Introduction

Atherogenic dyslipidemia, a condition often associated with obesity, is characterized by increased levels of plasma triglycerides (TG) and very-low-density lipoprotein cholesterol (VLDL-C) and decreased levels of high-density lipoprotein cholesterol (HDL-C)\(^1\). Epidemiological studies and clinical trials have shown a strong, independent inverse relationship between the plasma HDL-C level and the risk of atherosclerosis\(^2,3\). The anti-atherogenic potential of HDL is strongly associated with its function in reverse cholesterol transport (RCT), a process by which excess cholesterol in peripheral tissues is transported to the liver for removal from the body in the form of bile/bile acids\(^4\).

Several animal models have been used to study the mechanistic basis underlying the development of obesity and associated disorders. In particular, the WNIN/Ob mutant strain was developed from a...
90-year-old Wistar-inbred rat stock colony maintained at the National Institute of Nutrition, India and has both lean and obese phenotypes. Rats of the obese phenotype are euglycemic and typically exhibit hyperphagia, hypertriglyceridemia, hypercholesterolemia, hyperleptinemia and hyperinsulinemia5, 6 in addition to accelerated aging and degenerative disorders, such as impaired immunity, tumors, infertility, hypertension, polycystic ovaries, cataracts and retinal degeneration6-8, thus making them an ideal model to investigate metabolic syndrome as a whole. Studies are currently in progress to localize the mutation associated with the development of obesity in this strain. Recently, the unilocus mutation observed in these rats was localized to the 4.3 cm region with flanking markers D5Rat256 and D5Wox37 on chromosome 5 upstream of the leptin receptor9.

Vitamin A is known to play a major role in lipid metabolism10. Previously, we demonstrated that obese rats of the WNIN/Ob strain are hypercholesterolemic with abnormally high levels of plasma HDL-C, which are subsequently normalized by the upregulation of the scavenger receptor class B type 1 (SR-BI) expression in the liver (in which the basal expression is low) upon feeding a chronic non-toxic dose of vitamin A (129 mg/kg) for a period of two months5. However, the regulatory effects of vitamin A on several enzymes and proteins involved in HDL metabolism and the optimal dose required to bring about favorable changes were not addressed in that report. Therefore, in the present study, we assessed the role of key proteins involved in RCT and their possible link to hypercholesterolemia in obese rats and hypothesized that chronic feeding of a vitamin A-enriched diet at a dose of less than 129 mg/kg would be effective in increasing HDL-C clearance and thus lowering the plasma cholesterol level.

Methods and Procedures

Animals and Diet

For feeding experiment, 5-month-old male lean (n = 32; group A) and obese (n = 32; group B) rats of the WNIN/Ob strain were obtained from the National Centre for Laboratory Animal Sciences (NCLAS) of the National Institute of Nutrition (NIN), Hyderabad, India. Each group was further divided into four subgroups (AI, AII, AIII, AIV & BI, BII, BIII, BIV) consisting of eight rats each. Subgroups AI and BI received the stock diet, which provided 2.6 mg of vitamin A/kg diet, forming the control group. The stock diet consisted of 22.5% wheat flour, 4% casein, 4% salt mixture and 0.5% vitamin mixture. Subgroups AII and BII received 26 mg of vitamin A/kg as retinyl palmitate, subgroups AIII and BIII received 52 mg and subgroups AIV and BIV received 129 mg/kg diet. All diets were identical with regard to ingredients, except vitamin A content. The animal experiment was approved by the Institutional Animal Ethics Committee (IAEC). The rats were housed individually with an ambient temperature of 22.0 ± 1 ℃, relative humidity of 50-60% and 12:12-hour light-dark cycle and cared for in accordance with the principles of guidelines for the care and use of experimental animals. Food and water were provided ad libitum.

The animals were fed their respective diets for a period of 20 weeks. At the end of the experimental period, the rats were fasted for 12 hours, after which blood was collected from the supraorbital sinus via the inner canthus and the plasma was separated. The animals were then sacrificed via CO2 asphyxiation, and the livers were dissected, weighed, immersed in liquid nitrogen and stored at −80 ℃ for a further analysis.

Determination of the Serum and Liver Retinol Levels

The plasma and liver retinol levels were determined (using high-performance liquid chromatography) as previously described5.

Analysis of the Plasma and Hepatic Parameters

The plasma total and HDL-cholesterol levels were measured using commercially available kits (Biosystems, Spain). The plasma lipoprotein cholesterol profiles were obtained via fast protein liquid chromatography (FPLC) size fractionation of the lipoproteins, as previously described11, and the cholesterol level in each fraction was estimated using the enzymatic kit method (Biosystems, Spain). Total lipids of liver were then extracted and assayed for the cholesterol content, as previously described5. The plasma lecithin cholesterol acyltransferase (LCAT) activity was subsequently measured using a fluorescent LCAT activity kit (Roar Biomedical, New York, NY).

Hepatic Lipase Activity

The hepatic lipase (HL) activity in the liver was measured using an LPL activity assay kit (Roar Biomedical, New York, NY), according to the manufacturer’s instructions, and expressed as nmol/min/mg protein.

Western Blotting Analysis

Isolation of the total membrane fraction from
the liver and Western blotting were performed as previously outlined\textsuperscript{5}. Immunolabeling was conducted using either rabbit polyclonal anti-SR-BI (1:1,000; a gift from Salmon Azhar), goat polyclonal anti-ATP-binding cassette transporter protein I (ABCA1) (1:400; Santa Cruz Biotechnology, USA) or monoclonal anti-β-actin (1:30,000; Sigma-Aldrich, USA) as primary antibodies and horseradish peroxidase-conjugated goat anti-rabbit IgG, donkey anti-goat IgG and goat anti-mouse IgG (1:10,000; Santa Cruz Biotechnology, USA), respectively, as secondary antibodies. Immunoreactive SR-BI or ABCA1 bands were quantified and normalized to the intensity of β-actin.

Nuclear extracts of the liver samples were prepared according to the method of Sheng et al.\textsuperscript{12}. Equal amounts of protein (50 μg) were resolved on 10% SDS-PAGE and then transferred onto nitrocellulose membranes (GE healthcare, UK). Equal loading of the proteins and proper transfer were ensured by staining the membrane with Ponceau S. Immunoblotting was performed using either goat polyclonal anti-liver X receptor α (LXRα) or rabbit polyclonal anti-retinoid X receptor α (RXRα) (1:400; Santa Cruz Biotechnology, USA) as primary antibodies and donkey anti-rabbit IgG-HRP, goat anti-rabbit IgG-HRP (1:10,000; Santa Cruz Biotechnology, USA), respectively, as secondary antibodies. Immunoreactive proteins were detected with an ECL advance Western blotting detection kit (GE Healthcare, UK), and the band density was analyzed using the Quantity One software program (GS-710 Imaging Densitometer-Bio-Rad, Hercules, CA).

### Statistical Analysis

The data are presented as the mean ± SE. Statistical significance was determined according to the t-test or one-way ANOVA. Differences between means were assessed using a least-significance difference (LSD) post-hoc comparison, and a p value of <0.05 was considered to be significant. All statistical analyses were performed using the SPSS software program (Version 11.0).

### Results

#### Effects of Dietary Vitamin A on Weight Gain and Food Intake

The effects of vitamin A supplementation on weight gain and food intake are shown in Table 1. The pre (dietary)-treatment body weights were not different among the various groups, although the obese rats (BI) had significantly higher body weights than their lean counterparts (AI). The final body weights and amount of body weight gain were not significantly different between the lean groups. On the other hand, a significant reduction was observed in these parameters in the vitamin A-supplemented obese rats (BIII & BIV) treated with 52 and 129 mg of vitamin A/kg diet, respectively, whereas no such changes were observed in the obese rats fed 26 mg of vitamin A/kg diet (BII) compared with that noted in the control diet-fed obese rats (BI). However, the level of food intake was not altered in the vitamin A-supplemented lean or obese rats.

#### Effects of Dietary Vitamin A on the Plasma and Hepatic Retinol Levels

The male obese rats fed the control diet (BI) had higher plasma retinol levels than their age- and sex-matched lean counterparts (AI). However, the plasma retinol levels were not altered by feeding the vitamin A-enriched diet in either phenotype. In addition, the total retinol levels in the liver were lower in the obese control rats (BI) than in the control-diet fed lean rats (AI), while vitamin A feeding led to a dose-dependent increase in the liver total retinol levels in both the lean

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### Table 1. Effects of vitamin A supplementation on body weight gain and food intake in the lean and obese rats

<table>
<thead>
<tr>
<th>Parameters (g)</th>
<th>AI</th>
<th>AII</th>
<th>AIII</th>
<th>AIV</th>
<th>BI</th>
<th>BII</th>
<th>BIII</th>
<th>BIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body wt.</td>
<td>352 ± 13.3</td>
<td>374 ± 8.2</td>
<td>351 ± 21.8</td>
<td>362 ± 24.9</td>
<td>628 ± 48.3</td>
<td>594 ± 36.4</td>
<td>574 ± 27.9</td>
<td>591 ± 12.9</td>
</tr>
<tr>
<td>Final Body wt.</td>
<td>424 ± 19.8</td>
<td>455 ± 7.1</td>
<td>441 ± 19.2</td>
<td>454 ± 13.3</td>
<td>926 ± 56.2</td>
<td>883 ± 38.9</td>
<td>811 ± 33.4</td>
<td>778 ± 23.8</td>
</tr>
<tr>
<td>Body wt. gain</td>
<td>72 ± 18.9</td>
<td>80 ± 10.1</td>
<td>90 ± 25.1</td>
<td>92 ± 15.9</td>
<td>298 ± 22.1</td>
<td>288 ± 10.9</td>
<td>237 ± 20.8</td>
<td>189 ± 17.2</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>18.9 ± 0.57</td>
<td>17.4 ± 0.68</td>
<td>19.4 ± 2.15</td>
<td>18.8 ± 0.86</td>
<td>27.9 ± 0.82</td>
<td>29.3 ± 1.06</td>
<td>28.1 ± 1.33</td>
<td>26.8 ± 0.60</td>
</tr>
</tbody>
</table>

*Five-month-old male lean (A) and obese (B) rats were fed vitamin A at a dose of 2.6 (I), 26 (II), 52 (III) or 129 (IV) mg/kg diet for a period of 20 weeks. AI served as the control group for the lean phenotype, while BI served as the control group for the obese phenotype. The data represent the mean ± SE of seven animals in each group.\textsuperscript{§} p<0.05. Comparison was made between the control vitamin A groups of the lean (AI) and obese (BI) phenotypes.\textsuperscript{*} Significant at a p<0.05 level. Comparisons were made between the control vitamin A and vitamin A-enriched groups of each phenotype (by one-way ANOVA and LSD post-hoc analysis).
Fed the control diet (AI). Feeding a vitamin A-enriched diet to the obese rats significantly decreased the plasma cholesterol and HDL-C levels, whereas no such changes were noted in the lean rats fed the identical dietary regimen compared to that observed in the respective control groups treated with 2.6 mg of vitamin A/kg diet (BI and AI). This finding was further supported by lipoprotein fractionation of whole plasma using FPLC, wherein a marked decline

Table 2. Effects of vitamin A supplementation on the cholesterol and retinol levels in the lean and obese rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>AI</th>
<th>All</th>
<th>AIII</th>
<th>AIV</th>
<th>BI</th>
<th>BII</th>
<th>BIII</th>
<th>BIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma retinol (μg/dL)</td>
<td>28 ± 1.4</td>
<td>28 ± 1.3</td>
<td>26 ± 2.0</td>
<td>29 ± 3.4</td>
<td>40 ± 1.6</td>
<td>41 ± 2.5</td>
<td>46 ± 3.9</td>
<td>43 ± 5.7</td>
</tr>
<tr>
<td>Liver retinol (μg/g tissue)</td>
<td>957 ± 386</td>
<td>3795 ± 211</td>
<td>4556 ± 465</td>
<td>9842 ± 1151</td>
<td>375 ± 60</td>
<td>2840 ± 221</td>
<td>5031 ± 239</td>
<td>8133 ± 883</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dL)</td>
<td>79 ± 7.4</td>
<td>62 ± 2.7</td>
<td>62 ± 4.8</td>
<td>60 ± 3.6</td>
<td>162 ± 17.1</td>
<td>102 ± 7.3</td>
<td>107 ± 15.9</td>
<td>83 ± 7.6</td>
</tr>
<tr>
<td>Plasma HDL-C (mg/dL)</td>
<td>49 ± 4.9</td>
<td>36 ± 2.0</td>
<td>36 ± 3.1</td>
<td>35 ± 3.1</td>
<td>106 ± 10.1</td>
<td>71 ± 4.4</td>
<td>50 ± 7.1</td>
<td>53 ± 5.3</td>
</tr>
<tr>
<td>Hepatic cholesterol (mg/g tissue)</td>
<td>3.0 ± 0.06</td>
<td>3.6 ± 0.20</td>
<td>3.5 ± 0.27</td>
<td>3.5 ± 0.20</td>
<td>3.3 ± 0.21</td>
<td>3.6 ± 0.021</td>
<td>3.2 ± 0.27</td>
<td>4.0 ± 0.35</td>
</tr>
</tbody>
</table>

Five-month-old male lean (A) and obese (B) rats were fed vitamin A at a dose of 2.6 (I), 26 (II), 52 (III) or 129 (IV) mg/kg diet for a period of 20 weeks. AI served as the control group for the lean phenotype, while BI served as the control group for the obese phenotype. The data represent the mean ± SE of seven animals in each group.

* p < 0.05. Comparisons were made between the control vitamin A groups of the lean (AI) and obese (BI) phenotypes.

§ Significant at a p < 0.05 level. Comparisons were made between the control vitamin A and vitamin A-enriched groups of each phenotype (by one-way ANOVA and LSD post-hoc analysis).

Fig. 1. Plasma-lipoprotein cholesterol profiles of the WNIN/Ob strain rats by FPLC.

A total of 200 μL of pooled plasma samples from the lean (AI) and obese control rats (BI) treated with 2.6 mg of vitamin A/kg were compared against those of the animals treated with 129 mg of vitamin A/kg in the lean (AIV) and obese (BIV) groups upon size fractionation via FPLC. An aliquot of each fraction was analyzed for the cholesterol content and expressed as mg/dL.

Effects of Dietary Vitamin A on the Plasma and Hepatic Cholesterol Levels

The effects of feeding various doses of vitamin A on the plasma and hepatic cholesterol levels are shown in Table 2. The plasma cholesterol and HDL-C levels were higher in the obese rats (BI) than in the lean rats and obese rats compared to their respective controls (Table 2).
Vitamin A Improves Cholesterol Homeostasis

Impact of Dietary Vitamin A on the Plasma LCAT Activity

The plasma LCAT activity was higher in the obese rats (B1) than in their age- and sex-matched lean counterparts (A1). Vitamin A supplementation at a dose of 52 or 129 mg/kg diet resulted in a significant decrease in the LCAT activity in the obese rats (BII and BIV) compared to that observed in the control diet-fed obese rats (B1). However, in the lean rats, the LCAT activity remained unaltered upon feeding with the vitamin A-enriched diet (Fig. 2).

Dietary Vitamin A Elevates the HL Activity in Obese Rats

Compared to that noted in the lean rats (A1), a low HL activity was observed in the control-diet-fed obese rats (B1) (Fig. 1). Whereas vitamin A supplementation markedly increased the activity in the obese rats. On the other hand, feeding with the vitamin A-enriched diet did not elicit these changes in the lean rats (Fig. 3).

Dietary Vitamin A Increases the Hepatic SR-BI Receptor Expression in Obese Rats

The basal expression of hepatic SR-BI protein levels was low in the obese rats (B1) compared to that measured in the age- and sex-matched lean rats (A1) (Fig. 4A). The levels of hepatic SR-BI proteins were also similar between the lean rats treated with various doses of vitamin A (Fig. 4B). However, vitamin A feeding resulted in the overexpression of hepatic SR-BI in the obese phenotype, as compared to that observed in the obese control rats (B1) treated with 2.6 mg of vitamin A/kg diet (Fig. 4C).
In the present study, we investigated the regulatory effects of dietary vitamin A supplementation on various components associated with HDL metabolism in the setting of obesity-associated dyslipidemia by employing obese rats of the WNIIN/Ob strain. Previously, we reported that chronic vitamin A feeding (129 mg/kg) for two months normalized the abnormal plasma HDL-C levels in obese rats by upregulating the hepatic SR-BI levels (both protein and gene).

In the present report, we observed that dietary vitamin A supplementation ameliorated hypercholesterolemia in obese rats, as evidenced by reduced plasma total and HDL-cholesterol levels. It is interesting to note that the overall response of vitamin A supplementation was observed in the obese, but not lean rats, suggesting the role of genetic make-up in eliciting such divergent responses. In order to understand the underlying mechanisms, we studied various enzymes and cellular transporters involved in RCT.

**Impact of Dietary Vitamin A on the Hepatic ABCA1 Receptor Expression**

As shown in Fig. 5, the obese rats (BI) had comparable levels of hepatic ABCA1 proteins to their lean counterparts fed the control diet (AI). Further, the expression of hepatic ABCA1 was increased in the vitamin A-fed lean (AIV, 129 mg/kg) and obese (BIII & BIV, 52 mg and 129 mg/kg diet, respectively) rats compared to that noted in the respective control groups (AI and BI).

**Vitamin A Regulates the Hepatic LXRα and RXRα Expression**

A Western blot analysis revealed no significant differences in the hepatic LXRα and RXRα levels between the control diet-fed lean (AI) and obese (BI) rats. Vitamin A feeding at a dose of 129 mg/kg diet increased the hepatic LXRα and RXRα levels in both the lean (AIV) and obese rats (BIV) compared to that seen in the respective control groups (AI and BI) treated with 2.6 mg of vitamin A/kg diet, whereas no marked changes were evident among the animals treated with other doses (Fig. 6A, 6B).

**Discussion**

In the present study, we investigated the regulatory effects of dietary vitamin A supplementation on various components associated with HDL metabolism in the setting of obesity-associated dyslipidemia by employing obese rats of the WNIIN/Ob strain. Previously, we reported that chronic vitamin A feeding (129 mg/kg) for two months normalized the abnormal plasma HDL-C levels in obese rats by upregulating the hepatic SR-BI levels (both protein and gene). In the present report, we observed that dietary vitamin A supplementation ameliorated hypercholesterolemia in obese rats, as evidenced by reduced plasma total and HDL-cholesterol levels. It is interesting to note that the overall response of vitamin A supplementation was observed in the obese, but not lean rats, suggesting the role of genetic make-up in eliciting such divergent responses. In order to understand the underlying mechanisms, we studied various enzymes and cellular transporters involved in RCT.

Cholesterol effluxed from peripheral cells onto the surface of HDL is esterified by plasma LCAT.
Vitamin A Improves Cholesterol Homeostasis

Vitamin A supplementation decreased the LCAT activity in obese rats at doses of 52 and 129 mg of vitamin A/kg. This finding may be due to a reduction in the HDL-C level upon vitamin A feeding, as LCAT is associated with HDL molecules. Conversely, vitamin A supplementation had no effect on the enzymatic activity of LCAT in the lean rats, which further supports the findings of unaltered plasma HDL-C levels in this group.

It is known that the efficiency of cholesterol flux through the RCT pathway is a more important determinant of atherosclerosis than the steady-state plasma cholesterol concentration. The HDL-C metabolism in rats differs from that observed in other mammalian species, as rats lack cholesteryl ester transfer protein (CETP) and thus clearance of HDL cholesterol is accomplished via a selective uptake pathway mediated by SR-BI in the liver. SR-BI is an 82-kDa, highly homologous mammalian protein that shares 70-80% sequence identity with the CD-36 superfamily of proteins. These receptors are widely expressed in the liver, steroidogenic organs (adrenals and gonads) and intestines. In liver and steroidogenic tissues, these receptors bring about the selective uptake of CE from HDL particles, thereby completing the final step in RCT. In the intestines, SR-B1 is involved in the cellular uptake of a wide range of lipid molecules, including cholesterol and provitamin A carotenoids, but not retinol. Numerous studies have highlighted the role of SR-BI in the prevention/development of atherosclerosis in various rodent models. For example, SR-BI overexpression markedly reduces the plasma HDL-C level and decreases the rate of atherosclerosis, while SR-B1 knockout mice have high plasma HDL-C levels due to an impaired hepatic HDL-CE uptake and markedly increased atherosclerosis.

These observations can be explained by the fact that an increased hepatic SR-B1 expression promotes RCT, whereas ablation reduces RCT. Several studies in rodents have focused on the regulation of SR-B1, in which fatty acids, polyunsaturated fats, cholesterol, estradiol, vitamin E, lipopolysaccharide, TNFα and IL-1 have been shown to play an important role in inducing the differential expression of the SR-B1 receptor. However, few studies have been reported on the role of SR-B1 in vitamin A metabolism. Consistent with our previous findings, vitamin A feeding induced the hepatic SR-B1 expression in the obese rats in the present study. In keeping with these results, retinoic acid (RA) has been shown to induce the SR-B1 expression in HUVECs. In contrast, Lobo et al. demonstrated that the intestinal SR-B1 expression is increased in lecithin: retinol acyl transferase (LRAT) knock-out mice made vitamin A deficient compared to that noted in vitamin A-sufficient animals and that this effect is reversed upon RA treatment. The RA-induced downregulation of intestinal SR-B1 is mediated by ISX, a gut-specific homeodomain transcription factor that is not expressed in the liver, suggesting the tissue-specific role of RA.

Obesity being identified as an inflammatory condition, our current observations of a decreased SR-B1 expression and HL activity in the liver in obese rats of the WNIN/Ob strain are consistent with the fact that infection and inflammation impair the RCT pathway. HL enhances the selective uptake of CE via forming cholesteryl esters (CE), an essential step for the maturation of nascent HDL particles. Transgenic mice overexpressing LCAT show markedly higher plasma HDL levels, while LCAT deficiency results in both decreased plasma HDL levels and hepatic HDL production. In the present study, we found high LCAT activity levels in the obese rats compared to the lean rats. Interestingly, vitamin A supplementation decreased the LCAT activity in obese rats at doses of 52 and 129 mg of vitamin A/kg. This finding may be due to a reduction in the HDL-C level upon vitamin A feeding, as LCAT is associated with HDL molecules. Conversely, vitamin A supplementation had no effect on the enzymatic activity of LCAT in the lean rats, which further supports the findings of unaltered plasma HDL-C levels in this group.

**Fig.5.** Effects of vitamin A supplementation on the hepatic ABCA1 protein expression in the lean and obese rats. Five-month-old male lean (A) and obese (B) rats were fed vitamin A at a dose of 2.6 (I), 26 (II), 52 (III) or 129 (IV) mg/kg diet for a period of 20 weeks. Comparisons were made between the control vitamin A and vitamin A-enriched groups of each phenotype (i.e., A1 vs. AII, AIII & AIV and B1 vs. BII, BIII & BIV). β-actin was used as the internal control. The bars represent the mean ± SE of four animals in each group. *Significant at p<0.05 level (by one-way ANOVA and LSD post-hoc analysis).
SR-B1 by facilitating the binding and anchoring of HDL to the SR-B1 receptor. The increased SR-B1 expression and HL activity detected in the vitamin A-challenged obese rats led to a marked reduction in the HDL-C levels compared to that seen in the hypercholesterolemic control diet-fed obese rats. Furthermore, there were no differences in the hepatic cholesterol or cholesterol 7α-hydroxylase protein levels (data not shown) between the lean and obese phenotypes supplemented with vitamin A, suggesting the absence of defective cholesterol clearance.

Cholesterol taken up by hepatocytes is either secreted into the bile or effluxed into the circulation via the ABCA1 transporter to lipid-poor apoA-I in order to form nascent HDL particles, the latter being the rate-limiting step in HDL biogenesis, which determines the plasma HDL-C level. In transgenic mice, the specific deletion of ABCA1 in the liver has been shown to significantly reduce the circulating HDL-C level, while its overexpression raises the HDL-C level. A previous study in mice demonstrated a relationship between the hepatic ABCA1 expression and susceptibility to atherosclerosis, indicating that the liver serves as an important site for ABCA1-mediated atheroprotection. Furthermore, retinoic acid is known to upregulate ABCA1 in macrophages and monocytes, while the regulatory effects of vitamin A on hepatic ABCA1 are unknown. In the present study, we observed an increased hepatic ABCA1 expression in the lean and obese rats fed high doses of vitamin A, suggesting that vitamin A enhances the hepatic ABCA1-mediated efflux of cholesterol and phospholipids to apoA1-containing nascent HDL particles. We then investigated whether the effects of the vitamin A-induced ABCA1 expression are mediated via alterations in the levels of LXR and its obligatory partner RXR. The results showed that a high dose of vitamin A supplementation (129 mg/kg) significantly increased the hepatic LXR and RXR protein levels in both the lean and obese rats, while other doses had no significant effect. We therefore, speculate that the ABCA1 overexpression induced upon feeding a high dose of vitamin A promotes the relipidation of lipid-poor apoA-I particles that would otherwise be directed to catabolism and thus plays a pivotal role in regulating cholesterol homeostasis.

Moreover, our findings imply that the dietary intake of vitamin A impacts the development of obesity and associated dyslipidemia, as evidenced by the abnormal HDL-C levels and reduction in bodyweight in the vitamin A-supplemented obese rats. Numerous studies have shown that the administration of all-trans retinoic acid treatment at different doses and via dif-

**Fig. 6.** Effects of vitamin A supplementation on the hepatic LXR and RXR protein levels in the lean and obese rats.

Five-month-old male lean (A) and obese (B) rats were fed vitamin A at a dose of 2.6 (I), 26 (II), 52 (III) or 129 (IV) mg/kg diet for a period of 20 weeks. Representative Western blot showing the levels of (A) LXR and (B) RXR. Comparisons were made between the control vitamin A and vitamin A-enriched groups of each phenotype (i.e., AI vs. AII, AIII & AIV and BI vs. BII, BIII & BIV). Equal loading of proteins was ensured by staining the membranes with Ponceau S (image not shown). The bars represent the mean ± SE of three animals in each group. *Significant at p < 0.05 level (by one-way ANOVA and LSD post-hoc analysis).
different routes ameliorates obesity, dyslipidemia, insulin resistance and hepatosteatosis in normal adult mice and genetic mouse models of obesity.\textsuperscript{10} In humans, an inverse relationship has been reported between vitamin A intake and adiposity,\textsuperscript{36} as well as an inadequate vitamin A status in overweight and obese individuals,\textsuperscript{37, 38} suggesting that subclinical vitamin A deficiency is a contributing factor to obesity, fatty liver and insulin resistance.

In summary, the present study clearly established the potential of vitamin A supplementation to normalize the plasma HDL-C levels in hypercholesterolemic obese rats at a dose of 52 mg/kg diet, which is less than that previously reported.\textsuperscript{5} We further demonstrated that the mechanisms mediating this effect include an increase in the SR-BI, HL and ABCA1 levels in the liver in vitamin-A supplemented obese rats. These findings are of practical relevance as humans are more sensitive to changes in HDL, and early studies have demonstrated the presence of a selective HDL-CE uptake pathway in human liver tissue.\textsuperscript{39} The physiological role of hSR-B1 (human orthologue CLA-1) is generally assumed to be similar to that of rodent SR-B1 in terms of HDL lipid uptake and is regulated in human hepatic cells.\textsuperscript{40} Furthermore, SR-B1 gene polymorphisms associated with increased HDL levels have been reported.\textsuperscript{41-43} Recently, Vergeer et al.\textsuperscript{44} documented increased HDL-C levels and decreased cellular CE uptake in a family with a functional mutation in the hSR-B1 gene, suggesting that SR-B1 is relevant for maintaining the plasma HDL-C levels and metabolism in humans. Therefore, further studies are needed to address the role of dietary vitamin A in correcting obesity-associated dyslipidemia, which would be of unquestionable relevance to human health.

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Abbreviations

RCT, reverse cholesterol transport; LCAT, lecithin cholesterol acyltransferase; SR-BI, scavenger receptor class BI; HL, hepatic lipase; ABCA1, ATP-binding cassette transporter protein 1; LXR, liver X receptor; RXR, retinoid X receptor; CETP, cholesterol ester transfer protein; CE, cholesteryl ester

Conflicts of Interest

The authors have no conflicts of interest to declare.

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