Metabolic dysfunction-associated steatotic liver disease and metabolic abnormalities are associated with age and serum des-acyl ghrelin among apparently healthy females: Findings from a whole gastroenteropancreatic hormones survey with path analysis

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Abstract. Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent liver condition associated with metabolic disturbances, with high incidence in Asian regions. Numerous patients with MASLD, particularly females, are neither obese nor overweight. Although gastroenteropancreatic hormones (GEPHs) are key in metabolic regulation, current understanding is largely limited to a subset of these hormones. The present study aims to identify key factors influencing MASLD and associated metabolic abnormalities among female patients. GEPHs were assessed in 139 adult female volunteers. Using separate forward-selected regression to screen 12 candidate factors, the present study identified age and serum des-acyl ghrelin as the primary determinants of MASLD development. Pathway analysis validated their mechanistic roles. Predictive classification based on Mahalanobis distance using these two factors effectively stratified at-risk individuals. Body mass index and waist circumference were notable mediators. This cross-sectional study identified significant correlations between age, serum des-acyl ghrelin and metabolic complications, including MASLD, in adult females. Monitoring serum des-acyl ghrelin levels may offer predictive insights into metabolic risks in preventive medicine. However, to determine the accuracy and reliability of these predictions, future longitudinal studies are needed to determine the association between serum des-acyl ghrelin levels and the progression of metabolic disease.

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease, has become a global health concern (1), particularly in Asia, 41% of cases occur in people with BMI <25 kg/m^2, and 19% in those with BMI <23 kg/m^2, with no significant difference in liver histology compared with higher-BMI groups (2). In Taiwan, the prevalence of metabolic syndrome (MS), a condition associated with MASLD, increased from 11.5% during 2003-2004 (3,4) to more than one-third of adults in 2017-2020, increasing with age (5). Numerous studies have explored established factors, including being overweight, hyperglycemia, insulin resistance, energy imbalance, metabolic inflammation, an unhealthy diet and a sedentary lifestyle, that contribute

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to these conditions (1,3). However, reliable biomarkers for MASLD, particularly for early detection or risk prediction in asymptomatic individuals, remain elusive, as emphasized in the latest Asian Pacific Association for the Study of the Liver guidelines (2). This critical gap underscores the need to explore novel pathogenic mechanisms. The role of gastroenteropancreatic hormones (GEPHs) in the early pathogenesis of MASLD, especially among seemingly healthy females, a key target for prevention, is poorly understood. The present study aimed to investigate GEPH profiles in this population.

GEPHs serve a crucial role in the regulation of appetite, body weight and metabolic homeostasis, acting as key mediators between the central nervous system and peripheral tissue (6,7). Ghrelin exists in two forms: Acyl ghrelin stimulates appetite, while des-acyl ghrelin, previously thought to be inactive, suppresses appetite (8,9). Des-acyl ghrelin possesses broad metabolic protective properties beyond appetite suppression, including anti-inflammatory, anti-fibrotic and insulin-sensitizing effects in multiple tissues (10,11). In addition to ghrelin, other GEPHs, regulate satiety, energy balance and metabolic dysfunction (12-14), including obestatin, nesfatin-1, cholecystokinin (CCK), gastric inhibitory peptide (GIP), glucagon-like peptide 1 (GLP-1), oxyntomodulin (OXM), peptide YY (PYY), pancreatic polypeptide (PP) and amylin. Understanding the interactions between these hormones and their metabolic effects is key for designing preventive and targeted therapies for MASLD and metabolic disorder. However, comprehensive studies simultaneously evaluating multiple GEPHs to identify the most salient predictor of MASLD risk within a specific ethnic population are currently lacking.

The present study employed correlation, regression and path analysis to explore the connections and potential causal association between a broad panel of 11 GEPHs and MASLD in seemingly healthy females. The primary objective was to identify the most influential biomarkers predicting MASLD.

Materials and methods

Study design and data collection. The present study adopted a hospital-based prospective observational design, in accordance with the Helsinki Declaration, approved by the Institutional Review Board, Cheng Hsin General Hospital, Taipei, Taiwan [approval nos. (665) 107A-37, (732) 108A-48 and (736) 108A-52] and was performed according to the Association for the Accreditation of Human Research Protection Program guidelines (aahrpp.org/). Subjects were randomly selected from individuals undergoing voluntary health check-ups at the Cheng Hsin Healthy Management Center , Taipei, Taiwan between June 2019 and July 2020. Initial recruitment included 150 participants, comprising 139 females and 11 males. The objective was to identify the most influential biomarkers predicting MASLD in the apparently healthy population. However, the small number of male participants precluded meaningful sex-stratified analysis or reliable adjustment for sex as a potential confounder. Therefore, the present study investigated only the female patients aged 18-65 years old. The inclusion criteria were age ≥18 years and complete anthropometric measurement and laboratory data; exclusion criterion was a history of alcohol consumption.

'Apparently healthy' was operationally defined as: i) No prior diagnosis of chronic diseases requiring medication; ii) no hospitalization in the past 6 months and iii) voluntarily participating in health check-ups. A standardized questionnaire collected basic information, such as sex, age and medical history. Height, weight, waist circumference (WC) and blood pressure were measured. The following data were obtained from the database of the health examination center: Fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT) and laboratory tests for acyl ghrelin, des-acyl ghrelin, obestatin, nesfatin-1, CCK, GIP, GLP-1, OXM, PP, PYY and amylin. Body mass index (BMI), an indicator used to identify obese individuals, was calculated as body weight (kg)/[body height (m)]^2 (15). The TG/HDL-C ratio is indicative of insulin resistance (16,17). The diagnosis of MS criteria was made when at least three of the five following risk determinants were present: WC ≥80 cm; blood pressure >130/85 mmHg or use of antihypertensive medications; HDL-C <50 mg/dl; FBS ≥100 mg/dl or regular treatment for diabetes mellitus and TG ≥150 mg/dl (5). Lipid accumulation product (LAP) was employed as a blood-based surrogate marker for MASLD, serving as a non-invasive alternative to imaging or liver biopsy. LAP was calculated as: [(WC (cm)-58) x TG (mmol/l)] (18,19). WC values ≤58 cm were adjusted to 59 cm to ensure a non-positive LAP value. Abnormal metabolic indicators were defined as FBS ≥126 mg/dl, TG ≥150 mg/dl and HDL-C <50 mg/dl.

Measurement of serum GEPHs. Enzyme immunoassays for hormones were performed according to the manufacturers' protocols in a single, blinded batch using commercial kits. These included acyl ghrelin, des-acyl ghrelin (A05106, A05119; all Bertin Pharma), obestatin, CCK, GIP, GLP-1, OXM, PYY, amylin (S-1284, S-1205, S-1273, S-1359, S-1272, S-1187; all Bachem), nesfatin-1and OXM (EK-003-26, EK-028-22; bothPhoenix Pharmaceuticals, Inc) (19).

Statistical analysis. The analysis was conducted using Social Sciences Statistics Package version 20.0 (IBM Corp.). Data are presented as mean ± SD for continuous variables. Bivariate correlation analysis was performed using Spearman's correlation to assess the association between age, GEPHs (including acyl ghrelin, des-acyl ghrelin, obestatin, nesfatin-1, CCK, GIP, GLP-1, OXM, PYY, PP and amylin) and metabolic parameters (BMI, TG/HDL-C ratio, LAP and ALT). SAS 9.4 (SAS Institute, Inc.) software was applied for regression and path analysis. For multivariable analysis, separate forward-selected regression models were constructed for each metabolic outcome, including BMI, WC, FBS, TG, TC, LDL-C and HDL-C. A total of 12 candidate predictors, comprising age and 11 GEPHs, were evaluated. Variables were retained in the final model only if they met the criteria of $P<0.05$, $\Delta R^2 >3\%$ and variance inflation factor (VIF) <5 to ensure both statistical and explanatory robustness while minimizing multicollinearity. Notably, predictors showing significant bivariate correlations were excluded from the final multivariate models if they no longer contributed independently after adjusting for other covariates or if they exhibited

Table I. Characteristics of the study subjects.

Variable	All	Non-MS (n=126)	MS (n=13)	P-value
Age, years	38.4±10.1	37.9±10.1	43.6±9.3	0.050
BMI, kg/m^2	23.1±3.8	22.4±3.3	29.0±2.6	<0.001
WC, cm	73.2±9.4	71.4±7.9	89.8±5.0	<0.001
FBS, mg/dl	95.0±11.3	93.3±8.8	111.4±18.6	<0.001
TG, mg/dl	77.0±50.7	71.4±46.7	130.7±57.8	<0.001
TC, mg/dl	193.7±34.9	192.5±33.1	205.9±49.1	0.190
LDL-C, mg/dl	117.2±32.1	114.7±30.1	141.1±42.0	0.040
HDL-C, mg/dl	60.4±13.3	62.1±12.8	44.5±5.6	<0.001
ALT, U/l	19.2±14.7	17.1±7.9	40.2±36.4	<0.001
TG/HDL-C ratio	1.4±1.3	1.3±1.1	3.0±1.4	<0.001
LAP, cm mmol/l	14.9±17.0	11.6±11.8	47.6±24.3	<0.001
Acyl ghrelin, pg/ml	50.3±30.0	51.0±29.6	43.5±34.5	0.392
Des-acyl ghrelin, pg/ml	341.2±199.5	353.8±199.9	219.8±154.7	0.021
Obestatin, ng/ml	2.6±1.3	2.6±1.3	2.4±0.8	0.645
Nesfatin-1, ng/ml	1.0±6.1	1.0±6.4	0.2±0.2	0.630
CCK, ng/ml	2.4±5.5	2.4±5.8	2.1±1.9	0.824
GIP, ng/ml	0.3±0.2	0.3±0.2	0.3±0.2	0.843
GLP-1, ng/ml	0.05±0.05	0.05±0.04	0.08±0.11	0.022
OXM, ng/ml	0.9±0.3	0.9±0.3	0.9±0.2	0.655
PYY, ng/ml	0.6±0.3	0.6±0.3	0.6±0.2	0.782
PP, ng/ml	0.2±0.8	0.2±0.8	0.2±0.4	0.931
Amylin, ng/ml	0.8±0.7	0.8±0.8	0.7±0.6	0.828

BMI, body mass index; WC, waist circumference; FBS, fasting blood sugar; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; LAP, lipid accumulation product; CCK, cholecystokinin; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin; PYY, peptide YY; PP, pancreatic polypeptide.

excessive shared variance (VIF ≥5). Forward regression was employed to identify significant predictor variables for inclusion in the subsequent path analysis. Model fit was evaluated to determine how well the proposed structure aligned with the observed data (20,21).

GEPHs retained in the final model (des-acyl ghrelin, and obestatin) and age underwent exploratory path analysis, Mahalanobis distance-based classification was applied. P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of the study subjects. The study cohort consisted of 139 female participants with a mean age of 38.4±10.1 years, BMI of 23.1±3.8 kg/m^2 and WC of 73.2±9.4 cm (Table I). Notably, 9.4% of these participants met the Taiwanese MS criteria, despite being recruited from the healthy management center without prior diagnosis of major chronic disease. This finding highlights the potential underdiagnosis of metabolic risk in apparently healthy females and underscores the value of early metabolic screening in normal-weight individuals (mean BMI <24 kg/m^2).

Patients with MS were older and had significantly higher BMI, WC, FBS, TG, LDL-C, ALT, TG/HDL-C ratio, LAP and GLP-1, as well as lower HDL-C and des-acyl ghrelin levels, than non-MS subjects (Table I).

Association between GEPHs and metabolic factors. Bivariate correlation analysis revealed significant associations between age, GEPH and metabolic outcomes (Table II; Fig. 1). Age, serum acyl ghrelin and des-acyl ghrelin correlated positively with BMI and TG/HDL-C ratio, while age, serum acyl ghrelin, des-acyl ghrelin and obestatin were correlated with LAP. Age also had a significant relationship with ALT. No correlation existed between age and des-acyl ghrelin (Fig. 1G), while acyl-ghrelin declined significantly with age (Fig. 1H). Despite these bivariate associations, forward-selected multivariate regression models using age and all GEPHs as candidate variables identified independent predictors for metabolic abnormalities (Table III). Age positively predicted BMI (β=0.060), WC (β=0.223), FBS, TG, TC and LDL-C. Des-acyl ghrelin was independently associated with BMI (β=-0.004) and WC (β=-0.014) but positively associated with HDL-C (β=0.023). Obestatin predicted FBS (β=2.028). Notably, while significant in the bivariate correlations (Table II), acyl-ghrelin was not retained in the final forward-selected model (Table III) as it did not meet the statistical criterion for entry after accounting for variance explained by the other variables. Overall, age was the strongest predictor, associated with all

Table II. Correlations between variables and metabolic factors.

	BMI		TG/HDL-C ratio		LAP		ALT	
Factor	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
Age	0.238	0.005	0.257	0.002	0.353	<0.001	0.375	<0.001
Acyl ghrelin	-0.194	0.022	-0.245	0.004	-0.282	0.001	-0.049	0.567
Des-acyl ghrelin	-0.172	0.043	-0.245	0.004	-0.287	0.001	-0.046	0.595
Obestatin	-0.128	0.134	-0.162	0.057	-0.235	0.005	-0.035	0.685
Nesfatin-1	0.066	0.438	0.064	0.454	0.112	0.189	0.022	0.801
CCK	-0.052	0.547	-0.119	0.162	-0.129	0.131	-0.008	0.927
GIP	-0.058	0.497	-0.029	0.733	-0.082	0.339	-0.041	0.633
GLP-1	-0.126	0.138	-0.152	0.074	-0.155	0.068	-0.143	0.092
OXM	-0.025	0.769	0.059	0.487	-0.011	0.895	0.105	0.217
PYY	-0.063	0.462	0.023	0.791	-0.010	0.905	0.079	0.354
PP	-0.002	0.977	0.071	0.404	0.055	0.519	-0.103	0.229
Amylin	-0.118	0.165	0.039	0.648	-0.049	0.567	-0.093	0.278

BMI, body mass index; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; LAP, lipid accumulation product; CCK, cholecystokinin; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin; PYY, peptide YY; PP, pancreatic polypeptide.

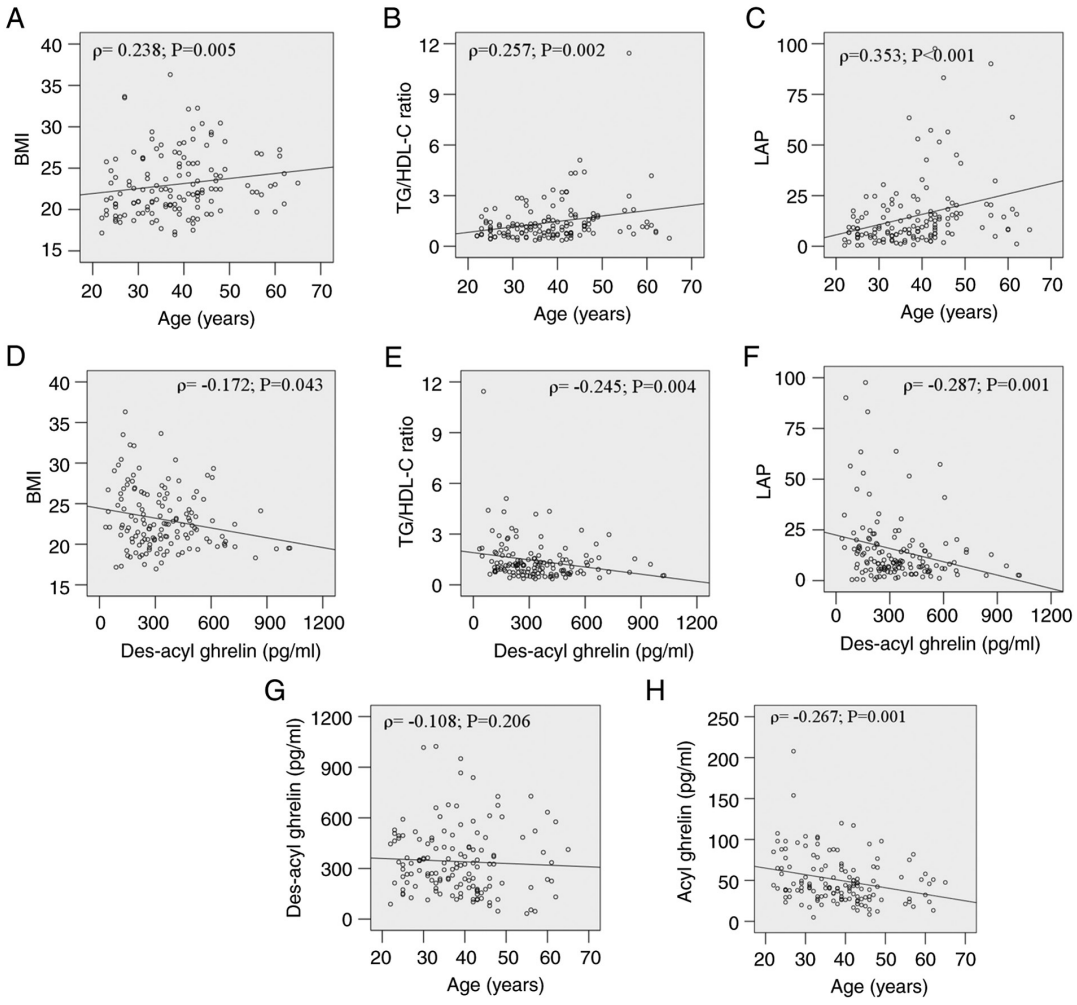

Figure 1. Correlations between age, ghrelin and metabolic markers. Age was positively correlated with (A) BMI, (B) TG/HDL-C ratio and (C) LAP. Des-acyl ghrelin levels were inversely correlated with (D) BMI, (E) TG/HDL-C ratio and (F) LAP. (G) Acyl ghrelin levels increased with age. (H) No significant association was found between age and des-acyl ghrelin levels. BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LAP, lipid accumulation product.

Table III. Variables significantly associated with age and gastroenteropancreatic hormones using forward-selected regression model.

	Age		Des-acyl ghrelin		Obestatin	
Variable	Estimated β	P-value	Estimated β	P-value	Estimated β	P-value
BMI	0.060	0.050	-0.004	0.015	-	-
WC	0.223	0.003	-0.014	<0.001	-	-
FBS	0.519	<0.001	-	-	2.028	0.042
TG	1.440	<0.001	-	-	-	-
TC	0.858	0.024	-	-	-	-
LDL-C	0.601	0.013	-	-	-	-
HDL-C	-	-	0.023	<0.001	-	-

BMI, body mass index; WC, waist circumference; FBS, fasting blood sugar; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; -, excluded from the forward selection regression model.

sevenmetabolic parameters . Among GEPHs, des-acyl ghrelin showed the significant associations with the highest number of metabolic parameters (3/7), while other GEPHs showed significant associations with ≤1 parameter (Table III).

Potential mechanism of metabolic abnormalities. Path analysis was performed to determine whether age, des-acyl ghrelin and obestatin influence metabolic abnormalities and contribute to the onset of MASLD through mediators such as BMI and WC. The discriminating criteria for metabolic abnormalities in female patients are BMI ≥24 kg/m^2, WC ≥80 cm, FBS ≥126 mg/dl, TG ≥150 mg/dl, TC ≥200 mg/dl, LDL-C ≥100 mg/dl and HDL-C <50 mg/dl (4,5). Serum des-acyl ghrelin levels were not significantly affected by age (β=-0.003, P=0.560; Table IV). The analyses for general metabolic abnormalities (Table IV) highlight the pervasive influence of age, which was a significant predictor in 4 out of 7 final models (for WC, FBS, TG, and TC). In comparison, des-acyl ghrelin was a significant predictor in 3 models (for BMI, WC, and HDL-C). These findings were derived from a systematically comparison of parallel models using either BMI or WC as the primary adiposity measure. This approach revealed that adiposity measures often served as both significant predictors and potential mediators.

HDL-C (Table IV), both BMI (β=-1.204) and WC (β=-0.480) were significant predictors alongside des-acyl ghrelin. Furthermore, in the case of TG (Table IV-E), the effect of age was partially mediated by WC, as evidenced by the attenuation of the direct effect co-efficient from β=1.424 to β=1.084 after including WC.

A consistent finding across all these analyses was that both the BMI-based and WC-based models demonstrated a poor overall goodness-of-fit, indicating that the simple linear structures proposed do not fully capture the complexity of the metabolic interrelationships.

Potential mechanism of MASLD. To investigate the potential mechanism of MASLD, path analyses were conducted for its key indicators, LAP and ALT, systematically comparing parallel models that incorporated either BMI or WC as the primary adiposity measure (Table V). The analyses for LAP revealed clear patterns of full mediation (Table V). The significant total effect of age on LAP (β=0.517) became non-significant in the final model that included WC as a mediator. Similarly, the significant total effect of des-acyl ghrelin on LAP (β=-0.024) became non-significant in the final model that included BMI as its mediator. For ALT (Table V-B), LAP itself emerged as the most significant direct predictor (β=0.338) and also functioned as a key mediator. The initial significant association between age and ALT (β=0.311) became non-significant after accounting for LAP. This finding was consistent in both the BMI-based and WC-based models, where LAP maintained a strong, direct path to ALT (final model β=0.338,), suggesting the effect of age on liver enzymes is transmitted through liver fat accumulation.In summary, these analyses elucidate the key role of age and serum des-acyl ghrelin in the progression of MASLD. Their influence, however, appears to be largely indirect. Adiposity measures like BMI and WC act as crucial mediators, connecting these initial factors to downstream LAP, which in turn impacts ALT. This highlights a potential cause for MASLD progression (Fig. 2).

Prediction of MASLD using age and serum des-acyl ghrelin. Mahalanobis distance analysis was performed to determine whether age and serum des-acyl ghrelin were key predictors of MASLD. LAP, a powerful tool for diagnosing metabolic disorders, was used with a threshold of 31.6 for MASLD (18). Sensitivity and specificity were 71.43 and 71.20%, respectively, for individuals with LAP >31.6.

Discussion

MASLD and metabolic abnormalities are increasingly recognized as notable health concerns, particularly in aging and hormonal regulation (10,12-15,17,22-24). To assess MASLD, the present study used the LAP, a simple and non-invasive measure that is associated with liver steatosis and fibrosis (18). Although liver biopsy is more accurate, its invasive nature limits its suitability for initial screening and monitoring. Therefore, LAP provides a practical and patient-friendly alternative

Table IV. Identification of metabolic abnormality pathways using path analysis.

A, Age

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Des-acyl ghrelin	-0.003	0.560	0.560
2	Obestatin	-1.685	0.011	0.011
3	Des-acyl ghrelin	-0.001	0.737	0.038
	Obestatin	-1.663	0.013	

B, BMI

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Des-acyl ghrelin	-0.004	0.012	0.012
2	Obestatin	-0.233	0.352	0.352
3	Age	0.061	0.056	0.056
4	Age	0.055	0.088	0.022
	Des-acyl ghrelin	-0.004	0.017	
	Obestatin	-0.081	0.748	

C, WC

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Des-acyl ghrelin	-0.014	<0.001	<0.001
2	Obestatin	-1.159	0.061	0.061
3	Age	0.241	0.002	0.002
4	Age	0.212	0.006	<0.001
	Des-acyl ghrelin	-0.013	<0.001	
	Obestatin	-0.592	0.314	

D, FBS

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Age	0.458	<0.001	<0.001
2	BMI	1.203	<0.001	<0.001
3	WC	0.481	<0.001	<0.001
4	Obestatin	0.950	0.025	0.025
5	Age	0.444	<0.001	<0.001
	BMI	1.064	<0.001	
	Obestatin	1.956	0.003	
6	Age	0.414	<0.001	<0.001
	WC	0.412	<0.001	
	Obestatin	2.134	0.001	

E, TG

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Age	1.424	0.007	0.007
2	BMI	3.710	0.001	0.001
3	WC	1.761	<0.001	<0.001
4	Des-acyl ghrelin	-0.042	0.056	0.056
5	Age	1.223	0.003	<0.001

Table IV. Continued.

E, TG

Model	Variable	Standardized β	P-value	Model fitting P-value
	BMI	2.882	0.010	
	Des-acyl ghrelin	-0.027	0.201	
6	Age	1.084	0.009	<0.001
	WC	1.33	0.005	
	Des-acyl ghrelin	-0.021	0.334	

F, TC

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Age	0.840	0.004	0.004
2	BMI	1.547	0.056	0.056
3	WC	0.454	0.155	0.155
4	Age	0.767	0.009	0.005
	BMI	1.215	0.118	
5	Age	0.784	0.009	0.012
	WC	0.233	0.469	

G, LDL-C

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Age	0.840	0.004	0.004
2	BMI	1.547	0.056	0.056
3	WC	0.454	0.155	0.155
4	Age	0.516	0.057	<0.001
	BMI	2.518	<0.001	
5	Age	0.480	0.076	0.0013
	WC	0.783	0.008	

H, HDL-C

Model	Variable	Standardized β	P-value	Model fitting P-value
1	BMI	-1.376	<0.001	<0.001
2	WC	-0.624	<0.001	<0.001
3	Des-acyl ghrelin	0.025	<0.001	<0.001
4	BMI	-1.204	0.004	<0.001
	Des-acyl ghrelin	0.015	<0.001	
5	WC	-0.480	0.018	<0.001
	Des-acyl ghrelin	0.023	<0.001	

BMI, body mass index; WC, waist circumference; FBS, fasting blood sugar; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

for evaluating the risk and severity of MASLD. Based on the screening results, subsequent analysis should prioritize hormones demonstrating the strongest statistical associations. The present GEPH survey of apparently healthy female patients demonstrated des-acyl ghrelin as the strongest and most consistent predictor of the risk of MASLD, along with age. While extensive literature implicates multiple GEPHs (GLP-1, PYY and CCK) in the pathogenesis of metabolic disorders and MASLD, predominantly in populations with higher body weight, diabetes or differing ethnic backgrounds (12-14), none

Table V. Proposed physiological pathways linking LAP and ALT identified through path analysis modeling.

A, LAP

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Age	0.517	<0.001	<0.001
2	Des-acyl ghrelin	-0.024	0.003	0.003
3	TG	0.269	<0.001	<0.001
4	TC	0.152	<0.001	<0.001
5	LDL-C	0.176	<0.001	<0.001
6	HDL-C	-0.572	<0.001	<0.001
7	BMI	2.931	<0.001	<0.001
8	WC	1.390	<0.001	<0.001
9	Age	0.076	0.235	<0.001
	TG	0.218	<0.001	
	TC	-0.008	0.916	
	LDL-C	0.014	0.845	
	HDL-C	-0.031	0.745	
	BMI	1.980	<0.001	
	Des-acyl ghrelin	-0.003	0.296	
10	Age	-0.035	0.452	<0.001
	TG	0.214	<0.001	
	TC	-0.029	0.591	
	HDL-C	0.056	0.423	
	LDL-C	0.043	0.409	
	WC	1.038	<0.001	
	Des-acyl ghrelin	0.001	0.604	

B, ALT

Model	Variable	Standardized β	P-value	Model fitting P-value
1	Age	0.311	0.012	0.012
2	BMI	1.562	<0.001	<0.001
3	WC	0.574	<0.001	<0.001
4	Des-acyl ghrelin	-0.013	0.013	0.014
5	LAP	0.389	<0.001	<0.001
6	Age	0.1328	0.2543	<0.001
	BMI	0.7436	0.050	
	Des-acyl ghrelin	-0.0065	0.094	
	LAP	0.2570	0.004	
7	Age	0.125	0.308	<0.001
	WC	0.0641	0.734	
	Des-acyl ghrelin	-0.0016	0.059	
	LAP	0.3378	0.001	

LAP, lipid accumulation product; ALT, alanine aminotransferase; BMI, body mass index; WC, waist circumference; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

showed predictive strength comparable with des-acyl ghrelin in the present cohort. This divergence may arises from two key factors: First, unlike earlier studies (12-14) that typically assess one or two hormones in isolation, the present approach integrated forward regression and path analysis to evaluate multiple GEPHs, capturing their interactive effects. Second, the key role of des-acyl ghrelin in the present cohort may reflect population-specific hormonal drivers associated with early MASLD development, consistent with its reported metabolic protective functions (9,10). Together, age and des-acyl ghrelin constitute key upstream factors determinants of MASLD and metabolic disturbance, primarily mediated through increased

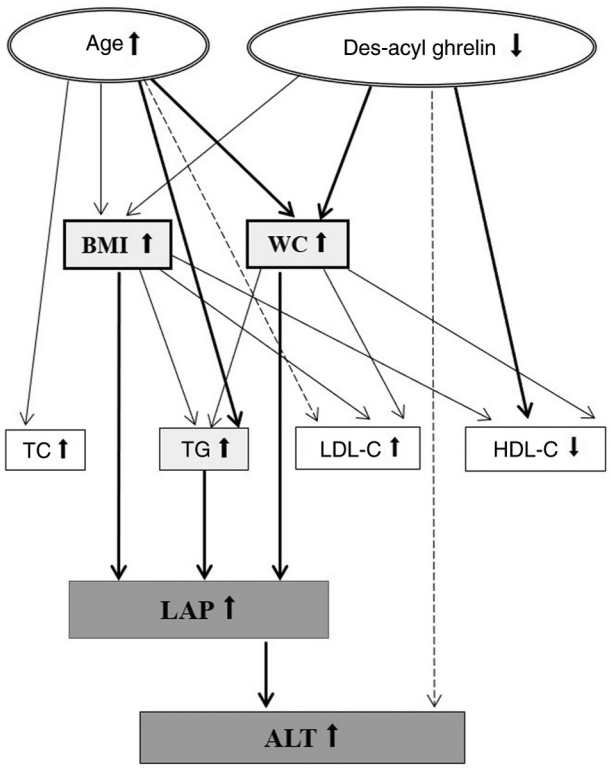

Figure 2. Pathways of MASLD development. Upstream factors such as age and des-acyl ghrelin on BMI and WC are associated with metabolic markers such as TG, LDL-C and HDL-C. BMI, WC and TG promote LAP (an indicator for MASLD), which impacts ALT levels and is an essential indicator of liver health. Thick lines represent the high correlation (R^2>0.6). The solid and dotted lines indicate significant (P<0.05) and borderline significant (0.05<P<0.06) results, respectively. BMI, body mass index; WC, waist circumference; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LAP, lipid accumulation product; ALT, alanine aminotransferase; MASLD, metabolic dysfunction-associated steatotic liver disease.

WC and BMI, and demonstrated utility in stratifying MASLD risk within this group. Therefore, monitoring des-acyl ghrelin levels in combination with age, even in individuals who are neither obese nor of normal weight, may offer a biologically grounded approach for screening early MASLD risk in East Asian female populations. This strategy may help identify people at higher risk for MASLD before symptoms appear, supporting the value of tracking body weight and WC for early prevention.

Age was a fundamental upstream driver of metabolic abnormalities and MASLD in the present cohort. Age showed a significant association with key metabolic indicators (BMI, TG/HDL-C ratio, LAP and ALT), suggesting as patients get older, they become more susceptible to metabolic dysfunction, such as MASLD. Consistently, Alexander *et al* (15) demonstrated that aging, independent of BMI, worsens metabolic dysregulation. Path analysis confirmed that age substantially increased MASLD risk via promoting central adiposity (elevated WC). This extends findings by Yuan *et al* (24) of aging as an independent MASLD risk factor in Chinese adults by delineating a central mechanism mediated by visceral adiposity. The present results are also consistent with Bilson *et al* (22), which demonstrated that age-related visceral fat dysfunction contributes to hepatic steatosis, even in patients who seem healthy.

Equally pivotal as a modifiable upstream factor, des-acyl ghrelin emerged with age as a core regulator within the network of metabolic dysregulation. Des-acyl ghrelin, once considered an inactive metabolite, serves distinct physiological roles compared with its acylated counterpart (25), particularly in anti-aging and anti-inflammatory processes (10,19,26-30). Unlike studies in populations with obesity (12,13), such as Ahmad *et al* (31), who investigated dysregulated fasting GLP1/ghrelin (using patrial correlation/logistic regression on five GEPHs, without distinguishing acyl/des-acyl isoforms), the present study targeted apparently healthy female patients and performed forward regression and path analysis on 11 GEPHs (including ghrelin isoforms) to delineate causal pathways. The present study revealed des-acyl ghrelin, not acyl ghrelin, as the primary upstream modulator of MASLD within a comprehensive GEPH framework, aligning with a cross-ethnic observation (32). In asymptomatic Korean men with MS, plasma des-acyl ghrelin and total ghrelin levels are significantly lower than in controls, and both are inversely correlated with insulin resistance (32). Transgenic models have confirmed the protective role of des-acyl ghrelin (27,28), attenuating age-associated wasting and enhancing muscle performance, while its deficiency accelerates aging and muscle wasting (28). Des-acyl ghrelin modulates inflammatory and oxidative stress markers, which is essential for slowing the aging process (26,28,33). Circulating ghrelin levels decrease with aging, which may impair endogenous ghrelin signaling (25,28,33). The present study similarly found that ghrelin was negatively correlated with age. In MASLD and MASH, insulin resistance leads to the accumulation of fatty acids and visceral fat in the liver, exacerbates hepatic insulin resistance and damages hepatocytes (21,29,35,36). Des-acyl ghrelin modulates blood glucose independent of growth hormone secretagogue receptor (12,25,29,30,36-38), and has been shown to enhance insulin sensitivity and prevent diabetes in high-fat diet-fed mice and rats (29,30). While the present study did not detect a direct association with FBS, the strong inverse association between des-acyl ghrelin and the TG/HDL-C suggested an indirect modulatory effect, potentially via mechanisms involving adipose tissue regulation (12,25,29,37), hormone balance or glycogen metabolism. Overexpression of des-acyl ghrelin in mice leads to decreased body size due to decreased food intake and delayed gastric emptying (39,40). Des-acyl ghrelin also stimulates basal autophagy via AMPK for lipid mobilization and counters TNF-α-induced cell damage, aiding in the improvement of MASLD (34,41). Beyond these cellular effects, recent evidence suggests des-acyl ghrelin may also act through a neuroendocrine pathway (42). Lv *et al* (42) identified a novel gut-brain-liver axis by which des-acyl ghrelin alleviates MASLD. Des-acyl ghrelin increases GLP-1 expression in the nucleus tractus solitarius and GLP-1 receptor in the paraventricular nucleus, leading to enhanced hepatic lipid metabolism (42). Disruption of this pathway via GLP-1R antagonism or hepatic vagotomy significantly attenuates therapeutic effects of des-acyl ghrelin, underscoring the relevance of this neural circuit (42). These findings reinforce the observation that des-acyl ghrelin is inversely associated with LAP and TG/HDL-C ratio, highlighting its systemic role in hepatic and

lipid regulation. Obestatin, derived from the ghrelin-obestatin precursor gene (*GHRL*), also exhibited a significant negative correlation with LAP and a strong positive association with fasting glucose. Obestatin shares functional overlap with des-acyl ghrelin in stimulating preadipocyte differentiation, promoting adipocyte fatty acid uptake, and inhibiting lipolysis (41). However, its role appears limited. Other GEPHs, such as OXM, PYY, PP and amylin, did not show any detectable effects on metabolic abnormalities or MASLD, suggesting the metabolic relevance is specific to ghrelin-derived peptides, particularly des-acyl ghrelin.

Incidence of MASLD is similar in postmenopausal females and males (19 and 22%, respectively), but lower in premenopausal females (9%) (44), suggesting sex hormones influence the prevalence of the disease. Estrogen enhances insulin sensitivity, while androgens increase insulin resistance (45). Sex-specific fat distribution patterns also affect the risk of metabolic disease, with males more prone to visceral fat accumulation, while postmenopausal patients exhibit increased central obesity and insulin resistance (46). The role of estrogen in regulating lipid metabolism, protecting the liver from oxidative damage and slowing the progression of liver fibrosis may provide partial protection for premenopausal patients against MS and MASLD (1). In females, ghrelin levels are naturally higher than in males and fluctuate with the menstrual cycle (47). Estrogen directly stimulates ghrelin-secreting cells in the stomach, increasing the expression and secretion of ghrelin (47). The interaction between ghrelin and sex hormones, such as estrogen, serves a crucial role in regulating appetite and energy balance. For example, the absence of ovaries increases ghrelin-induced effects, while estrogen therapy can counteract these (47,48). The interaction between sex hormones and GPEHs may be a key mechanism in the development of MASLD/MASH. The complex association between ghrelin and estrogen, especially in terms of food reward and stress eating, is an area of active research due to the importance of these hormonal interactions in metabolic diseases (47,48).

The present findings offer novel insight into the pathogenesis of MASLD and lay the groundwork for tailored prevention and therapeutic strategies. However, the study has limitations. First, its cross-sectional design precluded causal inference. While path analysis explored associations among GEPHs, metabolic parameters, and MASLD, it evaluates the statistical coherence of a prespecified model at a single time point and cannot establish a temporal sequence or rule out reverse causation. Second, the modest sample size (n=139), limited statistical power for subgroup analyses. Future longitudinal studies should include larger, multicenter, multiethnic cohorts encompassing both sexes and varying metabolic states (including obesity) along with analysis of sex hormones, to validate and expand the present findings. Third, using LAP as a surrogate for MASLD diagnosis, though non-invasive and scalable, may not accurately reflect the true severity of the disease compared with gold-standard methods (biopsy/FibroScan®). Fourth, the present findings are specific to apparently healthy females in Taiwan. Thus, caution is warranted in generalizing the results to other populations. Fifth, the data collection occurred in early 2020, before the global impact of the coronavirus disease-19 pandemic. While this timing may raise concerns about the applicability of the findings, it also provides a valuable pre-pandemic baseline for understanding metabolic health. As this study included only female participants, future research should enroll male subjects to enable analysis of sex differences. The core mechanistic pathway of age and des-acyl ghrelin influencing adiposity and MASLD reflects stable biological processes that are unlikely to be altered by temporal shifts. As such, the present data offer a meaningful reference point for studies comparing pre- and post-pandemic cohorts.

The present cross-sectional study identified significant correlations between age, serum des-acyl ghrelin and metabolic complications, including MASLD, in adult females in Taiwan. The findings suggest that age and des-acyl ghrelin could serve as a predictor of metabolic risks. Despite the limited number of participants, the data underscore the importance of monitoring serum des-acyl ghrelin levels in preventive medicine. Further longitudinal studies are essential to validate these associations, determine the precision and reliability of these predictions and clarify the specific role of des-acyl ghrelin in MASLD to develop more accurate predictive models and therapeutic approaches to manage metabolic diseases.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CYC conceived the study. CY, PYC and CYC performed the experiments. HHC and WCC confirm the authenticity of all the raw data. CPL, HHC and WCC analyzed and interpreted data. CPL, WCC and CYC wrote, reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Institutional Review Board of Cheng Hsin General Hospital [Taipei, Taiwan; approval nos. (665) 107A-37, (732) 108A-48 and (736) 108A-52] and conducted in compliance with the Declaration of Helsinki and institutional ethical guidelines. The rights and interests of all participants were fully protected by the Association for the Accreditation of Human Research Protection Program

guidelines. Written informed consent was obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO): EASL-EASD-EASO clinical practice guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). Obes Facts 17: 374-444, 2024.
2. Eslam M, Fan JG, Yu ML, Wong VW, Cua IH, Liu CJ, Tanwandee T, Gani R, Seto WK, Alam S, *et al*: The Asian Pacific association for the study of the liver clinical practice guidelines for the diagnosis and management of metabolic dysfunction-associated fatty liver disease. Hepatol Int 19: 261-301, 2025.
3. Chen CH, Huang MH, Yang JC, Nien CK, Yang CC, Yeh YH and Yueh SK: Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of Taiwan: Metabolic significance of nonalcoholic fatty liver disease in nonobese adults. J Clin Gastroenterol 40: 745-752, 2006.
4. Wang WS, Wahlqvist ML, Hsu CC, Chang HY, Chang WC and Chen CC: Age- and gender-specific population attributable risks of metabolic disorders on all-cause and cardiovascular mortality in Taiwan. BMC Public Health 12: 111, 2012.
5. Health Promotion Administration, Ministry of Health and Welfare: Report on the Changes in National Nutritional and Health Status 2017-2020: Survey on Changes in National Nutritional and Health Status, 2022 (In Chinese). https://www.hpa.gov.tw/EngPages/Detail.aspx?nodeid=3999&pid=15562
6. Chen CY, Fujimiya M, Laviano A, Chang FY, Lin HC and Lee SD: Modulation of ingestive behavior and gastrointestinal motility by ghrelin in diabetic animals and humans. J Chin Med Assoc 73: 225-229, 2010.
7. Chen CY and Tsai CY: From endocrine to rheumatism: Do gut hormones play roles in rheumatoid arthritis? Rheumatology (Oxford) 53: 205-212, 2014.
8. Chen CY, Asakawa A, Fujimiya M, Lee SD and Inui A: Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev 61: 430-481, 2009.
9. Iwakura H, Ensho T and Ueda Y: Desacyl-ghrelin, not just an inactive form of ghrelin? A review of current knowledge on the biological actions of desacyl-ghrelin. Peptides 167: 171050, 2023.
10. Gong Y, Qiu B, Zheng H, Li X, Wang Y, Wu M, Yan M and Gong Y: Unacylated ghrelin attenuates acute liver injury and hyperlipidemia via its anti-inflammatory and anti-oxidative activities. Iran J Basic Med Sci 27: 49, 2024.
11. Kim H, Ranjit R, Claflin DR, Georgescu C, Wren JD, Brooks SV, Miller BF and Ahn B: Unacylated ghrelin protects against age-related loss of muscle mass and contractile dysfunction in skeletal muscle. Aging Cell 23: e14323, 2024.
12. Koliaki C, Liatis S, Dalamaga M and Kokkinos A: The implication of gut hormones in the regulation of energy homeostasis and their role in the pathophysiology of obesity. Curr Obes Rep 9: 255-271, 2020.
13. Tarantino G and Balsano C: Gastrointestinal peptides and nonalcoholic fatty liver disease. Curr Opin Endocrinol Diabetes Obes 27: 11-15, 2020.
14. Bakar RB, Reimann F and Gribble FM: The intestine as an endocrine organ and the role of gut hormones in metabolic regulation. Nat Rev Gastroenterol Hepatol 20: 784-796, 2023.
15. Alexander CM, Landsman PB and Grundy SM: The influence of age and body mass index on the metabolic syndrome and its components. Diabetes Obes Metab 10: 246-250, 2008.
16. Baneu P, Văcărescu C, Drăgan SR, Cirin L, Lazăr-Höcher AI, Cozgarea A, Faur-Grigori AA, Crișan S, Gaiță D, Luca CT and Cozm D: The Triglyceride/HDL ratio as a surrogate biomarker for insulin resistance. Biomedicines 12: 1493, 2024.
17. Chen CY, Lee WJ, Asakawa A, Fujitsuka N, Chong K, Chen SC, Lee SD and Inui A: Insulin secretion and interleukin-1β dependent mechanisms in human diabetes remission after metabolic surgery. Curr Med Chem 20: 2374-2388, 2013.
18. Ebrahimi M, Seyedi SA, Nabipoorashrafi SA, Rabizadeh S, Sarzaeim M, Yadegar A, Mohammadi F, Bahri RA, Pakravan P, Shafiekhani P, *et al*: Lipid accumulation product (LAP) index for the diagnosis of nonalcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis. Lipids Health Dis 22: 41, 2023.
19. Chiang JK and Koo M: Lipid accumulation product: A simple and accurate index for predicting metabolic syndrome in Taiwanese people aged 50 and over. BMC Cardiovasc Disord 12: 78, 2012.
20. Lockie S, Lyons K, Lawrence G and Grice J: Choosing organics: A path analysis of factors underlying the selection of organic food among Australian consumers. Appetite 43: 135-146, 2004.
21. Senn TE, Espy KA and Kaufmann PM: Using path analysis to understand executive function organization in preschool children. Dev Neuropsychol 26: 445-464, 2004.
22. Bilson J, Sethi JK and Byrne CD: Non-alcoholic fatty liver disease: A multi-system disease influenced by ageing and sex, and affected by adipose tissue and intestinal function. Proc Nutr Soc 81: 146-161, 2022.
23. Muratsu J, Kamide K, Fujimoto T, Takeya Y, Sugimoto K, Taniyama Y, Morishima A, Sakaguchi K, Matsuzawa Y and Rakugi H: The combination of high levels of adiponectin and insulin resistance are affected by aging in non-obese old peoples. Front Endocrinol (Lausanne) 12: 805244, 2022.
24. Yuan Q, Wang H, Gao P, Chen W, Lv M, Bai S and Wu J: Prevalence and risk factors of metabolic-associated fatty liver disease among 73,566 individuals in Beijing, China. Int J Environ Res Public Health 19: 2096, 2022.
25. Delhanty PJ, Neggers SJ and van der Lely AJ: Mechanisms in endocrinology: Ghrelin: The differences between acyl- and des-acyl ghrelin. Eur J Endocrinol 167: 601-608, 2012.
26. Shimada T, Furuta H, Doi A, Ariyasu H, Kawashima H, Wakasaki H, Nishi M, Sasaki H and Akamizu T: Des-acyl ghrelin protects microvascular endothelial cells from oxidative stress-induced apoptosis through sirtuin 1 signaling pathway. Metabolism 63: 469-474, 2014.
27. Cappellari GG, Zanetti M, Semolic A, Vinci P, Ruozi G, Falcione A, Filigheddu N, Guarnieri G, Graziani A, Giacca M and Barazzoni R: Unacylated ghrelin reduces skeletal muscle reactive oxygen species generation and inflammation and prevents high-fat diet-induced hyperglycemia and whole-body insulin resistance in rodents. Diabetes 65: 874-886, 2016.
28. Agosti E, De Feudis M, Angelino E, Belli R, Teixeira MA, Zaggia I, Tamiso E, Raiteri T, Scircoli A, Ronzoni F, *et al*: Both ghrelin deletion and unacylated ghrelin overexpression preserve muscles in aging mice. Aging (Albany NY) 12: 13939-13957, 2020.
29. Yuan F, Zhang Q, Dong H, Xiang X, Zhang W, Zhang Y and Li Y: Effects of des-acyl ghrelin on insulin sensitivity and macrophage polarization in adipose tissue. J Transl Int Med 9: 84-97, 2021.
30. Alharbi S: Exogenous administration of unacylated ghrelin attenuates hepatic steatosis in high-fat diet-fed rats by modulating glucose homeostasis, lipogenesis, oxidative stress, and endoplasmic reticulum stress. Biomed Pharmacother 151: 113095, 2022.
31. Ahmad MA, Karavetian M, Moubareck CA, Wazz G, Mahdy T and Venema K: The association between peptide hormones with obesity and insulin resistance markers in lean and obese individuals in the United Arab Emirates. Nutrients 14: 1271, 2022.
32. Cho YH, Lee SY, Jeong DW, Cho AR, Jeon JS, Kim YJ, Lee JG, Yi YH, Tak YJ, Hwang HR, *et al*: Metabolic syndrome is associated with lower plasma levels of desacyl ghrelin and total ghrelin in asymptomatic middle-aged Korean men. J Obes Metab Syndr 26: 114-121, 2017.
33. Amitani M, Amitani H, Cheng KC, Kairupan TS, Sameshima N, Shimoshikiryo I, Mizuma K, Rokot NT, Nerome Y, Owaki T, *et al*: The role of ghrelin and ghrelin signaling in aging. Int J Mol Sci 18: 1511, 2017.
34. Ezquerro S, Mocha F, Frühbeck G, Guzmán-Ruiz R, Valentí V, Mugueta C, Becerril S, Catalán V, Gómez-Ambrosi J, Silva C, *et al*: Ghrelin reduces TNF-α-induced human hepatocyte apoptosis, autophagy, and pyroptosis: Role in obesity-associated NAFLD. J Clin Endocrinol Metab 104: 21-37, 2019.

35. Ezquerro S, Méndez-Giménez L, Becerril S, Moncada R, Valentí V, Catalán V, Gómez-Ambrosi J, Frühbeck G and Rodríguez A: Acylated and desacyl ghrelin are associated with hepatic lipogenesis, β-oxidation and autophagy: Role in NAFLD amelioration after sleeve gastrectomy in obese rats. Sci Rep 6: 39942, 2016.
36. Zang P, Yang CH, Liu J, Lei HY, Wang W, Guo QY, Lu B and Shao JQ: Relationship between acyl and desacyl ghrelin levels with insulin resistance and body fat mass in type 2 diabetes mellitus. Diabetes Metab Syndr Obes 15: 2763-2770, 2022.
37. Delhanty PJ, Sun Y, Visser JA, van Kerkwijk A, Huisman M, van Ijcken WF, Swagemakers S, Smith RG, Themmen AP and van der Lely AJ: Unacylated ghrelin rapidly modulates lipogenic and insulin signaling pathway gene expression in metabolically active tissues of GHSR deleted mice. PLoS One 5: e11749, 2010.
38. Liu X, Guo Y, Li Z and Gong Y: The role of acylated ghrelin and unacylated ghrelin in the blood and hypothalamus and their interaction with nonalcoholic fatty liver disease. Iran J Basic Med Sci 23: 1191-1196, 2020.
39. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM and Kasuga M: Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut 54: 18-24, 2005.
40. Ariyasu H, Takaya K, Iwakura H, Hosoda H, Akamizu T, Arai Y, Kangawa K and Nakao K: Transgenic mice overexpressing des-acyl ghrelin show small phenotype. Endocrinology 146: 355-364, 2005.
41. Rodríguez A, Gómez-Ambrosi J, Catalán V, Rotellar F, Valentí V, Silva C, Mugueta C, Pulido MR, Vázquez R, Salvador J, *et al*: The ghrelin O-acyltransferase-ghrelin system reduces TNF-α-induced apoptosis and autophagy in human visceral adipocytes. Diabetologia 55: 3038-3050, 2012.
42. Lv P, Li H, Li X, Wang X, Yu J and Gong Y: Intestinal perfusion of unacylated ghrelin alleviated metabolically associated fatty liver disease in rats via a central glucagon-like peptide-1 pathway. Am J Physiol Gastrointest Liver Physiol 326: G643-G658, 2024.
43. Pei XM, Yung BY, Yip SP, Chan LW, Wong CS, Ying M and Siu PM: Protective effects of desacyl ghrelin on diabetic cardiomyopathy. Acta Diabetol 52: 293-306, 2015.
44. Long MT, Pedley A, Massaro JM, Hoffmann U, Ma J, Loomba R, Chung RT and Benjamin EJ: A simple clinical model predicts incident hepatic steatosis in a community-based cohort: The Framingham heart study. Liver Int 38: 1495-1503, 2018.
45. Mumusoglu S and Yildiz BO: Metabolic syndrome during menopause. Curr Vasc Pharmacol 17: 595-603, 2019.
46. de Mutsert R, Gast K, Widya R, de Koning E, Jazet I, Lamb H, le Cessie S, de Roos A, Smit J, Rosendaal F and den Heijer M: Associations of abdominal subcutaneous and visceral fat with insulin resistance and secretion differ between men and women: The Netherlands epidemiology of obesity study. Metab Syndr Relat Disord 16: 54-63, 2018.
47. Smith A, Woodside B and Abizaid A: Ghrelin and the control of energy balance in females. Front Endocrinol (Lausanne) 13: 904754, 2022.
48. de Souza GO, Wasinski F and Donato J Jr: Characterization of the metabolic differences between male and female C57BL/6 mice. Life Sci 301: 120636, 2022.