PNPLA3 I148M Variant Influences Circulating Retinol in Adults with Nonalcoholic Fatty Liver Disease or Obesity1,2

Alison Mondul,3,9 Rosellina M Mancina,4,9 Andrea Merlo,5,6,9 Paola Dongiovanni,5,6 Raffaela Rametta,5,6 Tiziana Montalcini,7 Luca Valenti,5,6,10 Demetrius Albanes,3,10 and Stefano Romeo4,7,8,10*

3Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Bethesda, MD; 4Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden; 5Internal Medicine, Institution of Scientific Inpatient Care (Istituto di ricovero e cura a carattere scientifico, IRCCS) Ca’Granda Polyclinic Hospital, Milan, Italy; 6Department of Pathophysiology and Transplantation (DEPT), University of Milan, Milan, Italy; 7Department of Medical and Surgical Sciences, Clinical Nutrition Unit, University Magna Graecia of Catanzaro, Catanzaro, Italy; and 8Department of Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden

Abstract

Background: Retinol is a lipid-soluble essential nutrient that is stored as retinyl esters in lipid droplets of hepatic stellate cells. Patatin-like phospholipase domain-containing 3 (PNPLA3), through its retinyl-palmitate lipase activity, releases retinol from lipid droplets in hepatic stellate cells in vitro and ex vivo. We have shown that the genetic variant I148M (rs738409) reduces the PNPLA3 retinyl-palmitate lipase activity.

Objective: The aim of the present genetic association study was to test whether overweight/obese carriers of the PNPLA3 I148M mutant allele had lower circulating concentrations of retinol than individuals who are homozygous for the 148I allele.

Methods: PNPLA3 I148M (rs738409) was genotyped by Taqman assay in 76 overweight/obese individuals (BMI (kg/m²) ≥25; mean ± SD age: 59.7 ± 11.4 y; male gender: 70%) with a histologic diagnosis of nonalcoholic fatty liver disease (NAFLD; namely the Milan NAFLD cohort) and in 413 obese men (BMI ≥30; mean ± SD age: 57.1 ± 4.9 y) from the α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study. Serum concentrations of retinol and α-tocopherol were measured by HPLC in both cohorts. β-Carotene concentrations in the ATBC study were measured by using HPLC.

Results: The PNPLA3 I148M mutant allele was associated with lower fasting circulating concentrations of retinol (β = −0.289, P = 0.03) in adults with NAFLD (Milan NAFLD cohort). The PNPLA3 I148M mutant allele was also associated with lower fasting circulating concentrations of retinol in adults with a BMI ≥30 (ATBC study; β = −0.043, P = 0.04). Conclusion: We showed for the first time, to our knowledge, that carriers of the PNPLA3 I148M allele with either fatty liver plus obesity or obesity alone have lower fasting circulating retinol concentrations. J Nutr 2015;145:1687–91.

Keywords: PNPLA3, retinol, NAFLD, obesity, RBP4

Introduction

Retinol and its related compounds retinaldehyde and retinoic acid (collectively known as vitamin A or retinoids) are essential nutrients involved in important physiologic functions such as vision, regulation of gene expression, and cell differentiation. Retinol is a lipid-soluble molecule, which is absorbed by the intestine and transported to the liver in chylomicron remnants (1). Retinol is stored as retinyl palmitate in lipid droplets of stellate cells in the liver (2), which accounts for 50–80% of total vitamin A body stores in humans (3, 4).

We showed that patatin-like phospholipase domain-containing 3 (PNPLA3)11 is the enzyme responsible for the release of retinol from lipid droplets in hepatic stellate cells in vitro and ex vivo in response to insulin (5). We also showed that the retinyl-palmitate lipase activity is markedly reduced by a common genetic variant of

11Abbreviations used: ATBC, α-Tocopherol, β-Carotene Cancer Prevention; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing 3; RBP4, retinol binding protein 4.
PNPLA3, resulting in an isoleucine to methionine substitution at position 148 (I148M). Moreover, circulating concentrations of retinol binding protein 4 (RBP4), a marker of retinol stores secreted by hepatocytes, were lower in overweight/obese individuals with nonalcoholic fatty liver disease (NAFLD) carrying the PNPLA3 148M mutant than in those with the 148I wild-type allele (5). RBP4 concentrations are influenced by total body retinol and by inflammation, whereas fasting retinol concentrations more closely reflect retinol release by hepatic stellate cells (6–9).

We hypothesized that carriers of the PNPLA3 148M mutation have lower circulating concentrations of retinol due to a retention of this molecule in hepatic stellate cells. Therefore, we examined circulating retinol concentrations among overweight/obese carriers of the PNPLA3 148M mutant allele.

Methods

Study cohorts

The Milan NAFLD cohort. For this genetic study, we recruited 76 consecutive overweight/obese [BMI (in kg/m²) ≥ 25] patients with a histologic diagnosis of NAFLD who attended the outpatient clinic at the Metabolic Liver Disease Center, Institute of Scientific Inpatient Care, Ca’-Granda Polyclinic Hospital, University of Milan, Milan, Italy, between June 2013 and June 2014. Patients were excluded if they had coexistent causes of liver disease, including viral hepatitis, genetic liver diseases, and excessive alcohol intake (≥ 30 g/d in men and ≥ 20 g/d in women). Patients who were taking vitamin supplements were also excluded. Fasting blood samples were taken at the time of the biopsy (n = 10) or within a year (n = 66), after excluding those who had modified/begun pharmacologic treatment or had weight change ≥ 5%.

Demographic and anthropometric features, presence of diabetes and other comorbidities, and fasting serum glucose and lipoproteins were determined at the time of blood sample collection. Blood samples were collected after overnight fasting during routine clinical controls. Liver damage and the presence of nonalcoholic steatohepatitis (NASH) were assessed according to the NAFLD Clinical Research Network recommendations (10). DNA was extracted from peripheral blood by the phenol-chloroform method, and the rs738409 polymorphism (encoding PNPLA3 I148M) was determined by 5′-nuclease Taqman assays (Life Technologies) (11).

Serum concentrations of retinol and α-tocopherol (another liposoluble vitamin) were measured by HPLC (Waters) according to the manufacturer’s instructions (Chromsystems Diagnostics) (12). Serum RBP4 concentrations were measured in serum samples by ELISA according to the manufacturer’s instructions (Abcam) (5).

The study was approved by the Institutional Review Board of the Fondazione IRCCS Ca’-Granda and conforms to the Declaration of Helsinki. Each subject signed a written informed consent for participation in the study.

The α-Tocopherol, β-Carotene Cancer Prevention Study. The α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study was a randomized, double-blind, placebo-controlled primary prevention trial conducted in southwest Finland to test the effect of α-tocopherol and β-carotene supplements on the prevention of lung and other cancers (13). Briefly, between 1985 and 1993, a total of 29,133 male smokers aged 50 to 69 y at entry were recruited. Anthropometric, clinical, and biochemical variables were measured. Retinol, α-tocopherol, and β-carotene concentrations were measured in fasting serum samples by using reverse-phase LC with diode-array UV detection at the time the study was conducted at the National Public Health Institute in Helsinki, Finland. After ethanol-ether extraction and injection into a Hypersil octadecysilane column with an isotropic methanol mobile phase and flow rate of 0.9 mL/min for a 9-min run, retinol was monitored at a 305-nm, α-tocopherol at a 292-nm, and β-carotene at a 460-nm wavelength (14). All samples were protected from light and stored at −70°C until they were assayed.

For the present analysis, we randomly sampled 413 obese men (BMI ≥ 30) for whom germline DNA was previously extracted. Genotyping for the rs738409 polymorphism was performed by using the Taqman assay described above at the Cancer Genomics Research Laboratory of the National Cancer Institute at the NIH.

The ATBC Study was approved by institutional review boards at both the US National Cancer Institute and the Finnish National Public Health Institute. Written informed consent was obtained from all trial participants.

Statistical analyses

Genotype and allele frequencies were compared by chi-square test. Categorical variable distribution (namely gender, NASH, and diabetes incidence and percentage of individuals who were physically active and smokers) across the 3 genotypes [II (homozygous for the PNPLA3 148I allele), IM (heterozygous), and MM (homozygous for the PNPLA3 148M allele)] were compared by chi-square or Fisher’s exact test. \( P \) values for continuous variables (namely age; BMI; HOMA-IR; concentrations of insulin, glucose, retinol, α-tocopherol, RBP4, and β-carotene; and RBP4:retinol ratio) were calculated by linear regression analysis under an additive model after adjusting for appropriate confounding factors such as NASH, BMI, age, gender, smoking status, and diabetes or age at randomization and BMI for the Milan NAFLD cohort and ATBC study cohort, respectively. In obese individuals from the ATBC study, the \( P \) value for retinol concentration was calculated after adjusting for age, BMI, and dietary retinol intake. Nonnormally distributed variables were log-transformed before entering the model.

All statistical analyses were performed by using the Statistical Package for Social Sciences (SPSS, version 19.0) or the Statistical Analysis System (SAS Institute) for the Milan NAFLD cohort and for obese individuals from the ATBC study, respectively. \( P \) values <0.05 were considered significant.

Results

Characteristics of the cohort with NAFLD (n = 76) are shown in Table 1. Individuals were overweight/obese, 51% had NASH, and 39% were diabetic. On average, the NAFLD cohort was older at randomization and BMI for the Milan NAFLD cohort was lower than that for the ATBC study. Characteristics of overweight/obese participants with NAFLD (Milan NAFLD cohort) and obese participants from the ATBC study are shown in Table 1.

For this genetic study, we recruited 76 consecutive overweight/obese [BMI (in kg/m²) ≥ 25] patients with a histologic diagnosis of NAFLD who attended the outpatient clinic at the Metabolic Liver Disease Center, Institute of Scientific Inpatient Care, Ca’-Granda Polyclinic Hospital, University of Milan, Milan, Italy, between June 2013 and June 2014. Patients were excluded if they had coexistent causes of liver disease, including viral hepatitis, genetic liver diseases, and excessive alcohol intake (≥ 30 g/d in men and ≥ 20 g/d in women). Patients who were taking vitamin supplements were also excluded. Fasting blood samples were taken at the time of the biopsy (n = 10) or within a year (n = 66), after excluding those who had modified/begun pharmacologic treatment or had weight change ≥ 5%.

Demographic and anthropometric features, presence of diabetes and other comorbidities, and fasting serum glucose and lipoproteins were determined at the time of blood sample collection. Blood samples were collected after overnight fasting during routine clinical controls. Liver damage and the presence of nonalcoholic steatohepatitis (NASH) were assessed according to the NAFLD Clinical Research Network recommendations (10). DNA was extracted from peripheral blood by the phenol-chloroform method, and the rs738409 polymorphism (encoding PNPLA3 I148M) was determined by 5′-nuclease Taqman assays (Life Technologies) (11).

Serum concentrations of retinol and α-tocopherol (another liposoluble vitamin) were measured by HPLC (Waters) according to the manufacturer’s instructions (Chromsystems Diagnostics) (12). Serum RBP4 concentrations were measured in serum samples by ELISA according to the manufacturer’s instructions (Abcam) (5).

The study was approved by the Institutional Review Board of the Fondazione IRCCS Ca’-Granda and conforms to the Declaration of Helsinki. Each subject signed a written informed consent for participation in the study.

The α-Tocopherol, β-Carotene Cancer Prevention Study. The α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study was a randomized, double-blind, placebo-controlled primary prevention trial conducted in southwest Finland to test the effect of α-tocopherol and β-carotene supplements on the prevention of lung and other cancers (13). Briefly, between 1985 and 1993, a total of 29,133 male smokers aged 50 to 69 y at entry were recruited. Anthropometric, clinical, and biochemical variables were measured. Retinol, α-tocopherol, and β-carotene concentrations were measured in fasting serum samples by using reverse-phase LC with diode-array UV detection at the time the study was conducted at the National Public Health Institute in Helsinki, Finland. After ethanol-ether extraction and injection into a Hypersil octadecysilane column with an isotropic methanol mobile phase and flow rate of 0.9 mL/min for a 9-min run, retinol was monitored at a 305-nm, α-tocopherol at a 292-nm, and β-carotene at a 460-nm wavelength (14). All samples were protected from light and stored at −70°C until they were assayed.

For the present analysis, we randomly sampled 413 obese men (BMI ≥ 30) for whom germline DNA was previously extracted. Genotyping for the rs738409 polymorphism was performed by using the Taqman assay described above at the Cancer Genomics Research Laboratory of the National Cancer Institute at the NIH.

The ATBC Study was approved by institutional review boards at both the US National Cancer Institute and the Finnish National Public Health Institute. Written informed consent was obtained from all trial participants.

Statistical analyses

Genotype and allele frequencies were compared by chi-square test. Categorical variable distribution (namely gender, NASH, and diabetes incidence and percentage of individuals who were physically active and smokers) across the 3 genotypes [II (homozygous for the PNPLA3 148I allele), IM (heterozygous), and MM (homozygous for the PNPLA3 148M allele)] were compared by chi-square or Fisher’s exact test. \( P \) values for continuous variables (namely age; BMI; HOMA-IR; concentrations of insulin, glucose, retinol, α-tocopherol, RBP4, and β-carotene; and RBP4:retinol ratio) were calculated by linear regression analysis under an additive model after adjusting for appropriate confounding factors such as NASH, BMI, age, gender, smoking status, and diabetes or age at randomization and BMI for the Milan NAFLD cohort and ATBC study cohort, respectively. In obese individuals from the ATBC study, the \( P \) value for retinol concentration was calculated after adjusting for age, BMI, and dietary retinol intake. Nonnormally distributed variables were log-transformed before entering the model.

All statistical analyses were performed by using the Statistical Package for Social Sciences (SPSS, version 19.0) or the Statistical Analysis System (SAS Institute) for the Milan NAFLD cohort and for obese individuals from the ATBC study, respectively. \( P \) values <0.05 were considered significant.

Results

Characteristics of the cohort with NAFLD (n = 76) are shown in Table 1. Individuals were overweight/obese, 51% had NASH, and 39% were diabetic. On average, the NAFLD cohort was older at randomization and BMI for the Milan NAFLD cohort was lower than that for the ATBC study. Characteristics of overweight/obese participants with NAFLD (Milan NAFLD cohort) and obese participants from the ATBC study are shown in Table 1.
32% had type 2 diabetes, and 34% were smokers. The PNPLA3 I148M genotypes were in Hardy-Weinberg equilibrium (P = 0.25).

Characteristics of the subset of obese participants from the ATBC study (n = 413) are also shown in Table 1. Individuals had a BMI ≥30, and 6% had type 2 diabetes. Hardy-Weinberg equilibrium was conserved (P = 0.76).

In the Milan NAFLD cohort, the PNPLA3 148M mutant allele was associated with lower fasting circulating concentrations of retinol and RBP4 (β = −0.289, P = 0.03, and β = −0.292, P = 0.02, respectively), independently of other predictors of circulating retinol concentrations (namely age, gender, BMI, presence of diabetes, NASH, and smoking status) (Table 2). The P value for the association with retinol concentration remained significant (β = −0.265, P = 0.03) after adjusting for NASH, BMI, age, gender, diabetes, smoking status, and α-tocopherol, a lipid-soluble vitamin. In the Milan NAFLD cohort, we did not observe any associations between the PNPLA3 148M allele and anthropometric or clinical variables or concentrations of α-tocopherol (P = 0.40) (Table 2). No associations between the PNPLA3 148M allele and the RBP4:retinol ratio were detected (P = 0.63).

The PNPLA3 148M mutant allele associated with lower circulating retinol concentrations also in obese adults in the ATBC study (β = −0.043, P = 0.04; Table 2), independently of other predictors of circulating retinol concentrations (namely age, BMI, and dietary retinol intake). No associations between PNPLA3 I148M variant and α-tocopherol (P = 0.15) or β-carotene (P = 0.90) concentrations were observed in the ATBC study participants (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>PNPLA3 I148M genotype</th>
<th>Milan NAFLD cohort</th>
<th>Obese participants from the ATBC study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II (n)</td>
<td>IM (n)</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>16 (78)</td>
<td>27 (82)</td>
</tr>
<tr>
<td>Age, y</td>
<td>55.6 ± 10.31</td>
<td>57.4 ± 11.02</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.0 ± 5.0</td>
<td>28.1 ± 5.6</td>
</tr>
<tr>
<td>Serum insulin, μU/mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dietary β-carotene intake, μg/d</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dietary retinol intake, μg/d</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Serum retinol, μg/L</td>
<td>588 ± 1421</td>
<td>482 ± 1362</td>
</tr>
<tr>
<td>Serum α-tocopherol, mg/L</td>
<td>14.0 ± 3.5</td>
<td>13.5 ± 4.4</td>
</tr>
<tr>
<td>Serum RBP4, μg/L</td>
<td>54 ± 114</td>
<td>52 ± 12b</td>
</tr>
<tr>
<td>Serum β-carotene, μg/L</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RBP4-retinol, μg/L</td>
<td>0.09 ± 0.02</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>NASH, n (%)</td>
<td>8 (30)</td>
<td>18 (65)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>6 (29)</td>
<td>10 (30)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>6 (29)</td>
<td>12 (36)</td>
</tr>
<tr>
<td>Physically active, n (%)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1. Values are means ± SDs unless otherwise indicated. ATBC, α-Tocopherol, β-Carotene Cancer Prevention; II, homozygous for the PNPLA3 148I allele; IM, heterozygous; MM, homozygous for the PNPLA3 148M allele; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing protein 3; RBP4, retinol-binding protein 4. Superscript letters are used to indicate specific differences between mean values (specifically a>b>c where “a” is the largest value).
2. Categorical variable distribution across the genotypes was compared by chi-square or Fisher’s exact test; P values for continuous variables were calculated by linear regression analysis under an additive model after adjusting for confounding factors (NASH, BMI, age, gender, smoking status, and diabetes or age at randomization and BMI for the Milan NAFLD cohort and for obese participants from the ATBC study respectively). Nonnormally distributed variables were log-transformed before entering the model; β is shown only for significant P values.
3. Values for insulin, glucose, and HOMA-IR (measured in a subset of the ATBC study cohort; n = 101) are means ± SEs.
4. In the Milan NAFLD cohort, the P value for retinol concentration remained significant (P = 0.03) after adjusting for NASH, BMI, age, gender, diabetes, smoking status, and α-tocopherol.
5. In the obese participants from the ATBC study, the P value for retinol concentration was calculated after adjusting for age, BMI, and dietary retinol intake. The P value remained significant (P = 0.05) after adjusting for age, BMI, glucose, insulin, physical activity, dietary retinol, and dietary β-carotene. P = 0.05 after adjusting for age, BMI, physical activity, and dietary or for age, BMI, and dietary retinol plus insulin and glucose for those available.

### Discussion

In this study, we show that the PNPLA3 148M mutant allele is associated with lower circulating concentrations of retinol in adults with nonalcoholic fatty liver or obesity.

We started by examining the association between the PNPLA3 I148M genetic variant and circulating retinol, RBP4, and α-tocopherol in overweight/obese individuals with NAFLD. The PNPLA3 148M mutant allele was associated with lower circulating retinol concentrations, along with lower concentrations of RBP4, the major protein carrying retinol in the body. Although serum retinol concentrations were lower in carriers of the mutant allele, mean concentrations of retinol among PNPLA3 genotypes were within the normal range. No associations between genotype and serum α-tocopherol and serum β-carotene were found, supporting a specific association with retinol and not with other lipid-soluble vitamins more generally. Furthermore, the association was independent of demographic/anthropometric features, the presence of diabetes, and the severity of liver damage.

**PNPLA3 I148M affects retinol concentration**
The association with RBP4 is consistent with that previously found in an independent group of individuals with NAFLD (5). However, RBP4 concentrations are influenced by total body retinol stores as well as by inflammation (6–9), whereas fasting retinol more closely reflects retinol release by hepatic stellate cells. For each RBP4 protein secreted by the hepatocyte, 1 molecule of retinol is also secreted (15). We found no association between the PNPLA3 I148M variant and RBP4:retinol ratio. PNPLA3 has a retinyl-esterase activity and the mutation induces a loss of function (5). Therefore, we speculate that overweight/obese carriers of the PNPLA3 148M mutation have lower circulating retinol due to the reduced enzymatic activity of this protein and therefore subsequent intracellular retention and lower circulating RBP4 that is directly dependent on retinol bioavailability. Alternatively, differences in retinol concentrations observed in these cohorts may result from changes in retinol kinetics (e.g., more uptake and storage of retinol in nonhepatic tissue).

We observed a relatively small, but significant difference in circulating retinol concentrations in the ATBC cohort. Hepatic stellate cells represent only a small fraction of cells in the liver. This could explain the small difference observed in the study. These small differences may not have any physiologic implication for the whole organism, but they may contribute to the pathogenesis of NAFLD in the liver. We and others have shown in vitro that PNPLA3 148M mutant form determines a loss of function (16, 17) and overexpression of this mutant protein in immortalized hepatic stellate cells results in intracellular retinol retention (5). In the present study, we observed lower circulating retinol concentrations in carriers of the PNPLA3 148M allele compared with noncarriers, which is likely due to the retention of retinol in hepatic stellate cells.

To assess whether PNPLA3 I148M influences retinol metabolism in other conditions, we examined circulating retinol concentration stratified by PNPLA3 genotype in obese individuals from the ATBC study. The reason for selecting a subset of obese individuals was based on the consideration that the effect of PNPLA3 is specifically present when this variant interacts with this condition. We and others showed an interaction between the PNPLA3 genetic variant and obesity in determining increased transamine concentrations in adults and children (18–21). In obese adults after sustained weight loss due to bariatric surgery, the interaction was lost (22). Moreover, this interaction was also confirmed in a population-based sample study (23). A possible mechanism uncovering the interaction between the PNPLA3 genotype and obesity in retinol metabolism is that in obese individuals more retinol needs to be released.

The differences in the effect size found between the ATBC study and NAFLD cohort may be due to a more aggressive liver disease in individuals who underwent liver biopsy than in those from the ATBC study with simple obesity.

Insulin resistance is implicated as a mechanism for reduced retinol concentrations in obesity. It would have been of interest to examine differences in retinol concentrations specifically in insulin-resistant individuals. Unfortunately, fasting insulin concentrations were not measured at the same time of retinol measurements in the Milan cohort and were present only in 24% of the ATBC cohort.

Carriers of the PNPLA3 148M allele have a higher risk of progressing throughout the entire spectrum of liver disease from steatohepatitis to cancer (24). The current findings support the likelihood that they also have intracellular retention of retinol in hepatic stellate cells. Whether the PNPLA3 I148M variant also influences retinol after liver damage and hepatic stellate cell activation/transdifferentiation and whether retinol retention is involved in mediating the progression of liver damage and favors hepatic carcinogenesis associated with the I148M variant remain to be determined (25–27).

Limitations of the study include that it is difficult to distinguish whether the findings of the study in the Milan cohort depend on NAFLD or obesity because of the absence of lean individuals and that, although individuals taking vitamin supplements were excluded, in the Milan cohort the assessment of retinol and β-carotene dietary intakes was not available. Another limitation of the study is that the diet could be very different between the 2 cohorts examined. On the other hand, this dissimilarity could be considered a strength, suggesting that the results can be generalized to different diet conditions.

In conclusion, we showed for the first time, to our knowledge, that the PNPLA3 148M allele associates with lower fasting circulating retinol concentrations in adults with either nonalcoholic fatty liver or obesity alone. We also observed that, in these individuals, the PNPLA3 I148M variant associates with lower RBP4. The differences detected in retinol concentrations may not have any physiologic implication for the whole organism, but they may contribute to the pathogenesis of NAFLD in the liver. Future studies are warranted to understand the impact of the retinol release from hepatic stellate cells in liver inflammation, repair, fibrosis, and carcinogenesis.

Acknowledgments

We thank Rosie Perkins for editing the manuscript. SR designed the research and wrote, reviewed, and edited the manuscript; A Mondul and RMM performed statistical analysis and wrote, reviewed, and edited the manuscript; A Merlo, PD, RR, and TM contributed to the discussion and reviewed the manuscript; and LV, DA, and SR had primary responsibility for final content. All authors read and approved the final manuscript.

References


