Increased Fetuin-A Concentrations in Impaired Glucose Tolerance with or without Nonalcoholic Fatty Liver Disease, But Not Impaired Fasting Glucose

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Context: Fetuin-A, a liver-derived glycoprotein that impairs insulin signaling, is associated with nonalcoholic fatty liver disease (NAFLD), diabetes, and the risk of cardiovascular diseases. Both prediabetes and NAFLD are associated with increased cardiovascular risk, and their concurrence significantly impairs hepatic and adipose tissue insulin sensitivity.

Objective: Our objective was to investigate the relationship between serum fetuin-A levels and prediabetes in subjects with or without NAFLD.

Design: This was a cross-sectional case-control study.

Patients: A total of 510 age- and sex-matched subjects with normal glucose tolerance (NGT), impaired fasting glucose (IFG), and impaired glucose tolerance (IGT) with or without NAFLD were recruited. Each subject was assessed by abdominal ultrasound to diagnose NAFLD.

Main Outcome Measures: Serum fetuin-A concentrations were compared between groups. The association with clinico-metabolic parameters was examined.

Results: The presence of NAFLD significantly increases fetuin-A levels in subjects with NGT and prediabetes. As compared with NGT, IGT, but not IFG, significantly increases fetuin-A levels in subjects with or without NAFLD. Serum fetuin-A concentrations were positively related to postload 2-h glucose, body mass index, triglyceride, and homeostasis model assessment of insulin resistance but negatively associated with age, high-density lipoprotein cholesterol, and adiponectin. In multiple regression analysis, age, IGT *vs.* NGT, and IGT with NAFLD *vs.* NGT were independently associated with fetuin-A levels after adjustment for cardiovascular risk factors and adiponectin.

Conclusions: IGT with or without NAFLD was independently associated with fetuin-A levels after adjustment for cardiometabolic risk factors. The elevated fetuin-A levels could have a clinical implication in the increased cardiovascular risk and insulin resistance associated with NAFLD and IGT. **(*J Clin Endocrinol Metab* 97: 4717–4723, 2012)**

Nonalcoholic fatty liver disease (NAFLD) and prediabetes are highly prevalent in the general population (1–3), and both conditions are risk factors of type 2 diabetes and cardiovascular diseases (4–6). Because liver fat continuously increases from normal glucose tolerance (NGT) to isolated impaired fasting glucose (IFG), isolated

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2012–2414 Received June 6, 2012. Accepted September 24, 2012.
First Published Online October 12, 2012

Abbreviations: A1C, Hemoglobin A1c; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CV, coefficient of variation; FFA, free fatty acid; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; hsCRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; NGT, normal glucose tolerance; TLR4, toll-like protein receptor 4; WC, waist circumference.

impaired glucose tolerance (IGT), and IFG+IGT (7), the prevalence of NAFLD is therefore significantly higher in subjects with prediabetes than those with NGT (8), and prediabetes is more common in patients with NAFLD than those without it (9). Furthermore, both NAFLD and prediabetes are closely related to insulin resistance (IR) in their pathogenesis, and their concurrence significantly impairs hepatic and adipose tissue insulin sensitivity (9). However, the mechanism underlying IR is complex and may involve hepatic and systemic inflammation, oxidative stress, and humoral factors released by the adipose tissue and liver (10).

Some recent studies reveal that circulating factors, such as leptin, adiponectin, retinol-binding protein 4, and fetuin-A, contribute significantly to the IR in both NAFLD and prediabetes (10, 11). Among these factors, fetuin-A is peculiar because it is a glycoprotein produced exclusively in the liver and then secreted into circulation in high concentrations (12). Fetuin-A *per se* is an endogenous inhibitor of insulin receptor tyrosine kinase in the liver and skeletal muscle (13). One very recent study showed that fetuin-A is crucial in lipid-induced IR. The authors demonstrated that fetuin-A, being a major carrier of free fatty acids (FFAs) in circulation, functions as an adaptor between FFAs and toll-like protein receptor 4 (TLR4) signaling in adipocytes and, thus, increases inflammatory cytokine expression and IR (14). On the other hand, fetuin-A knockout mice showed improved insulin signaling, less adiposity, and lower FFA and were resistant to weight gain upon a high-fat diet compared with wild-type controls (15). In human studies, serum fetuin-A levels are associated with various types of metabolic dysregulation. A cross-sectional study showed that serum fetuin-A correlates positively with liver fat in nondiabetic individuals (16). In addition, high fetuin-A levels are associated with subjects with metabolic syndrome (17) and IR in nondiabetic subjects (18), prediabetic subjects, and diabetic individuals (19). In addition, fetuin-A is also strongly associated with increased risk of myocardial infarction and ischemic stroke independent of standard risk factors in the general population (20). Furthermore, IGT and newly diagnosed diabetes are positively associated with serum fetuin-A concentrations in subjects without NAFLD independent of cardiometabolic risk factors (21). However, given the important role that NAFLD and prediabetes play in the deterioration of IR (9), the association among serum fetuin-A concentration, prediabetes, and NAFLD is not well understood. Therefore, the aim of this study was to investigate the relationship between serum fetuin-A levels and prediabetes in subjects with or without NAFLD.

Patients and Methods

The study protocol was approved by the Human Experiment and Ethics Committee of National Cheng Kung University Medical Center, and all eligible subjects gave written informed consent before participation. From June 2007 to July 2009, all subjects who had been admitted for a physical checkup at the Preventive Health Center of National Cheng-Kung University Hospital were screened (a total of 4077 cases). All healthy subjects who did not have a medical history of diabetes received a standard 75-g oral glucose tolerance test after a 10-h overnight fast, a normal diet including a minimum of 150–200 g carbohydrate per day for 3 d before the test, and abstention from smoking for more than 24 h. None of the women were pregnant when tested. To avoid the confounding effects of age and sex, we selected subjects based on the following approach. The study subjects were classified into six groups, NGT, IFG, IGT, NGT+NAFLD, IFG+NAFLD, and IGT+NAFLD, in the order of their admission to the checkup. NGT, IFG, and IGT were defined according to American Diabetes Association criteria: NGT, if fasting plasma glucose was less than 100 mg/dl and 2-h postload glucose was less than 140 mg/dl without a history of diabetes; IFG, if fasting plasma glucose was 100–125 mg/dl and 2-h postload glucose was less than 140 mg/dl; and IGT, if fasting plasma glucose was less than 100 mg/dl and 2-h postload glucose was 140–199 mg/dl. Each consecutive index IGT subject was matched to the first subject of the same gender in the other five groups from the list who had the same age. If an exact age match could not be found, then the first subject closest to the age of the index subject (within ±1 yr) was picked. Using this method, we were able to select 255 subjects in the NAFLD-free groups (NGT, n = 90; IFG, n = 75; IGT, n = 90) and 255 subjects in the NAFLD groups (NGT+NAFLD, n = 90; IFG+NAFLD, n = 75; IGT+NAFLD, n = 90).

After an overnight 12-h fast, all subjects received a blood test, including routine biochemistry, fasting plasma glucose, hemoglobin A1c (A1C), adiponectin, and fetuin-A. Wearing light indoor clothes, each subject's body height (to the nearest 0.1 cm), weight (to the nearest 0.1 kg), and waist circumference (WC) (to the nearest 0.1 cm) were measured. WC measurement was performed at the end of normal expiration in duplicate on bare skin midway between the lower rib margin and the iliac crest. Body mass index (BMI) (in kilograms per square meter) was calculated as weight (in kilograms) divided by height (in meters) squared. For the blood pressure measurement, subjects were resting in a supine position in a quiet ambience, and measurements were obtained in a fasting state between 0800 and 1000 h. Two blood pressure readings, separated by intervals of at least 5 min, were taken with an appropriate-sized cuff wrapped around the right upper arm using a DINAMAP vital sign monitor (model 1846SX; Critikon Inc., Irvine, CA). Subjects with a systolic blood pressure at least 140 mm Hg or diastolic blood pressure at least 90 mm Hg were defined as having hypertension. Liver ultrasound was performed by an experienced radiologist with high-resolution ultrasonography (Xario SSA-660A; Toshiba, Nasu, Japan) using a 3.5-MHz linear transducer. The NAFLD diagnostic criteria included characteristic echo patterns of hepatorenal echo contrast, bright liver, deep (posterior beam) attenuation, and vascular blurring (22). Smoking habits were categorized as current, former, or never.

After venipuncture, the blood was collected in sodium fluoride (NaF) collection tubes for plasma glucose determination.

Within 30 min, the tubes were centrifuged at 3000 × *g* for 10 min, and the measurement was then performed by a hexokinase method (Roche Diagnostic GmbH, Mannheim, Germany). The intraassay and interassay coefficients of variation (CV) were 1.5 and 1.7%, respectively. Serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol levels were determined in the central laboratory of National Cheng Kung University Medical Center with an autoanalyzer (Hitachi 747E; Hitachi, Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. A1C was measured with a HPLC method (Tosoh automated glycohemoglobin analyzer HLC-723 GHbV A1c 2.2, Tokyo, Japan; intraassay CV of 0.5%, interassay CV of 2.0%). Serum insulin was measured by Mercodia ultrasensitive insulin ELISA (Mercodia AB, Uppsala, Sweden; intraassay CV of 4%, interassay CV of 2.6%). IR was estimated by using the homeostasis model assessment (HOMA) for IR (HOMA-IR) index defined as [fasting insulin (microunits per milliliter) × fasting plasma glucose (millimoles per liter)]/22.5. Serum fetuin-A was determined by an ELISA method (Biovendor Laboratory Medicine, Brno, Czech Republic; intraassay CV of 2.7%, interassay CV of 3.2%). The determination of serum adiponectin was carried out using AssayMax human adiponectin (Acrp30) ELISA kits (AssayPro, St. Charles, MO; intraassay CV of 2.5%, interassay CV of 6.5%). High-sensitivity C-reactive protein (hsCRP) was measured with a highly sensitive ELISA kit (Immunology Consultants Laboratory, Newberg, OR; intraassay CV of 2.9%, interassay CV of 4.7%). Subjects with the following conditions or diseases were excluded: 1) alcohol consumption of 20 g/d or more in the last year; 2) serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels higher than two times the normal limit; 3) a positive test for hepatitis B surface antigen, hepatitis C antibody, and other causes of liver disease; 4) serum creatinine over 1.5 mg/dl; 5) any acute or chronic inflammatory disease as determined by a leukocyte count over 10,000/mm^3 or clinical signs of infection; and 6) any other major diseases, including generalized inflammation or advanced malignant diseases contraindicating this study.

Statistical analysis

SPSS software (version 17.0; SPSS, Chicago, IL) was used for statistical analysis. All normally distributed continuous variables are expressed as means ± SD. Study subjects were divided into six groups: NGT, IFG, IGT, NGT+NAFLD, IFG+NAFLD, and IGT+NAFLD. The continuous variables among groups were compared using ANOVA or a Kruskal-Wallis test when the distribution of the variable was not normal. Serum fetuin-A, adiponectin, hsCRP levels, and HOMA-IR of groups with the same glycemic status were compared with a *t* test. The associations between serum fetuin-A and individual variables were examined using simple linear regression analysis. Multiple linear regression analysis was conducted to identify variables that best predicted serum fetuin-A concentrations, of which the variable selection strategies were stepwise and backward. The independent variables included age, sex, IFG *vs.* NGT, IGT *vs.* NGT, NGT+NAFLD *vs.* NGT, IFG+NAFLD *vs.* NGT, IGT+NAFLD *vs.* NGT, BMI, hypertension, triglyceride, HDL-cholesterol, creatinine, adiponectin, hsCRP, and HOMA-IR. A P value <0.05 was considered statistically significant.

Results

A total of 510 age- and sex-matched subjects with NGT (n = 90), IFG (n = 75), IGT (n = 90), NGT+NAFLD (n = 90), IFG+NAFLD (n = 75), and IGT+NAFLD (n = 90) were included. Table 1 shows the clinical characteristics of the study subjects in the final analysis. There were significant differences in WC, BMI, systolic/diastolic blood pressure, fasting and 2-h postload plasma glucose, A1C, ALT, AST, hsCRP, adiponectin, triglyceride, HDL cholesterol, LDL/HDL cholesterol ratio, and HOMA-IR

TABLE 1. Clinical characteristics among study subjects with NGT, IFG, or IGT with or without NAFLD

	NGT	IFG	IGT	NGT+NAFLD	IFG+NAFLD	IGT+NAFLD	*P*
n	90	75	90	90	75	90	
Age (yr)	63 ± 12	61 ± 12	62 ± 13	62 ± 10	60 ± 10	61 ± 11	NS
Sex (female/male)	39/51	31/46	37/55	41/51	35/40	38/52	NS
WC (cm)	79.5 ± 8.8	83.9 ± 8.9	81.9 ± 8.6	88.6 ± 9.3	90.8 ± 9.2	90.7 ± 8.6	<0.001
BMI (kg/m^2)	22.4 ± 2.5	24.0 ± 2.9	23.5 ± 2.9	26.1 ± 3.1	27.3 ± 3.5	26.8 ± 2.6	<0.001
Former/current smoker (%)	7.8/0	7.8/1.3	4.3/1.1	5.4/5.4	4.0/2.7	8.9/2.2	NS
Systolic blood pressure (mm Hg)	122 ± 18	127 ± 18	128 ± 17	129 ± 17	13. ± 16	130 ± 16	<0.05
Diastolic blood pressure (mm Hg)	71 ± 10	73 ± 10	74 ± 11	75 ± 10	73 ± 10	80 ± 11	<0.01
Fasting plasma glucose (mg/dl)	85 ± 7	105 ± 5	89 ± 12	89 ± 7	106 ± 5	93 ± 10	<0.001
Postload 2-h glucose (mg/dl)	96 ± 22	106 ± 23	161 ± 17	108 ± 19	111 ± 18	162 ± 15	<0.001
A1C (%)	5.7 ± 0.3	5.8 ± 0.3	5.8 ± 0.3	5.8 ± 0.3	5.9 ± 0.4	5.9 ± 0.4	<0.001
ALT (U/liter)	22 ± 10	25 ± 16	23 ± 11	31 ± 15	32 ± 18	36 ± 21	<0.001
AST (U/liter)	26 ± 8	25 ± 42	25 ± 8	28 ± 10	28 ± 9	30 ± 11	<0.01
Creatinine (mg/dl)	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	NS
Total cholesterol (mg/dl)	198 ± 32	202 ± 37	198 ± 37	208 ± 35	209 ± 38	207 ± 35	NS
Triglyceride (mg/dl)[a]	95 ± 37	110 ± 63	118 ± 62	151 ± 77	153 ± 94	155 ± 79	<0.001
HDL cholesterol (mg/dl)	59 ± 19	54 ± 15	54 ± 15	48 ± 11	46 ± 12	46 ± 11	<0.001
LDL cholesterol (mg/dl)	120 ± 32	126 ± 33	121 ± 34	130 ± 35	133 ± 34	129 ± 30	NS
LDL/HDL cholesterol ratio	2.3 ± 1.0	2.5 ± 0.9	2.4 ± 0.9	2.9 ± 1.0	3.0 ± 0.9	2.9 ± 0.8	<0.001

Data are expressed as means ± SD. NS, Not significant.

[a] Kruskal-Wallis test.

among subjects with NGT, IFG, IGT, NGT+NAFLD, IFG+NAFLD, and IGT+NAFLD.

Serum fetuin-A concentrations were 293 ± 62, 305 ± 62, 328 ± 77, 328 ± 76, 326 ± 56, and 366 ± 85 μg/ml in subjects with NGT, IFG, IGT, NGT+NAFLD, IFG +NAFLD, and IGT+NAFLD, respectively (Fig. 1A). NAFLD subjects had significantly higher fetuin-A, hsCRP, and HOMA-IR values (Fig. 1, A, C, and D) but lower adiponectin levels (Fig. 1B) than those of NAFLD-free subjects with the same glycemic status. As compared with NGT subjects, only serum fetuin-A levels were elevated in IGT subjects with or without NAFLD ($P = 0.001$ and $P = 0.002$, respectively), but no significant difference was found in adiponectin, hsCRP, and HOMA-IR values. The difference in fetuin-A concentrations remained significant after adjustment for age, gender, and BMI. However, there were no significant differences in fetuin-A, adiponectin, hsCRP levels, and HOMA-IR between IFG and NGT subjects.

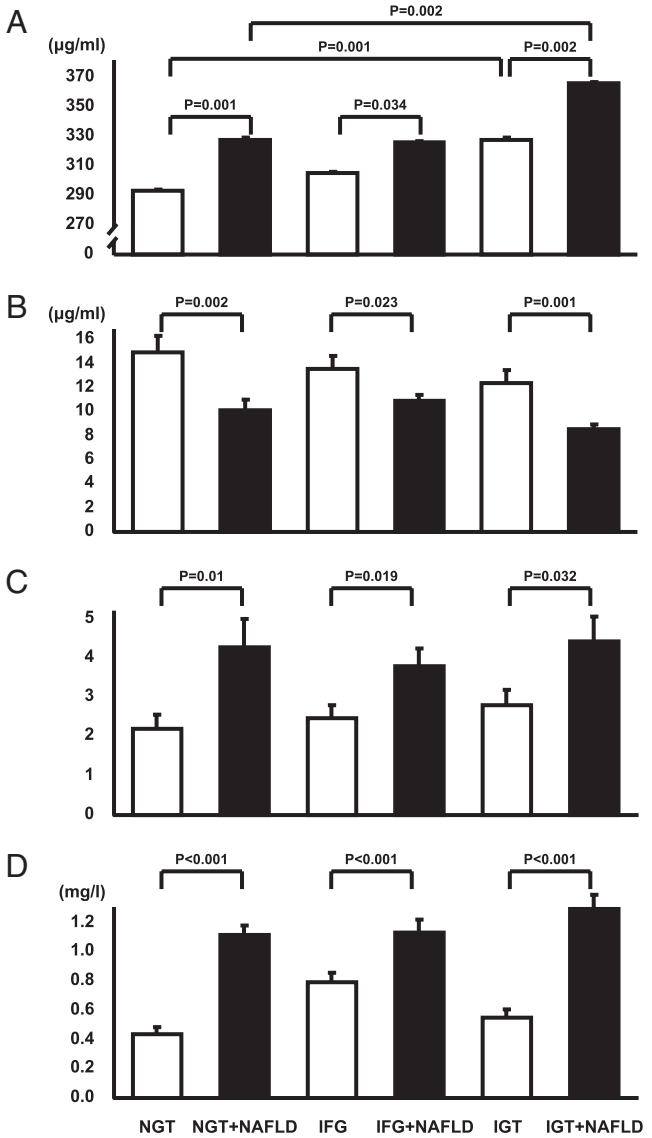

FIG. 1. Comparison of serum fetuin-A (A), adiponectin (B), and hsCRP (C) levels and HOMA-IR (D) among subjects with NGT, IFG, and IGT in the absence or presence of NAFLD.

The simple regression analysis shows that serum fetuin-A concentrations were positively related to postload 2-h glucose, BMI, triglyceride, and HOMA-IR but negatively associated with age, HDL cholesterol, and adiponectin (Table 2). To further determine the independent factors associated with serum fetuin-A levels, we performed multiple linear regression analysis. Both stepwise and backward selection strategies showed a consistent model, which is summarized in Table 2. Using NGT as a reference group, we found that age ($P < 0.001$), IGT ($P < 0.01$), NGT+NAFLD ($P < 0.001$), IFG+NAFLD ($P < 0.01$), and IGT+NAFLD ($P < 0.001$) were independently associated factors of fetuin-A concentrations after adjustment for age and sex (model 1). Further adjustment for BMI, hypertension, triglyceride, HDL cholesterol, creatinine, adiponectin, hsCRP, HOMA-IR, and smoking in model 2 reduced the association of fetuin-A with NGT+NAFLD and IFG+NAFLD but did not appreciably alter the association with IGT and IGT+NAFLD shown in model 1. The results were still the same when substituting BMI with WC in the multiple linear regression analysis.

Discussion

To the best of our knowledge, this is the first study to explore the independent effects of NAFLD and prediabetes on serum fetuin-A levels. We found that IGT, with or without NAFLD, is independently associated with serum fetuin-A concentrations after adjusting for cardiometabolic risk factors, insulin resistance (HOMA-IR), and adiponectin.

Previous studies have shown that fetuin-A is closely related to IR (13, 14, 18). In addition, one study by Ishibashi *et al.* (19) found that fetuin-A concentration was independently associated with HOMA-IR, BMI, LDL cholesterol, leptin, and adiponectin concentrations. Consistent with this earlier study, we demonstrated in the present work that after adjusting for cardiometabolic variables and adiponectin, fetuin-A is significantly associated with HOMA-IR. Although the effect of fetuin-A on insulin sensitivity has been well established, little is known about the mechanisms regulating the expression of fetuin-A. Two recent studies report that palmitate stimulates nuclear factor-κB binding to the fetuin-A promoter to increase the expression of fetuin-A (23), and high glucose enhances fetuin-A expression through activation of ERK1/2 (24). Recently, our group demonstrated that endoplasmic reticulum stress induced by high glucose and palmitate sig-

TABLE 2. Regression analysis between serum fetuin-A concentrations and clinical variables

	Simple regression		Multiple regression			
			Model 1		Model 2	
	Regression coefficient	95% CI	Regression coefficient	95% CI	Regression coefficient	95% CI
Age (yr)	−1.1[c]	−1.6–−0.5	−1.0[c]	−1.5–−0.4	−1.0[c]	−1.6–−0.4
Sex, male *vs.* female	−3.2	−16.3–9.9	−5.6	−18.0–6.8	−2.7	−21.1–15.7
Compared with NGT						
IFG			10.5	−11.1–32.0	1.3	−21.2–23.9
IGT			34.4[b]	13.9–54.9	31.4[b]	10.3–52.5
NGT+NAFLD			33.8[c]	13.3–54.3	12.3	−11.2–35.8
IFG+NAFLD			28.7[b]	6.9–50.5	6.9	−18.6–32.4
IGT+NAFLD			71.6[c]	50.9–92.2	50.7[c]	26.4–75.0
Fasting plasma glucose (mg/dl)	0.37	−0.21–0.94				
Postload 2-h glucose (mg/dl)	0.43[c]	0.24–0.62				
BMI (kg/m^2)	3.8[c]	1.9–5.6			0.8	−1.5–3.2
Hypertension, yes *vs.* no	−10.4	−26.1–5.3			−9.9	−25.5–5.7
Triglyceride (mg/dl)	65.5[c]	35.2–95.9			22.4	−13.3–58.2
HDL cholesterol (mg/dl)	−0.62[b]	−1.06–−0.19			−0.04	−0.57–0.50
Creatinine (mg/dl)	−10.9	−45.0–23.3			−6.8	−52.0–38.4
Adiponectin (μg/ml)	−26.1[a]	−49.5–−2.7			5.5	−20.5–31.4
hsCRP (mg/liter)	0.9	−0.4–2.3			0.5	−0.8–1.9
HOMA-IR	21.5[c]	13.1–30.0			15.0[b]	4.9–25.1
Smoking (former *vs.* never)					3.7	−23.9–31.3
Smoking (current *vs.* never)					15.8	−29.4–61.0

Triglyceride was log transformed before analysis. The regression coefficient represents the change, in micrograms per milliliter, in serum fetuin-A concentrations per unit increase in the independent variables or the presence (*vs.* absence) of the independent variables. CI, Confidence interval.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

nificantly increases the expression of fetuin-A and further contributes to the development of IR in the human hepatoma cell line (HepG2) and high-fat diet–induced insulin-resistant diabetic mice (25). Therefore, it is possible that in the presence of IR, the expression and secretion of fetuin-A may be up-regulated indirectly by the increased circulating FFA and glucose and that this increase in fetuin-A further deteriorates IR.

Our result that IGT, irrespective of NAFLD, is an independently associated factor of the level of serum fetuin-A, is compatible with Kantartzis *et al.* (7), which found that in overweight/obese nondiabetic subjects, IGT subjects have significantly higher liver fat and fetuin-A levels than those with IFG, but similar adiponectin levels. Moreover, the regression analysis in their study showed that fetuin-A is a stronger independent determinant of the glucose categories than adiponectin. Similarly, in another study assessing adipokine profiles in obese patients with different glycemic statuses, Tönjes *et al.* (11) suggested that IGT subjects had significantly higher fetuin-A levels than NGT subjects, but not IFG ones. However, this result was not adjusted for the possible confounding effect of fatty liver, which is reported to be frequent among obese subjects (75%) (10). To further clarify the separate effects of NAFLD and prediabetes on fetuin-A levels, we recruited IFG and IGT subjects with or without NAFLD for this study. This work had a significantly larger sample size than earlier research and found that IGT, both with and without NAFLD, was an independent predictor of the level of fetuin-A after adjusting for measures of adiposity, adiponectin, inflammation (hsCRP), and IR (HOMA-IR). In contrast, IFG, both with and without NAFLD, was not an independent predictor of the level of fetuin-A after also adjusting for these factors.

In addition, we also found that the NAFLD superimposed on IGT significantly increased serum fetuin-A levels. Previous studies in both humans and animals have suggested that fetuin-A is associated with fatty liver (16, 26). In nondiabetic subjects, fetuin-A levels are associated with high liver fat, as measured by ^{1}H magnetic resonance spectroscopy independent of body fat (16). Similarly, in obese children with NAFLD, fetuin-A concentrations are significantly higher than in obese and normal-weight children without NAFLD (26). Moreover, lifestyle interventions, including increased physical activity, nutrition education, and behavior therapy, can induce a parallel decrease in liver fat and fetuin-A concentrations (16, 26). Likewise, the fetuin-A mRNA expression is also significantly increased in mice with fatty liver (16). The mechanism responsible for the synergistic effects of NAFLD

and IGT with regard to increasing serum fetuin-A levels, as seen in the current study, remains unclear. One explanation is that through the induction of endoplasmic reticulum stress, hepatic steatosis and hyperglycemia may additively increase the fetuin-A expression (25). Alternatively, it is likely that in NAFLD subjects with higher fetuin-A levels, FFAs would stimulate adipose tissue inflammation through the TLR4 pathway, resulting in chronic low-grade inflammation that impairs insulin sensitivity and thus glucose tolerance. However, more studies are needed to further clarify this issue.

The clinical implications of elevated fetuin-A levels in IGT subjects with NAFLD remain unknown and may be related to the worsening IR and increased cardiovascular risk. One recent study shows that NAFLD patients with newly diagnosed prediabetes had worse hepatic insulin sensitivity than NAFLD subjects with NGT and those without NAFLD (9). Based on the finding that fetuin-A inhibits insulin signaling in hepatocytes (13), it is reasonable to assume that the elevated fetuin-A in these subjects may contribute to the deteriorated hepatic IR. With regard to cardiovascular risk, although there have been no studies exploring the cardiovascular outcome of subjects with concurrent NAFLD and IGT, previous works do show that both IGT (27, 28) and NAFLD (29, 30) are associated with an increased cardiovascular disease risk that is independent of classical risk factors. Similarly, high serum fetuin-A levels are associated with subclinical atherosclerosis (31) and the risk of cardiovascular events independent of traditional cardiovascular risk factors, hsCRP, as well as adiponectin (20). Therefore, we speculate that elevated fetuin-A levels may contribute significantly to the increased incidence of cardiovascular disease among subjects with both IGT and NAFLD, although a prospective study is required to confirm the causal relationship between fetuin-A and cardiovascular disease in the future. In addition, our finding that serum fetuin-A levels are inversely associated with age is compatible with previous studies (17, 20, 32). However, the explanation for this is not clear and may be related to decreased hepatic synthesis in the older subjects.

There are some limitations in this work. First, because this study used a cross-sectional design, it does not allow for causal inference between serum fetuin-A concentrations and the development of prediabetes. Second, in this work, the diagnosis of NAFLD was made by ultrasound but was not confirmed pathologically. Although a liver biopsy is still considered the gold standard in diagnosing NAFLD, it is difficult to perform in clinical practice. However, ultrasound is an established noninvasive tool used as a screening modality with acceptable sensitivity and specificity (33). The major drawbacks of ultrasound include operator dependency and insensitivity with regard to small amounts of fat. To minimize the interobserver variability, in this work, the ultrasound was performed by a single experienced radiologist.

Taken together, the results show that both NAFLD and IGT are important associated factors of serum fetuin-A concentrations, independent of gender and cardiometabolic risk factors. Future studies are needed to explore the possible mechanisms involved in elevating fetuin-A levels and its clinical implications in the cardiovascular risk associated with NAFLD and IGT.

Acknowledgments

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This study was supported by grants from the National Science Council, Taipei, Taiwan (NSC-94-2314-B-006-114, NSC-95-2314-B-006-048, NSC98-2314-B-006-012, and NSC-99-2314-B-006-049-MY3) and National Cheng Kung University Hospital (NCKUH-9501005, NCKUH-9702005, and NCKUH-9903034).

Disclosure Summary: No potential conflicts of interest relevant to this article were reported.

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