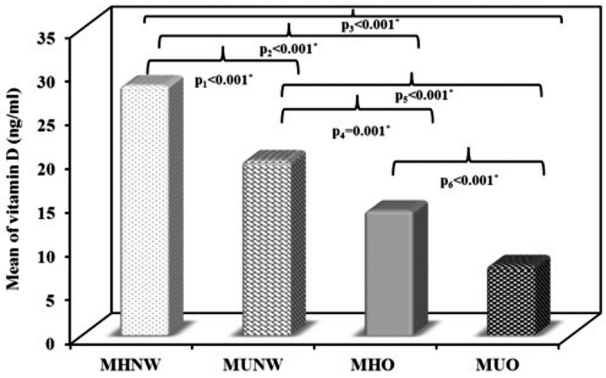

Fig. (1): Serum 25(OH) vitamin D level (ng/ml) in the four studied subgroups.

Metabolically healthy normal weight (MHNW), metabolically unhealthy normal weight (MUNW), metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO). ANOVA test, pairwise comparison done using post hoc test (Tukey). Data stated as mean ± SEM. Statistically significant at p<0.001.

Fig. (2) shows the percentages of presence fatty liver in the studied subgroups. The majority of obese subjects had fatty liver as diagnosed by B mode ultrasound 90% (n=9) and 93.3% (n=14) in MHO and MUO respectively. While only 7.7% of MHNW (n=1) and 33.3% of MUNW (n=4) had fatty liver.

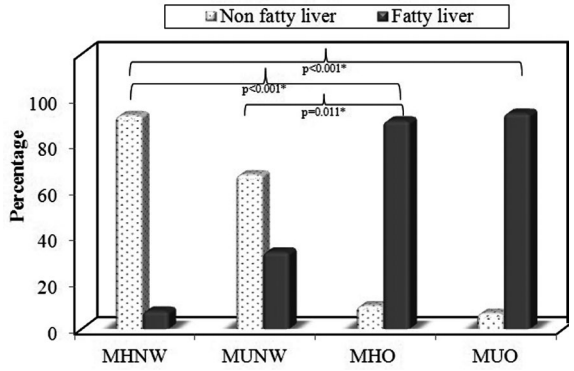

Fig. (2): Percentage of fatty liver as diagnosed by B mode ultrasound in the four studied subgroups.

Metabolically healthy normal weight (MHNW), metabolically unhealthy normal weight (MUNW), metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO).

Biochemical indicators of serum liver function tests, FLI and FIB-4 are shown in Table (2). Coinciding with our ultrasonographic diagnosis of fatty liver in the studied subgroups, FLI was significantly higher in both obese subgroups than normal weight ones (p<0.001). Fib-4 was found to be significantly higher in MUO as compared to MUNWbut within normal ranges.

Table (2): Biochemical Indicators of Liver Function, Fatty Liver Index (FLI) and Fibrosis-4 (FIB-4) in the studied subgroups.

	Normal weight (n = 25)		Obese (n = 25)		F	*p*
	MHNW (n = 13) 52%	MUNW (n=12) 48%	MHO (n=10) 40%	MUO (n=15) 60%		
ALB (g/dl)	3.92±0.05	3.95±0.03	3.89±0.03	3.97±0.05	0.527	0.666
ALT (U/L)	37.85±3.67	37.75±1.44	34.70±1.03	41.33±1.28	1.494	0.229
AST (U/L)	37.15±0.93	42.17±1.59	37.90±1.26	45.67**ac**±2.04	6.524*	0.001*
GGT (U/L)	40.23±6.77	37.25±9.04	35.80±6.11	26.0±2.59	1.091	0.362
FLI	44.91±3.96	53.79±2.53	86.54**ab**±4.21	90.32**ab**±2.01	54.012*	<0.001*
Fib-4	1.03±0.07	0.89±0.07	0.98±0.11	1.22**b**±0.05	4.245*	0.010*

Data was stated by using Mean ± SE.
SE: Standard Error of Mean.
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey).
p: *p*-value for comparing between the studied groups.

a: Significant with MHNW.
b: Significant with MUNW.
c: Significant with MHO.
*: Statistically significant at $p \leq 0.05$.

Metabolically healthy normal weight (MHNW), metabolically unhealthy normal weight (MUNW), metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO). Albumin (ALB), Alanine aminotransferase (ALT), Aspartate transaminase (AST), Gamma-glutamyl transferase (GGT), Fatty liver index (FLI), Fibrosis-4 (FIB-4).

Correlation studies: Correlation between serum level of Vitamin D (ng/ml) and BMI, waist circumference, metabolic measures as well as FLI in the three studied subgroups (MUNW, MHO and MUO) are shown in Fig. (3). There were significantly negative correlations between serum vitamin D levels and BMI, TG, LDL, HOMA-IR, hs-CRP, and FLI in the three subgroups. Whereas, the negative correlation with waist circumference was noticed only in the unhealthy subgroups MUNW and MUO.

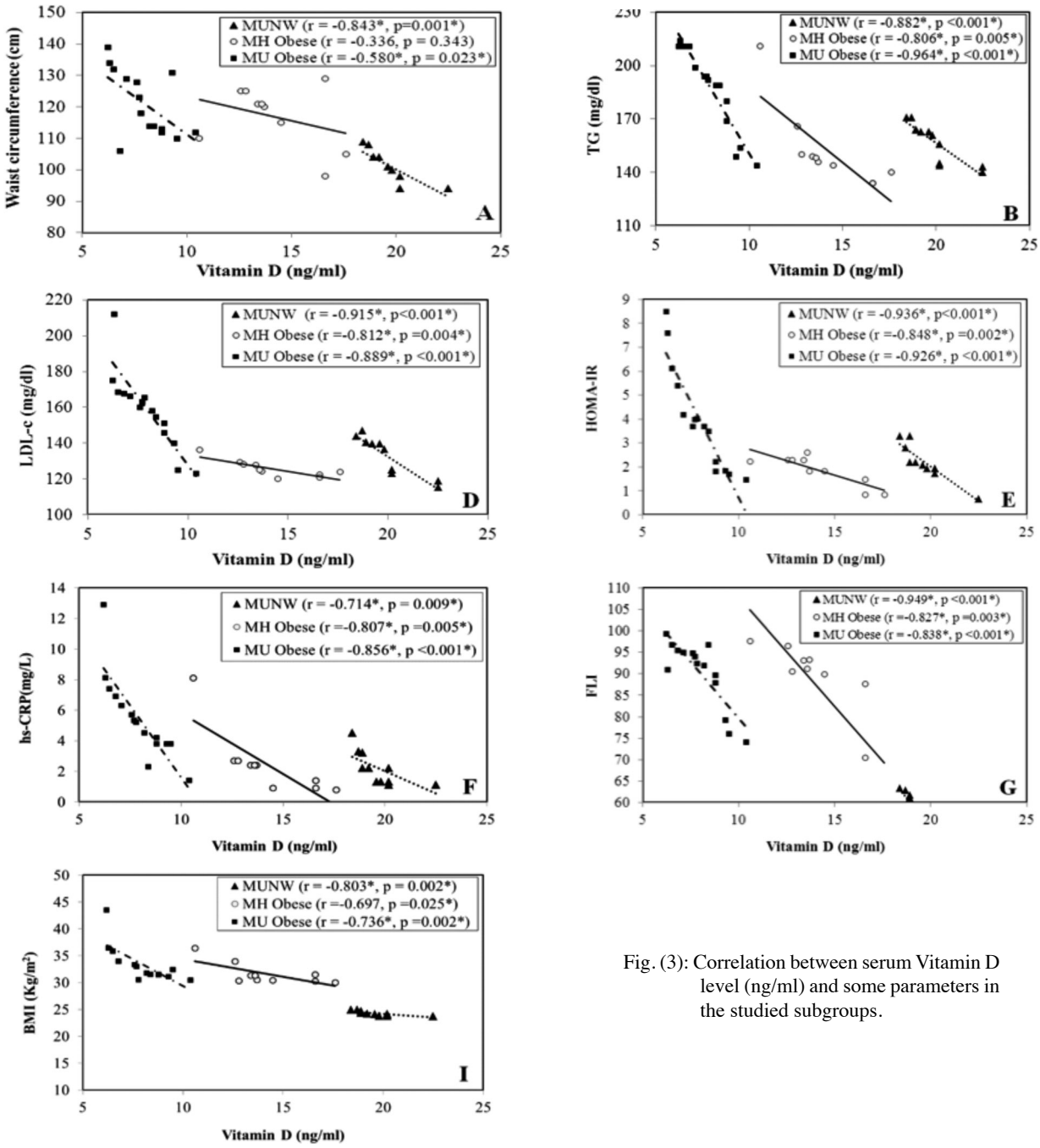

Fig. (3): Correlation between serum Vitamin D level (ng/ml) and some parameters in the studied subgroups.

Waist circumference (WC) (3.A), Serum Triglycerides (TG) (3.B), Low density lipoprotein (LDL) (3.D), Insulin resistance (HOMA-IR) (3.E), High sensitive C-reactive protein (hs-CRP) (3.F), and Fatty Liver Index (FLI) (3.G), Body mass Index (BMI) (3.I) in the three groups (MUNW, MHO and MUO). *r* Pearson's coefficient; *statistically significant at $p<0.05$.

Discussion

The association between Vitamin D level with obesity and NAFLD was recently studied. However, the underlying pathophysiologic mechanism is still unclear **[11]**.

Metabolic health is an important predictor of cardiometabolic consequences in obesity. Meanwhile, some studies have reported that obesity is not always associated with metabolic abnormalities **[12]**. Findings of the present study demonstrated disturbed metabolic profile in MUO subjects showing dyslipidemia, higher CRP andHOMA-IR as compared to MHO. Dysregulated lipolysis of stored TG is the result of adipose tissue IR. IR is linked to defective transport of free fatty acids (FFA) into adipocytes, as well as impaired insulin suppression of stored TG breakdown. This could exacerbate dyslipidemia, ectopic lipid accumulation, and tissue damage, characteristic of MUO **[22]**.

In obese subjects, the pattern of fat storage and the metabolic response vary depending on the hereditary and acquired susceptibility characteristics of the individual.Some people develop the MHO phenotype, which makes them unaffectedby obesity-related metabolic illnesses for a while,whereas some develop easily MUO phenotype **[22]**. The presence of the following pathophysiological characteristics is commonly used to determine MHO status: On comparison with obese patients having coexisting metabolic syndrome (MetS), there was less intra-abdominal visceral fat, preserved insulin sensitivity, and less systemic and adipose tissue inflammation **[23]**. Previous research have shown that about 50% of MHO individuals may progress to MUO **[24]**.

In addition, our findings showed dyslipidemia in MUNW as compared toMHNW subjects. The MUNW phenotype, according to reports, refers to people of normal weight who exhibit various metabolic problems prevalent in obese people. This was explained on the basis that compared to MHNW individuals, MUNW individuals have greater abdominal fat distribution, worse inflammatory state, and higher dyslipidemia **[21]**.

Vitamin D, could be linkedto metabolic changes in obesity. However, The link between 25-hydroxy vitamin D concentrations and different metabolic phenotypes of obesity is controversial **[25]**. Vitamin D deficiency has been connected to an increased hazard of cardiometabolic diseases in research **[26]**. Hong et al., on the contrary, detected no significant difference in vitamin D level between the MHO and MUO groups **[27]**.

Findings of the current study support the hypothesis that vitamin D level could be influenced by metabolic health status and metabolic obesity phenotypes. The MUNW and MUO showed lower vitamin D level as compared to metabolic healthy subgroups but MUO showed the lowest levels. In addition, serum vitamin D showed inverse correlation with BMI, WC, dyslipidemia, CRP and HOMA-IR in MHO, MUO and MUNW subgroups confirming the role of metabolic health as an important factor.

Our findings support those of the Third National Health and Nutrition Examination Survey (NHANESIII), which discovered a link between vitamin D and metabolic health. Vitamin D was also found to be inversely related to cardiometabolic mortality **[28]**.

Volumetric dilution **[29]**, sequestration into adipose tissue **[30]**, inadequate sunlight exposure, and impaired vitamin D synthesis in the adipose tissue and liver are among the clarifications linking low vitamin D to obesity **[31]**. Research supported the hypothesis that rather than just being a consequence of obesity, vitamin D may also contribute to its development.According to experimental research, elevated parathyroid hormone levels caused by Vitamin D deficiency stimulate lipogenesis via increasing calcium inflow in adipocytes **[32]**. Another more plausible theory, is that the active form of Vitamin D, 1, 25 (OH) D, suppresses adipogenesis via effects mediated by Vitamin D receptors (VDR) **[33]**.

Abdominal fat has been suggested to be a predictor of Vitamin D deficiency. In obesity,hypertrophic expansion of adipose tissue (AT) causes imbalanced blood flow, which leads to inflammation and macrophage infiltration, resulting in a drop in adiponectin secretion and arise in proinflammatory cytokines, in addition to down regulation of anti-inflammatory molecules **[34]**. Yarparvar A. et al., **[35]** explained that Tumor Necrosis Factor (TNF) alpha and nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB), both significant active components of inflammation, can be suppressed by vitamin D3. In our study, higher CRP was noticed in MUO subgroup which was negatively correlated with serum vitamin D thus, confirming the reduced anti-inflammatory effect of vitamin D in obese subjects with unhealthy metabolic status.

In the present study, the negative correlation between WC and serum vitamin D levels, suggests that fat distribution is more relevant than total body fat, and the presence of abdominalfat could be a predictor of worsening Vitamin D deficiency.

Both obesity and insufficiency of Vitamin D interact synergistically to impact the risk of IR. Vitamin D deficiency has been related to a decrease in insulin production, insulin receptor dysfunction, and the development of subclinical inflammation. Vitamin D deficiency induced IR due to anupsurge in the gene expression of liver resistin and the

up-regulation of hepatic inflammatory and oxidative stress genes [36,37].

NAFLD is a widespread metabolic disorder with serious clinical implications [38]. In our study, we noticed higher percentage of NAFLD diagnosed by ultrasonography in both obese subgroups as well as in MUNW subgroup compared to MHNW subgroup. In addition, FLI showed significantly higher levels in both obese subgroups as compared to normal weight ones. The relationship between vitamin D level and NAFLDhas been previously studied. Research connecting low serum vitamin D levelwith NAFLD have reported that the two disordersmay be associated with obesity [39]. Serum 25(OH) D concentrations are significantly lower in NAFLD patients [40]. Vitamin D decreases FFA-induced insulin resistance in vitro by modulating the metabolism of free fatty acids (FFAs) via the peroxisome proliferator-activated receptor (PPAR-). As a result, higher FFAs in the blood stream may stimulate fat deposition in hepatocytes and the development of NAFLD in those who are deficient in Vitamin D [41].

Our findings showed that NAFLD appears to be related to vitamin D deficiency in both subgroups of obesity MHO, MUO and also in MUNW. The lowest vitamin D level was detected in the MUO with significantly highest percentage of NAFLD detected by US and highest level of FLI. Patients with vitamin D deficiency showed greater serum levels of proinflammatory cytokines, which promoted the progression of NAFLD [42]. Researchers found a link between serum vitamin D content and NAFLD, which was validated by a meta-analysis that included 12,794 people from 17 different investigations [7,43]. This is in accordance with our study which detected a significant negative correlation between vitamin D level and FLI especially in the obese groups. The negative correlation between serum vitamin D and FLI confirms this association, implying that inhibition of vitamin D's anti-inflammatory properties may play a role in the development and progression of NAFLD.

Targher et al., revealed that biopsy-proven NAFLD patients had reduced vitamin D level, compared to healthy individuals [40]. Knowing that vitamin D undergoes a crucial phase of its activation in the liver, chronic liver disorders, such as NAFLD, have the potential to modify vitamin D metabolism and reduce its levels [44].

It was reported that lower levels of 25-hydroxycholecalciferol are found in NAFLD and non-alcoholic steatohepatitis (NASH) patients than in people without these conditions [8], and its level was negatively associated with NAFLD severity, suggesting that vitamin D deficiency may play a role in the development of NAFLD [41]. In addition, as VDR expression is low or absent in hepatocytes, a deficit in vitamin D-VDR axis signaling could be a provoking factor for NAFLD [45].

Vitamin D deficiency could enhance the metabolic pathways of NAFLD pathogenesis, including immunological, hormonal, and cellular differentiation pathways, influencing adipocytokines and proinflammatory cytokines, which are secreted by AT and are significant in the development of NAFLD [46].

Conclusions:

Vitamin D deficiency is associated with disturbed metabolic health and NAFLD. Screening for vitamin D status and metabolic health parameters should be routinely performed in NAFLD patients for early risk stratification. Further studies are recommended to evaluate the potential role of vitamin D supplementation in the management of NAFLD patients. It is also recommended to study genetic factors that may distinguish metabolic unhealthy from healthy individuals. Follow-up is needed to clarify if the transition from the MHO to the MUO phenotypes has an effect on severity of NAFLD.

Declarations:

Ethics approval and consent to participate: A written informed consent was collected from each participant as privacy of their personal data was followed. The study was approved by the Ethical Committee of MRI, Alexandria University, Egypt. Approval serial number: E/C S/N.R 3/2022.

The ethics committee of the Medical Research Institute, Alexandria University is constituted and operating according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern IRB operation. IORG#:IORG0008812.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: Ola Salama and Manal-Mahmoud performed material preparation, data collection and analysis. Azza Saad analyzed and interpreted the data. Ola Salama and Manal Mahmoud wrote the first draft of the manuscript. Azza Saad revised the paper. All authors have approved the final version of the manuscript.

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دراسة مستوى فيتامين د ومرض التدهن الكبدى اللاكحولى فى الاشخاص البدناء وذوى الاوزان الطبيعيه فى الانماط الايضيه الظاهرية المختلفة

تعد البدانه من أمراض الاضطراب الأيضي الشائعه. يهدف هذا البحث الى تعيين مستوى ٢٥ هيدروكسى فيتامين د وحدوث مرض التدهن الكبدى اللاكحولى فى الأشخاص البدناء وذوى الاوزان الطبيعيه فى الانماط الأيضية الظاهرية المختلفه. تم اختيار ٢٥ شخصاً من البدناء و٢٥ شخصاً من اصحاب الوزن الطبيعى كلهم من الذكور البالغين المترددين على العيادات الخارجيه بمستشفى معهد البحوث الطبيه جامعة الأسكندريه فى الفتره من يونيو ٢٠٢٢ إلى ديسمبر ٢٠٢٢ وتم تقسيمهم تبعا لمؤشر كارليس لأربعة مجموعات حسب الأنماط الأيضيه الظاهريه المختلفه. تم تسجيل القياسات الأنثروبومترية وقياس مستوى ٢٥ هيدروكسى فيتامين د فى الدم وكذلك التحاليل الخاصه بالحالة الصحيه الأيضية. وتم تشخيص مرض التدهن الكبدى اللاكحولى باستخدام الموجات فوق الصوتية وحساب مؤشر تدهن الكبد لجميع المشاركين فى البحث تم تحديد الحالة الصحية الأيضية فى جميع الاشخاص مع تصنيف كل مجموعة إلى مجموعات فرعية صحية وغير صحية.

النتائج: لوحظ انخفاض ذو دلالة احصائية فى مستوى فيتامين د فى المصل واضطراب فى التمثيل الغذائى فى المجموعة الفرعية التى تعانى من السمنة غير الصحية (MUO) مقارنة بالمجموعات الفرعية الأخرى المدروسة ($p<0,001$). أظهرت كل من المجموعات الفرعية ذات الوزن الطبيعى غير الصحى الأيضى (MUNW) والتى تعانى من السمنة غير الصحية (MUO) مستويات أقل مقارنة بالمجموعات الصحية المقابلة لها ($p<0,001$) وكانت نسبه حدوث التدهن الكبدى اللاكحولى ٩٠ ٪ من المصابين بالسمنة الأيضية الصحية (MHO) و٩٣,٣٪ من المجموعة الفرعية التى تعانى من السمنة غير الصحية (MUO) فى حين أن ٧,٧٪ فقط فى أصحاب الوزن الطبيعى الصحى الأيضى (MHNW) و٣٣,٣٪ من المجموعة الفرعية ذات الوزن الطبيعى غير الصحى الأيضى (MUNW). وقد لوحظت ارتباطات سلبية ذات دلالة احصائية بين مستويات فيتامين د فى الدم ومؤشر كتلة الجسم (BMI)، والدهون الثلاثية فى الدم (TG)، والبروتين الدهنى منخفض الكثافة (LDL)، وتقييم نموذج التوازن لمقاومة الأنسولين (HOMA-IR)، والبروتين التفاعلى C عالى الحساسية (hs-CRP) ومؤشر الكبد الدهنى (FLI) فى المجموعات الفرعية المصابين بالسمنة الأيضية الصحية وذات الوزن الطبيعى غير الصحى الأيضى والتى تعانى من السمنة غير الصحية.

وقد خلص البحث إلى انه يمكن أن تكون الصحة الأيضية المضطربة مرتبطة بانخفاض فيتامين د ومرض التدهن الكبدى اللاكحولى فى الأشخاص الذين يعانون من السمنة وكذلك اصحاب الوزن الطبيعى.