Published in final edited form as:
J Gastroenterol Hepatol. 2014 March ; 29(3): 589–596.

The relationship between serum circulating IGF-1 and liver fat in the United States

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Abstract

Background and Aim—Nonalcoholic fatty liver disease (NAFLD), circulating insulin-like growth factor-1 (IGF-1) and IGF-1/IGF binding protein-3 (IGFBP-3) concentrations are associated with adiposity and insulin resistance. We aimed to determine whether serum IGF-1, IGFBP-3 and IGF-1/IGFBP-3 are associated with presence or severity of NAFLD independent of potential confounding.

Methods—We performed a cross-sectional analysis of data from the Third National Health and Nutrition Examination Survey, 1988–1994, a representative sample of the United States adult population. Among participants who had a fasting blood draw and ultrasound examination we excluded those with missing data, viral hepatitis, iron overload, excessive alcohol intake, pregnancy or taking glucose-lowering therapy, yielding 4172 adults for this analysis.

Results—In logistic regression analyses adjusted for age, gender, and race/ethnicity, higher IGF-1 and IGF-1/IGFBP-3 quartiles were associated with lower likelihood of NAFLD and lower grade steatosis. These associations became non-significant when further adjusted for adiposity (BMI, waist circumference) with the exception of the association between IGF-1/IGFBP-3 and severity of NAFLD which remained significant after adjustment for HOMA-IR (OR (95% CI): Q3:0.71 (0.53–0.96), Q4:0.62 (0.43–0.89)) and adiposity (Q4: 0.67 (0.47–0.96)). Full adjustment (age, gender, race/ethnicity, adiposity, HOMA-IR, A1C%) further attenuated associations between IGF-1 or IGF-1/IGFBP-3 and liver fat such that they were no longer significant.

Conclusions—Adiposity explains much of the observed association between IGF-1 or IGF-1/IGFBP-3 and liver fat. These findings do not support a direct role for the GH-IGF-1/IGFBP-3 axis in the pathophysiology of NAFLD.

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DISCLOSURE STATEMENT
The authors have nothing to disclose.

Key terms

Insulin-Like Growth Factor I; Insulin-Like Growth Factor Binding Protein-3; Fatty Liver; National Health and Nutrition Examination Survey

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a condition of increased fat in the liver in the absence of significant alcohol intake. Although the etiology of NAFLD remains unclear, the growth hormone (GH)-IGF-1 axis may be involved. NAFLD is often coexistent with conditions of obesity, insulin resistance and the metabolic syndrome[1] as well as untreated GH deficiency in adults[2]. Replacement with GH therapy is reported to improve liver steatosis[3,4] and in epidemiological studies, an inverse association between serum IGF-1 concentration and fatty liver disease has been observed [5–9]. IGF-1 is primarily synthesized in the liver in response to GH [10]. Exogenous IGF-1 prevents excess liver fat in GH deficient rats [11] by an underlying mechanism that is still unknown. The observed reduction in liver fat in association with GH treatment in humans may likewise be due to an increase in circulating IGF-1.

At this time it is unknown whether low IGF-1 levels are a cause or consequence of accumulation of excess liver fat [12], or simply an innocent bystander. A direct effect of IGF-1 on hepatic fatty acid metabolism is unlikely as there are very few hepatic IGF-1 receptors [13], but this possibility cannot be ruled out. Lower IGF-1 concentration in NAFLD may simply reflect decreased synthesis in the presence of liver disease. Low IGF-1 levels have been associated with fibrotic stages of non-alcoholic steatohepatitis (NASH) [6,14] and also observed in patients with other causes of hepatic fibrosis [15]. They do not appear to be related to the etiology of cirrhosis [16,17]. However, IGF-1 levels have also been shown to be lower at earlier stages of NAFLD [8,9]. Alternatively, lower IGF-1 levels may have indirect effects that contribute to development of NAFLD, such as excess adiposity or insulin resistance, both of which are strongly associated with NAFLD [18].

IGF binding protein-3 (IGFBP-3) is the primary binding protein for circulating IGF-1 and the ratio of IGF-1/IGFBP-3 provides an indication of IGF-1 bioavailability (free IGF-1 is considered the bioactive form) [19]. Several cross-sectional studies, varying in study population and statistical approach, have evaluated NAFLD in association with circulating levels of IGF-1 and IGF-1/IGFBP-3 [5–9,14]. Validation of the association between IGF-1 and NAFLD in a large population-based, multi-ethnic sample is needed with additional determination of whether this association persists after appropriate adjustment for potential confounders such as adiposity and insulin resistance.

METHODS

Study design

We performed a cross-sectional analysis using data from the Third National Health and Nutrition Examination Survey (NHANES III) conducted among the non-institutionalized US population from 1988 to 1994 [20]. NHANES used a complex, weighted survey design to obtain a representative sample of the US population and included deliberate oversampling of the elderly and certain racial/ethnic minorities. The survey included a home interview, physical examination, laboratory measurements and ultrasonography of the liver and gallbladder performed in a mobile examination center.

Subjects

Data for this analysis included subjects who had both a morning fasting blood draw and an interpretable hepatic ultrasound examination (n=6069) performed at the mobile examination center. After excluding subjects with missing data; positive Hepatitis C antibody and/or Hepatitis B surface antigen or borderline status; possible iron overload (serum ferritin > 500 mg/mL for men or > 400 ng/mL for women and transferrin saturation > 45%); excessive alcohol intake defined as > 2 drinks daily for men and > 1 drink daily for women; pregnancy; or those taking glucose-lowering medications, data on 4172 adults were included.

Laboratory methods

Fasting blood was obtained by venipuncture, processed locally and stored. Samples were shipped to a centralized laboratory for analysis. Procedures for NHANES laboratory quality control measures have been previously reported [21]. ELISA quantification of serum IGF-I (ng/ml) and IGFBP-3 (ng/ml) were performed using Diagnostic Systems Laboratories Inc (DSL, Webster TX) reagents and standard protocols [22]. Serum insulin was quantified by radioimmunoassay using a Berthold model multi-crystal gamma counter (Berthold, Nasua, NH) and Pharmacia Insulin RIA kit (Pharmacia Diagnostics AB, Uppsala, Sweden). Serum glucose was quantified using the hexokinase method (Roche Diagnostic Systems, Inc., Montclair, New Jersey). Measurement of glycated hemoglobin A1C% was performed by automated high-performance liquid chromatography system (Bio-Rad DIAMAT glycosylated hemoglobin analyzer system, Bio-Rad Laboratories, Hercules, CA).

Ascertainment of hepatic steatosis, insulin resistance and diabetes mellitus

Ultrasound images for assessment of hepatic steatosis were obtained from archived liver and gall bladder ultrasound images originally obtained between 1988 and 1994 using a Toshiba (Tustin, CA) SSA-90A machine using two 3.75 and one 5.0 MHz transducers. Details of the protocol are published elsewhere [23–25]. The presence of fat within the hepatic parenchyma was graded “normal, mild, moderate or severe” by 3 trained ultrasound readers under quality controlled supervision of a board certified hepatic radiologist from 2009 to 2010; readings were standardized using quality assurance procedures. We defined NAFLD as having any grade of liver fat (mild, moderate or severe). An estimate of insulin resistance, Homeostasis model assessment for insulin resistance (HOMA-IR), was calculated by taking the product of fasting insulin (μU/mL) and glucose (mg/dL) and dividing this by 405 [26]. Diabetes was defined as hemoglobin A1C% ≥ 6.5% [27].

Statistical Methods

We used bivariate linear models and chi-square analyses to assess for associations between NAFLD severity, IGF-1, IGFBP-3, IGF-1/IGFBP-3 quartiles and subject characteristics. The purpose of our initial statistical analyses was to show crude (unadjusted) associations and minimally-adjusted associations (age, gender, and race/ethnicity) between IGF-1, IGFBP-3, IGF-1/IGFBP-3 and the presence or severity of NALFD. To further evaluate the role of adiposity, insulin resistance or diabetes in associations between IGF-1, IGFBP-3 or IGF-1/IGFBP-3 and NAFLD, we utilized the following additional multivariate models:

- **a.** HOMA-IR as well as age, gender, race/ethnicity, in order to adjust for insulin resistance.
- **b.** BMI and waist circumference as well as age, gender, race/ethnicity, in order to adjust for adiposity.
- **c.** A1C% as well as age, gender, race/ethnicity, in order to adjust for diabetes.

d. BMI, waist circumference, HOMA-IR and A1C%, as well as age, gender, race/ethnicity, our "fully adjusted model", in order to test for any association that is independent of potential confounding by the above factors.

NAFLD was the main outcome variable and was dichotomized as absent or present (mild, moderate or severe) or modeled as an ordered categorical variable. Odds ratios were calculated with the lowest quartile of IGF-1, IGFBP-3 or IGF-1/IGFBP-3 serving as the reference category. To evaluate the association between IGF-1, IGFBP-3 or IGF-1/IGFBP-3 and liver fat grade, we performed ordered logistic regression analysis using the ologit STATA command with grade of liver fat (1=mild, 2=moderate or 3=severe liver fat) versus none as the outcome (dependent) variable. We performed a test for effect modification by adiposity by assessing significance of interaction terms (IGF, IGFBP-3 or IGF-1/IGFBP-3 quartiles × BMI) inserted in the models. Finally, we performed tests for trend for IGF, IGFBP-3 or IGF-1/IGFBP-3 quartiles (independent variables) and presence/absence of NAFLD or NAFLD severity (dependent variables) with above adjustments. In order to assess potential selection bias in our findings, we compared those subjects with an interpretable ultrasound who fasted overnight (n=5481) and non-fasting subjects with an interpretable ultrasound (n=4659) after making exclusions for positive hepatitis status, possible iron overload, excessive alcohol intake, pregnancy and use of glucose-lowering medications. Per the NHANES protocol, households were randomly assigned to a morning versus an afternoon/evening exam time. Analyses were performed using STATA SE 12 (College Station, TX). All analyses were performed using survey commands to account for sampling weights and the complex survey design stratum and cluster. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 4172 adults included in this analysis, approximately 33% (n=1390) had NAFLD. Unadjusted analyses are presented in Tables 1 and 2. The amount of liver fat by ultrasound was positively associated with age, BMI, waist circumference, HOMA-IR, A1C%, presence of diabetes and Mexican-Hispanic race/ethnicity and was negatively associated with IGF-1, IGF-1/IGBP-3 and Black race/ethnicity. Liver fat grade was not associated with IGFBP-3 levels (Table 1).

Higher IGF-1 quartiles were associated with male sex, younger age, lower BMI, lower waist circumference, lower HOMA-IR, lower A1C%, absence of diabetes, absence of NAFLD and race/ethnicity other than Mexican-Hispanic (Table 2). Higher IGFBP-3 quartiles were associated with younger age, lower waist circumference, lower A1C%, White race/ethnicity, and race/ethnicity other than Mexican-Hispanic or Black. Higher IGF-1/IGFBP-3 quartiles were associated with male sex, younger age, Black race/ethnicity, lower BMI, lower waist circumference, lower HOMA-IR, lower A1C%, absence of diabetes, and absence of NAFLD.

In unadjusted logistic regression analyses, increasing serum IGF-1 quartile was associated with a declining trend in odds of NAFLD (Table 3). These associations remained statistically significant within the upper two IGF-1 quartiles after adjustment for age, gender, race/ethnicity and additionally A1C%. However, adjustment for HOMA-IR or BMI and waist circumference in place of A1C% resulted in loss of significance between IGF-1 quartile and odds of NAFLD. Further analyses considered IGFBP-3 and IGF-1/IGFBP-3 quartiles in relation to NAFLD prevalence. Serum IGFBP-3 quartile was not associated with odds of NAFLD in crude or adjusted models (Table 3). In unadjusted logistic regression analyses, increasing IGF-1/IGFBP-3 quartile was associated with a significant declining trend in odds of NAFLD (Table 3). These associations remained significant after adjustment for age, gender, race/ethnicity and A1C%. The association between IGF-1/IGFBP-3 quartile

and NAFLD became non-significant after additional adjustment for BMI and waist circumference and in all but the highest quartile after adjustment for HOMA-IR (Q4: p=0.032). In fully adjusted (waist circumference, BMI, HOMA and A1C%, age, gender, race/ethnicity) models, there was no significant association between level of IGF-1, IGFBP-3 or IGF-1/IGFBP-3 and the presence of NAFLD. There was no significant effect modification by BMI for the association between IGF-1 or IGF/IGFBP-3 quartiles and presence of NAFLD.

The test for trend of the inverse relationship between IGF-1 or IGF-1/IGFBP-3 quartiles and NAFLD remained significant after adjustment for age, gender, race/ethnicity and additional adjustment for HOMA (p=0.04 and p=0.03, respectively) and A1C% (p=0.02 and p<0.01, respectively), but was lost after additional adjustment for adiposity. The test for trend was not significant in the fully adjusted model. The test for trend between IGFBP-3 quartile and NAFLD was not significant in any model.

The upper two IGF-1 quartiles were inversely associated with severity of NAFLD in ordered logistic regression analyses adjusted for age, gender, race/ethnicity and additionally for A1C %. These associations became non-significant in all quartiles after additional adjustment for waist circumference and BMI, but remained significant in the highest quartile after adjustment for HOMA-IR (Table 4, p=0.04). All IGF-1/IGFBP-3 quartiles were inversely associated with severity of NALFD in ordered logistic regression analyses adjusted for age, gender, race/ethnicity and after additional adjustment for A1C%. These associations remained significant in the upper two IGF-1/IGFBP-3 quartiles after additional adjustment for HOMA-IR and in the highest IGF-1/IGFBP-3 quartile after adjustment for BMI and waist circumference (Table 4).

The test for trend of the inverse relationship between severity of NAFLD and IGF-1 or IGF-1/IGFBP-3 quartiles remained significant after adjustment for age, gender, race/ethnicity and additional adjustment for HOMA-IR (p=0.03 and p<0.01 for IGF-1 and IGF-1/IGFBP-3, respectively) and A1C% (p<0.01 for both IGF-1 and IGF-1/IGFBP-3). The test for trend for the inverse relationship between severity of NAFLD and IGF-1/IGFBP-3 quartiles remained marginally significant after additional adjustment for BMI and waist circumference (p=0.04), but was no longer significant for IGF-1. The test for trend between severity of NAFLD and IGFBP-3 quartile was not significant in any model. Furthermore, the test of trend of the inverse relationship between severity of NAFLD and IGF-1 or IGF-1/IGFBP-3 quartile was not significant in any fully adjusted model.

The comparison of subject characteristics between those with an interpretable ultrasound who fasted overnight and the non-fasting subjects with an interpretable ultrasound who were excluded from these analyses showed that, on average, those in the fasting group as compared to those in the non-fasting group were slightly older (average age of 43 versus 39 years old, p<0.01), had a higher BMI (average BMI was 26.6 versus 25.7 p=0.02) and had some differences in race/ethnicity (the fasting group had a higher percentage of white and fewer black subjects than the non-fasting group, p=0.03).

DISCUSSION

Previous studies have found a significant relationship between IGF-1 and liver fat [5–9,14], suggesting that the GH-IGF-1 axis may play an important role in the pathophysiology of NAFLD. In this large, U.S. population-based multi-ethnic cross-sectional study, we noted a strong trend, in the OR for presence or severity of NAFLD across quartiles of IGF-1 and IGF-1/IGFBP-3, after adjusting for age, gender, race/ethnicity and A1C%. However, these associations became non-significant after further adjustment for waist circumference and

BMI. The strongest association and most robust trend in adjusted models were seen between severity of liver fat and IGF-1/IGFBP-3 quartile. Despite confounding attributable to adiposity's strong positive relationship with NAFLD and its inverse relationship with IGF-1/IGFBP-3 level, an independent association remained between the highest IGF-1/IGFBP-3 level and mildest NAFLD after adjustment for BMI and waist circumference. This finding suggests that there may still be an important underlying etiological connection between the GH-IGF-1 axis and hepatic steatosis. However, in all fully adjusted models this association and trend were not significant, highlighting the importance of metabolic factors (related to glucose homeostasis and adiposity) in this relationship.

The complex interaction of the GH-IGF-1 axis and its relationship to the pathophysiology of hepatic steatosis is not yet clear. In those with GH deficiency, glucose tolerance and insulin sensitivity temporarily worsen and then improve with GH replacement, concurrent with favorable effects on free fatty acid metabolism [28,29] as well as changes in body composition such as increased muscle mass and decreased fat mass [30]. Conversely, states of GH excess are accompanied by glucose intolerance and insulin resistance despite increased IGF-I levels [28]. While some suggest NAFLD and hepatic insulin resistance may modulate circulating IGF-I levels [5], the reverse hypothesis is also possible [31]. Both GH and IGF-1 have been shown to prevent NASH in growth-hormone deficient rodent models [4] and treatment with GH improves NASH in GH deficient adult humans [4]. It is not clear if the observed reduction in liver fat associated with GH replacement in adults with GH deficiency is mediated indirectly by circulating IGF-1, by direct effects of GH [31] or by another mechanism related to changes in body composition (decreased fat and increased lean mass [30]) and insulin sensitivity [3,4].

Our findings suggest that circulating IGF-1 and IGF-1/IGFBP-3 levels are related to presence of NAFLD through confounding by age, gender, ethnicity, insulin resistance and adiposity. Adiposity may affect IGF-1 levels by decreasing binding protein levels (some of which were not measured in this study) [32] and by modifying the ghrelin (a GH secretagogue) / obestatin ratio [33]. Although findings regarding the relationship between adiposity and IGF-1 level are mixed, most epidemiological studies show an inverse relationship between IGF-1 and measures of adiposity [34]. Lower IGF-1/IGFBP-3 has been associated with obesity, diabetes and other components of metabolic disease in the NHANES III population and other epidemiological studies [34–37].

Although model adjustment for measures of adiposity affected the significance of the association between IGF-1 quartile and odds of liver fat, we noted no significant statistical interaction between adiposity and the association between NAFLD and IGF-1 quartile in these models. That is, the non-association (or marginal association) between IGF-1 quartile and odds of liver fat in adiposity-adjusted models is expected to hold at all levels of adiposity within the subjects' BMI range. Findings of another study showed that a significant relationship between IGF-1 and liver fat existed even at extreme adiposity. Serum IGF-1 level was found to be inversely associated with degree of hepatic steatosis on liver biopsy in 36 morbidly obese (BMI $\geq$ 40 kg/m^2) patients awaiting bariatric surgery [7]. In contrast, two other studies of biopsy proven-NAFLD patients found no significant difference in IGF-1 levels by amount of liver fat but did observe an inverse association with fibrosis [6,14]. These studies were limited by their small size (n= 92 and 55 respectively) and lack of adjustment for confounders such as adiposity, glycemia and insulin resistance. Thus, our study, which included a large number of subjects and adjusted for the main IGF-1 binding protein and confounders, confirmed only a weak association between degree of liver fat and IGF-1.

Our study contrasts with two other similarly large cross-sectional studies: the West Pomeranian study (n=3863) and the CATAMERI study (n=503). The West Pomeranian study was a population-based study in Germany [8] that examined the relationship between IGF-1/IGFBP-3 and severity of liver fat by ultrasound. They found that presence of hepatic steatosis by ultrasound was associated with lower IGF-1 and IGF-1/IGFBP-3 levels after adjusting for age, sex, BMI, waist circumference and presence of diabetes. Their findings differ from ours for several possible reasons. In addition to including subjects on diabetes medications (an exclusion in our study because of possible medication effects on liver fat), the West Pomeranian study used alternate statistical methodology. They modeled IGF-1 and IGF-1/IGFBP-3 as dependent categorical variables, BMI and waist circumference as categorical independent variables, did not adjust for insulin sensitivity, and defined outcomes based on presence or absence of ALT elevations with or without evidence of sonographic liver fat. Additionally, our population-based sample was inclusive of multiple ethnicities, whereas the West Pomeranian study presumably included only Caucasian subjects of German ethnicity.

The smaller Italian CATAMERI study also found significantly lower IGF-1 levels in individuals with sonographic evidence of NAFLD after adjusting for age, gender and BMI [5]. In contrast to our sampled population, the CATAMERI study specifically enrolled subjects with at least one risk factor for diabetes or cardiovascular disease. Additionally, in their statistical models, the CATMERI study did not report IGF-1/IGFBP-3, an indicator of bioavailable IGF-1 hormone levels, nor IGF binding protein levels. These differences in exclusion criteria and laboratory methods may have contributed to the dissimilarity of our findings.

A major strength of our study is its large, nationally representative sample inclusive of diverse ethnic/racial groups, thereby allowing for generalizability of our findings. Our study has several potential weaknesses. Cross-sectional analyses does not allow for examination of temporal sequence, and therefore no causal assessment can be made in this study. Approximately half of the subjects with interpretable ultrasounds were excluded as they were nonfasting and did not have blood samples measured for IGF-1. Despite the fact that morning vs. afternoon/evening exams were randomized by household, there was a small but statistically significant difference in age, BMI and ethnicity between the fasting and non-fasting groups. This may have introduced a selection bias in our results. However, because these differences were of small magnitude, but statistically significant due to the large NHANES sample size, the potential for bias is unlikely. The use of sonographic techniques for assessment of liver fat is not as sensitive as CT or magnetic resonance spectroscopy to detect lower levels of liver fat [38], but such techniques and the more invasive liver biopsy are simply not feasible for large cross-sectional studies. Despite lower sensitivity, the prevalence of some degree of hepatic steatosis in this large US population-based sample, within a dataset now more than 20 years old, was nearly a third and is likely to be even higher now.

In summary, we found that among a large population of adults including different racial/ethnic minorities, the association between IGF-1 or IGF-1/IGFBP-3 and presence of liver fat is explained by factors, namely age, sex, and adiposity. Our findings do not support a direct role for the GH-IGF-1/IGFBP-3 axis in the pathophysiology of NAFLD.

Acknowledgments

Grants and fellowships supporting the writing of this paper: T32HL007028, NIDDK DRC grant number P30DK017047

REFERENCES

1. Ioannou GN, Weiss NS, Boyko EJ, Kahn SE, Lee SP. Contribution of metabolic factors to alanine aminotransferase activity in persons with other causes of liver disease. Gastroenterology. 2005 Mar. 128:627–635. [PubMed: 15810122]
2. Ichikawa T, Hamasaki K, Ishikawa H, Ejima E, Eguchi K, Nakao K. Non-alcoholic steatohepatitis and hepatic steatosis in patients with adult onset growth hormone deficiency. Gut. 2003 Jun.52:914. [PubMed: 12740357]
3. Takahashi Y, Iida K, Takahashi K, et al. Growth hormone reverses nonalcoholic steatohepatitis in a patient with adult growth hormone deficiency. Gastroenterology. 2007 Mar.132:938–943. [PubMed: 17324404]
4. Nishizawa H, Iguchi G, Murawaki A, et al. Nonalcoholic fatty liver disease in adult hypopituitary patients with GH deficiency and the impact of GH replacement therapy. Eur J Endocrinol. 2012 Jul. 167:67–74. [PubMed: 22535644]
5. Arturi F, Succurro E, Procopio C, et al. Nonalcoholic fatty liver disease is associated with low circulating levels of insulin-like growth factor-I. J Clin Endocrinol Metab. 2011 Oct.96:E1640–E1644. [PubMed: 21816784]
6. Colak Y, Senates E, Ozturk O, et al. Serum concentrations of human insulin-like growth factor-1 and levels of insulin-like growth factor-binding protein-5 in patients with nonalcoholic fatty liver disease: association with liver histology. Eur J Gastroenterol Hepatol. 2012 Mar.24:255–261. [PubMed: 22157234]
7. Garcia-Galiano D, Sanchez-Garrido MA, Espejo I, et al. IL-6 and IGF-1 are independent prognostic factors of liver steatosis and non-alcoholic steatohepatitis in morbidly obese patients. Obes Surg. 2007 Apr.17:493–503. [PubMed: 17608262]
8. Volzke H, Nauck M, Rettig R, et al. Association between hepatic steatosis and serum IGF1 and IGFBP-3 levels in a population-based sample. Eur J Endocrinol. 2009 Nov.161:705–713. [PubMed: 19690083]
9. Mallea-Gil MS, Ballarino MC, Spiraquis A, et al. IGF-1 levels in different stages of liver steatosis and its association with metabolic syndrome. Acta Gastroenterol Latinoam. 2012 Mar.42:20–26. [PubMed: 22616493]
10. Daughaday WH, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev. 1989 Feb.10:68–91. [PubMed: 2666112]
11. Nishizawa H, Takahashi M, Fukuoka H, Iguchi G, Kitazawa R, Takahashi Y. GH-independent IGF-I action is essential to prevent the development of nonalcoholic steatohepatitis in a GH-deficient rat model. Biochem Biophys Res Commun. 2012 Jun 29.423:295–300. [PubMed: 22659415]
12. Bonefeld K, Moller S. Insulin-like growth factor-I and the liver. Liver Int. 2011 Aug.31:911–919. [PubMed: 21733081]
13. Caro JF, Poulos J, Ittoop O, Pories WJ, Flickinger EG, Sinha MK. Insulin-like growth factor I binding in hepatocytes from human liver, human hepatoma, and normal, regenerating, and fetal rat liver. J Clin Invest. 1988 Apr.81:976–981. [PubMed: 2832449]
14. Ichikawa T, Nakao K, Hamasaki K, et al. Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. Hepatol Int. 2007 Jun.1:287–294. [PubMed: 19669352]
15. Okan A, Comlekci A, Akpinar H, et al. Serum concentrations of insulin-like growth factor-I and insulin-like growth factor binding protein-3 in patients with chronic hepatitis. Scand J Gastroenterol. 2000 Nov.35:1212–1215. [PubMed: 11145295]
16. Colakoglu O, Taskiran B, Colakoglu G, Kizildag S, Ari Ozcan F, Unsal B. Serum insulin like growth factor-1 (IGF-1) and insulin like growth factor binding protein-3 (IGFBP-3) levels in liver cirrhosis. Turk J Gastroenterol. 2007 Dec.18:245–249. [PubMed: 18080921]
17. Vyzantiadis T, Theodoridou S, Giouleme O, Harsoulis P, Evgenidis N, Vyzantiadis A. Serum concentrations of insulin-like growth factor-I (IGF-I) in patients with liver cirrhosis. Hepatogastroenterology. 2003 May-Jun;50:814–816. [PubMed: 12828091]

18. Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2006 Dec.91:4753–4761. [PubMed: 16968800]
19. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev. 1997 Dec.18:801–831. [PubMed: 9408744]
20. Plan and operation of the Thirth National Health and Nutrition Examination Survey, 1988–1994. Vital and health statistics 1994. Vol Publication No (PHS) 94–1308: National Center for Health Statistics, Department of Health and Human Services; 1994.
21. Gunter EW, McQuillan G. Quality control in planning and operating the laboratory component for the Third National Health and Nutrition Examination Survey. J Nutr. 1990 Nov; 120(Suppl 11): 1451–1454. [PubMed: 2243286]
22. Berrigan D, Potischman N, Dodd KW, et al. Serum levels of insulin-like growth factor-I and insulin-like growth factor-I binding protein-3: quality control for studies of stored serum. Cancer Epidemiol Biomarkers Prev. 2007 May.16:1017–1022. [PubMed: 17507631]
23. Lazo M, Hernaez R, Bonekamp S, et al. Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. BMJ. 2011; 343:d6891. [PubMed: 22102439]
24. Third National Health and Nutrition Examination Survey: Gallbladder Ultrasonography Procedure Manual. 1988 Sep. http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/gallblad.pdf
25. Third National Health and Nutrition Examination Survey: hepatic steatosis assessment procedure manual. 2010. http://www.cdc.gov/nchs/data/nhanes/nhanes3/Hepatic_Steatosis_Ultrasound_Procedures_Manual.pdf
26. Rudenski AS, Matthews DR, Levy JC, Turner RC. Understanding “insulin resistance”: both glucose resistance and insulin resistance are required to model human diabetes. Metabolism. 1991 Sep.40:908–917. [PubMed: 1895955]
27. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010 Jan; 33(Suppl 1):S62–S69. [PubMed: 20042775]
28. Moller N, Jorgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. Endocr Rev. 2009 Apr.30:152–177. [PubMed: 19240267]
29. Krusenstjerna-Hafstrom T, Clasen BF, Moller N, et al. Growth hormone (GH)-induced insulin resistance is rapidly reversible: an experimental study in GH-deficient adults. J Clin Endocrinol Metab. 2011 Aug.96:2548–2557. [PubMed: 21613350]
30. Amato G, Mazziotti G, Di Somma C, et al. Recombinant growth hormone (GH) therapy in GH-deficient adults: a long-term controlled study on daily versus thrice weekly injections. J Clin Endocrinol Metab. 2000 Oct.85:3720–3725. [PubMed: 11061530]
31. Takahashi Y. Essential roles of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in the liver. Endocr J. 2012; 59:955–962. [PubMed: 22986486]
32. Ahmed RL, Thomas W, Schmitz KH. Interactions between insulin, body fat, and insulin-like growth factor axis proteins. Cancer Epidemiol Biomarkers Prev. 2007 Mar.16:593–597. [PubMed: 17372257]
33. Hassouna R, Zizzari P, Tolle V. The ghrelin/obestatin balance in the physiological and pathological control of growth hormone secretion, body composition and food intake. J Neuroendocrinol. 2010 Jul.22:793–804. [PubMed: 20456603]
34. Parekh N, Roberts CB, Vadiveloo M, Puvananayagam T, Albu JB, Lu-Yao GL. Lifestyle, anthropometric, and obesity-related physiologic determinants of insulin-like growth factor-1 in the Third National Health and Nutrition Examination Survey (1988–1994). Ann Epidemiol. 2010 Mar. 20:182–193. [PubMed: 20159489]
35. Lam CS, Chen MH, Lacey SM, et al. Circulating insulin-like growth factor-1 and its binding protein-3: metabolic and genetic correlates in the community. Arterioscler Thromb Vasc Biol. 2010 Jul.30:1479–1484. [PubMed: 20378848]
36. Rowlands MA, Holly JM, Gunnell D, et al. The relation between adiposity throughout the life course and variation in IGFs and IGFBPs: evidence from the ProtecT (Prostate tes ting for cancer and Treatment) study. Cancer Causes Control. 2010 Nov.21:1829–1842. [PubMed: 20652394]

37. Faupel-Badger JM, Berrigan D, Ballard-Barbash R, Potischman N. Anthropometric correlates of insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3) levels by race/ethnicity and gender. Ann Epidemiol. 2009 Dec.19:841–849. [PubMed: 19944347]

38. Tobari M, Hashimoto E, Yatsuji S, Torii N, Shiratori K. Imaging of nonalcoholic steatohepatitis: advantages and pitfalls of ultrasonography and computed tomography. Intern Med. 2009; 48:739–746. [PubMed: 19443967]

Table 1

Subject characteristics by NAFLD grade

		NAFLD Grade				
	N all	**Normal**	**Mild**	**Moderate**	**Severe**	**P value**
Age (y) (mean ± SE)	4172	41 ± 0.5	43 ± 0.9	45 ± 1.0	48 ± 1.2	*
F/M (%)	2391/1781	55.4/44.6	57.6/42.4	52.5/47.6	46.9/53.2	NS
Black/Mex-His/ Other/White (%)	1163/1182/ 175/1652	10.9/4.5/ 7.3/77.2	8.6/5.7/ 9.0/76.8	7.0/7.2/ 10.8/75.0	7.9/8.0/ 6.1/78.0	**
BMI (kg/m^2) (mean ± SE)	4172	25.5 ± 0.2	26.7 ± 0.4	30.0 ± 0.5	31.7 ± 0.6	*
Waist (cm) (mean ± SE)	4172	88.3 ± 0.4	90.9 ± 1.1	100.2 ± 1.3	106.8 ± 1.4	*
HOMA-IR (mean ± SE)	4172	2.1 ± 0.0	2.6 ± 0.2	4.1 ± 0.3	4.9 ± 0.5	*
A1C% (mean ± SE)	4172	5.2 ± 0.0	5.3 ± 0.0	5.4 ± 0.0	5.7 ± 0.1	*
Diabetes mellitus present (%)	162	1.1	2.8	3.8	7.3	*
IGF-1 (ng/ml) (mean ± SE)	4172	287.1 ± 4.0	270.2 ± 6.8	256.2 ± 6.0	233.2 ± 8.9	*
IGFBP-3 (ng/ml) (mean ± SE)	4172	4542 ± 41	4550 ± 65	4450 ± 65	4410 ± 96	NS
Ratio IGF-1/IGFBP-3 (mean ± SE)	4172	0.063 ± 0.001	0.060 ± 0.001	0.058 ± 0.001	0.053 ± 0.001	*

* for $p < 0.001$;

** Black and Mexican/Hispanic p value = 0.001, Other and White NS; NS for $p > 0.05$

Abbreviations: Mex-His, Mexican-Hispanic; Diabetes Mellitus (defined by A1C% ≥ 6.5%)

Table 2

Subject characteristics by quartiles of serum IGF-1, IGFBP-3 and ratio IGF-1/IGFBP-3

		Serum IGF-1 Level				
	N	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	**P value**
Age (y) (mean ± SE)	4172	52 ± 0.8	45 ± 0.7	39 ± 0.6	32 ± 0.4	*
F/M (%)	2391/1781	70.1/29.9	55.7/44.3	44.1/55.9	49.5/50.6	*
Black/Mex-His/ Other/White (%)	1163/1182/ 175/1652	9.6/6.2/ 8.2/76.1	9.1/5.6/ 10.7/74.7	10.4/4.6/ 5.9/79.1	11.0/4.3/ 6.7/77.9	**
BMI (kg/m2) (mean ± SE)	4172	28.0 ± 0.3	26.7 ± 0.3	26.2 ± 0.2	25.4 ± 0.3	*
Waist circumference (cm) (mean ± SE)	4172	95.0 ± 0.7	92.1 ± 0.8	90.8 ± 0.7	86.7 ± 0.7	*
HOMA-IR (mean ± SE)	4172	3.1 ± 0.2	2.6 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	*
A1C% (mean ± SE)	4172	5.5 ± 0.0	5.3 ± 0.0	5.2 ± 0.0	5.1 ± 0.0	*
Diabetes mellitus present (%)	162	4.8	1.1	1.1	1.0	*
NAFLD present (%)	1390	39.9	32.9	28.1	24.3	*
		Serum IGFBP-3 Level				
	N all	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Age (y) (mean ± SE)	4172	48 ± 0.7	42 ± 0.8	40 ± 0.8	38 ± 0.7	*
F/M (%)	2391/1781	51.5/48.5	54.2/45.8	54.4/45.6	59.3/40.7	NS
Black/Mex-His/ Other/White (%)	1163/1182/175/1652	14.0/7.0/ 9.6/69.4	11.8/5.5/ 8.2/74.5	7.8/4.7/ 5.2/82.4	6.5/3.6/ 8.4/81.5	***
BMI (kg/m2) (mean ± SE)	4172	26.9 ± 0.2	26.3 ± 0.2	26.6 ± 0.3	26.4 ± 0.3	NS
Waist circumference (cm) (mean ± SE)	4172	92.7 ± 0.6	90.8 ± 0.7	91.0 ± 0.7	90.0 ± 0.8	**
HOMA-IR (mean ± SE)	4172	2.8 ± 0.2	2.4 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	NS
A1C% (mean ± SE)	4172	5.4 ± 0.0	5.2 ± 0.0	5.2 ± 0.0	5.2 ± 0.0	*
Diabetes mellitus present (%)	162	2.0	1.5	2.5	2.2	NS
NAFLD absent/present (%)	1390	32.9	29.8	33.5	28.9	NS
		Ratio IGF-1/IGFBP-3				

	Serum IGF-1 Level					
	N	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	**P value**
	N all	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Age (y) (mean ± SE)	4172	50 ± 0.7	44 ± 0.8	40 ± 0.6	34 ± 0.4	*
F/M (%)	2391/1781	77.1/22.9	52.1/47.9	47.0/53.0	43.2/56.8	*
Black/Mex-His/ Other/White (%)	1163/1182/175/1652	7.6/5.6/6.9/79.9	8.2/5.2/9.9/76.6	9.0/5.4/8.1/77.5	15.2/4.5/ 6.5/73.8	****
BMI (kg/m2) (mean ± SE)	4172	27.8 ± 0.3	27.1 ± 0.3	26.2 ± 0.3	25.1 ± 0.2	*
Waist circumference (cm) (mean ± SE)	4172	94.1 ± 0.8	93.2 ± 0.7	90.3 ± 0.9	87.0 ± 0.4	*
HOMA-IR (mean ± SE)	4172	3.0 ± 0.1	2.7 ± 0.2	2.4 ± 0.1	2.2 ± 0.0	*
A1C% (mean ± SE)	4172	5.4 ± 0.0	5.3 ± 0.0	5.2 ± 0.0	5.1 ± 0.0	*
Diabetes mellitus present (%)	162	4.1	2.0	0.8	1.2	*
NAFLD absent/present (%)	1390	40.0	33.2	28.7	23.4	*

IGF-1 quartiles: **1** <205.8 ng/ml, **2** 205.8–267.1 ng/ml, **3** 267.2–339.4 ng/ml, **4** >339.4 ng/ml; IGFBP-3 quartiles: **1** <3915 ng/ml, **2** 3915–4513.2 ng/ml, **3** 4513.3–5087 ng/ml, **4** >5087 ng/ml; IGF-1/ IGFBP-3 ratio quartiles: **1** <0.048, **2** 0.048–0.060, **3** 0.061–0.073, **4** >0.073;

* for $p < 0.001$; NS for $p > 0.05$;

** Waist circumference and Mexican/Hispanic p value = 0.02, Black, Other and White NS;

*** Black, Mexican/Hispanic and White p value < 0.001, Other NS;

**** Black p value = 0.001, White, Mexican/Hispanic and Other NS;

Abbreviations: Mex-His, Mexican-Hispanic; Diabetes mellitus (defined by A1C% ≥ 6.5%)

Table 3

Logistic regression models of OR[1] for presence of any NAFLD coded as mild or greater versus none by serum IGF-1 and IGFBP-3 quartiles

	IGF-1 Quartile 2		IGF-1 Quartile 3		IGF-1 Quartile 4		All quartiles
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Unadjusted	0.74*	0.55–0.99	0.59*	0.45–0.77	0.48*	0.33–0.70	<0.01
Age, gender, race/ethnicity	0.79	0.57–1.10	0.67*	0.49–0.93	0.61*	0.41–0.92	0.01
Age, gender, race/ethnicity, HOMA-IR	0.87	0.64–1.19	0.75	0.53–1.07	0.67	0.44–1.01	0.04
Age, gender, race/ethnicity, BMI, waist circumference	0.87	0.64–1.19	0.77	0.54–1.11	0.76	0.50–1.15	NS
Age, gender, race/ethnicity, A1C%	0.82	0.60–1.14	0.69*	0.50–0.96	0.64*	0.43–0.95	0.02
Age, gender, race/ethnicity, HOMA-IR, BMI, waist circumference, A1C%	0.90	0.66–1.23	0.79	0.55–1.14	0.74	0.49–1.11	NS
	IGFBP-3 Quartile 2		**IGFBP-3 Quartile 3**		**IGFBP-3 Quartile 4**		**All quartiles**
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Unadjusted	0.87	0.62–1.22	1.03	0.77–1.37	0.83	0.58–1.20	NS
Age, gender, race/ethnicity	0.98	0.70–1.38	1.23	0.90–1.67	1.04	0.72–1.49	NS
Age, gender, race/ethnicity, HOMA-IR	0.98	0.70–1.38	1.15	0.84–1.57	0.87	0.59–1.29	NS
Age, gender, race/ethnicity, BMI, waist circumference	0.98	0.70–1.39	1.18	0.86–1.63	0.97	0.66–1.44	NS
Age, gender, race/ethnicity, A1C%	0.99	0.71–1.39	1.20	0.89–1.62	1.02	0.71–1.47	NS
Age, gender, race/ethnicity, HOMA-IR, BMI, waist circumference, A1C%	1.00	0.70–1.41	1.15	0.84–1.58	0.89	0.60–1.32	NS

	IGF-1 Quartile 2		IGF-1 Quartile 3		IGF-1 Quartile 4		All quartiles
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
	Ratio IGF-1/IGFBP-3 Quartile 2		**Ratio IGF-1/IGFBP-3 Quartile 3**		**Ratio IGF-1/IGFBP-3 Quartile 4**		**All quartiles**
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Unadjusted	0.75*	0.60–0.93	0.60*	0.45–0.80	0.46*	0.33–0.64	<0.01
Age, gender, race/ethnicity	0.76*	0.59–0.97	0.64*	0.47–0.89	0.54*	0.37–0.77	<0.01
Age, gender, race/ethnicity, HOMA-IR	0.84	0.65–1.09	0.75	0.54–1.04	0.66*	0.45–0.96	0.03
Age, gender, race/ethnicity, BMI, waist circumference	0.80	0.62–1.05	0.74	0.53–1.03	0.70	0.48–1.01	NS
Age, gender, race/ethnicity, A1C%	0.78*	0.61–0.99	0.67*	0.49–0.92	0.56*	0.39–0.81	<0.01
Age, gender, race/ethnicity, HOMA-IR, BMI, waist circumference, A1C%	0.84	0.65–1.10	0.78	0.56–1.08	0.72	0.50–1.05	NS

[1] Odds ratio for presence of mild, moderate or severe liver fat. IGF-1 quartiles: **1** <205.8 ng/ml, **2** 205.8–267.1 ng/ml, **3** 267.2–339.4 ng/ml, **4** >339.4 ng/ml; IGFBP-3 quartiles: **1** <3915 ng/ml, **2** 3915–4513.2 ng/ml, **3** 4513.3–5087 ng/ml, **4** >5087 ng/ml; IGF-1/IGFBP-3 ratio quartiles: **1** <0.048, **2** 0.048–0.060, **3** 0.061–0.073, **4** >0.073;

* for $p < 0.05$; NS for $p > 0.05$. N = 4172

Table 4

Ordered logistic regression models[1] for severity of NAFLD by serum IGF-1 and IGF-1/IGFBP-3 quartiles

	IGF-1 Quartile 2		IGF-1 Quartile 3		IGF-1 Quartile 4		All quartiles
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Unadjusted	0.73*	0.54–0.97	0.56*	0.43–0.72	0.46*	0.32–0.66	<0.01
Age, gender, race/ethnicity	0.78	0.57–1.06	0.64*	0.47–0.87	0.58*	0.39–0.87	<0.01
Age, gender, race/ethnicity, HOMA-IR	0.87	0.65–1.16	0.76	0.56–1.04	0.67*	0.46–0.99	0.03
Age, gender, race/ethnicity, BMI, waist circumference	0.88	0.65–1.19	0.76	0.54–1.07	0.73	0.49–1.10	NS
Age, gender, race/ethnicity, A1C%	0.82	0.60–1.12	0.66*	0.48–0.90	0.61*	0.42–0.90	<0.01
Age, gender, race/ethnicity, HOMA-IR, BMI, waist circumference, A1C%	0.92	0.69–1.23	0.82	0.59–1.14	0.76	0.51–1.13	NS
	IGFBP-3 Quartile 2		**IGFBP-3 Quartile 3**		**IGFBP-3 Quartile 4**		**All quartiles**
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Unadjusted	0.87	0.62–1.22	1.03	0.77–1.37	0.83	0.58–1.20	NS
Age, gender, race/ethnicity	0.98	0.70–1.38	1.23	0.90–1.67	1.04	0.72–1.49	NS
Age, gender, race/ethnicity, HOMA-IR	0.98	0.70–1.38	1.15	0.84–1.57	0.87	0.59–1.29	NS
Age, gender, race/ethnicity, BMI, waist circumference	0.98	0.69–1.39	1.20	0.88–1.64	0.95	0.65–1.39	NS
Age, gender, race/ethnicity, A1C%	0.99	0.71–1.39	1.20	0.89–1.62	1.02	0.71–1.47	NS
Age, gender, race/ethnicity, HOMA-IR, BMI, waist circumference, A1C%	1.04	0.74–1.47	1.24	0.91–1.69	0.94	0.65–1.37	NS
	Ratio IGF-1/IGFBP-3 Quartile 2		**Ratio IGF-1/IGFBP-3 Quartile 3**		**Ratio IGF-1/IGFBP-3 Quartile 4**		**All quartiles**

	IGF-1 Quartile 2		IGF-1 Quartile 3		IGF-1 Quartile 4		All quartiles
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Covariates in the Model	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**OR**	**CI (95%)**	**P trend**
Unadjusted	0.73*	0.59–0.91	0.58*	0.45–0.76	0.43*	0.32–0.60	<0.01
Age, gender, race/ethnicity	0.74*	0.59–0.93	0.62*	0.46–0.83	0.51*	0.36–0.72	<0.01
Age, gender, race/ethnicity, HOMA-IR	0.82	0.63–1.07	0.71*	0.53–0.96	0.62*	0.43–0.89	<0.01
Age, gender, race/ethnicity, BMI, waist circumference	0.81	0.63–1.04	0.75	0.55–1.02	0.67*	0.47–0.96	0.04
Age, gender, race/ethnicity, A1C%	0.77*	0.61–0.96	0.65*	0.48–0.88	0.53*	0.37–0.76	<0.01
Age, gender, race/ethnicity, HOMA-IR, BMI, waist circumference, A1C%	0.84	0.65–1.09	0.78	0.57—1.06	0.70	0.49–1.01	NS

[1] Odds ratio by ordered logistic analyses; 2=mild, 3=moderate or 4= severe liver fat versus 1=none. **1** <205.8 ng/ml, **2** 205.8–267.1 ng/ml, **3** 267.2–339.4 ng/ml, **4** >339.4 ng/ml; IGFBP-3 quartiles: **1** <3915 ng/ml, **2** 3915–4513.2 ng/ml, **3** 4513.3–5087 ng/ml, **4** >5087 ng/ml; IGF-1/IGFBP-3 ratio quartiles: **1** <0.048, **2** 0.048–0.060, **3** 0.061–0.073, **4** >0.073;

* for $p < 0.05$; NS for $p > 0.05$. N = 4172