The role of serum retinol in nonalcoholic fatty liver disease

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Background Retinol has been involved in the regulation of lipid metabolism and hepatic steatosis. Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) have emerged as the most common chronic liver diseases. A minority of affected patients develop subsequently hepatic fibrosis, whereas most of them exhibit simple steatosis. Indeed, the relation between retinol and NAFLD and NASH is still incomplete and unknown.

Objective This study aimed to identify the clinical relevance of retinol in patients with NAFLD and NASH.

Patients and methods This study enrolled 90 individuals who were selected from the outpatient clinic of Al Zahraa University Hospital, Egypt, which comprised 30 patients with NAFLD, 30 with NASH and 30 healthy persons as a control group. Serum glucose, lipid profiles, markers of liver damage, serum retinol, and abdominal ultrasound were studied.

Results Serum retinol concentrations were significantly lower in NAFLD and NASH than in control, where the mean serum retinol concentration in patients with NAFLD was 23.02±2.9 and NASH was 11.7±2.3, and it was significantly lower than those in controls, with 36.1±2.7 ($P<0.01$).

Conclusion Circulating retinol concentrations were lower in patients with NAFLD and were associated with hepatic lipid metabolism and insulin resistance.

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Introduction

Vitamin A is required for important physiological process. Many of vitamin A ?s functions are performed through retinoic acids, which activate transcriptional networks controlled by retinoic acid receptors and retinoid X receptor [1].

The liver plays a central role in retinol metabolism as follows: (a) it produces bile, supporting efficient intestinal absorption of fat-soluble nutrients like retinol; (b) it produces retinol-binding protein 4, which distributes vitamin A as retinol to peripheral tissues; and (c) it harbors the largest body supply of retinol, mostly as retinyl esters, in hepatic stellate cells (HSCs). Liver injury triggers HSCs to differentiate into myofibroblasts, which produce excessive amount of extracellular matrix, leading to fibrosis [2]. Recently, owing to new, very potent antiviral drugs that can eradicate or control viral replication in patients with chronic hepatitis C and B infections, a change in focus has been directed from chronic viral hepatitis to fatty liver disease. Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome and is a spectrum of conditions ranging from benign hepatic steatosis to nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and liver cancer [3].

NASH is suspected to be the main cause of liver failure occurrence in the near future. Retinoic acids are key regulators of glucose and lipid metabolism in the liver and adipose tissue, but it is unknown whether impaired vitamin A homeostasis contributes to or suppresses the development of NAFLD, although recent studies showed that retinol deficiency contributes toward pathogenesis of NAFLD and is considered as new a therapeutic approach to NAFLD. Thus, the current study aims to identify the clinical relevance of retinol in patients with NAFLD and NASH [4].

Patients and methods

Patients

This study was conducted on 90 Egyptian individuals, comprising 30 patients with NAFLD, 30 with NASH, and 30 apparently healthy persons as a control group. These individuals were aged 35–45 years and were selected from the outpatient clinic of Internal Medicine Department at Al Zahraa University Hospital, Egypt, within the period from March 2016 till December 2017.

Declaration

This study conforms to the principles outlined in the declaration of Helsinki. Approval of the local ethical committee of Faculty of Medicine, Al Azhar University, Egypt, was obtained, and informed consents were obtained from all participants.

Methods

All patients and controls were subjected to full medical history, physical examination, and routine laboratory investigation. The exclusion criteria included acute and chronic viral hepatitis, diabetes mellitus and hyperlipidemia, total parental nutrition, postgastroenterology operation, obstructive biliary diseases, current use of systemic corticosteroid, current pregnancy, and thyroid dysfunction.

Anthropometric measurements

These included BMI and waist-to-hip ratio [5].

Biochemical measurements

These included complete blood count; fasting and postprandial blood glucose levels; liver enzymes test comprising asprtate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase, and serum bilirubin, total and direct; overnight fasting serum lipid (12–14 h) measurements, including serum triglycerides, total cholesterol, and high-density and low-density lipoprotein cholesterol; antinuclear antibody; erythrocyte sedimentation rate; renal function tests (serum creatinine and blood urea); fasting serum insulin level; insulin resistance evaluated using the HOMA-IR, calculated as fasting glucose (mmol/l), fasting insulin (mU/l)/22.5 (23) [6]; hepatitis B surface antigen; anti-hepatitis C virus antibody; and thyroid function tests, including FT3, FT4, and TSH.

Serum retinol concentration

Serum retinol was determined by high-performance liquid chromatography assay kit supplied by Eagle Biosciences Inc. (Amherst, New Hampshire, USA). This assay was highly sensitive and specific for the detection of human retinol (normal value, 31.6–82.0 μg/dl) [7].

Radiological examination

Hepatic steatosis was determined by abdominal ultrasound performed by an experienced radiologist to evaluate five ultrasound parameters including parenchymal echogenicity, far gain attenuation, gallbladder wall blurring, portal vein wall blurring, and hepatic vein blurring to distinguish between NAFLD and NASH, as shown in.

Diagnosis of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis

(1) HAIR score (hypertension, elevation of ALT, and elevation of insulin resistance) more than or equal to 2 was used to diagnose NASH [8].
(2) Grading of steatosis by abdominal ultrasonography provides guidance in selecting those patients: score 0 (FS$<$7 and MFS$<$3) indicates simple steatosis, score 1 (FS$\geq$7 or MFS$\geq$3) indicates early detection of NASH, and score 2 (FS$\geq$7 and MFS$\geq$3) indicates presence of NASH [9].

Statistical analysis

Data were analyzed using statistical program for the social science (SPSS, version 15.0; IBM Corp., Released 2017, IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Quantitative data were expressed as mean±SD, and qualitative data were expressed as frequency and percentage. *t* test, χ^2 test, one-way analysis of variance, post-hoc test (least significance difference), and Pearson's correlation coefficient (*r*) test were used for analytical research. *P* value less than 0.001 was considered as highly significant, and *P* value more than 0.05 was considered insignificant.

Results

Baseline characteristics of the study participants

The clinical and biochemical characteristics of the study population showed no significant differences in age, sex, BMI, and waist-to-hip ratio between patients in the control, NAFLD, and NASH groups. Moreover, a highly statistical significant difference ($P<0.001$) was found between NAFLD and NASH and between NASH and control regarding white blood cells, red blood cells, hemoglobin, % platelets, and erythrocyte sedimentation rate. A statistical significant difference ($P<0.05$) was seen between NASH and control groups regarding RDW. A highly statistically significant difference was found among the three groups regarding ALT, AST, gamma-glutamyl transferase, insulin, and HOMA-IR. No statistically significant difference was found among the three groups regarding total bilirubin (BIL T), albumin (ALB), prothrombin time (PT), kidney function tests (KFTs), serum glucose, and hormonal and lipid profiles, as shown in Table 1.

A highly statistically significant difference was found among the three groups regarding retinol. After adjustment for age, sex, and BMI, serum retinol concentrations in patients with NAFLD (23.02±2.9) and NASH (11.7±2.3) were significantly lower than

those in controls (36.1±2.7) ($P<0.01$), as shown in Table 2.

Correlation between serum retinol concentrations with insulin, HOMA-IR, alanine aminotransferase, and aspartate aminotransferase in nonalcoholic fatty liver disease

We analyzed the correlation between serum retinol concentration and the biochemical variables in patients with NAFLD, and found that serum retinol concentrations were negatively associated with insulin, HOMA-IR, ALT, and AST (all $P<0.01$), as shown in Figs 1–5, respectively.

Association of serum retinol concentration with insulin HOMA-IR, ALT, and AST in NASH group.

Notably, we observed that serum retinol concentration was inversely correlated with serum insulin, HOMA-IR, ALT, and AST in NASH group, as shown in Figs 6–9, respectively.

Discussion

We found that serum concentrations of retinol decreased in patients with NAFLD when compared with control and decreased more in patients with NASH when compared with those with NAFLD. Our finding is in agreement with many studies [10–13].

The power of retinol in predicting the degree of liver involvement in biopsy-proven NAFLD has been reported, and it was also found that low level of retinol was associated significantly with necroinflammatory activity and that retinol was negatively correlated with the grade of steatosis and stage of fibrosis; moreover, serum retinol was significantly lower in patients with NASH compared with those with NAFLD [14–17].

However, other studies displayed no differences between control and NAFLD adults [18,19] or even higher serum retinol in patients with NAFLD compared with a control group [20].

NAFLD is characterized by hepatic triglycerides accumulation-enhanced VLDL production, and secretion leading to hypertriglyceridemia. Vitamin A metabolites especially retinol are involved in regulatory networks that affect all these processes either directly or indirectly [21].

On the contrary, our patients in the NAFLD group had a high level of insulin and IR as measured by serum fasting insulin and HOMA-IR as compared with the control group. Moreover, patients in the NASH group had higher levels of insulin and IR as compared with NAFLD group, which was the same observation in other studies [22,23]. The HOMA-IR index showed statistically significant values for differentiating NASH from simple fatty liver.

HOMA-IR was statistically related to NASH. Using ultrasound to detect fatty liver in obese children and

Table 1 Comparison between all groups regarding cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein

Groups	Variables					
				One-way ANOVA (*P* value)		
	NAFLD (*N*=30) (mean±SD)	NASH (*N*=30) (mean±SD)	Control (*N*=30) (mean±SD)	NAFLD vs. NASH	NAFLD vs. control	NASH vs. control
Cholesterol (mg/dl)	150.7±15.9	154.5±12.8	155.1±16.8	0.3	0.2	0.8
TG (mg/dl)	123.7±13.2	132.9±16.7	125.4±16.4	0.07	0.6	0.06
LDL (mg/dl)	80.4±15.1	81.5±13.2	87.1±16.2	0.8	0.08	0.1
HDL (mg/dl)	43.5±6.5	42.6±6.4	43.1±5.9	0.5	0.8	0.7

ANOVA, analysis of variance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TG, triglyceride.

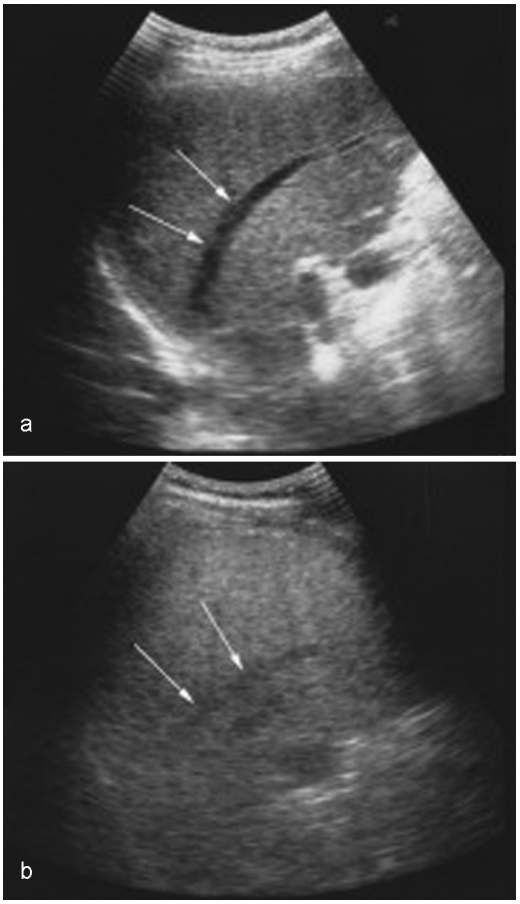
Table 2 Comparison between all groups regarding insulin, HOMA-IR, and retinol

Groups	Variables					
				One-way ANOVA (*P* value)		
	NAFLD (*N*=30) (mean±SD)	NASH (*N*=30) (mean±SD)	Control (*N*=30) (mean±SD)	NAFLD vs. NASH	NAFLD vs. control	NASH vs. control
Insulin (μIU/ml)	21.6±2.6	34.7±3.1	11.4±2.6	<0.001	<0.001	<0.001
HOMA-IR	4.4±0.6	7.3±0.8	2.3±0.5	<0.001	<0.001	<0.001
Retinol (μg/ml)	23.02±2.9	11.7±2.3	36.1±2.7	<0.001	<0.001	<0.001

ANOVA, analysis of variance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

adolescents, different studies have reported that patients with NAFLD who had more prevalent IR should have NASH, and the severity of fatty liver was positively related to hyperinsulinemia and IR; therefore, HOMA-IR has been suggested as a marker of insulin sensitivity and could be a useful screening parameter for NAFLD and NASH [24–29]. Our results also suggested that insulin resistance plays a significant role in pathogenesis of NAFLD in nonobese nondiabetic patients.

Figure 1



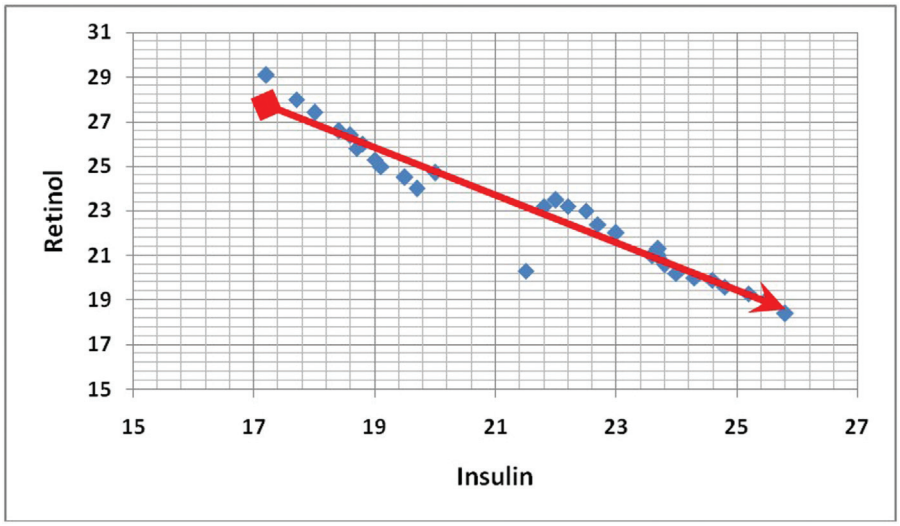
Difference between NAFLD and NASH by abdominal ultrasonography in our patients (NASH). NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis. $P<0.01$.

Insulin controls the expression of a variety of genes involved in glycolysis, glycogenesis, lipogenesis, and gluconeogenesis in the liver, and generally hepatic lipid and glucose metabolism are altered with the development of insulin resistance. Some studies showed that insulin was profibrogenic; furthermore, the molecular mechanisms underlying how insulin mediates hepatic fibrogenesis in NASH remain uncertain [30].

Although we found a negative correlation between retinol level and IR, which was estimated by HOMA-IR, it agrees with other reports [10–32], which found an association of retinol serum level with HOMA-IR. Inadequate serum retinol levels were found in morbidly obese adults with ultrasonography-proven NAFLD, and a significant association between low retinol levels and insulin resistance was found [33]. A similar trend was observed, but others did not find such a correlation in adults with NAFLD [15–20].

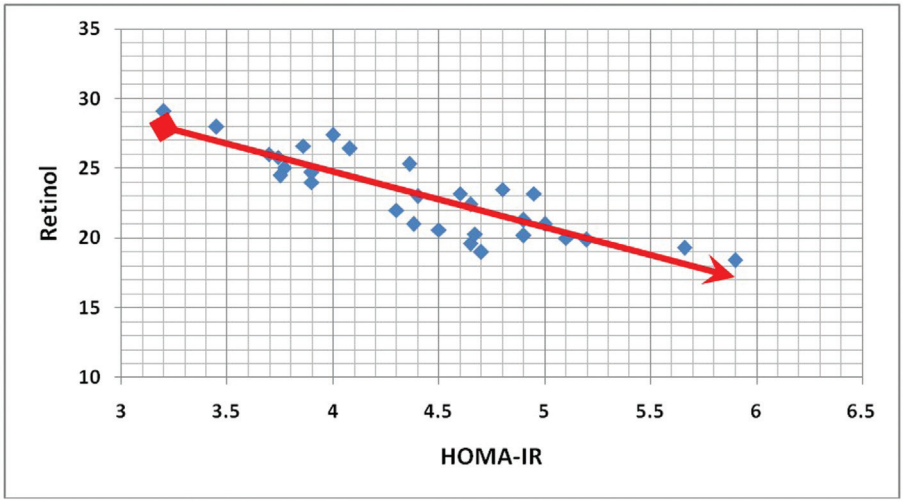
Retinol levels correlate highly with IR. Deficiency of serum retinol causes IR, but the molecular mechanisms are unknown. Another study showed that retinol deficiency induces expression of proinflammatory

Figure 2



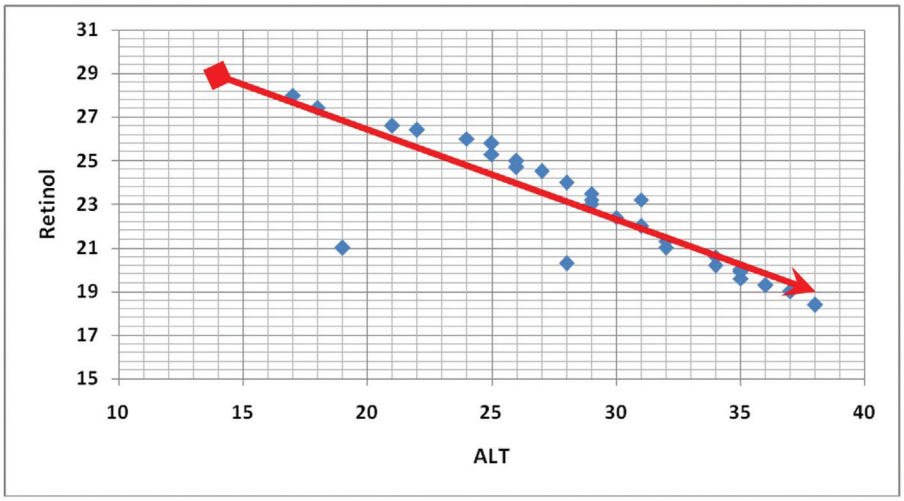
Negative correlation between retinol and insulin in NAFLD group. NAFLD, nonalcoholic fatty liver disease.

Figure 3



Negative correlation between retinol and HOMA-IR in NAFLD group. NAFLD, nonalcoholic fatty liver disease.

Figure 4

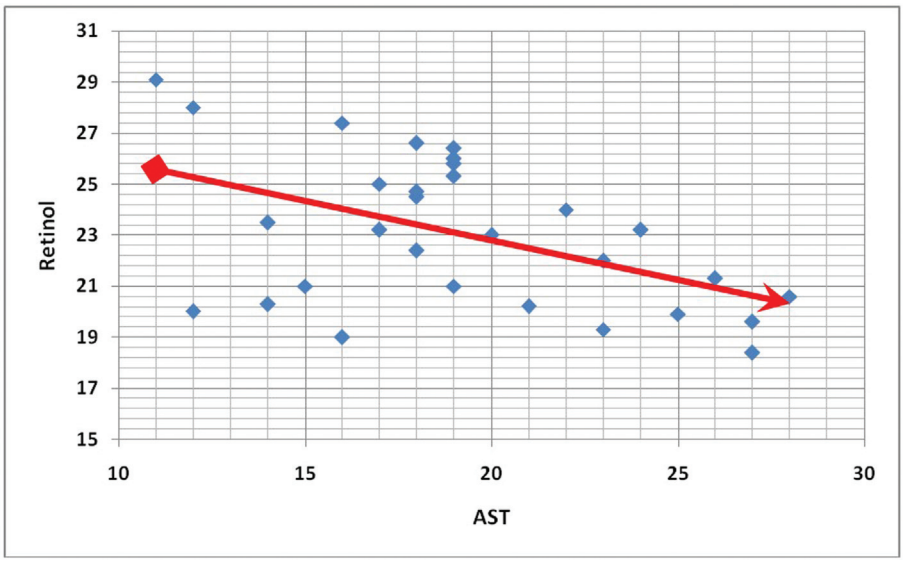


Negative correlation between retinol and ALT in NAFLD group. ALT, alanine aminotransferase; NAFLD, nonalcoholic fatty liver disease.

cytokines in mouse and human macrophages and thereby indirectly inhibits insulin signaling in cocultured adipocytes [34]. This occurs through activation of toll-like receptor 4 pathways. Results indicated that lower retinol is associated with insulin resistance independent of obesity, and this may be because regions near the retinol locus on human chromosome 10q have been linked to hyperinsulinemia or early onset of NAFLD, consistent with a pathogenic role for retinol deficiency in insulin resistance and incidence of NAFLD [35]. It is not known whether insulin action are retinol (vitamin A) dependent, and retinol has important biologic effect in insulin action and integrity of hepatocyte and HSCs.

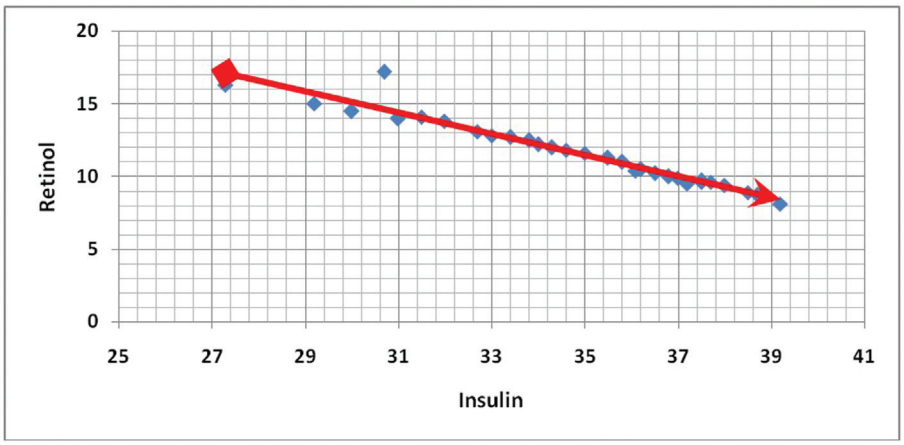
When interpreting our results, some limitation should be considered. The first limitation is the presence of NAFLD, which was assessed by ultrasound instead of liver biopsy, which was not performed in our patients because of it being invasive and owing to some limitations such as sampling error. Although ultrasound is regarded reasonable and accurate, it cannot identify fatty infiltration of the liver below the threshold of 30%. Therefore, there is the possibility of discrepancy between ultrasonography finding and real NAFLD; however, it was inappropriate to perform invasive test in a population-based epidemiological study. The second limitation of this study is the small number of NAFLD patients, and the third limitation is the controversy as to the clinical acceptability of cutoff

Figure 5



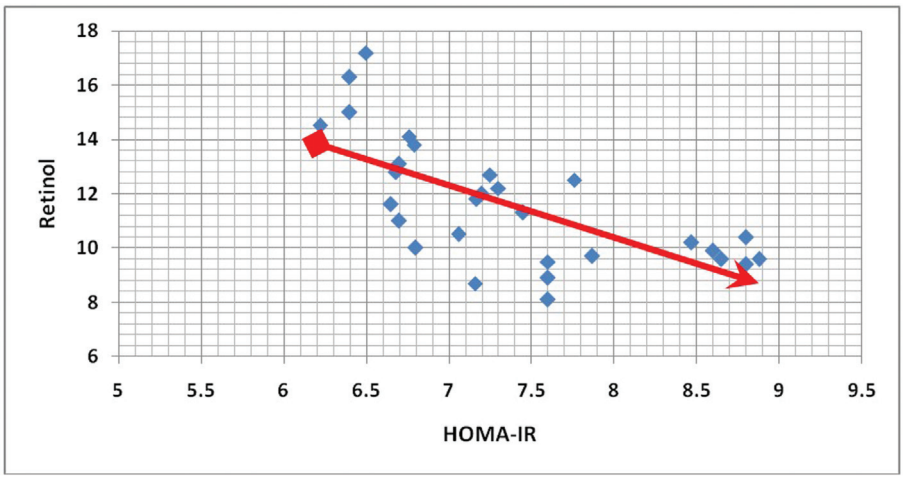
Negative correlation between retinol and AST in NAFLD group. AST, asprtate aminotransferase; NAFLD, nonalcoholic fatty liver disease.

Figure 6



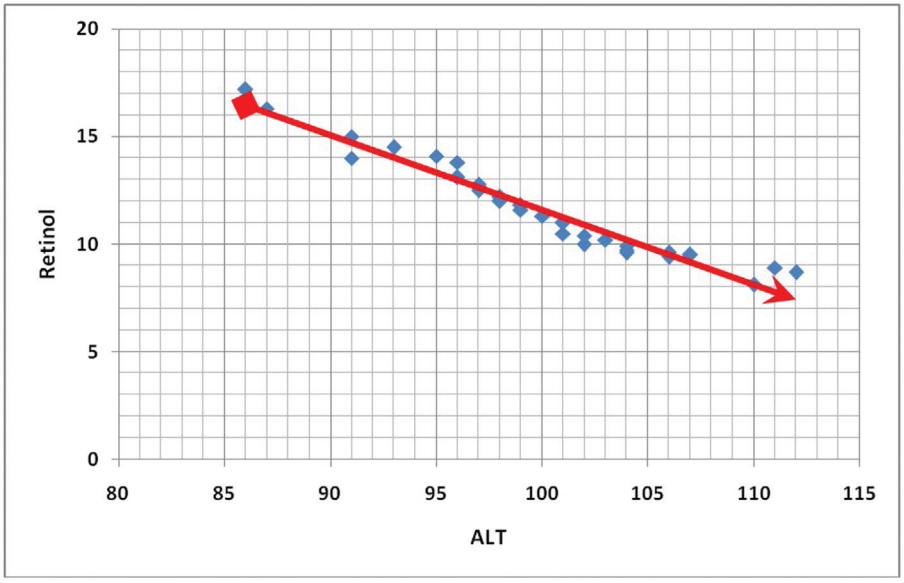
Negative correlation between retinol and insulin in NASH group. NASH, nonalcoholic steatohepatitis.

Figure 7



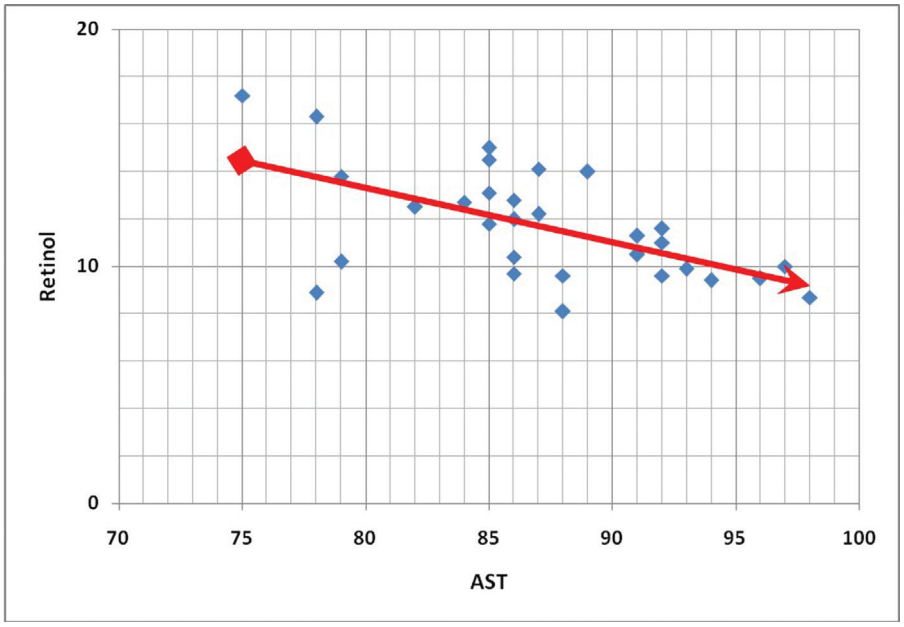
Negative correlation between retinol and HOMA-IR in NASH group. NASH, nonalcoholic steatohepatitis.

Figure 8



Negative correlation between retinol and ALT in NASH group. ALT, alanine aminotransferase; NASH, nonalcoholic steatohepatitis.

Figure 9



Negative correlation between retinol and AST in NASH group. AST, asprtate aminotransferase; NASH, nonalcoholic steatohepatitis.

value of IR in this study, as we adopted HOMA-IR more than or equal to 2.9 as an indicator of IR.

Conclusion

It can be concluded that in patients with NAFLD there is a strong association between deficiency of serum retinol and occurrence of NAFLD, and the subsequent development of IR retinoid (nutritional) and insulin (hormonal) signals converge to regulate the FA synthesis in the liver. Serum retinol can considered as a serum biomarker of intrahepatic lipid content. Retinol could lead to development of new prevention and treatment approaches of NAFLD and NASH. Our study can be helpful to understand the clinical association between NAFLD and metabolic disease.

We recommended that, retinol can be regarded as a noninvasive serum biomarker of NAFLD. This finding needs to be confirmed in larger studies with biopsy-proven NAFLD, and further intervention studies are needed to verify the protective effects of retinol on the development of NAFLD and NASH. Retinol supplementation

might be a promising therapeutic nutrient for fatty liver disease.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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