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Circulating alanine transaminase (ALT) and γ-glutamyl transferase (GGT), but not fetuin-A, are associated with metabolic risk factors, at baseline and at two-year follow-up: The prospective Cyprus Metabolism Study

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ARTICLE INFO

Article history:
Received 14 January 2014
Accepted 13 March 2014

Keywords:
Obesity
Insulin resistance
Metabolic syndrome

ABSTRACT

Objective. To comparatively evaluate traditional liver tests and fetuin A as predictors of cardiometabolic risk, we studied associations between serum alanine transaminase (ALT), γ-glutamyl transferase (GGT), aspartate aminotransferase (AST) and fetuin-A and anthropometric, metabolic, and cardiovascular parameters cross-sectionally at baseline, and prospectively, after 2-years of follow-up.

Research Design and Methods. 616 randomly enrolled young healthy participants in the Cyprus Metabolism Study, including all 93 subjects who participated in the follow-up study 2 years after baseline assessment, were included in this study.

Results. In the cross-sectional study, serum ALT and GGT were strongly correlated with anthropometric, cardiovascular, and metabolic variables, while serum AST was only correlated with waist circumference and waist-to-hip ratio. Fetuin-A was correlated with anthropometric variables, systolic blood pressure (SBP), insulin, and homeostasis model of

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; AUC, Area under the curve; BMI, Body mass index; BMR, Basal metabolic rate; BP, Blood pressure; DBP, Diastolic blood pressure; ELISA, Enzyme-linked immunosorbent assay; GGT, Gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, Homeostasis model of assessment of insulin resistance; hsCRP, High sensitivity C-reactive protein; IDF, International Diabetes Federation; LDL, Low-density lipoprotein; LBP, Leptin-binding protein; LFTs, Liver function tests; NAFLD, Non-alcoholic fatty liver disease; NGAL, Neutrophil gelatinase-associated lipocalin; NHANES III, Third National Health and Nutrition Examination Survey; ROC, Receiver operating characteristic; SBP, Systolic blood pressure; SE, Standard error; VAT, Visceral adipose tissue; WHR, Waist-to-hip ratio.

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http://dx.doi.org/10.1016/j.metabol.2014.03.008

assessment-insulin resistance (HOMA-IR) in the unadjusted model. In the fully adjusted model, both serum ALT and GGT levels remained positively correlated with total and low-density lipoprotein (LDL) cholesterol. GGT levels also remained correlated with triglycerides. ALT levels remained strongly positively correlated with insulin ($r = 0.17$, $p < .0001$) and HOMA-IR ($r = 0.16$, $p = 0.0001$). Serum fetuin-A levels were no longer significantly correlated with any variables.

Prospectively, ALT and GGT were predictors of anthropometric variables and LDL cholesterol, while baseline levels of AST and fetuin-A were not predictors of any variables at 2-year follow-up.

Conclusions. We confirmed associations of ALT and GGT levels but failed to demonstrate an independent association between fetuin-A and cardiometabolic risk factors in young healthy men. Traditional liver tests (LFTs) are thus better than fetuin-A predictors of metabolic risk factors cross-sectionally and prospectively in young healthy adults.

1. Introduction

Obesity is a risk factor for the development of insulin resistance, hypertension and dyslipidemia, which increase an individual's eventual risk of developing type 2 diabetes mellitus and cardiovascular disease [1–3]. The mechanisms underlying these adverse metabolic effects of obesity have been the subject of intense investigation. The clustering of disturbed glucose and insulin metabolism, overweight and abdominal fat distribution, hypertension, and dyslipidemia, i.e. the metabolic syndrome, has also been associated with non-alcoholic fatty liver disease (NAFLD) [4–6]. Similar to NAFLD, abnormal liver function tests are considered markers of the metabolic syndrome and may be stronger predictors of a worse metabolic milieu than other clinical measurements [7–9].

Alanine transaminase (ALT) is a widely used serum marker of liver disease and even a minor elevation of ALT is a good predictor of mortality from liver disease [10,11]. γ-glutamyltransferase (GGT) and aspartate aminotransferase (AST) are mainly derived from the liver and hence often considered liver function tests (LFTs), although a significant contribution from other tissues makes them imperfect markers of liver function alone. Recently, a number of observational studies have suggested that abnormal LFTs are associated with obesity, insulin resistance, and metabolic syndromes [4,12–14]. Several prospective studies have also shown that high levels of ALT and GGT are independently associated with increased risk for incident metabolic syndrome and diabetes [12,15–19]. In non-diabetic subjects, some studies suggested that GGT might be a stronger predictor of development of type 2 diabetes than AST or ALT [19–21] while others have suggested that ALT is the only predictor [12]. All prior studies have been focused on middle to old-aged populations and thus data on young adults are lacking.

Fetuin-A, also known as alpha-2-Heremans-Schmid glycoprotein, is another serum protein that is mostly derived from liver, with limited contribution from the tongue and placenta [22]. Fetuin-A has attracted much attention since recent cross-sectional human studies have found that levels of fetuin-A are associated with insulin resistance [23,24], metabolic syndrome [25,26], visceral adipose tissue [27], fatty liver, body mass index (BMI), waist circumference, and an atherogenic lipid profile. Weight loss induced by bariatric surgery has also been shown to lead to a decrease in fetuin-A levels [28]. In prospective studies, even after adjusting for markers of body composition, increased levels of fetuin-A have been linked to increased amount of visceral adipose tissue at five-years of follow-up in a group of 508 older subjects [29], as well as increased incidence of type 2 diabetes mellitus in both older and middle-aged subjects [27,30]. Prospective studies have also found a higher risk of clinical manifestations of atherosclerotic disease with elevated fetuin-A levels, including myocardial infarction and stroke, potentially through its association with insulin resistance and associated adverse metabolic findings [31,32].

No study to date has studied associations between fetuin-A, anthropometrics and metabolic risk factors in young adults. Moreover, no prior studies have comparatively assessed fetuin-A and traditional LFTs as predictors of cardiovascular and metabolic disease in either young or older individuals.

Thus, we investigated fetuin-A as a marker of cardiovascular and metabolic risk factors in a population of young men, both cross-sectionally and prospectively. We then comparatively evaluated traditional liver enzymes ALT, AST and GGT vs. fetuin-A as predictors of cardiovascular and metabolic diseases.

2. Materials and Methods

2.1. Subjects

The full design of the Cyprus Metabolism Study has been published elsewhere [33,34,34]. This research has received institutional approval from both Harvard School of Public Health and the Cyprus National Bioethics Committee.

2.1.1. Cross-sectional study

In brief, 1056 eighteen-year-old candidates for recruitment in the Cypriot Army were enrolled in the study in July 2006 and 2007. For this study, a random subgroup of 616 participants was studied.

2.1.2. Prospective study

All participants who enrolled in the study in July 2006 were contacted for the two-year follow-up study. One hundred and

fifteen out of 417 eligible study participants expressed an interest in participating and could make the appointment to be seen at the Nicosia General Hospital for the follow-up evaluation two years later in July 2008. Twenty-one participants were excluded because of lack of a baseline sample for fetuin-A assays. One other participant was excluded because no follow-up weight was available. This resulted in 93 participants eligible for inclusion in the prospective analyses. No major differences were detected between the initial group of subjects and the 93 participants in the prospective analysis [34].

2.2. Measurements

Baseline and follow-up anthropometric and metabolic measures were ascertained using standardized methods [33,34]. Body composition was measured using the TanitaTBF-300A Body Composition Analyzer; basal metabolic rate was calculated by the analyzer using a Tanita proprietary formula. Activity level was assessed using questionnaires and includes both exercise and habitual activities such as walking or farm work. Baseline liver function tests including albumin, ALT, AST, alkaline phosphatase, total bilirubin, and GGT were assessed at Nicosia General Hospital using routine automated laboratory methods (Olympus AU2700™ Chemistry-Immuno Analyzer, Olympus, Center Valley, PA). De-identified frozen samples were shipped to Beth Israel Deaconess Medical Center and serum fetuin-A levels were measured using sandwich enzyme immunoassay (ELISA, BioVendor, Candler, NC). All samples were analyzed in duplicate in the same assay. Assay sensitivities as well as interassay and intraassay coefficients of variation were similar to those reported by the manufacturer.

2.3. Statistical analysis

All the variables were tested for normal distribution and logarithmically transformed if not normally distributed. Results were presented as mean values ± S.E.M., or for categorical variables, number and percentage, were used for the descriptive statistics. SAS (version 9.1, SAS Institute, Cary, NC) was used for statistical analysis. $P < 0.0125$ was considered statistically significant based on Bonferroni correction testing four dependent or independent hypotheses at the same time on one set of data.

Baseline characteristics of the follow-up group were compared to those of the entire cohort using t-test for continuous variables and chi-square test for categorical variables. Characteristics of participants at baseline and at follow-up were compared by repeated measures analysis of variance for both continuous and categorical variables.

Pearson correlation coefficients and the probabilities associated with this statistic were obtained between variables in the cross-sectional study. Pearson partial correlation coefficients were obtained after controlling for the effects of age, smoking status, activity, BMI, body fat percentage, and waist-to-hip ratio (WHR) between variables. General linear models and logistic regression analysis were used in the prospective study. The area under the receiver operating characteristic (ROC) curves was used to compare accuracy of logistic regression models.

3. Results

3.1. Cross-sectional study

Descriptive characteristics of the study population are presented in Table 1. As expected from a young healthy population, all mean values were within the normal range. However, 29.5% of the participants had a BMI greater than 25 kg/m^2. Dyslipidemia was found in 30.0% of the participants, mainly due to elevated low-density lipoprotein (LDL) or low high-density lipoprotein (HDL) cholesterol.

Findings from the cross-sectional portion of the study are summarized in Table 2. In the unadjusted model, we found that serum ALT and GGT levels were strongly positively correlated with anthropometric, cardiovascular, and metabolic variables, except for height, HDL cholesterol, urea and creatinine. On the other hand, AST levels were only correlated with waist circumference and WHR. Serum fetuin-A levels were significantly positively correlated with most anthropometric variables including body weight ($r = 0.15$, $p = 0.0002$), BMI ($r = 0.15$, $p = 0.0004$), total body fat ($r = 0.14$, $p = 0.0009$), body fat mass ($r = 0.13$, $p = 0.001$), body fat-free mass ($r = 0.15$, $p = 0.0003$), waist ($r = 0.16$, $p < .0001$) and hip circumferences ($r = 0.16$, $p < .0001$), WHR ($r = 0.11$, $p = 0.010$), and BMR ($r = 0.15$, $p = 0.0003$). Serum fetuin-A levels were also significantly correlated with some cardiovascular and metabolic variables, such as systolic blood pressure (SBP, $r = 0.12$, $p = 0.004$), insulin ($r = 0.15$, $p = 0.0002$), homeostasis model of assessment-insulin resistance (HOMA-IR, $r = 0.14$, $p = 0.0005$), and leptin ($r = 0.10$, $p = 0.012$).

After controlling for the effects of age, smoking status, activity, BMI, body fat percentage, and WHR, serum ALT and GGT levels remained strongly positively correlated with total and LDL cholesterol. ALT levels also remained strongly positively correlated with heart rate ($r = 0.14$, $p = 0.0003$), insulin ($r = 0.17$, $p < .0001$) and HOMA-IR ($r = 0.16$, $p = 0.0001$), and serum GGT levels remained positively correlated with triglycerides and leptin. In the fully adjusted model, serum AST and fetuin-A levels were not significantly correlated with any cardiovascular and metabolic variables.

In multi-variable linear regression analysis including ALT, GGT, AST and fetuin-A as dependent variables, ALT has the strongest association with anthropometric variables, cardiovascular risk factors (except for DBP, HDL cholesterol and triglycerides), leptin, uric acid, urea, insulin, and HOMA-IR. GGT has strongest association with triglycerides and LBP. GGT also showed less strong association with anthropometric variables, cardiovascular risk factors, and leptin. AST has much less strong association with anthropometric variables, insulin, HOMA-IR and no association with cardiovascular variables in these models. Fetuin-A does not have any significant association with any variables in these models. After controlling for age, smoking status, activity, BMI, body fat percentage, and WHR, only serum ALT levels are independently strongly associated with insulin, HOMA-IR, and heart rate. Both serum GGT and ALT levels are

Table 1 – Baseline characteristics of participants in the cross-sectional study.

Variables	All subjects (n = 616)
Age (year)	18.3 ± 0.02
Activity (h/week)	11.6 ± 0.5
Anthropometric variables	
Height (cm)	175.0 ± 0.2
Weight (kg)	72.6 ± 0.6
BMI (kg/m^2)	23.7 ± 0.2
Total body fat (%)	14.2 ± 0.3
Fat mass (kg)	11.2 ± 0.3
Fat-free mass (kg)	61.5 ± 0.3
Waist circumference (cm)	82.0 ± 0.4
Hip circumference (cm)	97.3 ± 0.4
Waist-to-hip ratio	0.84 ± 0.002
BMR (kcal/day)	1822 ± 9
Cardiovascular risk factors	
Heart rate (beats/min)	69.8 ± 0.4
SBP (mmHg)	108.1 ± 0.4
DBP (mmHg)	63.8 ± 0.3
Total cholesterol (mg/dL)	154.8 ± 1.3
HDL cholesterol (mg/dL)	48.1 ± 0.4
LDL cholesterol (mg/dL)	103.8 ± 1.1
Triglycerides (mg/dL)	60.4 ± 1.0
Fasting glucose (mg/dL)	81.6 ± 0.3
Insulin (ng/mL)	9.1 ± 0.2
HOMA-IR	1.88 ± 0.05
Leptin (ng/mL)	2.55 ± 0.11
LBP (ng/mL)	23.4 ± 0.2
Fetuin-A (μg/mL)	295.5 ± 2.8
Uric Acid (ng/dL)	6.7 ± 0.1
Urea (ng/dL)	35.6 ± 0.3
Creatinine (ng/dL)	1.0 ± 0.004
Liver Function test	
GGT (U/L)	25.2 ± 0.6
ALT (U/L)	27.7 ± 0.9
AST (U/L)	27.8 ± 1.1
Categorical variables	
Smoking status	
Never	368 (59.7)
Previous	26 (4.2)
Current	222 (36.1)
BMI >25 kg/m^2	182 (29.5)
Waist circumference >94 cm	96 (15.6)
Total cholesterol >200 mg/dL	48 (7.9)
HDL <40 mg/dL	103 (16.9)
LDL >130 mg/dL	94 (15.4)
Dyslipidemia	183 (30.0)

Results are presented as mean ± SEM, or as n (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, leptin-binding protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; SE, standard error.

independently associated with total, and LDL cholesterol. AST and fetuin-A are no longer associated with any variables in the adjusted model.

3.2. *Prospective study*

Table 3 shows the baseline and follow-up characteristics of the 93 participants who took part in the prospective study. Interestingly, most of the anthropometric variables and cardiovascular risk factors significantly worsened over the 2-year period between visits.

Findings from the prospective portion of the study are summarized in Table 4. In the unadjusted model, baseline serum ALT and GGT levels were significant predictors of BMI, body weight, total body fat, body fat mass, waist circumference, hip circumference, WHR, body fat-free mass, BMR, and LDL cholesterol at 2-years of follow-up (for ALT, p = 0.0004–0.01, β=0.27–0.37; for GGT, p = <0.0001–0.003, β=0.32–0.52). In the models adjusted for baseline age, smoking status, activity, BMI, body fat percentage, and WHR, the baseline serum GGT level remained a significant predictor of LDL cholesterol (p = 0.003, β=0.32), whereas serum ALT was not predictive of any cardiovascular or metabolic variable. Unlike ALT and GGT, baseline serum AST levels were not predictive of any anthropometric, cardiovascular, and metabolic variables in either unadjusted or adjusted models.

Similarly, the changes of serum ALT and GGT levels from baseline to 2-year follow-up were strong predictors of changes of all anthropometric variables in the unadjusted model. The change of serum ALT levels was also a significant predictor of change of triglycerides (p = 0.005, β=0.28), and the change of serum GGT levels was also a significant predictor of change of total (p < 0.0001, β=0.41), HDL (p = 0.002, β=0.31) and LDL (p = 0.01, β=0.26) cholesterol in unadjusted models. In the model adjusted for baseline age, smoking status, activity, BMI, body fat percentage, and WHR, the change of serum GGT levels remained significantly associated with the change of total (p < 0.0001, β=0.41), HDL (p = 0.002, β=0.33) and LDL (p = 0.01, β=0.26) cholesterol while the change of serum ALT levels were only significantly associated with HDL cholesterol (p = 0.008, β=0.31).

Baseline levels of serum fetuin-A were not a significant predictor of any anthropometrics, cardiovascular, and metabolic variables at two-year follow-up in either unadjusted or adjusted models.

Using logistic regression analysis, serum ALT has fair accuracy to significantly predict a total cholesterol level of >200 mg/dL vs. <200 mg/dl, as measured using the area under the ROC curve method (ROC AUC =0.67, p = 0.02, and/or OR = 0.949 [0.909, 0.992]) indicating that each 1 U/L decrease of ALT conveys an approximately 5% lower probability of having total cholesterol >200 mg/dL. Serum AST and fetuin-A levels were not significant predictors of categorically analyzed cholesterol levels.

4. Discussion

In this study, we demonstrated that the liver-derived enzymes ALT and GGT are strongly positively correlated to BMI, adiposity, fat free mass, BMR, blood pressure, total and LDL cholesterol, triglycerides, dyslipidemia, fasting glucose, insulin, and HOMA-IR in young healthy adults. In addition, after adjusting for various confounders, ALT remained associated with heart rate, total and LDL cholesterol, insulin, and HOMA-IR, suggesting that these correlations are independent from any underlying correlation with body composition, age, smoking status, and activity level. Conversely, GGT predicts only LDL cholesterol whereas ALT predicts no parameters at two-year

Table 2 – Correlations between liver function tests, fetuin-A and baseline values of study variables.

	ALT				GGT				AST				Fetuin-A			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.01	0.74			−0.02	0.61			0.11	0.01			−0.04	0.31		
Activity	−0.02	0.67			−0.03	0.44			−0.03	0.47			0.02	0.70		
Smoking status	0.06	0.16			0.13	0.001			0.02	0.58			−0.03	0.53		
Anthropometrics																
Height	0.06	0.12			−0.04	0.34			0.02	0.70			0.05	0.22		
Weight	0.44	<.0001			0.39	<.0001			0.09	0.02			0.15	0.0002		
BMI	0.46	<.0001			0.45	<.0001			0.10	0.02			0.15	0.0004		
Total body fat	0.46	<.0001			0.46	<.0001			0.10	0.01			0.14	0.0009		
Fat mass	0.47	<.0001			0.44	<.0001			0.10	0.01			0.13	0.001		
Fat free mass	0.36	<.0001	0.03	0.44	0.30	<.0001	−0.08	0.06	0.08	0.06	0.03	0.51	0.15	0.0003	0.06	0.16
Waist circumference	0.47	<.0001			0.45	<.0001			0.11	0.006			0.16	<.0001		
Hip circumference	0.41	<.0001			0.39	<.0001			0.18	0.06			0.16	<.0001		
WHR	0.39	<.0001			0.40	<.0001			0.13	0.001			0.11	0.010		
BMR	0.42	<.0001	0.05	0.21	0.36	<.0001	−0.07	0.08	0.09	0.03	0.03	0.53	0.15	0.0003	0.05	0.23
Cardiovascular risk factors																
Heart rate	0.22	<.0001	0.14	0.0003	0.15	0.0003	0.06	0.13	0.04	0.29	0.01	0.75	0.04	0.39	0.01	0.73
SBP	0.31	<.0001	0.10	0.015	0.29	<.0001	0.08	0.06	0.05	0.21	0.003	0.95	0.12	0.004	0.04	0.30
DBP	0.14	0.0004	−0.001	0.97	0.12	0.002	−0.01	0.76	0.03	0.47	−0.01	0.81	0.07	0.08	0.03	0.54
Total cholesterol	0.32	<.0001	0.19	<.0001	0.35	<.0001	0.24	<.0001	0.08	0.04	0.04	0.32	0.06	0.12	0.03	0.40
HDL	−0.03	0.50	0.08	0.04	−0.07	0.07	0.04	0.33	0.01	0.74	0.04	0.28	−0.02	0.71	0.02	0.61
LDL	0.34	<.0001	0.21	<.0001	0.38	<.0001	0.26	<.0001	0.07	0.06	0.04	0.38	0.10	0.02	0.07	0.11
Triglycerides	0.19	<.0001	−0.002	0.95	0.31	<.0001	0.13	0.001	−0.01	0.76	−0.06	0.13	0.04	0.28	−0.005	0.91
Dyslipidemia	0.18	<.0001	0.05	0.18	0.17	<.0001	0.04	0.33	0.02	0.70	−0.01	0.74	0.01	0.74	−0.02	0.62
Hormonal and metabolic variables																
Fasting glucose	0.13	0.001	0.01	0.77	0.14	0.0003	0.04	0.30	0.02	0.60	−0.003	0.93	0.01	0.74	−0.03	0.53
Insulin	0.37	<.0001	0.17	<.0001	0.30	<.0001	0.09	0.02	0.07	0.09	0.02	0.62	0.15	0.0002	0.08	0.07
HOMA-IR	0.36	<.0001	0.16	0.0001	0.30	<.0001	0.09	0.02	0.07	0.10	0.02	0.63	0.14	0.0005	0.06	0.12
Leptin [a]	0.41	<.0001	0.04	0.31	0.46	<.0001	0.15	0.0002	0.07	0.09	−0.03	0.43	0.10	0.012	−0.02	0.54
LBP	−0.16	<.0001	0.06	0.14	−0.21	<.0001	−0.002	0.97	−0.02	0.56	0.03	0.48	−0.08	0.06	−0.01	0.88
Uric acid	0.13	0.001	0.05	0.26	0.14	0.0003	0.06	0.14	0.06	0.13	0.04	0.27	0.02	0.56	0.001	0.99
Urea	−0.02	0.60	0.02	0.59	−0.04	0.35	0.002	0.95	0.08	0.06	0.09	0.03	0.01	0.87	0.03	0.50
Creatinine	−0.05	0.19	−0.03	0.45	−0.05	0.24	−0.02	0.62	0.02	0.67	0.02	0.55	−0.05	0.26	−0.04	0.35
Liver function tests																
ALT					0.54	<.0001	0.41	<.0001	0.52	<.0001	0.53	<.0001	0.11	0.006	0.06	0.13
GGT	0.54	<.0001	0.42	<.0001					0.11	0.008	0.06	0.13	0.15	0.0002	0.10	0.02
AST	0.52	<.0001	0.53	<.0001	0.11	0.01	0.06	0.13					−0.005	0.91	−0.02	0.69

Model 1: Pearson correlation coefficients and the probabilities associated with this statistics.
Model 2: Pearson partial correlation coefficients were obtained after controlling for the effects of age, smoking status, activity, BMI, body fat percentage, and WHR.
$P < 0.0125$ was considered statistically significant based on Bonferroni correction testing four dependent hypotheses at same time on one set of data.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, leptin-binding protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; WHR, waist-to-hip ratio.
[a] Values of leptin were logarithmically transformed for analysis.

follow-up in the adjusted models. Similarly, when compared to ALT and GGT, we found that fetuin-A was less strongly associated with anthropometric parameters, SBP, insulin, HOMA-IR, and leptin. In addition, we showed for the first time, that fetuin-A is not an independent predictor of metabolic risk factors in young healthy men since these correlations became nonsignificant in this group of lean insulin-sensitive 18-year-old subjects after adjusting for various confounders. This suggests that fetuin-A is not as closely correlated to these parameters when compared with traditional LFTs and that to a large extent, its associations with cardiometabolic risk factors may be due to an underlying correlation with body composition.

Our data are in agreement with prior cross-sectional studies of mostly middle-aged participants, linking ALT and GGT to components of the metabolic syndrome, as well as type 2 diabetes mellitus [4,9,12,35]. For example, in a large population-based study analyzing data from the Third National Health and Nutrition Examination Survey (NHANES III), ALT elevation

Table 3 – Baseline and follow-up characteristics of participants in the prospective study.

Variables	At baseline (n = 93)	At follow-up (n = 93)	P [a]
Age (year)	18.2 ± 0.01		
Activity (h/week)	11.3 ± 1.4		
Anthropometric variables			
Height (cm)	175.0 ± 0.6	175.2 ± 0.6	<.0001
Weight (kg)	68.9 ± 1.3	72.9 ± 1.3	<.0001
BMI (kg/m^2)	22.5 ± 0.4	23.7 ± 0.4	<.0001
Total body fat (%)	12.8 ± 0.6	15.6 ± 0.6	<.0001
Fat mass (kg)	9.5 ± 0.7	12.1 ± 0.8	<.0001
Fat-free mass (kg)	59.4 ± 0.7	60.9 ± 0.7	<.0001
Waist circumference (cm)	79.1 ± 1.0	83.8 ± 1.0	<.0001
Hip circumference (cm)	95.7 ± 0.8	97.1 ± 0.8	0.0005
Waist-to-hip ratio	0.82 ± 0.004	0.86 ± 0.004	<.0001
BMR (kcal/day)	1770 ± 20	1814 ± 20	<.0001
Cardiovascular risk factors			
Heart rate (beats/min)	66.1 ± 0.9	71.6 ± 1.0	<.0001
SBP (mmHg)	106.2 ± 1.1	111.7 ± 1.0	<.0001
DBP (mmHg)	61.7 ± 0.8	71.6 ± 0.7	<.0001
Total cholesterol (mg/dL)	144.2 ± 2.7	159.6 ± 2.8	<.0001
HDL cholesterol (mg/dL)	47.1 ± 0.9	46.0 ± 0.9	0.10
LDL cholesterol (mg/dL)	101.9 ± 2.4	98.4 ± 2.4	0.05
Triglycerides (mg/dL)	58.7 ± 2.3	75.8 ± 4.3	<.0001
Fasting glucose (mg/dL)	78.2 ± 0.7	86.9 ± 1.0	<.0001
Insulin (ng/mL)	7.4 ± 0.3		
HOMA-IR	1.44 ± 0.07		
Leptin (ng/mL)	1.76 ± 0.20		
LBP (ng/mL)	25.0 ± 0.5		
Fetuin-A (μg/mL)	333 ± 8.1		
Uric Acid (ng/dL)	6.6 ± 0.1	6.0 ± 0.1	<.0001
Urea (ng/dL)	35.8 ± 0.7	30.4 ± 0.6	<.0001
Creatinine (ng/dL)	1.05 ± 0.01	1.1 ± 0.01	0.002
Liver Function test			
GGT (U/L)	22.6 ± 0.8	22.2 ± 1.0	0.62
ALT (U/L)	24.1 ± 1.4	21.5 ± 1.3	0.07
AST (U/L)	28.1 ± 1.9	20.3 ± 0.5	0.007
Categorical variables			
Smoking status			
Never	60 (64.5)		
Previous	2 (2.2)		
Current	31 (33.3)		
BMI >25 kg/m^2	17 (18.3)	22 (23.7)	0.35
Waist circumference >94 cm	7 (7.5)	12 (12.9)	0.21
Total cholesterol >200 mg/dL	2 (2.2)	7 (7.5)	0.09
HDL <40 mg/dL	14 (15.1)	22 (23.7)	0.14
LDL >130 mg/dL	12 (12.9)	9 (9.7)	0.49
Dyslipidemia	26 (28.0)	34 (36.6)	0.21

Results are presented as mean ± SEM, or as n (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model of assessment-insulin resistance; LBP, leptin-binding protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure; SE, standard error.
[a] P for difference of follow-up group between baseline and 2-year follow-up; repeated measure analysis was applied for both continuous and categorical variables.

was associated with a number of risk factors of NAFLD, such as impaired glucose metabolism, insulin resistance, central obesity, high leptin and triglycerides, even when patients with known diabetes (who are at high risk for NAFLD) were excluded [36]. Others have found similar cross-sectional correlations between GGT and components of the metabolic syndrome [37]. Both GGT and ALT have also been shown prospectively to predict the development of insulin resistance and diabetes mellitus [12,15,17,38,39], which we did not find in this population of healthy 18-year-old men.

In the present study, we found that fetuin-A was significantly correlated with body fat distribution, but not with insulin resistance in young healthy men. Similar to our findings, prior studies in middle-aged or older populations have found that fetuin-A is correlated with truncal obesity [40]. However, many studies have reported a significant

Table 4 – Univariable and multivariable linear regression models of liver function tests and fetuin-A in predicting anthropometrics, cardiovascular and metabolic risk factors at 2-year follow-up.

	ALT						GGT						AST						Fetuin-A					
	Model 1			Model 2			Model 1			Model 2			Model 1			Model 2			Model 1			Model 2		
	β	P	R^2	β	P	R^2	β	P	R^2	β	P	R^2	β	P	R^2	β	P	R^2	β	P	R^2	β	P	R^2
Anthropometrics																								
Height	0.15	0.17	0.02				0.05	0.63	0.00				0.10	0.38	0.009				0.22	0.04	0.050			
Weight	0.37	0.0004	0.14				0.44	<.0001	0.19				0.08	0.46	0.007				0.15	0.17	0.022			
BMI	0.35	0.001	0.13				0.47	<.0001	0.22				0.04	0.70	0.002				0.06	0.56	0.004			
Total body fat	0.34	0.001	0.12				0.49	<.0001	0.24				0.09	0.43	0.008				0.02	0.83	0.001			
Fat mass	0.35	0.001	0.12				0.48	<.0001	0.23				0.08	0.45	0.007				0.05	0.66	0.002			
Fat free Mass	0.34	0.001	0.12	0.02	0.76	0.62	0.33	0.002	0.11	−0.003	0.97	0.62	0.06	0.56	0.004	−0.05	0.48	0.62	0.25	0.02	0.060	0.14	0.05	0.63
Waist circumference	0.36	0.001	0.13				0.52	<.0001	0.27				0.03	0.81	0.001				0.11	0.33	0.011			
Hip circumference	0.33	0.002	0.11				0.43	<.0001	0.19				0.07	0.53	0.005				0.10	0.36	0.010			
Waist-to-hip ratio	0.30	0.01	0.09				0.51	<.0001	0.26				−0.06	0.60	0.596				0.03	0.43	0.007			
BMR	0.37	0.001	0.13	0.02	0.80	0.74	0.41	<.0001	0.17	0.02	0.78	0.74	0.09	0.42	0.008	−0.03	0.64	0.74	0.17	0.11	0.029	0.07	0.25	0.74
Cardiovascular risk factors																								
Heart rate	0.23	0.03	0.05	0.21	0.09	0.08	0.25	0.02	0.06	0.24	0.08	0.09	0.06	0.61	0.00	0.05	0.68	0.05	−0.17	0.12	0.03	−0.18	0.11	0.08
SBP	0.07	0.50	0.01	−0.10	0.38	0.23	0.17	0.114	0.03	0.06	0.63	0.23	−0.02	0.84	0.00	−0.12	0.23	0.24	−0.05	0.63	0.00	−0.09	0.39	0.23
DBP	0.16	0.15	0.02	0.04	0.72	0.21	0.06	0.55	0.00	−0.04	0.76	0.21	0.04	0.70	0.002	−0.02	0.85	0.20	−0.05	0.62	0.003	−0.13	0.22	0.22
Total Cholesterol	0.22	0.04	0.05	0.23	0.06	0.10	0.27	0.01	0.07	0.29	0.03	0.12	−0.07	0.55	0.004	−0.07	0.57	0.07	−0.04	0.70	0.002	−0.09	0.42	0.07
HDL	−0.04	0.71	0.00	−0.04	0.77	0.03	−0.02	0.87	0.00	−0.04	0.78	0.03	−0.06	0.58	0.004	−0.06	0.63	0.04	0.03	0.78	0.001	0.04	0.73	0.03
LDL	0.27	0.01	0.07	0.29	0.02	0.12	0.32	0.003	0.10	0.39	0.003	0.16	−0.05	0.67	0.00	−0.04	0.75	0.06	−0.05	0.65	0.00	−0.11	0.33	0.07
Triglycerides	0.02	0.86	0.00	−0.03	0.80	0.11	0.00	0.99	0.00	−0.10	0.43	0.11	−0.02	0.84	0.001	−0.06	0.62	0.11	−0.03	0.80	0.001	−0.03	0.80	0.11
Hormonal and metabolic variables																								
Fasting glucose	0.06	0.58	0.00	0.00	0.97	0.12	0.06	0.61	0.003	0.04	0.78	0.12	0.17	0.11	0.03	0.11	0.31	0.13	−0.07	0.55	0.00	−0.08	0.47	0.12
Uric Acid	0.02	0.87	0.00	−0.15	0.18	0.22	0.10	0.36	0.01	−0.05	0.69	0.20	−0.05	0.66	0.00	−0.11	0.30	0.21	0.03	0.81	0.00	−0.04	0.71	0.20
Urea	0.04	0.69	0.00	0.07	0.55	0.08	−0.08	0.48	0.006	−0.04	0.79	0.07	0.14	0.21	0.02	0.16	0.16	0.09	0.23	0.03	0.05	0.19	0.09	0.10
Creatinine	−0.09	0.42	0.01	−0.06	0.64	0.08	−0.21	0.05	0.05	−0.16	0.24	0.09	0.08	0.49	0.006	0.08	0.46	0.08	0.06	0.60	0.003	0.05	0.63	0.08

Model 1: unadjusted model.
Model 2: adjusted for age, smoking status, activity, BMI, body fat percentage, and waist to hip ratio.
β denotes the adjusted regression coefficient.
P < 0.0125 was considered statistically significant based on Bonferroni correction testing four dependent hypotheses at the same time using one set of data.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; DBP, Diastolic blood pressure; GGT, gamma-glutamyl transferase; HDL, High-density lipo protein; LDL, Low-density lipoprotein; SBP, Systolic blood pressure.

correlation between fetuin-A and impaired fasting glucose [41,42], metabolic syndrome [43], insulin resistance [24], type 2 diabetes [42,44–46] and prospective studies have found that fetuin-A has an independent association with type 2 diabetes mellitus [27,30] in middle-aged or old overweight populations. Interestingly, Jenkins et al. found that there was no significant relationship between plasma fetuin-A and insulin or HOMA-IR in the combined groups of older individuals and young participants in their study. However, plasma fetuin-A levels trended to be correlated with insulin and HOMA-IR in older but not in younger participants, suggesting effect modification by age [47]. We also found that fetuin-A is not an independent predictor of metabolic risk factors or dyslipidemia in our younger cohort. It has been shown that higher fetuin-A levels are associated with visceral adipose tissue (VAT) as opposed to overall body fat [29]. Deposition of VAT may play a more important role with advancing age and increasing BMI, explaining the negative result in this young healthy cohort. Jenkins et al. found in their younger participants that plasma fetuin-A was significantly related to blood pressure and blood lipid variables; in our study, fetuin-A was associated with SBP but no other blood pressure or lipid variables at baseline.

In summary, the novel findings of our study are that fetuin-A levels are not independently associated with any metabolic or cardiovascular risk factor at baseline and are not a better than traditional LFTs predictor of these variables cross-sectionally and prospectively in young adults.

The strengths of this study are that it is the first cross-sectional and prospective study comparing associations between serum liver enzymes, serum fetuin-A levels and cardiovascular and metabolic characteristics in young men. We also adjusted for known potential confounders, such as smoking status and activity in our analysis, thus eliminating bias or confounding by these variables. Measurements were performed under code using de-identified specimens and state of the art methodology by technicians who were blinded to the study hypotheses eliminating bias from these sources. Random assay variability could have resulted in misclassification but this random misclassification would have suppressed effect estimates and hence should not have resulted in statistical significance where this does not exist.

The limitations of our study include the relatively short follow-up time of only 2 years; this period of time has been shown to be adequate in terms of evaluation of cardiometabolic predictors of risk in prior studies and in this study in terms of traditional LFTs. Despite the large number of subjects in the cross sectional study, the prospective study included only a relatively small follow-up group (93 subjects) but numbers of subjects were sufficient to demonstrate significant associations between serum liver enzymes levels and outcomes of interest. The results may not be directly generalizable to other populations since we focused on a young and healthy population of Mediterranean descent. Future prospective studies are needed to confirm our data in cohorts of women and/or older subjects in the same and other ethnic groups. Moreover, interventional, mechanistic studies are needed to interpret our findings that fetuin-A may not be a better indicator of the metabolic syndrome, diabetes, and cardiovascular disease compared to traditional LFTs in younger adults as this study clearly demonstrates.

Grants

The study was supported by the Cyprus Research Promotion Foundation (ΕΡΥΕΞ/0205/10). The Mantzoros Lab is supported by grants DK58785, DK79929, DK81913, AG032030, a VA Merit award and a discretionary grant from Beth Israel Deaconess Medical Center. This work was conducted with support from Harvard Catalyst | The Harvard Clinical and Translational Science Center (NIH Award #UL1 RR 025758 and financial contributions from Harvard University and its affiliated academic health care centers).

Author contributions

CSM derived the hypothesis and conceived the study design; MP, CAC, SNK, and DCC planned and organized the collection of the data; XL, HG, and JPC performed the laboratory analyses; XL and CSM planned and did the statistical analyses and XL collated the data and run the statistical analyses, XL, OPRH, MP, CAC, SNK, DCC, and CSM contributed to the interpretation and discussion of results; XL, OPRH wrote initial versions of the manuscript and CSM completed the manuscript. This report was critically reviewed and subsequently approved by all authors.

Acknowledgments

We would like to thank the Harvard Cyprus Program, Nicosia General Hospital, all volunteers for participating in the study, and especially Maria Michael, Androula Chasikou-Constantinidou, and Christos Tapakoudes for their expert technical assistance.

Conflict of interest

The authors have no relevant conflict of interests.

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