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• *BRIEF REPORTS* •

Serum leptin levels and insulin resistance are associated with gallstone disease in overweight subjects

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Abstract

AIM: To establish an association between the serum leptin levels and the development of gallstone disease (GD).

METHODS: We carried out a non-matched case-controlled study in a university hospital in Mexico City. Two hundred and eighty-seven subjects were included: 97 cases with gallstones and 190 controls. Body mass index (BMI), fasting plasma leptin, insulin, serum lipid, and lipoprotein levels were measured. Insulin resistance was calculated by homeostasis model assessment (HOMA-IR). Unconditional logistic regression analysis (univariate and multivariate) stratified by BMI was used to calculate the risk of GD.

RESULTS: The multivariate conditional regression analysis revealed a model for those patients with BMI <30. The selected variables in the model were HOMA-IR index with OR = 1.31, P = 0.02 and leptin higher than median with OR = 2.11, P = 0.05. In the stratum of BMI ⩾30, we did not find a useful model.

CONCLUSION: We concluded that insulin resistance and the development of GD appears to be associated with serum leptin levels in subjects with overweight, but not in obese subjects with similar metabolic profiles.

Key words: Obesity; Leptin; Gallstones; Cholesterol; Insulin resistance

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INTRODUCTION

The prevalence of obesity has been increasing progressively worldwide, and is closely associated with the increased morbidity caused by several of the most common diseases in the Western world, including diabetes, hypertension, cardiovascular disease, cancer, and gallstone disease (GD)[1]. Obesity is one of the main risk factors for cholesterol gallstone formation and has been associated with the supersaturation of bile with cholesterol as a result of the increased hepatic secretion of this sterol[2-5].

On the other hand, leptin, the product of the *ob* gene, is an adipose-tissue-derived hormone considered to regulate the limitation of food intake and increased energy expenditure, and thus adiposity[6]. These findings are similar in human beings mainly failure to produce adequate amounts of leptin, or resistance to its central action, which may result in the development of obesity[7].

To identify a link between leptin and the effects of obesity on gallstone formation, Duggirala *et al*.[8], in a previous study explored the role of leptin as an indicator of adiposity and its relation to GD in a Mexican-American population. They reported that leptin levels correlated significantly with the incidence of GD in both sexes, especially in women. Ruhl and Everhart[9] evaluated the possibility that serum leptin levels are better predictors of GD than the body mass index (BMI). They found that leptin concentrations were associated with GD in both sexes (P<0.001), but this association disappeared after controlling BMI and waist-to-hip circumference in both women (P = 0.29) and men (P = 0.65). They concluded that serum leptin concentrations are not better predictors of GD than anthropometry.

Mendez-Sanchez *et al*.[10], demonstrated a significant and positive correlation between plasma leptin levels and biliary cholesterol saturation in a group of obese subjects. This correlation emerged following a modest degree of weight loss. We believe that one additional mechanism with obesity and high risk for GD is serum leptin level and insulin resistance. The aim of the present study was to find whether there is an association between serum leptin levels and the development of GD.

MATERIALS AND METHODS

Populations and sample

This study was carried out at the check-up unit of the Diagnostic Clinic at the Medica Sur Clinic and Foundation. This hospital provides care mainly for middle- and high-income individuals from Mexico City and other metropolitan Mexican areas. The study was approved by the Human Subjects Committee at The Medica Sur Clinic and Foundation as conforming to the ethical guidelines of the 1975 Declaration of Helsinki, and written informed consent was obtained from all participants before entry. A total of 287 subjects were included in this study: 97 cases with gallstones (42 women and 55 men) and 190 controls (79 women and 111 men). Ages were similar in both groups (52.9±11.8 and 52.7±11.7 years). GD cases and controls were a series of consecutive asymptomatic subjects who were referred to the unit by their companies as an annual requirement, but not for symptomatic disease. Abdominal ultrasound was performed on all subjects using a Sonoline Elegra instrument (Siemens Medical System, Germany) with a 3.5 MHz transducer. Ultrasound diagnosis of GD was assessed by the presence of strong intraluminal echoes that were gravity-dependent or attenuated ultrasound transmission (acoustic shadowing). At the completion of each patient's participation in the study, all ultrasonographic studies were evaluated by the same radiologist. No discrepancies were found between the results of the first and second evaluations ($\kappa = 0.93$).

Physical examination

Body weight was measured, in light clothing and without shoes, to the nearest 0.10 kg. Height was measured to the nearest 0.5 cm. BMI was calculated as weight in kilograms divided by height in square meters.

Analytical techniques

Plasma leptin levels were determined by radioimmunoassay using a human leptin RIA kit (Linco Research, St. Charles, MO, USA). Both the intra- and inter-assay coefficients of variation were less than 5%. Insulin levels were measured using an immunoenzymometric assay (MEIA; Abbott Diagnostics), with inter- and intra-assay coefficients of variation less than 3%. Plasma glucose in the fasting state was measured in duplicate with an automated analyzer. The coefficient of variation for a single determination was 1.5%. Cholesterol, HDL-cholesterol, and triglycerides were measured by enzymatic colorimetric methods, using CHOL, HDL-C plus (second generation) and TG assays (Roche Diagnostics Co., Indianapolis, IN, USA). Low-density lipoprotein (LDL) cholesterol concentrations were calculated using the Friedewald formula[11]. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol, as surrogate in cases where LDL cholesterol was inaccurate[12]. Insulin resistance was calculated by means of the homeostasis model assessment (HOMA-IR). HOMA-IR = [fasting insulin (μU/mL) ·fasting glucose (mmol/L)]/22.5, high index of insulin resistance a value >2.5[13].

Statistical analysis

We examined the relationship between anthropometric measures and serum leptin and concentrations by first comparing means of these variables in persons with and without GD. To further study these relations while controlling the effects of covariates related to GD, we used logistic regression for the univariated and multivariated analysis stratifying by BMI (EGRET: Epidemiological Graphics, Estimation, and Testing Package v 1.02.10. Cytel Software Corporation, USA, 1997). Multivariate analyses excluded persons with missing values for any factor were included in the model. The comparison of single frequencies and crossing variables, χ^2 test, *F* exact test, and Mann-Whitney U, and clustering by using SPSS/PC 10.0 program (Chicago, IL, USA, 1999). $P<0.05$ was considered to indicate statistical significance.

RESULTS

We collected information from 97 cases and 190 matched controls (control/patient ratio of 1.96). Cases and their respective controls did not differ in age or lipid values (Table 1). Patients and controls tended to differ in BMI values (28.2 *vs* 27, $P = 0.12$). The index for resistance to insulin (HOMA-IR) was higher among patients than controls (2.9 *vs* 2.4, $P = 0.04$, Table 1).

We stratified subjects according to overweight or obesity, with a BMI value of 30 as the cut-off point, according to World Health Organization[14]. We observed a statistically

Table 1 Comparison of variables between cases and controls

Variable	Cases *n* = 97					Controls *n* = 190					*P*[1]
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max	
Age (yr)	52.1	12.1	51.0	19.0	85.0	50.5	11.7	50.0	18.0	85.0	0.25
BMI (kg/m^2)	28.2	5.5	27.7	18.6	54.9	27.0	3.9	26.9	19.0	40.1	0.12
Leptin (ng/mL)	18.2	44.7	10.2	1.9	431.0	11.0	6.5	9.5	1.3	40.7	0.11
Insulin (μU/mL)	11.1	7.4	9.1	1.8	35.7	9.4	6.3	7.6	2.0	43.7	0.07
T-chol (mmol/L)	5.43	1.09	5.46	2.48	8.38	5.38	1.03	5.4	3.36	9.44	0.95
LDL (mmol/L)	3.44	0.85	3.36	1.11	5.61	3.49	0.85	3.41	1.60	6.13	0.73
HDL (mmol/L)	1.19	0.36	1.14	0.47	2.72	1.22	0.36	1.14	0.52	2.56	0.42
NHDL (mmol/L)	4.27	1.03	4.34	1.78	7.34	4.27	0.98	4.24	2.40	8.53	0.90
TGC (mmol/L)	1.89	1.15	1.70	0.43	6.78	1.71	0.96	1.46	0.06	7.38	0.18
HOMA-IR	2.9	2.2	2.3	0.4	11.7	2.4	1.8	1.8	0.4	13.2	0.04

[1]Mann-Whitney *U* test. BMI, body mass index; T-chol, total cholesterol; LDL, low-density cholesterol; HDL, high-density cholesterol; NHDL, non-HDL cholesterol; TGC, triglycerides.

significant difference in rate between cases and controls according to the tercile distribution in those patients who were not obese (34.7% *vs* 22.9%, $P = 0.04$, Table 2). In the same stratum (BMI<30), cases had a higher prevalence of insulin resistance, according to HOMA-IR, than controls, with borderline statistical significance (40.3% *vs* 27.5%, $P = 0.07$, Table 2). In a multivariate model, adjusted for BMI and insulin levels did not change the association related with leptin levels (data not shown). In the stratum of obese patients (BMI≥30), we observed no statistical differences between control and patient variables, except for hypertriglyceridemia, which had a higher incidence among cases than among controls.

Because of the matched design of the investigation, we analyzed the data under conditional logistic regression analyses. With univariate conditional logistic regression, we observed a higher probability of GD in those patients with BMI <30 according to HOMA-IR index value (OR = 1.33; 95%CI 1.06-1.68, $P = 0.02$) and to both leptin levels (by median or tercile distribution with an OR=2.25; 95%CI 1.08-4.69, $P = 0.03$ and OR = 1.67; 95%CI 1.03-2.69, $P = 0.04$, respectively; Table 3). In the stratum of obese patients, we identified a lower probability of GD associated with high levels of leptin, with both median and tercile distributions (OR = 0.12; 95%CI 0.01-1.0, $P = 0.05$ and OR = 0.14; 95%CI 0.02-0.98, $P = 0.05$, respectively; Table 3). Other variables were not significantly associated.

Multivariate conditional regression analysis (92 cases and 188 controls) identified a correlation for those patients with BMI <30. The selected variables in the model were HOMA-IR with OR = 1.31; 95%CI 1.04-1.66, $P = 0.02$ and

Table 2 Comparison of variables between cases and controls, stratified by BMI

Variable	BMI <30				P^1	BMI ≥30				P^1
	Cases $n = 72$		Controls $n = 153$			Cases $n = 25$		Controls $n = 37$		
	No	%	No	%		No	%	No	%	
Gender (male)	42	58.3	89	58.2	1.0	13	52.0	22	59.5	0.61
Age >50 yr	38	52.8	69	45.1	0.32	13	52.0	21	56.8	0.80
Leptin ≥9.78 ng/mL	37	51.4	61	39.9	0.11	16	64.0	30	81.1	0.15
Leptin					0.04[2]					0.25[2]
$Tercile_1$<7.67 ng/mL	22	30.6	64	41.8		5	20.0	4	10.8	
$Tercile_2$7.6–12.4 ng/mL	25	34.7	54		35.3		7	28.0	9	24.3
$Tercile_3$>12.4 ng/mL	25	34.7	35	22.9		13	52.0	24	64.9	
Insulin≥20 μU/mL	9	12.5	0	0	<0.0001	4	16.0	12	32.4	0.24
HOMA-IR (>2.5)	29	40.3	42	27.5	0.07	15	60.0	24	64.9	0.79
T-chol≥6.21 mmol/L	19	26.4	45	29.4	0.75	6	24.0	6	16.2	0.52
LDL≥4.14 mmol/L[3]	19	27.1	40	26.7	1.0	5	21.7	7	18.9	1.0
HDL<1.03 mmol/L	27	37.5	50	32.7	0.55	8	32.0	13	35.1	1.0
NHDL ≥4.91 mmol/L	20	27.8	43	28.1	1.0	5	20.0	7	18.9	1.0
TGC ≥1.70 mmol/L	32	44.4	62	40.5	0.66	17	68.0	14	37.8	0.04

[1]*F* exact test. [2]χ^2 for linear trend. [3]Five missing values for cases and 2 for controls. BMI, body mass index; T-chol, total cholesterol; LDL, low-density cholesterol; HDL, high density cholesterol; NHDL, non-HDL cholesterol; TGC, triglycerides.

Table 3 Univariated analysis of conditional logistic regression, stratified by BMI

Variable	BMI <30			BMI ≥30		
	OR	95%CI	*P*	OR	95%CI	*P*
Age (yr)	1.20	0.97–1.49	0.09	0.93	0.68–1.26	0.62
Leptin (ng/mL)	1.06	0.99–1.13	0.09	0.91	0.81–1.03	0.15
Insulin (μU/mL)	1.09	1.02–1.16	0.01	0.94	0.86–1.03	0.17
HOMA-IR	1.33	1.06–1.68	0.02	0.85	0.61–1.17	0.32
T-chol (mmol/L)	1.0	0.99–1.00	0.32	1.02	0.99–1.05	0.27
LDL (mmol/L)	1.0	0.99–1.01	0.51	1.01	0.98–1.05	0.39
HDL (mmol/L)	0.99	0.97–1.01	0.44	1.02	0.95–1.09	0.66
NHDL (mmol/L)	1.0	0.99–1.01	0.44	1.02	0.99–1.05	0.30
TGC (mmol/L)	1.0	1.0–1.0	0.86	1.0	1.0–1.01	0.30
Leptin ≥9.78 ng/mL	2.25	1.08–4.69	0.03	0.12	0.01–1.0	0.05
Leptin terciles (ng/mL)	1.67	1.03–2.69	0.04	0.14	0.02–0.98	0.05
Insulin ≥20 μU/mL		Non convergence		0.47	0.12–1.88	0.29
HOMA-IR >2.5	1.46	0.75–2.83	0.27	0.44	0.11–1.84	0.26
T-chol ≥6.21 mmol/L	0.86	0.42–1.74	0.67	3.88	0.38–39.5	0.25
LDL ≥4.14 mmol/L	1.06	0.55–2.04	0.87	1.50	0.36–6.30	0.58
HDL <1.03 mmol/L	1.26	0.66–2.41	0.49		Non convergence	
NHDL ≥4.91mmol/L	1.07	0.54–2.13	0.84	0.78	0.07–8.88	0.84
TGC ≥1.7 mmol/L	1.09	0.60–1.99	0.77	3.03	0.76–12.1	0.12

OR, odds ratio; 95%CI, 95% confidence intervals; BMI, body mass index; T-chol, total cholesterol; LDL, low-density cholesterol; HDL, high density cholesterol; NHDL, non-HDL cholesterol; TGC, triglycerides.

leptin levels higher than the median value, with OR = 2.11; 95%CI 0.99-4.53, $P = 0.05$ (Table 4). In the stratum with BMI ⩾30, we did not identify any useful correlation.

Table 4 Multivariated analysis of conditional logistic regression, stratified by BMI

Variables	BMI <30			BMI ⩾30		
	OR	95%CI	*P*	OR	95%CI	*P*
HOMA-IR	1.31	1.04–1.66	0.02	No model founded		
Leptin ⩾9.78 ng/mL	2.11	0.99–4.53	0.05			

OR, odds ratio; 95%CI, 95% confidence intervals.

DISCUSSION

This study was designed to examine the role of serum leptin levels as a risk factor for developing GD. We observed that plasma leptin levels and the HOMA-IR are highly associated in the group of subjects with BMI <30, because they increase the probability of GD, but that the same plasma leptin levels are associated with a lower probability of GD in the group of subjects with BMI >30.

How can we explain these findings? Bile is the route by which cholesterol is eliminated from the body, and reverse cholesterol transport is the metabolic pathway by which cholesterol is moved from peripheral tissues to the liver for biliary secretion[15,16]. Van Patten *et al.*[17], reported that obesity in leptin-receptor-defective Zucker (*fa/fa*) rats is associated with decreased biliary cholesterol secretion due to the uncoupling of cholesterol and phospholipid from bile-salt secretion. High-dose leptin partially normalized cholesterol secretion in these obese rats without altering lipid composition, implying that both the chronic effects of obesity and a relative resistance to leptin contributed to the impaired biliary cholesterol elimination. From these data, it is clear that leptin plays a role in the elimination of cholesterol from the body.

In the present study, the question arises: Why is BMI important in the association between serum leptin levels and GD, especially in subjects with BMI <30. It has been suggested that in human obesity, leptin levels adapt to changes in energy balance. During fasting[17] or weight loss[18], leptin concentrations decrease, whereas they increase during overfeeding or weight gain. Obese human beings have high leptin concentrations[18]. Leptin mRNA expression in fat cells correlates significantly with body fat mass[18]. Hyperleptinemia is thought to be indicative of "leptin resistance", and may play a role in the pathogenesis of obesity[18,19]. We believe that leptin levels are important risk factors for GD, before "leptin resistance" is apparent, i.e., when the leptin levels are not as high as those observed in obese subjects. In accordance with this hypothesis, Hyogo *et al.*[20], showed that weight loss in chow-fed C57BL/6J *ob/ob* mice induced by chronic intraperitoneal administration of high-dose leptin (10 μg/g per d) is associated with cholesterol gallstone formation.

A 25% reduction in body weight over a 28-d period was sufficient to produce cholesterol crystals and gallstones in the gallbladder bile of all lithogenic-diet-fed mice treated with low-dose leptin. Nevertheless, neither cholesterol crystals nor stones were observed in any chow-fed mouse. These findings indicate that, whereas leptin replacement is required for cholesterol cholelithiasis in *ob/ob* mice, a lithogenic diet accelerates this process[21].

The other variable associated with GD in the present study was the HOMA-IR. Recently, we have found a strong relationship between GD and metabolic syndrome, of which the cardinal feature is hyperinsulinemia[22]. In fact, hyperinsulinemia has been proposed as a risk factor for GD, and some studies support this hypothesis. For example, Scragg *et al.*[23], found that mean fasting insulin levels to be higher in patients of both sexes with GD, independent of age and triglyceride levels. Laakso *et al.*[24], also found that subjects with GD had significantly higher levels of insulin than controls. Haffner *et al.*[25], in the San Antonio Heart Study, observed increasing hyperinsulinemia and a high incidence of GD in both Mexican-Americans and non-Hispanic whites. In Mexico, Gonzalez Villalpando *et al.*[26], found higher fasting insulin levels in women with GD than in controls, but no such relationship was observed in men. Ruhl and Everhart[27], in an well-presented study, have confirmed this association of GD with higher fasting serum insulin and C-peptide levels in women. The association was independent of fasting glucose levels and other covariates related to GD.

Increasing cholesterol saturation of the bile and decreasing gallbladder motility are two possible mechanisms by which insulin plays a role in gallstone formation. At present, these are considered very important in the pathogenesis of gallstones. It has also been suggested that high concentrations of insulin increase the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase[28,29], the rate-limiting enzyme in hepatic synthesis of new cholesterol, or by activating LDL receptors, resulting in greater hepatic uptake of LDL cholesterol[30]. By inhibiting basal and cholecystokinin-stimulated gallbladder motility, insulin might also increase the risk of developing gallstones through an effect on motility[31].

There are several unresolved issues concerning the biological effects of leptin and its relationship to GD about the importance of motility[32], secretion or both. Tran *et al.*[33,34], included other factors in the link between obesity and gallstone formation, particularly an impaired response to neurotransmitters (mainly neuropeptide Y). The present work shows that there is probably a threshold to the biological activity of leptin, and the differences between our experimental groups indicate that leptin has a "lithogenic effect" in selected overweight subjects. Wauters *et al.*[35], analyzed how polymorphisms in the leptin-receptor gene influence fat topography and levels of abdominal fat in human beings, providing further evidence for the wide response spectrum to several grades of obesity and several grades of leptin-resistance. Similar data on the effects of leptin on lipid metabolism demonstrate differences between sexes and grades of obesity[36], including evidence for the independent effects of leptin on lipid metabolism[37]. These complex data were observed in both healthy subjects and disease models[38,39]. Recently, it was observed that besides fat mass and gender, which are the main determinants of leptin levels in type 2 diabetic and healthy subjects, insulin

secretion and the degree of insulin resistance also contribute significantly to leptin levels[40]. In the present study, there were more insulin-resistant subjects in the BMI >30 group, and these data indicate a correspondence between greater obesity, higher resistance, and a lack of biological effects (in this case GD). This study contribute to show one side of a wide clinical response of a metabolic disturbance, in subjects with similar grade of insulin resistance, but different phenotype (BMI) we observe an unexpected response of leptin effect. However, this is only a hypothesis drawn from the data on the relationship between leptin and insulin resistance[41].

Due to the sample analyzed (mainly employees) we loose randomization effect, and could not reflect general population behavior. By otherwise a lack of metabolic difference between cases and controls (HOMA-IR), could explain contradictory data in obese group; however, this unexpected finding could represent similar leptin response even do differences in body composition.

In conclusion, the results of the present study show that, as with insulin resistance, the development of GD appears to be associated with serum leptin levels in overweight subjects.

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