Hormonal and metabolic profiles of obese and nonobese type 2 diabetes patients: implications of plasma insulin, ghrelin, and vitamin D levels

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Background **Type 2 diabetes (T2D) is associated with obesity whereas loss of weight is a feature of the disease; however, the two states are not mutually exclusive. Obesity is linked with changes in hormonal activity and overall body metabolism.**

Materials and methods **In this study, 408 T2D patients were recruited in three distinct studies conducted in Bahrain, Saudi Arabia, and Kuwait in three different intervals between 2001 and 2019. In addition to demographics, glycemic and lipid profiles were obtained in all studies, whereas plasma insulin and HOMA-IR, vitamin D, and ghrelin were analyzed in Saudi Arabia. Different techniques such as chemical auto-analyzer, ELISA, chemiluminescent immunoassay, radioimmunoassay were used.**

Results **The obese (BMI $\geq$ 30 kg/m^2) compared with nonobese (BMI 18.5 to <30) patients with diabetes were more likely to be women ($P < 0.001$), smaller in age ($P = 0.028$), and with shorter disease duration ($P = 0.018$). Unexpectedly, the glycemic and lipid profiles were consistently comparable between the two groups in the three sites. Furthermore, vitamin D was strikingly lower in obese patients with diabetes ($P = 0.007$). Finally, plasma ghrelin ($P = 0.163$), insulin ($P = 0.063$), and HOMA-IR ($P = 0.166$) were comparable between obese and nonobese patients with diabetes.**

Conclusion **Diabetic obesity was significantly associated with female sex, young age, short disease duration, and noticeably low vitamin D, and a trend of high insulin levels. However, the obese and nonobese patients had comparable metabolic profiles with no differences in insulin resistance and ghrelin levels. Further studies, especially at a molecular level, are needed to explore this topic which is barely investigated. *Cardiovasc Endocrinol Metab* 11: e256 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

Type 2 diabetes mellitus (T2DM), is a complex metabolic disorder with varying degrees of disturbance of different metabolic pathways, as a result, the disease can present with different clinical scenarios but a common cardinal feature of hyperglycemia, dyslipidemia and intracellular glucose depletion [1]. Insulin resistance is believed to be the hallmark of the disease [2]. The relative contribution of each biochemical pathway determines the disease outcome and predicts its evolution, importantly lipid metabolism and obesity. Metabolism is controlled largely by the hormonal system that involves ligands, receptors, and signal transduction cascade intermediates, which are subject to genetic polymorphism [3,4]. However, environmental factors including diet, exercise, etc., are equally important regulators as stimulants for hormones and neural transmitters which influence the metabolism [5]. Insulin is a central player in both T2D and obesity [1]. The other hormonal systems associated with both disorders include ghrelin, the gut hormone, and vitamin D, the fat-soluble vitamin which is defined as a hormone as well [6]. The last two hormones were previously analyzed in T2D [7] and obesity [8] in this setting and were reanalyzed in this study to examine their role in diabetic obesity.

The association of T2D with obesity is well documented [9]. Some studies claimed that obesity is a risk factor for T2D [10], whereas others believe that obesity is an inevitable event during the course of T2D [9]. In contrast, T2D can be associated with weight loss specifically before diagnosis [11].

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Obesity is graded by BMI which is calculated using body weight and height parameters. Isolated obesity as a separate symptomatic clinical disorder, characterized by elevated plasma insulin, insulin resistance, and dyslipidemia, is common [12], although healthy obesity also exists [13]. Obesity is linked with the hunger hormone, ghrelin [14], which is secreted in substantial amounts by the gastrointestinal tract [15]. Furthermore, low ghrelin was found to be associated with T2D and insulin resistance as reported before in the region [7] and elsewhere [16,17].

The third hormonal system that influences metabolism is vitamin D. Vitamin D (cholecalciferol) is a fat-soluble vitamin; it is also described as a hormone. It is best estimated in the body by measurement of plasma 25-hydroxycholecalciferol (25OH-D_3). Hypovitaminosis D is a very common disorder worldwide, especially in the Gulf Cooperation Council (GCC) region. Vitamin D is well known to be implicated in T2D and obesity development [8,18,19].

The following is the hypothesis of the present study: In addition to patients' genetic background, the metabolic profile of obese and nonobese T2D patients is likely to be different, and the differences are likely to be endorsed by hormonal metamorphosis. The inference of categorization of T2D patients based on BMI could help in the prediction of complications and management strategy.

Materials and methods

Study area and subjects

This study included data obtained from three Gulf Corporation Countries: Bahrain [20], Saudi Arabia [7], and Kuwait [21], collected at different times. A total of 405 subjects known to be T2D patients were enrolled from outpatients' diabetes clinics during routinely scheduled follow-up visits in each country. The patients' distribution was as follows: 187 from Bahrain (95 men and 92 women; median age 53.0, 45.0–60.0 years), 104 from the Kingdom of Saudi Arabia (KSA) (59 men and 45 women; median age 47.0, 41.0–55.0 years), and 112 from Kuwait (53 men and 59 women, median age 56.5, 52.0–64.0 years). Acute and chronic severe illnesses, pregnancy, and lactation were exclusion criteria. The Bahraini study was carried out in the period between 2001 and 2004 [20]. The Saudi study was conducted in the Eastern Region of the KSA, King Abdulaziz Hospital, and Primary Health Centers (PHC) of the Ministry of National Guard Health Affairs in Al Ahasa, Eastern province in 2014 [7]. The Kuwaiti study was carried out in 2019, in Kuwait hospital and affiliated health centers [21].

Study design

This was a cluster of prospective case–control cross-sectional studies.

Blood sampling

The blood samples were obtained from donors after 10–12 h of overnight fasting. Samples collected in gel/dry plain tube were used for lipid profile, and blood collected in EDTA tubes was used for HBA1c and ghrelin analysis, whereas plasma collected in fluoride tubes was used for fasting blood glucose (FBG) and insulin measurement. Buffy coats were retained for DNA extraction. Samples were stored at –20 to –80 °C until used.

Clinical data collections

Medical history, physical examination, and routine laboratory results including the fasting lipid and glycemic profiles were collected in questionnaires and transferred into electronic databases. Obesity was graded using the BMI classification as follows: Underweight, less than 18.5 kg/m^2; normal, at least18.5 to less than 25; overweight (OW), at least 25 to less than 30; obese class I (OBS), at least 30 to less than 35; obese class II, at least 35 to less than 40; and obese class III, at least 40 [22]. For simplification, obese classes II and III were merged as severely obese (SOBS), and the patients were recategorized into obese (BMI ≥30 kg/m^2) and nonobese (BMI ≥18.5 to <30) T2D patients. The numbers of the underweights in all study sites were too low (1–4 patients), and were thus excluded from the analysis.

Blood chemistry

In the Saudi and Bahraini studies, FBG was measured by an auto-analyzer, Architect c8000 (Abbott Laboratories, Inc., Abbott Park, Illinois, USA). HbA1c was estimated by G8 analyzer (Tosoh, Tessenderlo, Belgium), and the lipid profile [total serum cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglyceride (TG)] was analyzed by Cobas c501, Roche, Hitachi analyzer (High Technologies Corporation, Tokyo, Japan). While in Kuwait, the glycemic and lipid profile parameters were measured by UniCel1 DxC Synchron 800 analyzer (Beckman Corporation, Brea, California, USA) and TOSOH G8 HPLC Analyzer (Tosoh Bioscience, California, USA).

Plasma insulin

Plasma insulin was quantitatively assayed using a chemiluminescence procedure with an auto-analyzer, Architect i2000 (Abbott Laboratories, Inc.) in the Saudi study. Insulin resistance (IR) was calculated using the homeostasis model assessment 2 (HOMA-IR), HOMA-IR index = [fasting glucose (mmol/L) × fasting insulin (mIU/L)]/22.5.

Measurement of serum vitamin D level

Vitamin D adequacy was assessed by measuring serum 25-hydroxyvitamin D_3 (25OH-D_3) concentration. Abbott Architect 25 OH Vitamin D assay, a chemiluminescent immunoassay technology, was used as previously described [8,23].

Blood ghrelin measurement

The active form of plasma ghrelin was measured by a radioimmunoassay kit (Millipore Research, St Charles, Missouri, USA), as described before [7,24]. The plasma ghrelin levels were then determined by the interpolation

of the reference curve drawn using standard concentrations of ghrelin.

Research ethics

The studies were ethically approved by the Research and Ethics Committee of College of Medicine and Medical Sciences, Arabian Gulf University, approval letter (E28-PI-01/20). Informed consent was obtained from each patient before inclusion in the study.

Statistical analysis

For data analysis, SigmaStat software was used. Comparisons between obese and nonobese T2D patients were conducted by *t*-test or Mann–Whitney rank-sum test (MW), whereas for comparisons between the four BMI grades, one-way analysis of variance or Kruskal–Wallis one-way analysis of variance on ranks (KW) was used. Chi-square test was used for comparison of rates and proportion and Pearson product-moment correlation was used for correlations between the countable variables. The significant *P* value was less than 0.05.

Results

Study subjects' characteristics

A total of 403 T2D patients from Bahrain (187), KSA (104), and Kuwait (112) with a mean (±SD) age of 53.1 ± 10.5 years were included in the study; 51.4% were men and 54.6% were obese (BMI ≥30), with an overall mean (±SD) BMI of 31.5 ± 6.9 kg/m^2. Further details about the sites' parameters are shown in (Table 1).

Associations of the different grades of obesity in type 2 diabetes with patients' age, age of onset, disease duration, and comorbidity with hypertension

As shown in (Table 2), considering all patients together, the obese (OBS and SOBS) were significantly younger compared with nonobese (normal and OW) T2D patients, 52.0 ± 10.0 vs. 54.3 ± 10.9 years, $P = 0.028$, *t*-test; the comparison remained significant even when the four grades of obesity were considered separately, $P = 0.047$. However, taking each site separately, the differences of age between the obesity grades were NS and there was no specific trend. Although the age of the disease onset was relatively late in obese (43.0, 37.0–50.0 years, median, 25–75%) compared to the nonobese patients (41.0, 36.0–50.0 years), the difference was NS, $P = 0.673$, but the data were obtained from Bahrain only. Regarding disease duration, it was significantly shorter in obese compared to nonobese patients (6.0, 2.0–12.0 vs. 8.0, 4.0–15.0, $P = 0.018$), when patients from all sites (Bahrain and KSA) were taken together but not when each country was considered separately; though, there was a trend of short disease duration in obese T2D patients in each site.

In Bahrain, the BMI was significantly negatively correlated with age [correlation coefficient (CC) 0.183, $P = 0.012$] and age of disease onset (CC–0.194, P 0.003), but not with disease duration, whereas in KSA and Kuwait, none of the above variables were correlated with BMI; nevertheless, the all-sites analysis revealed borderline inverse correlation of BMI with age (CC 0.094, $P = 0.059$) (Table 3). The other related parameters were not available for all sites.

Finally, the comorbidity with hypertension (HTN) was comparable between all grades of obesity in all sites (Table 2).

Association of the BMI with sex

As shown in (Fig. 1a), T2D women had significantly higher BMI than T2D men in Bahrain (median, 25–75%) 31.4, 27.5–35.3 vs. 27.0, 25.0–31.6 kg/m^2, $P < 0.001$, and in all study subjects together (32.2, 27.6–36.5 vs. 29.6, 26.0–33.0, $P < 0.001$). Although women with diabetes had higher BMI in KSA (33.8, 30.7–39.3 vs. 31.7, 29.0–34.0; $P = 0.069$) and Kuwait (32.0, 27.6–36.5 vs. 29.7, 26.5–35.5, $P = 0.130$) but the differences were NS.

The BMI and drug treatment

Figure 1b shows the BMI of T2D patients in KSA under different treatment regimes. Patients treated with metformin alone had the lowest BMI (29.9, 27.0–32.4 kg/m^2), followed by patients treated with metformin and insulin (32.1, 28.1–36.5), and then patients treated by metformin and gliclazide (32.9, 31.0–38.6); however, the differences were borderline, $P = 0.060$. The drug treatment in the other two sites was underreported.

The metabolic profile of obese and nonobese type 2 diabetes patients

The glycemic and lipid profiles parameters were compared between the four grades of obesity in T2D patients in each site separately, then collectively and finally between all nonobese (normal and OW together) and all obese (OBS and SOBS together) T2D patients, as shown in Table 4. The only significant observations were the relatively low LDL-C in OBS Bahraini patients ($P = 0.024$) and low TG in the OBS Saudi patients ($P = 0.036$) compared with the other BMI grades in each site and a trend of low LDL-C in OBS people with diabetes when all study subjects were taken together ($P = 0.082$). The HbA1c was consistently slightly higher in obese patients but always below the significant levels. Otherwise, there were no significant differences or consistent trends of differences in the biochemical profile parameters between the different BMI grades of obesity or the obese and nonobese T2D patients in all study sites (Table 4).

Neither the glycemic parameters (FBG and HbA1c) nor the lipid profile parameters (HDL-C, LDL-C, total cholesterol, and TG), except TG in Bahrain, were correlated with BMI in any of the sites or all sites taken together (Table 3).

Table 1 Description of the study subjects

Characteristics	Bahrain	KSA	Kuwait	*P* value
Data collection date	2001–2004	2013	2019	
Number	187	104	112	
Men to women ratio	50.8% (95/92)	56.7% (59/45)	47.3% (53/59)	0.376 χ^2
Obese to nonobese ratio	46.0% (86/101)	71.1% (74/30)	53.6% (60/52)	<0.001 χ^2
BMI (kg/m^2)	29.0, 25.8–34.1	32.5, 29.7–36.4	30.8, 27.1–35.7	<0.001 KW
Age (years)				
Mean ± SD	53.0 ± 10.3	48.3 ± 10.8	57.6 ± 8.3	
Median	53.0, 45.0–60.0	47.0, 41.0–55.0	56.5, 52.0–64.0	<0.001 KW
Range	88.0–29.0 (59.0)	75.0–26.0 (49.0)	84.0–39.0 (45.0)	

KSA, Kingdom of Saudi Arabia; KW, Kruskal–Wallis one-way analysis of variance on ranks; χ^2, chi-square.

The hormonal profile of the obese and nonobese type 2 diabetes patients in the Kingdom of Saudi Arabia

The plasma insulin level (pmol/L) was significantly different between the four BMI grades of obesity in Saudi T2D patients: normal (36.9, 20.2–55.8), OW (43.4, 35.0–84.8), OBS (57.2, 37.0–76.2), and SOBS (75.3, 51.0–104.0), $P = 0.033$, KW (Fig. 2a-i). While there was a clear escalation of the insulin level with increasing BMI, the difference in levels between the obese (63.0, 40.2–85.9) and nonobese (42.4, 33.4–70.3) patients was borderline, $P = 0.063$, MW (Fig. 2a-ii).

Though the HOMA-IR index was highest in SOBS (4.3, 2.6–6.9), followed by OW (2.9, 1.8–5.0) and OBS (2.7, 2.2–4.6) patients than normal BMI patients (2.0, 1.4–2.7), the differences between the four BMI grades were NS, $P = 0.097$, KW (Fig. 2b-i), and that between obese and nonobese (3.4, 2.3–5.5 vs. 2.8, 1.7–4.6) T2D patients was also NS, $P = 0.166$, MW (Fig. 2b-ii).

On the contrary, the plasma ghrelin level (pmol/L) was highest in the normal BMI patients (16.6, 14.2–17.8), followed by the OW (14.5, 9.3–19.4) and OBS (13.6, 9.5–15.6) than the SOBS (12.2, 7.4–16.9) T2D patients but the differences were NS, $P = 0.404$, KW (Fig. 2c-i). Similarly, the plasma ghrelin level in obese (12.8, 9.1–16.3) and nonobese (14.5, 9.7–18.4) T2D patients was comparable, $P = 0.163$, MW (Fig. 2c-ii).

Finally, the plasma cholecalciferol (D3) level (nmol/L) was significantly different between the T2D patients in the different BMI grades of obesity with the highest level in the OW patients (37.4, 29.2–44.3), followed by OBS (33.5, 24.3–47.9) and normal (31.2, 17.8–33.9), whereas the lowest levels were seen in SOBS patients (27.3, 22.1–38.3); the difference was significant, $P = 0.045$, KW (Fig. 2d-i). The obese compared to the nonobese T2D patients had comparable D3 levels (29.3, 22.4–44.9 vs. 34.0, 28.9–43.4, $P = 0.214$, MW, data not shown in Fig. 2d); however, the levels in SOBS compared with OW grades was markedly significantly low, $P = 0.007$, MW (Fig. 2d-ii).

However, none of the hormonal parameters [plasma insulin, HOMA-IR-index, ghrelin, and vitamin D (D3)], was correlated with the BMI; but, these parameters were tested in KSA only (Table 3).

Discussion

The observations that T2D patients experience changes in body weights during the disease course, and that obesity is associated with T2D although the loss of weight is a feature of the disease [25], altogether impose the need for study of obesity in T2D. In the present study, unexpectedly, both glycemic and lipid profiles were comparable between the obese and nonobese T2D patients except for the inconsistently low LDL-C and TG in the OBS patients in Bahrain and Saudi Arabia, respectively, but not in the other sites or in all sites taken together. Reproducibility of the metabolic profile findings in the three different settings over different periods spanning over almost 20 years using different laboratory protocols, is strong evidence for the reliability of this data. Peculiarly, the similarity of the biochemical parameters between obese and nonobese T2D patients was never tested or reported before.

Several factors could contribute to obesity in T2D, such as sex and time-related variables, for example, age, age of disease onset, and disease duration [26]. In this study, we found that women with diabetes were significantly more obese than men with diabetes in Bahrain as reported elsewhere [26]; however, this association was borderline in KSA as well as in all study subjects grouped together but not in Kuwait (Fig. 1a). We previously showed a sex-dependent association of obesity with hypovitaminosis D in Bahraini subjects with no diabetes [8], which emphasize the role of sex in both obesity and hypovitaminosis D. The role of age as a contributory factor to diabetic obesity was controversial; in the present study, there was no consistency across the three sites though obese people with diabetes were significantly younger than nonobese ones when patients from all the sites were grouped (Table 2). Furthermore, there was a consistent trend of a shorter disease duration among the obese compared to nonobese people with diabetes in each site and it was significantly shorter in the obese patients when data from all sites were pooled. For the age of disease onset, data were obtained from Bahrain only, but it did not show any significant difference between the obese and nonobese patients, although there was an inverse correlation of the BMI and age of onset, that is, the obese patients developed

Table 2 Association of the different grades of obesity (BMI) in type 2 diabetes in Bahrain, Saudi Arabia, and Kuwait with patient age, age of onset, disease duration, and hypertension comorbidity

BMI	*N*	OW	OBS	M-OBS	*P* value
Age (years)					ANOVA
BH (186)	**57.0 ± 10.4** (37)	52.5 ± 10.4 (63)	52.0 ± 10.4 (45)	**51.2 ± 9.2** (41)	**0.057**
KSA (104)	43.0 (33.0–65.0) (6)	47.0 (41.5–63.0) (24)	47.0 (41.5–55.0) (43)	50.0 (41.3–53.8) (31)	0.972[a]
KU (112)	58.8 ± 7.2 (12)	57.2 ± 8.8 (40)	57.6 ± 8.2 (29)	57.5 ± 8.4 (31)	0.947
Total	**56.4 ± 11.1 (55)**	53.4 ± 10.7 (127)	**51.9 ± 10.5 (117)**	52.2 ± 9.4 (103)	**0.047**
n-O vs. O	**54.3 ± 10.9 (182)**		**52.0 ± 10.0 (220)**		**0.028**[b]
Onset age (years)					ANOVA
BH (187)	46.4 ± 12.3 (37)	41.4 ± 10.3 (63)	43.1 ± 9.2 (45)	43.3 ± 8.8 (41)	0.135
n-O vs. O	41.0 (36.0–50.0)		43.0 (37.0–50.0)		0.673[c]
Disease duration (years)					KW
BH	8.0 (4.0–15.8) (37)	9.0 (3.3–16.0) (63)	6.0 (3.0–12.3) (45)	6.0 (2.0–12.3) (41)	0.359
KSA	6.5 (6.0–8.0) (6)	6.5 (3.0–9.5) (24)	5.0 (1.6–10.0) (43)	5.0 (2.0–9.8) (31)	0.912
Total	7.0 (4.3–13.5) (43)	8.0 (3.3–15.0) (87)	5.0 (2.0–12.0) (88)	6.0 (2.0–11.0) (72)	0.133
n-O vs. O	8.0 (4.0–15.0) (130)		6.0 (2.0–12.0) (160)		**0.018**[c]
Hypertension					χ^2
BH	56.8% (21/37)	56.8% (21/37)	59.1% (26/44)	60.0% (24/40)	0.988
KSA	16.7% (1/6)	33.3% (8/24)	34.9% (15/43)	32.3% (10/31)	0.849
KU	41.7% (5/12)	62.5% (25/40)	69.0% (20/29)	77.4% (24/31)	0.151
Total	49. 1% (27/55)	53.5% (54/101)	52.6% (61/116)	56.9% (58/102)	0.818
n-O vs. O	51.9% (81/156)		54.6% (119/218)		0.686

Note that values between brackets are the number of patients. Bold indicates significance or borderline *P* value, and the compared values.
BH, Bahrain; KSA, Kingdom of Saudi Arabia; KU, Kuwait; KW, Kruskal–Wallis one-way analysis of variance on ranks; n-O vs. O, nonobese people with diabetes vs. obese people with diabetes; N, normal; OBS, obese; OW, overweight; SOBS, severely obese; ANOVA, one-way analysis of variance; χ^2, chi-square.
[a]KW;
[b]*t*-test;
[c]Mann-Whitney rank-sum test.

Table 3 Correlation of the BMI of T2DM patients with age, age of onset, disease duration, and metabolic profile in the different study sites

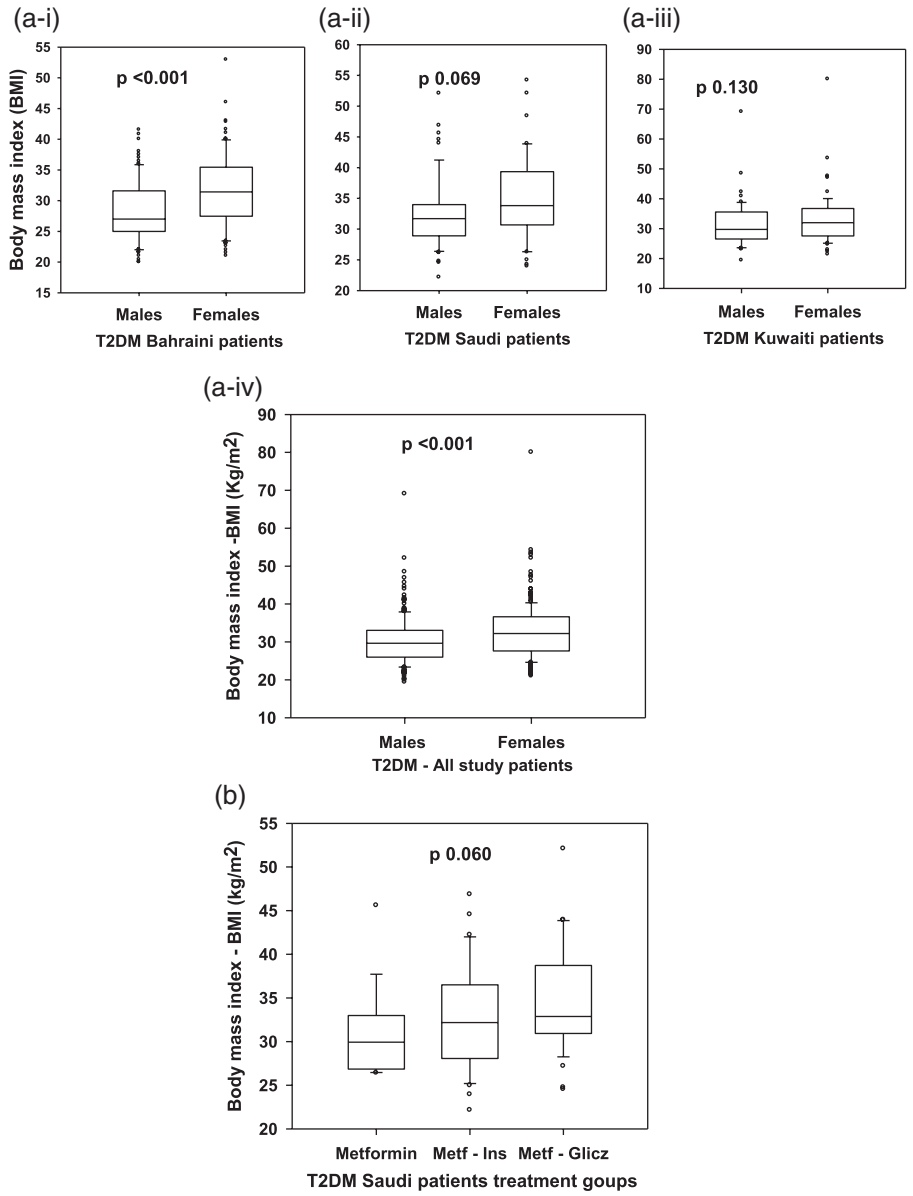
Study site	BMI			
Time-variables	Age	Age of onset	Disease duration	
BH	**−0.254, 0.000** (254)	**−0.194, 0.003** (228)	−0.099, 0.116 (251)	
KSA	−0.054, 0.589 (104)	ND	ND	
KU	0.0385, 0.687 (112)	ND	ND	
Total	−0.168, 0.000 (470)			
Glycemic profile	FBG	HbA1c		
BH (140)	0.016, 0.846 (142)	0.028, 0.753 (131)		
KSA (104)	−0.079, 0.425 (104)	0.061, 0.540 (104)		
KU (103)	0.145, 0.143 (103)	0.080, 0.431 (100)		
Total	−0.023, 0.675 (349)	0.015, 0.780 (335)		
Lipid profile	LDL-C	HDL-C	Total-C	TG
BH (140)	0.002, 0.980 (112)	−0.046, 0.643 (104)	0.066, 0.461 (129)	0.215, 0.012 (137)
KSA (104)	−0.045, 0.651 (104)	0.149, 0.130 (104)	−0.057, 0.564 (104)	−0.052, 0.598 (104)
KU (103)	0.002, 0.988 (95)	0.061, 0.548 (101)	0.050, 0.618 (101)	0.022, 0.827 (101)
Total	−0.028, 0.625 (311)	−0.051, 0.377 (309)	−0.018, 0.745 (334)	0.086, 0.114 (342)
Hormonal profile	Insulin level	HOMA-IR	Vitamin D	Ghrelin
KSA	−0.027, 0.783 (104)	−0.008, 0.939 (104)	−0.160, 0.104 (104)	−0.120, 0.249 (95)

The data are presented as CC, *P* (no.). Bold indicates significance or borderline *P* value, and the compared values.
BH, Bahrain; CC, correlation coefficient; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; KSA, Kingdom of Saudi Arabia; KU, Kuwait; ND, not done; No., number of patients; TG, triglycerides.

T2D at a smaller age (Tables 2 and 3). Taken together, the three parameters of time indicate that the obesity in T2D was associated with smaller age, and it declined with increasing age after onset, as mentioned before [27]. Furthermore, the prevalence of HTN in obese and nonobese diabetics was not significantly different although it was consistently higher in the obese diabetics in the 3 sites, however, a larger and multicenter study is needed to approve or disprove this observed trend of association of HTN with diabetic obesity. The HTN together with obesity and T2D are the main components of metabolic syndrome (MetS) [28]. Finally, the T2D therapeutic management is likely to play a major role in the metabolic profiles; in this study, data from KSA showed that treatment with metformin only was associated with the lowest BMI, as compared to the treatment when insulin or gliclazide was added ($P = 0.06$); however, the small sample size was a limiting factor for drawing a reliable conclusion. Metformin has been shown and is used to induce weight loss [29,30].

Not unexpectedly, there were pieces of evidence that the plasma insulin level was incriminated in T2D obesity in the present study, as shown in Fig. 2a-i. The higher plasma insulin levels were seen with increasing BMI grade of obesity, although the difference in insulin levels between the obese and nonobese T2D

Fig. 1



The BMI (kg/m^2): (a) Comparisons between men and women with T2D in Bahrain (a-i), Saudi Arabia (a-ii), Kuwait (a-iii), and all men vs. all women in the three sites (a-iv). (b) Comparisons between Saudi patients treated by metformin only, metformin plus insulin (Metf-Ins), and metformin plus gliclazide (Meft-Glicz), $P = 0.06$. T2D, type 2 diabetes.

patients was NS, $P = 0.063$ (Fig. 2a-ii). Moreover, there was no correlation between BMI and plasma insulin level when all patients were grouped (Table 3). The association of high insulin levels with obesity and T2D is well known as reviewed before [31]; however, the differences in insulin levels between the T2D patients based on differences in BMI were not reported previously to the best of our knowledge. On the contrary, the insulin resistance as estimated by the HOMA-IR index showed only a trend of association with obesity grades (Fig. 2b-i) but it was not significantly different between the obese and nonobese T2D (Fig. 2b-ii) or correlated with the BMI (Table 3). Worth noting, the association between obesity and insulin resistance was well documented [32], and the cause of T2D is known to be due to insulin resistance [33], and the latter is attributed to obesity. However, the role of obesity in the development of insulin resistance cannot explain the cause of insulin resistance in nonobese T2D patients. Thus, the recently proposed refined diabetes classification, which is based on analysis of six measured variables including metabolic, genetic, and clinical factors, in addition to

Table 4 The metabolic profiles in the different classes of obesity of type 2 diabetes patients in Bahrain, Saudi Arabia, and Kuwait

	N	OW	OBS	SOBS	*P* value
FBG (mmol/L)					
BH (140)	9.3 (7.1–11.5) (33)	10.7 (7.8–3.6) (45)	9.1 (7.4–1.3) (35)	8.9 (7.0–2.8) (27)	0.543
KSA (104)	7.4 (6.1–17.9) (6)	8.5 (6.9–11.4) (24)	7.5 (6.2–10.5) (43)	7.8 (6.5–10.1) (31)	0.514
KU (103)	7.2 (6.7–7.9) (12)	7.6 (6.2–8.9) (38)	7.8 (6.5–11.3) (27)	8.7 (7.0–10.0) (26)	0.196
Total	8.3 (6.8–10.9) (51)	8.5 (7.0–11.4) (107)	8.1 (6.6–10.9) (105)	8.5 (6.9–10.4) (84)	0.699
n-O vs. O	8.5 (7.0–11.1) (158)		8.4 (6.7–10.7) (189)		0.507[a]
Glycated hemoglobin (HbA1c%)					
BH (129)	7.2 (6.5–8.6) (28)	8.5 (7.2–9.7) (44)	8.6 (6.6–10.6) (33)	8.0 (6.8–10.5) (24)	0.634
KSA (104)	7.2 (5.7–10.5) (6)	7.7 (6.8–8.8) (24)	8.0 (6.9–8.7) (43)	8.1 (6.9–9.4) (31)	0.861
KU (103)	7.0 (6.7–7.7) (11)	7.5 (6.8–8.2) (36)	8.0 (7.0–9.6) (25)	8.0 (7.0–8.7) (28)	0.179
Total	7.1 (6.5–8.3) (45)	7.8 (7.0–9.2) (104)	8.0 (6.9–9.6) (101)	8.1 (6.9–9.5) (83)	0.214
n-O vs. O	7.6 (6.7–9.1) (149)		8.0 (6.9–9.5) (184)		0.188[a]
HDL-C (mmol/L)					
BH (102)	1.1 (1.0–1.4) (21)	1.2 (1.0–1.7) (36)	1.1 (1.0–1.4) (26)	1.2 (1.0–1.5) (19)	0.636
KSA (104)	0.9 (0.8–1.2) (6)	0.9 (0.8–1.1) (24)	1.0 (0.9–1.2) (43)	1.0 (0.9–1. 2) (31)	0.268
KU (101)	1.4 ± 0.4 (12)	1.2 ± 0.3 (38)	1.2 ± 0.4 (26)	1.2 ± 0.3 (25)	0.351[b]
Total	1.2 (1.0–1.4) (39)	1.1 (0.9–1.3) (98)	1.1 (0.9–1.3) (95)	1.1 (0.9–1.4) (75)	0.59
n-O vs. O	1.1 (0.9–1.4) (137)		1.1 (0.9–1.3) (170)		0.559[a]
LDLC (mmol/L)					
BH (110)	3.7 (2.8–4.1) (22)	3.4 (2.5–4.2) (37)	**2.9 (2.4–3.5)** (30)	3.7 (3.5–4.3) (21)	**0.024**
KSA (104)	2.7 (2.1–3.4) (6)	3.0 (2.5–3.8) (24)	2.6 (2.1–3.3) (43)	2.6 (2.2–3.2) (31)	0.325[b]
KU (95)	2.9 ± 0.9 (11)	2.2 ± 0.9 (35)	2.5 ± 1.1 (25)	2.7 ± 1.1 (24)	0.154
Total	3.4 (2.4–3.9) (39)	2.8 (2.1–3.7) (96)	2.8 (2.1–3.4) (98)	3.0 (2.2–3.7) (76)	0.082
n-O vs. O	3.0 (2.1–3.8) (135)		2.8 (2.2–3.6) (174)		0.370[a]
Total cholesterol (mmol/L)					
BH (137)	5.0 ± 1.3 (29)	5.1 ± 1.5 (45)	4.8 ± 0.9 (30)	5.3 ± 1.1 (23)	0.504[b]
KSA (104)	5.0 ± 1.2 (6)	5.0 ± 0.9 (24)	4.4 ± 1.0 (43)	4.6 ± 1.1 (31)	0.176[b]
KU (101)	4.7 ± 1.3 (12)	4.2 ± 1.1 (38)	4.4 ± 1.3 (26)	4.7 ± 1.2 (25)	0.294[b]
Total	4.9 ± 1.263 (47)	4.7 ± 1.298 (107)	4.5 ± 1.0 (99)	4.9 ± 1.153 (79)	0.194[b]
n-O vs. O	4.8 ± 1.3 (154)		4.7 ± 1.1 (178)		0.480[c]
TG (mmol/L)					
BH (135)	1.4 (1.0–2.0) (30)	1.9 (1.2–2.4) (46)	2.0 (1.3–2.6) (34)	1.7 (1.2–2.3) (25)	0.185
KSA (104)	**2.1 (1.1–2.8)** (6)	1.8 (1.4–2.3) (24)	**1.3 (1.0–1.7)** (43)	1.7 (1.0–2.1) (31)	**0.036**
KU (101)	1.2 (0.9–1.4) (12)	1.5 (0.9–2.3) (38)	1.5 (1.1–2.2) (26)	1.7 (1.1–2.3) (25)	0.281
Total	1.3 (1.0–2.0) (48)	1.7 (1.2–2.3) (108)	1.5 (1.1–2.1) (103)	1.7 (1.1–2.3) (81)	0.218
n-O vs. O	1.6 (1.0–2.3) (156)		1.6 (1.1–2.2) (184)		0.936[a]

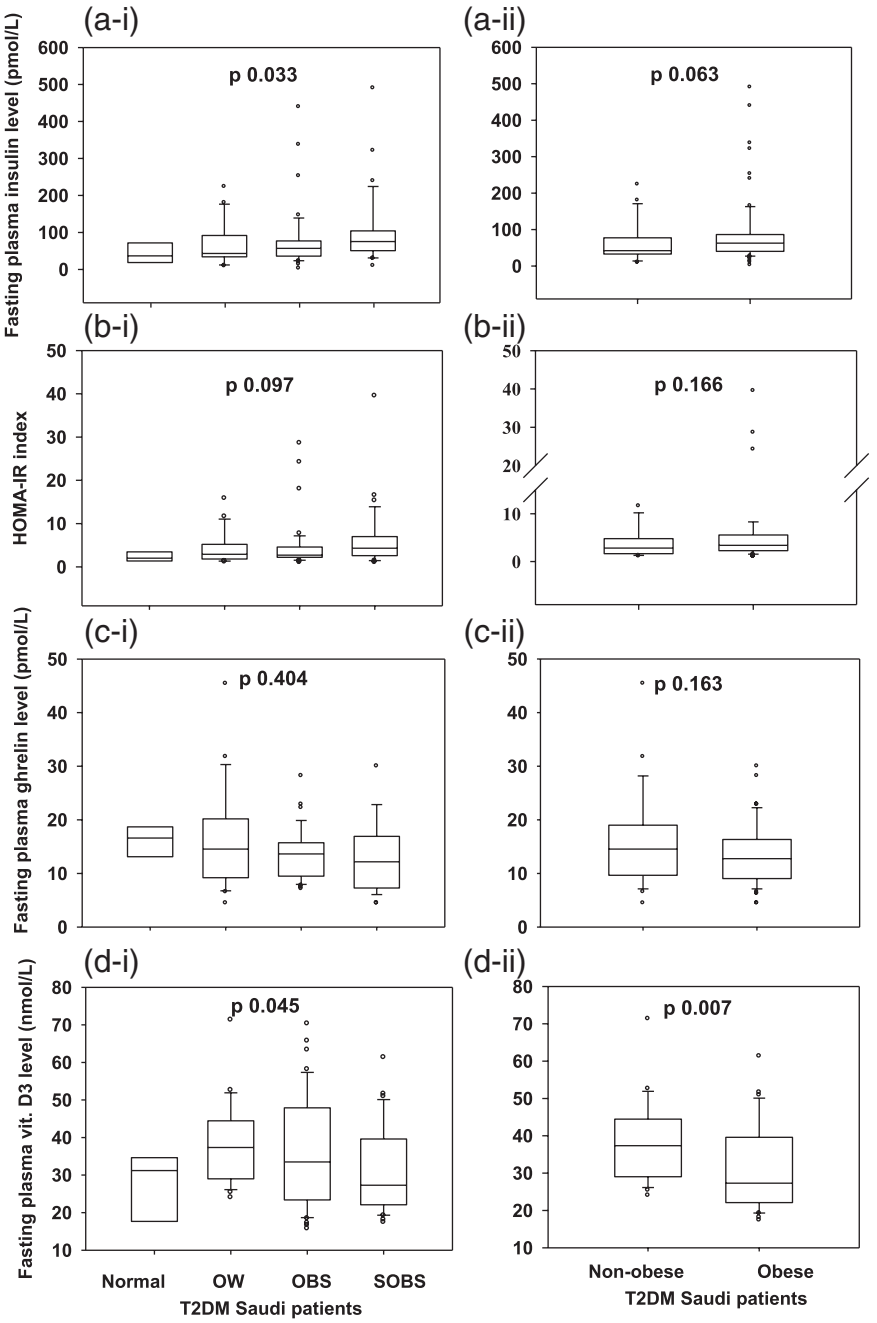
Bold indicates significance or borderline *P* value, and the compared values.
BH, Bahrain; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; KSA, Kingdom of Saudi Arabia; KU, Kuwait; N, normal; OBS, obese; OW, overweight; SOBS, severely obese; TG, triglycerides.
Note: the statistical test used for analysis was Kruskal–Wallis one-way analysis of variance on ranks, except for "a", "b", and "c" where Mann–Whitney rank-sum test , ANOVA, and *t*-test, respectively, were used.

obesity and insulin resistance, is worth consideration [34].

Another hormonal parameter, the ghrelin, was investigated in the present study, where plasma ghrelin levels were found to be comparable between obese and nonobese T2D and between the four BMI grades of obesity (Fig. 2c) with no correlation with BMI (Table 3). Low ghrelin was previously found to be significantly associated with T2D and insulin resistance in KSA [7] and elsewhere [16,17] as well as with obesity [14], satiety, and eating habits [35]. But whether or not ghrelin levels vary between T2D patients, based on their obesity, is not previously reported.

Finally, of the hormonal systems that are involved in T2D, obesity, and metabolism, is vitamin D. In this study, we demonstrated the association of obesity in T2D with decreased vitamin D, and the most marked reduction was noticed in patients with severe obesity (Fig. 2d-i). Interestingly, the overweight patients had the highest levels of vitamin D (Fig. 2d-ii); a similar finding was previously reported but from nondiabetic subjects in Bahrain where the overweight subjects were found to have the highest levels of vitamin D [8]. In this study, vitamin D level was not correlated with the BMI when all patients were taken together (Table 3), probably because vitamin D insufficiency and deficiency are too high in the region [8]. The associations of low vitamin D with obesity [18], T2D [36], insulin resistance [19], and MetS [37] were formerly documented. However, these disorders coexist with each other; thus, it is worth knowing with which one hypovitaminosis D is principally associated. Based on the present data, the low vitamin D that is associated with T2D and MetS is more likely to be due to the obesity component in both disorders. The previous claim that obesity is behind insulin resistance and thus T2D and MetS [37] supports the present finding that low vitamin D was more associated with severe obesity among T2D patients. However, the controversy was about the comparable vitamin D levels in the normal BMI and the OBS-BMI T2D patients (Fig. 2d-i). A recent large European population meta-analysis study denied the association of vitamin D and its metabolites with T2D [38], which further supports our interpretation for the present data.

Fig. 2



Comparisons of different hormonal parameters: insulin (a-i and a-ii), HOMA-IR index (b-i and b-ii), ghrelin (c-i and c-ii), and vitamin D (d-i and d-ii) between Saudi patients with T2D grouped based on (i) four BMI grades – normal, overweight (OW), obese (OBS), and severely obese (SOBS), (ii) two BMI grades – nonobese people with diabetes (BMI 18.5 to <30 kg/m^2) and obese people with diabetes (BMI ≥30 kg/m^2). *P* values are shown within the figures boxes. Note that (d-ii) comparison was limited between OW vs. SOBS only, $P = 0.007$. HOMA-IR, homeostasis model assessment of insulin resistance; T2D, type 2 diabetes.

Some of the limitations of this study were that the hormones were not tested in all three sites and the relatively small number of patients, more specifically the patients with normal BMI. Finally, larger multicenter studies involving different ethnic groups and testing more biochemical and molecular parameters are needed to reveal the cause(s) and consequences of diabetic obesity for a better understanding, prevention and management.

In conclusion, this study revealed that the obese compared to nonobese T2D in the GCC region were younger in age with shorter disease duration and were more frequently women. The biochemical profile including the

glycemic and lipids parameters, except LDL-C, and TGs were consistently comparable between the obese and nonobese T2D patients. However, the hormonal profile showed that high insulin and low vitamin D were associated with diabetic obesity, unlike ghrelin which was comparable between the patients.

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The data are available upon genuine request.

The studies were ethically approved by the Research and Ethics Committee of College of Medicine and Medical Sciences, Arabian Gulf University; approval letter (E28-PI-01/20).

Informed consent was obtained from each patient before inclusion in the study. Consent for publication was also obtained.

Conflicts of interest

There are no conflicts of interest.

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