Changes in Plasma Ghrelin, Leptin, Insulin and Glucagon-Like Peptide-1 Levels Are Correlated to Body Mass Index among Patients with Early Asymptomatic Non-Alcoholic Fatty Liver

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ABSTRACT

Objectives: Saudi Arabia has high prevalence of obesity and Non-Alcoholic Fatty Liver (NAFL). Accurate diagnosis and dissection of early pathogenic events for fatty liver is important to furnish better grounds for early life-style intervention and therapy. We aimed to cross-sectionally assess the utility of ghrelin, leptin, insulin and glucagon-like peptide-1 (GLP-1) as early pathogenetic culprits for asymptomatic obesity-comorbid NAFL.

Methods: Age- and gender-matching healthy lean controls with normal body mass index (BMI) [(Group A; n = 40) with BMI = 18.5-25], otherwise healthy asymptomatic overweight NAFL [(Group B; n = 64) with BMI = 25.1 - 30], and, otherwise healthy asymptomatic obese NAFL [(Group C; n = 76) with BMI > 30 - < 40] participants were enrolled. NAFL was transabdominally ultrasonographically characterized, and, non-alcoholic individuals with normal liver size and liver plasma enzymes were included. Fasting plasma levels of ghrelin, leptin, insulin and GLP-1 were quantitatively immunoassayed.

Results: Ghrelin level was highest in Group A followed by Group C then Group B. Leptin was highest in Group A followed by Group B then Group C. Insulin was obviously elevated in Group C followed by Group B then Group A. GLP-1 negatively significantly related to BMI and was highest in group A followed by group B and then group C participants. Significant positive correlations were also observed between GLP-1 and leptin in all groups and between ghrelin and insulin in overweight NAFL participants. In overweight and obese NAFL individuals, ghrelin, leptin and GLP-1 were decreased whereas insulin was increased.

Conclusion: Our results reflect the relative dysfunction and imbalanced equilibrium of the investigated hormones, and, suggest their early pathogenetic implication in asymptomatic NAFL in the targeted population.

KEY WORDS
body mass index, non-alcoholic fatty liver, ghrelin, leptin, insulin, glucagon-like Peptide-1

INTRODUCTION

Obesity and overweight have several comorbidities, particularly Non-Alcoholic Fatty Liver (NAFL), dyslipidemia, insulin resistance, diabetes and cardiovascular diseases. Although obesity is a preventable condition, its comorbidities are dreadful. Out of the epidemic overweight/obese 1.9 billion persons aging ≥ 18 years worldwide in the year 2016. ~13% were obese, with a female predominance). Beside the excess energy violation due to the socioeconomic and life style changes in Saudi Arabia, hereditary factors, family history, racial/ethnic variations, obesogenic environmental, etc. are also implicated.

The relationships between obesity and metabolic hormones such as ghrelin, leptin and insulin are well established. And, these hormones actively inter-act. The central and peripheral resistance to these hormones is fundamental for the neuroendocrine control of energy homeostasis and peripheral metabolism. In a fast-acting manner, orexigenic ghrelin plays a major role in meal initiation and promotion of weight gain. Through activating the growth hormone secretagogue receptor, ghrelin regulates glucose and fat metabolism and decreases energy...
expenditure and promotes adiposity\(^4\). Contrastingly, the anorexigenic slow-acting leptin induces long-term weight loss by suppressing food intake. Surprisingly, obese people show high levels of leptin and reduced levels of ghrelin. Nowadays, leptin-resistance is well-recognized\(^5\). Insulin is a major regulator of adipogenesis and adipocyte-triglycerides metabolism, by its action on the differentiation of preadipocytes to adipocytes, and, stimulation of glucose and fatty acids uptake for triglyceride synthesis\(^6\). On the contrary, obesity is a risk factor for insulin resistance, type 2 diabetes mellitus and NAFLD\(^5\).

Ghrelin is anti-inflammatory, regulates glucose homeostasis, and, modulates insulin secretion and signaling. On the other hand, insulin affects the systemic concentrations of ghrelin\(^7,8\). The preprandial increase and postprandial decrease may be associated with specific ghrelin gene polymorphism\(^9,10\). The incretin, glucagon-like peptide-1 (GLP-1) is secreted by intestinal L-cells and neurons of the solitary tract nucleus of brainstem following food intake. It plays an important role in glucose homeostasis via stimulation of glucose-dependent insulin secretion. Furthermore, GLP-1 is also associated with protective effects on pancreatic β-cells and the cardiovascular system, decreased appetite, and increases weight loss, making GLP-1 and derivatives a rational treatment for type-2 diabetes and obesity. The pathogenesis of NAFLD involves alteration of these hormones and neurotransmitters on the energy balance among individuals with NAFL needs more research, particularly among a population not previously investigated. NAFL is the hepatic manifestation of metabolic syndrome that is not due to alcohol abuse. NAFL disease is a spectrum of histologically defined liver disorders. The disease can progress from benign hepatic macro-vesicular lipid accumulation (steatosis), the form that we are targeting, to nonalcoholic steatohepatitis (NASH) to outright fibrosis, cirrhosis, and even hepatocellular carcinoma\(^11\).

Because of the overlapping roles of our targeted hormones (both individually and as a group) and the known pathogenic factors implicated in NAFL, we hypothesized that changes in the homeostasis of these hormones are early events in causation and/or severity in early stages of NAFL. Immunoassays were used to correlate changes in ghrelin, leptin, insulin and GLP-1 with BMI in overweight and obese, otherwise healthy, early asymptomatic NAFL participants as compared to normal BMI healthy lean individuals.

**RESULTS**

The present study investigated 180 apparently healthy Saudi volunteers with a variable range of BMI. Table 1 summarizes the distribution of the means of the biochemical measurements among the three studied groups; healthy controls with normal BMI, and, overweight asymptomatic NAFL participants and obese NAFL participants. Plasma ghrelin as a dependent variable was highest in the lean controls followed by the obese NAFL individuals and lowest in the overweight NAFL participants. Plasma leptin with a variable range of BMI. Table 1 summarizes the distribution of the means of the biochemical measurements among the three studied groups; healthy controls with normal BMI, and, overweight asymptomatic NAFL participants and obese NAFL participants. Plasma ghrelin as a dependent variable was highest in the lean controls followed by the obese NAFL individuals and lowest in the overweight NAFL participants. Plasma leptin with a variable range of BMI. Table 1 summarizes the distribution of the means of the biochemical measurements among the three studied groups; healthy controls with normal BMI, and, overweight asymptomatic NAFL participants and obese NAFL participants. Plasma ghrelin as a dependent variable was highest in the lean controls followed by the obese NAFL individuals and lowest in the overweight NAFL participants.

For plasma leptin as a dependent variable, controls were the highest followed by overweight asymptomatic NAFL participants then obese NAFL participants with mean values of 9.323 ± 8.362, 5.074 ± 7.571 and 3.153 ± 6.328 pg/mL, respectively. Multiple comparison (using Tukey’s HSD as a Post Hoc test) of the three groups showed a global statistical significance difference amongst them - pointing to the strong association of BMI and ghrelin (F = 4.885, and, p = 0.009). Controls were non-significantly different from obese NAFL participants (p = 0.563), while, overweight NAFL individuals were significantly different compared to controls and almost significantly different from obese NAFL participants (p = 0.011 and 0.038, respectively).

For plasma insulin as a dependent variable, controls were the highest followed by overweight asymptomatic NAFL participants then obese NAFL participants with mean values of 9.323 ± 8.362, 5.074 ± 7.571 and 3.153 ± 6.328 pg/mL, respectively. Multiple comparison of the three groups, showed a global significant difference amongst them that strongly correlates plasma leptin significantly with BMI (F = 9.550 and p < 0.001). However, the two NAFL groups were lowest in overweight significantly different (p < 0.262) in their plasma leptin levels, but each of them was significantly lower vs. controls (p < 0.011 and < 0.001, respectively).

For plasma insulin as a dependent variable, it was highest in the obese asymptomatic NAFL participants followed by the overweight NAFL individuals and lowest in the normal controls with mean values of 27.256 ± 16.724, 27.222 ± 31.866, and 15.662 ± 7.458 μIU/mL, respectively. Multiple comparison of the three groups, there was a global significant difference amongst them indicating moderate increases in plasma insulin with increases in BMI (F = 4.211 and p < 0.016). These values revealed significant difference amongst the two NAFL groups (p = 0.036) but a non-significant difference amongst the two NAFL groups (p < 0.10).

**Participants and methods**

**Participants and Setting:** The present cross-sectional study consecutively enrolled 180 adult consented Saudi volunteering participants. Volunteering participants were randomly selected by simple sequentially inclusion from Aljouf community members, Sakaka, Saudi Arabia, undergoing a routine health checkup at Amas Poly-Clinic Center, Sakaka, Aljouf, Saudi Arabia, in the period from January 1 to June 1, 2019. They comprised age- and gender-matching Healthy Lean Control Group A (n = 40 and BMI = 18.5 ± 25), otherwise healthy asymptomatic overweight NAFL Group B (n = 64 and BMI = 25.1 ± 30), and, otherwise healthy asymptomatic obese NAFL Group C (n = 76 and BMI > 30 ± 40). The male/female ratio was 1:1. Their age ranged from 24 ± 50 years (37.0 ± 7.85) without statistically significant differences among groups or within each group (male/female). Fatty liver participants with increased liver size or increased liver function biomarkers were excluded. Known alcoholics (of any amount), morbid obese, diabetes, and those with fatty liver disease due to drug exposure and abuse (amiodarone, methotrexate, tamoxifen, glucocorticoids, valproate, anti-retroviral agents for HIV), Wilson disease, lipodystrophy, abetalipoproteinemia, Reye syndrome, HELLP syndrome, inborn errors of metabolism (LCAT deficiency, cholesterol ester storage disease, Wolman disease), pregnancy (acute fatty liver of pregnancy), viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, thyroid disease and hemochromatosis, hypertensive and those on parenteral nutrition, dieting regimens or starvation were excluded. Participants having signs or symptoms of cirrhosis or at high risk for advanced fibrosis or cirrhosis were also excluded. The participants were free of other chronic metabolic, autoimmune, endocrine and renal diseases. Following the tenets of the Declaration of Helsinki, each participant signed a written bioethical declaration. Non-significant differences were excluded. Participants were free of other chronic metabolic, autoimmune, and hemochromatosis, hypertensive and those on parenteral nutrition, dieting regimens or starvation were excluded. Participants having signs or symptoms of cirrhosis or at high risk for advanced fibrosis or cirrhosis were also excluded. The participants were free of other chronic metabolic, autoimmune, endocrine and renal diseases. Following the tenets of the Declaration of Helsinki, each participant signed a written bioethical declaration.

**Statistical analysis:** Statistical analysis was conducted using SPSS version 20.0 (IBM). Continuous variables were expressed as mean ± standard deviation (SD). Normal distribution of variables was checked by Kolmogorov-Smirnov test. Variables with normal distribution were compared with analysis of variance (ANOVA) test. Variables with asymmetric distribution were analyzed by Kruskal-Wallis and Mann-Whitney U tests. Correlation between variables was assessed by 2-tailed Pearson correlation coefficient test. P value of ≥ 0.05 was considered statistically significant.
controls (25.877 ± 22.747 pg/mL) followed by overweight asymptomatic NAFL participants (16.634 ± 19.990 pg/mL) and then obese NAFL subjects (9.34 ± 15.01 pg/mL). The multiple comparison of the three groups showed a global statistical significance difference amongst them; confirming a moderate association between GLP-1 and BMI (F = 8.170 and p < 0.016). The two NAFL groups were non-significantly different (p = 0.159), while, the difference was almost significant comparing controls vs. overweight NAFL participants (p = 0.051) and was highly significant different comparing controls vs. obese NAFL subjects (p < 0.001), where GLP-1 level was more than halved.

In Table 2, the correlation was analyzed to determine the relationship between the biomarkers within each group. Most correlations were positive but non-significant. In the healthy controls, only GLP-1 and leptin correlated significantly positively (r = 0.794 and p < 0.001). Among the overweight NAFL subjects, there were two significant positive correlations between ghrelin and insulin (r = 0.713 and p < 0.001) and between GLP-1 and leptin (r = 0.987 and p < 0.001). Among the obese NAFL participant, there was one significant positive correlations between GLP-1 and leptin (r = 0.991 and p < 0.001), as depicted in Figure 1.

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Feeding suppression and weight loss induced by GLP-1 receptor activation\(^{46}\). Adiposity is not only connected to NAFL disease through such hormonal disturbances, but rather, it is a pathogenetically multifactorial disease, including gut microbiome dysbiosis, endotoxins, immunomodulation and endogenous alcohol production\(^{46}\). This may suggest that the disease indeed is not a simple one. Limitations we faced include the small size of the study sample due to the stringent exclusion criteria we implemented. Although histopathological analysis of a liver biopsy specimen is still the gold standard for the diagnosis and staging of fibrosis/inflammation, due to its known relevance limitations and complications we did not utilize. The other alterations in magnetic resonance spectroscopy and 2D-SWE ultrasound echotomography, were not available for our use. With criteria used for characterizing our participants, still those with low degree of steatosis could have been missed - despite excluding morbid obese. Although we excluded participants with diabetes or prediabetes, we did not correlate our finding with insulin resistance.

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