

## IGF-1 levels in different stages of liver steatosis and its association with metabolic syndrome

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### Summary

**Background and aims.** Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome related to insulin resistance. Insulin-like growth factor 1 (IGF-1) is mainly produced by hepatocytes and its secretion is stimulated by growth hormone. Our aim was to assess possible changes in IGF-1 levels in patients with different ultrasonography stages of NAFLD and its association with hyperlipidemia, impaired glucose tolerance, non-insulin dependent type 2 diabetes, waist circumference, obesity and arterial hypertension. **Methods.** One hundred and ten consecutive patients were evaluated. **Results.** IGF-1 levels decreased as liver steatosis worsened. There was a statistically significant difference between mild-moderate steatosis on one hand, and severe steatosis on the other (142 vs. 110,  $P < 0.05$ ). Homeostasis model assessment of insulin resistance (HOMA) and insulin levels showed a tendency to inverse association with IGF-1, but it was not statistically significant. HOMA significantly increased in severe liver steatosis when compared with mild-moderate steatosis (6.20 vs. 3.99,  $P < 0.05$ ). Insulin levels also showed a significant increase ( $3.01 \pm 0.61$  vs.  $2.59 \pm 0.56$ ,  $P < 0.05$ ). Body mass index showed a significant inverse correlation with IGF-1 level ( $r = -0.19$ ,  $P < 0.05$ ) and a tendency to increase as liver steatosis worsened. Waist circumference increased significantly as liver steatosis worsened (severe vs. mild-moderate: 114 vs. 100,  $P <$

0.05). **Conclusions.** IGF-1 levels showed a decrease as liver steatosis worsened. This difference was statistically significant between mild-moderate and severe steatosis. Inverse correlation between IGF-1 levels and BMI was also statistically significant. There was no statistically significant correlation between IGF-1 levels and HOMA and insulin levels.

**Key Words.** IGF-1, liver steatosis, metabolic syndrome.

### Niveles de IGF-1 en diferentes estadios de esteatosis hepática y su relación con el síndrome metabólico

#### Resumen

**Antecedentes y objetivos.** La esteatosis hepática no alcohólica es la manifestación hepática del síndrome metabólico relacionado con la insulinoresistencia. El factor de crecimiento insulínico tipo 1 (IGF-1) es producido principalmente por los hepatocitos y su secreción está estimulada por la hormona de crecimiento. Nuestro objetivo fue evaluar posibles cambios en los niveles de IGF-1 en pacientes con diferentes estadios ecográficos de esteatosis hepática y su relación con hiperlipidemia, intolerancia a la glucosa, diabetes tipo 2 no insulino-dependiente, circunferencia de cintura, obesidad e hipertensión arterial. **Métodos.** Fueron evaluados 110 pacientes consecutivos. **Resultados.** Los niveles de IGF-1 fueron disminuyendo a medida que la esteatosis hepática empeoraba. Encontramos una diferencia estadísticamente significativa entre los grados leve-moderado por un lado, y severo por el otro (142 vs. 110,  $P < 0,05$ ). El índice de resistencia insulínica (HOMA) y los niveles de insulina mostraron una tendencia de correlación inversa con IGF-1, pero no fue estadísticamente significativo. El HOMA aumentó significativamente en la esteatosis severa cuando se comparó con el estadio leve-moderado (6,20

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vs. 3,99,  $P < 0,05$ ). Los niveles de insulina también mostraron un aumento significativo ( $3,01 \pm 0,61$  vs.  $2,59 \pm 0,56$ ,  $P < 0,05$ ). El índice de masa corporal (IMC) mostró una significativa correlación inversa con los niveles de IGF-1 ( $r = -0,19$ ,  $P < 0,05$ ). La circunferencia de cintura aumentó significativamente a medida que la esteatosis empeoraba. **Conclusiones.** Los niveles de IGF-1 disminuyeron a medida que la esteatosis hepática empeoraba. Esta diferencia fue estadísticamente significativa entre los estadios leve-moderado y severo. La correlación inversa entre IGF-1 e IMC también fueron significativos.

**Palabras claves.** IGF-1, esteatosis hepática, síndrome metabólico.

#### Abreviaturas.

NAFLD. non-alcoholic fatty liver disease.

IR. insulin resistance.

IGF-1. insulin-like growth factor 1.

GH. growth hormone, IGT: impaired glucose tolerance.

HOMA. homeostasis model assessment of insulin resistance.

BMI. body mass index.

NASH. non-alcoholic steatohepatitis.

IGFBPs. IGF1-binding proteins.

T2 DM. type 2 diabetes mellitus.

FFA. free fatty acids, ALT: alanine aminotransferase.

IFT. impaired fasting glucose.

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of a broad metabolic syndrome that includes different disorders related to insulin resistance (IR), such as obesity, type 2 diabetes (T2 DM), hypertension and hyperlipidemia.<sup>1</sup> Liver steatosis is the most frequent hepatic lesion in Western countries, with an estimated prevalence of 10% to 25%. The most severe forms (non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis) occur in about 2% to 7%.<sup>2</sup>

Hepatocytes are the main source of circulating insulin-like growth factor 1 (IGF-1), whose secretion is stimulated by growth hormone (GH).<sup>3</sup> Circulating IGF-1 levels could be important in the pathogenesis of T2 DM, either through the regulation of insulin sensitivity or the maintenance of  $\beta$ -cell mass.<sup>4</sup>

The presence of fatty liver is currently considered as a very early and sensitive indicator of insulin resistance.<sup>5</sup> Insulin resistance leads to an excessive accumulation of triglyceride in the hepatocyte through an increased release of free fatty acids (FFA) from the adipose tissue, as a result of acce-

lerated lipolysis. Hyperinsulinemia also favours the formation of triglycerides instead of mitochondrial oxidation.<sup>6</sup> Sandhu et al, in a prospective observational study, showed that high circulating IGF-1 levels were associated with reduced risk of development of impaired glucose tolerance (IGT) or T2DM in individuals with normal glucose concentrations at baseline.<sup>7</sup> In cross-sectional studies, Efstratiadis et al<sup>8</sup> found low concentrations of IGF-1 in subjects with metabolic syndrome, regardless of the presence of diabetes mellitus, and García-Galiano et al<sup>9</sup> showed that IGF-1 is an independent prognostic factor of liver steatosis and non-alcoholic steatohepatitis in morbidly obese patients and that its level decreases throughout the progression of NASH.

The aim of the present study was to assess possible changes in IGF-1 levels in patients with different ultrasonography stages of non-alcoholic liver steatosis and its association with the components of metabolic syndrome, hyperlipidemia, impaired glucose tolerance, non-insulin dependant type 2 diabetes, waist circumference, obesity, arterial hypertension and impaired liver function tests such as elevated alanine aminotransferase (ALT).

## Patients and methods

### Patients

Patients were consecutively enrolled in one medical center from January 10<sup>th</sup> to December 20<sup>th</sup> 2008. A descriptive cross-sectional study was conducted in 110 patients (41 to 85 years-old, 82 women) with liver steatosis confirmed by abdominal ultrasonography. In all patients IGF-1 was measured together with other related biochemical and clinical variables. According to ultrasonographic criteria, liver steatosis is present when the accumulation of fat exceeds 5% of the liver weight, and it is classified in mild, moderate and severe. In grade 1 (mild), echogenicity is slightly increased, with normal visualization of the diaphragm and intrahepatic vessel borders. In grade 2 (moderate), echogenicity is moderately increased, with slightly impaired visualization of the diaphragm or intrahepatic vessels. In grade 3 (severe), echogenicity is markedly increased, with poor or no visualization of the diaphragm, intrahepatic vessels and posterior portion of the right lobe.<sup>10-11</sup> All the ultrasonographies were performed by the same physician in order to avoid operator dependence. An Echotomography Toshiba Aplio plus with a

2-5 MHz transducer was used. We compared two groups (mild and moderate vs. severe liver steatosis) in order to show the difference in IGF-1 levels when the function of hepatic cellular mass was extremely diminished.

Body mass index (BMI) was calculated and considered normal when it was lower than 25 kg/m<sup>2</sup>, overweight between 25 and 29.9 kg/m<sup>2</sup> and obesity when it was higher than 30 kg/m<sup>2</sup>. Waist circumference and blood pressure were also measured. T2DM was defined when glucose levels were higher than 126 mg/dL (7.0 mmol/L). Impaired fasting glucose (IFT) was diagnosed when glucose levels were between more than 110 mg/dL (6.1 mmol/L) and less than 125 mg/dL (6.9 mmol/L). IGT was diagnosed when glucose levels 2 hours after a 75 g glucose challenge were between 140 and 199 mg/dL in the oral glucose tolerance test (OGTT).<sup>12</sup> Insulin resistance was determined when homeostasis model assessment of insulin resistance (HOMA), calculated as insulin (mU/L) x glucose (umol/L) /22.5, was higher than 3.<sup>13</sup> Metabolic syndrome was defined by any three of the following five features: waist circumference higher than 102 cm in men or 88 cm in women, triglycerides higher than 150 mg/dL, HDL-cholesterol lower than 40 mg/dL in men or 50 mg/dL in women, blood pressure higher than 130/85 mmHg and glucose higher than 110 mg/dL (6.1 mmol/L).<sup>14</sup> We excluded: acromegaly, insulin dependant diabetes, uncontrolled hypothyroidism, viral hepatitis and pregnancy.

An informed consent was obtained from each patient and was approved by the Ethical Committee of Hospital Militar Central.

### Biochemical analysis

Total IGF-1 level was measured with a two-site immunoradiometric assay with ethanol-acid extraction (IRMA-DSL). We used the reference "normal" according to the normal range given by manufacturers, adjusted by age and gender. Calibration RIR for IGF-1 (OMS 87/518) was performed, coefficient of variation (CV) was 8% intra-assay and 11% inter-assay, and analytical sensitivity was 12 ng/mL. Glucose was measured by hexokinase method (Roche Diagnostic) with a 6.1% CV inter-assays at 100 mg%. Liver function tests: ALT, gamma-glutamyl-transpeptidase, total bilirubin, HDL and LDL cholesterol, and triglycerides were measured with

routine methods. Insulin was measured by an electrochemiluminescence immunoassay "ECLIA" (Roche Diagnostics) with a 4.5% and 3.3% CV inter-assays at 23 and 76.5 UI/mL, respectively.

Antibody to hepatitis C was assessed by an enzyme-linked immunoassay test of second generation (ELISA, Abbott Laboratories, Chicago, IL) in accordance with the manufacturer's instructions.

### Statistic analysis

Data on continuous variables are expressed as mean  $\pm$  standard deviation. Data on qualitative characteristics are given as percent values. Measurements performed in mild to moderate and severe liver steatosis were compared by means of unpaired-sample t tests, while relationships between IGF-1 and other clinical and humoral variables were assessed by their linear correlation coefficients. Before analysis, care was taken to check for normality of sample distributions and the appropriate corrections were performed when required. Statistical significance was set at  $P < 0.05$ . The SSPS for Windows 13.0 statistical package was used for the analysis.

### Results

One hundred and ten patients were evaluated. Clinical and biochemical variables are shown in Tables 1 and 2. ALT was elevated in 14% of patients, diabetes was present in 19% and IGT was found in 12%. Obesity was observed in 68% of patients and overweight in 25%. Waist circumference ranged from 77 to 120 cm in women and from 82 to 153 cm in men. Thirty-four percent of women presented HDL lower than 50 mg% and 72% of men HDL lower than 40 mg%.

IGF-1 levels decreased as liver steatosis worsened, there was a statistically significant difference between mild and moderate steatosis on one hand and severe steatosis on the other (142 vs. 110,  $P < 0.05$ ) (Figure 1).

Total, HDL and LDL cholesterol, triglycerides, glycemia, insulin level, waist circumference, age, arterial hypertension and ALT were not correlated with IGF-1 levels. HOMA and insulin levels showed tendency to inverse association with IGF-1, but it was not statistically significant. HOMA significantly increased in severe liver steatosis when compared with mild and moderate (6.20 vs. 3.99,  $P$

**Table 1.** Clinical and biochemical variables in 110 individuals with liver steatosis.

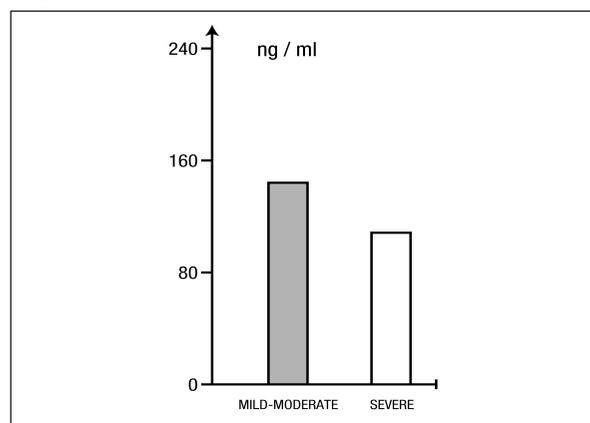
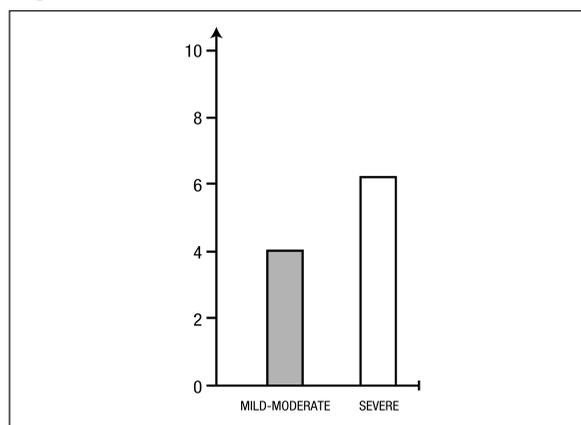
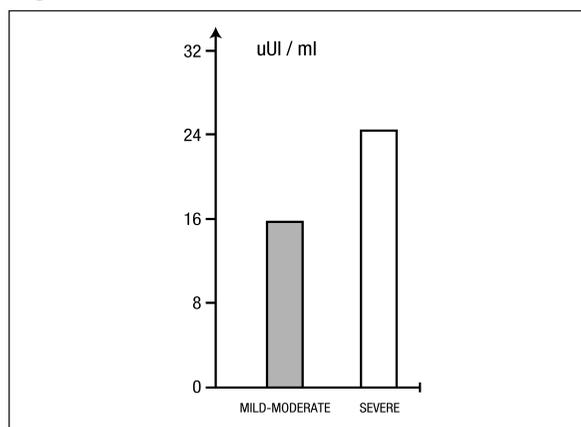
Age (years)		60±9.4 [41 - 85]
Gender (female/male)		75% / 25%
BMI (kg/m <sup>2</sup> )		33±6.1 [20 - 53]
Waist Circumference (cm)	female	101±10 [77 - 120]
	male	106±19 [82 - 153]
Diabetes (%)		19%
Hypertension (%)		48%
Total cholesterol (mg/dL)		210±45 [110 - 318]
HDL cholesterol (mg/dL)	female	55±14 [30 - 89]
	male	43±14 [27 - 89]
LDL cholesterol (mg/dL)		124±38 [24 - 213]
Triglycerides (mg/dL)		170±94 [59 - 681]
Glucose (mg/dL)		104±14 [62 - 179]
Insulin (uIU/ml)		16.5±10.6 [2 - 78]
HOMA		4.25±2.90 [0.20 - 19.4]

Continuous variables are given as mean ± SD [range].  
The other variables are expressed as percentages

**Table 2.** Clinical and biochemical variables in 110 individuals according to their degree of liver steatosis.

	Liver steatosis	
	Mild and moderate	Severe
Age (years)	60±9.6	60±8.2
Body mass index (kg/m <sup>2</sup> )	32.7±6.1	35.9±5.6 *
Waist circumference (cm)	100±12	114±18 **
Total cholesterol (mg/dL)	211±46	199±39
HDL cholesterol (mg/dL)	52±14	51±22
LDL cholesterol (mg/dL)	125±39	117±35
Triglycerides (mg/dL)	172±98	154±58
Glucose (mg/dL)	104±14	103±16
Insulin (uIU/ml)	15.6±9.9	23.7±13.5 *
HOMA	3.99 ± 2.62	6.20 ± 4.11 *
Hypertension	46%	62%

\*  $P < 0.05$  vs mild and moderate, \*\*  $P = 0.07$  vs mild and moderate.

**Figure 1.** IGF-1 levels in liver steatosis.**Figure 2.** HOMA in liver steatosis.**Figure 3.** Insulin levels in liver steatosis.

< 0.05). Insulin levels also showed a significant increase ( $3.01 \pm 0.61$  vs.  $2.59 \pm 0.56$ ,  $P < 0.05$ ) (Figures 2 and 3). BMI showed a significant inverse correlation with IGF-1 levels ( $r = -0.19$ ,  $P < 0.05$ ) and a tendency to increase as liver steatosis worsened (severe vs. mild and moderate:  $35.9$  vs.  $32.7$ ,  $P < 0.07$ ). Waist circumference increased significantly as liver steatosis worsened (severe vs. mild and moderate:  $114$  vs.  $100$ ,  $P < 0.05$ ).

## Discussion

It is not well defined how the sequence of events and the mediators involved in the association of visceral fat with insulin resistance and progression of liver disease are triggered. Lipotoxicity describes the damage that occurs when fat supply surpasses the storage and oxidative capacity of skeletal muscle, li-

ver or pancreatic beta-cells. Lipid flux in excess is redirected into harmful pathways of non-oxidative metabolism with intracellular accumulation of toxic metabolites that renders these tissues resistant to the action of insulin and elicits damaging inflammatory responses. It is important to emphasize that triglycerides accumulation per se is not the unique harmful event, but rather are the lipid-derived metabolites that trigger the formation of reactive oxygen species (ROS) and activation of inflammatory pathways with cellular apoptosis.<sup>15-16</sup>

Under conditions of long-term energy excess intake, carbohydrates and fatty acids convert to triglycerides for storage. The visceral adipose tissue has more metabolic activity than the more abundant subcutaneous adipose tissue, and the release of FFAs and adipokines from visceral fat depots, secreted directly into the portal circulation, is one of the mechanisms of hepatic injury, acting through the increase of lipids in the hepatic cells.<sup>17</sup> We believe that this is the most important pathogenic mechanism involved in liver steatosis. FFAs and adipokines released from visceral fat into portal circulation cause peripheral hyperinsulinemia by retarding insulin clearance and increasing lipid synthesis.<sup>13</sup>

In advanced liver disease, the differentiation between severe NAFLD and NASH depends on liver lesions which may progress with the association of variable degree of necro-inflammation and fibrosis through altered adipocytokine profiles (low adiponectin, and high TNF- $\alpha$ , interleukin-6, C-reactive protein and others).<sup>8,9,17,18</sup> The presence of NAFLD might also predict the development of metabolic disorders due to insulin resistance, thus heralding the development of central obesity, high triglycerides, low HDL cholesterol, hypertension and glucose abnormalities. We believe that obesity, through a reduction of the mass of hepatic cells in severe steatosis, is the principal cause of NAFLD.

IGF-1 is a peptide with structural homology to proinsulin, physiologically similar to insulin, but it is a much more potent mitogen and antiapoptotic agent, and its insulin-like action is modulated by six specific binding proteins that are produced in the liver. IGF-1 is the only acutely regulated IGF-1 and its production is inhibited by insulin.<sup>7</sup> In the circulating blood, most of the IGF-1 is bound to serum IGF binding protein-3 (IGFBP-3).<sup>20-22</sup>

Skeletal muscle appears to be particularly sensitive to insulin-like actions, whereas mature adipo-

cytes and liver seem to be very insensitive to IGF-1.<sup>23</sup> Insulin suppresses production of IGFBP-1 and increases sensitivity of growth hormone receptor in the liver, except in chronic hyperinsulinemia where the expression of GH receptor decreases because there is a reduction in GH receptor expression and signaling in the liver.<sup>24</sup> GH exerts direct effects on insulin action and indirect effects through increased lipolysis, resulting in elevated FFAs levels which impair insulin sensitivity of both the liver and muscle.<sup>4</sup> On the other hand, suppression of IGFBP-1 by insulin might increase bioavailability of IGF-1 which could lead to inhibition of GH release by the pituitary gland and a consequent reduction of IGF-1 in the liver.<sup>7</sup>

Völzke et al showed that hepatic steatosis is associated with low serum IGF-1 levels and its association is independent of alcohol consumption.<sup>22</sup> The progressive reduction of IGF-1 concentration in blood from patients with severe obesity might be the consequence of the reduction of functional liver cells by the steatosis.<sup>9</sup> This finding agrees with our findings that showed a statistically significant difference between IGF-1 levels in mild-moderate steatosis on one hand and severe on the other, with a decrease of IGF-1 as liver steatosis worsened. Similarly there was an inverse correlation between IGF-1 levels and BMI: when weight increased, IGF-1 decreased.

Our results showed that there was not statistically significant correlation between IGF-1 levels and insulin resistance parameters, such as HOMA and insulin levels, in accordance with NHANES study.<sup>20</sup> Our study also showed that there was a statistically significant association between HOMA and the severity of liver steatosis.

The risk of steatosis at ultrasonography is increased by a factor of 3 in the presence of overweight and peaks at a factor of approximately 15 in the presence of obesity. Most patients with ultrasonographically detectable liver disease may have normal liver enzymes. Therefore, liver enzymes are considered poor predictors of the severity of liver disease.<sup>17</sup> The prevalence of unexplained elevations in ALT in individuals from the National Health and Nutrition Survey (NHANES III) with metabolic syndrome was 7%.<sup>18</sup> Liver biopsy is the most sensitive test for the differential diagnosis between NAFLD and NASH because it has 100% of specificity.<sup>25</sup> The accuracy of ultrasonography for the diagnosis of liver

steatosis and its severity is about 92%. However, this difference does not justify the use of biopsy for epidemiological purposes.<sup>19-25</sup> Therefore, the ultrasonography would be the simplest and most reliable diagnostic method for the detection of NAFLD, mainly because it is not invasive and easy to perform as many times as necessary in order to follow up the evolution of liver disease and to determine the optimal timing for a liver biopsy. Ultrasonography enabled us to differentiate mild and moderate steatosis from severe steatosis because there is a stark decrease in IGF-1 levels in severe steatosis.

This study has two limitations. First, we did not assess IGF-1 binding proteins which regulate bioavailability of circulating IGF-1. Second, we used only one assay (IRMA) for the determination of total IGF-1 that is subjected to binding proteins interference.

In conclusion, IGF-1 levels showed a decrease as liver steatosis worsened, and this difference was statistically significant between mild and moderate steatosis and severe steatosis. Inverse correlation between IGF-1 levels and BMI was also statistically significant. The lower level of IGF-1 might be related to the reduction in the hepatic cell mass due to the worsening of liver steatosis when obesity increases. Our results showed that there was not statistically significant correlation between IGF-1 levels and HOMA and insulin levels. Although liver biopsy remains the gold standard in the evaluation of NAFLD, ultrasonography would be the first diagnostic method for its detection because it is noninvasive, reliable, inexpensive and easily repeatable.

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