

Original Article

Relationship between retinol and risk of diabetic retinopathy: a case-control study

Chunmei Zhang MD¹, Kelei Li PhD², Jiayu Zhang BM¹, Xiaotong Kuang BM², Chunxiao Liu BM², Qingxue Deng BM², Duo Li PhD²

¹Beijing Luhe Hospital, Capital Medical University, China

²Institute of Nutrition and Health, Qingdao University, Qingdao, China

Background and Objectives: The aim of the present study was to evaluate the relationship between retinol and risk of diabetic retinopathy in Chinese adults. **Methods and Study Design:** Eighty-six subjects with type 2 diabetes mellitus (T2DM) and 40 healthy subjects (healthy comparison group, HCG) were recruited in Beijing Luhe Hospital. Of the 86 T2DM subjects, 43 subjects were diagnosed with diabetic retinopathy (DRG), and 43 subjects had no retinopathy (DNRG). **Results:** Dietary intake of retinol ($p<0.001$) and retinol equivalent ($p<0.05$) was significantly higher but serum retinol and ($p<0.001$) retinol binding protein 4 (RBP4) ($p<0.05$) were significantly lower in DNRG compared with HCG. Dietary intake of retinol ($p<0.05$) and retinol equivalent ($p<0.05$) was significantly lower, and serum retinol and ($p<0.01$) retinol binding protein 4 (RBP4) ($p<0.01$) were significantly higher in DRG compared with DNRG. In T2DM subjects, per 100 $\mu\text{g}/\text{day}$ higher dietary retinol intake was associated with 17% lower risk of diabetic retinopathy (odds ratio (OR), 0.83; 95% CI, 0.70 to 0.98; $p=0.032$), and after adjusting for potential confounding factors, the OR was 0.82 (95% CI, 0.69 to 0.99; $p=0.036$); per 100 $\mu\text{g}/\text{day}$ higher in dietary retinol equivalent intake was associated with 12% lower risk of diabetic retinopathy (OR, 0.88; 95% CI, 0.79 to 0.97; $p=0.010$), and after adjusting for potential confounding factors, the OR was 0.88 (95% CI, 0.79 to 0.98; $p=0.025$). **Conclusions:** Higher dietary intake of retinol or retinol equivalent is associated with lower risk of diabetic retinopathy.

Key Words: retinol, diabetes, diabetic retinopathy, retinol binding protein 4, human

INTRODUCTION

Diabetic retinopathy is a common microvascular complication of diabetes, and will affect nearly all patients with sufficient duration of disease.¹ With increasing global prevalence of diabetes, diabetic retinopathy becomes a major cause of vision impairment affecting approximately 4.2 million people worldwide.²

Retinol plays an important role in visual cycle by transformation between all-trans-retinal and 11-cis-retinal (in retinal pigment epithelium (RPE), retinol is reisolomerized by means of cis-trans isomerase to 11-cis-retinal).³ In human body, carotene can also be transformed into retinol, and dietary retinol equivalents include all substances having retinol activity. One previous animal study indicated that 11-cis-retinal in the retina was significantly lower in diabetic rats than healthy ones.⁴ In addition to its role in vision, retinol is also involved in glycometabolism. One previous case-control study indicated that dietary retinol intake was significantly lower in subjects with type 2 diabetes mellitus (T2DM) than healthy controls.⁵ Previous animal study indicated that all-trans retinoic acid (the active form of retinol) intake could effectively suppress insulin resistance and thus decrease blood glucose level.⁶ Mice fed with retinol deficient diet displayed abnormal β -cell function, while reintroduction of dietary retinol could effectively reverse this adverse effect.⁷ Retinol binding protein 4 (RBP4) is the only specific transport protein for

retinol in the circulation.^{8,9} The complex of RBP4 and retinol is always bonded to prealbumin by means of a noncovalent link.¹⁰ Elevated RBP4 level was thought to be associated with insulin resistance and abnormal glycometabolism.¹¹ One observational study found that serum RBP4 was significantly higher in subjects with diabetic retinopathy than those free of diabetic retinopathy.¹² All these data above indicated that retinol may be involved in the development of diabetic retinopathy. However, limited studies have been conducted to evaluate the relationship between retinol intake and risk of diabetic retinopathy in humans.

The aim of the present study was to evaluate the relationship between dietary retinol and retinol equivalent intake, serum retinol, RBP4 and prealbumin with the risk of diabetic retinopathy.

Corresponding Author: Prof Duo Li, Institute of Nutrition and Health, Qingdao University, 308 Ningxia Road, Qingdao, China.

Tel:+86-0532-82991018; Fax: +86-0532-82991018

Email: duoli@qdu.edu.cn; duoli@zju.edu.cn

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METHODS

Subjects

The research was conducted according to the Declaration of Helsinki. The protocol was approved by Ethics Committee of Beijing Luhe Hospital, Capital Medical University. Eighty-six subjects with T2DM (diabetes group, DG) were recruited from Beijing Luhe Hospital. T2DM was identified if subjects had a fasting glucose level ≥ 7 mmol/L or had been previously diagnosed with T2DM. Of the 86 T2DM subjects, 43 subjects were diagnosed with diabetic retinopathy (diabetic retinopathy group, DRG), and 43 subjects had no retinopathy (diabetes with no retinopathy group, DNRG). Forty healthy subjects (healthy comparison group, HCG) were recruited by a health check program in Beijing Luhe Hospital. Subjects were excluded if they were diagnosed with infection, severe renal, liver, heart, or psychiatric diseases, had a history of cancer, thyroid disease, or were pregnant or lactating women. Their habitual dietary intake in the past 3 months was collected by a validated quantitative food frequency questionnaire (FFQ), which has been used for the China Nutrition and Health Survey in 2002.¹³ Dietary intake of retinol, carotene, retinol equivalent, macronutrients and energy were calculated by Nutrition System of Traditional Chinese Medicine Combining with Western Medicine, version 11.0 (Medical College, Qingdao University, Shandong, China). Basic characteristics of subjects, including age, sex, body mass index (BMI), smoking and alcohol consumption, were obtained by face-to-face interview. Written consent was obtained from all subjects.

Laboratory analysis

Overnight fasting venous blood samples were collected in the morning. Serum samples were prepared after blood collection as soon as possible, aliquoted into separated tubes and stored at -80°C until analysis. Serum glucose and prealbumin were analyzed by a biochemical autoanalyzer (Beckman AU5800, Beckman Coulter, Inc., Brea, CA, USA). Serum retinol was analyzed by high-performance liquid chromatography (HPLC) with a C18 column (4.6mm \times 25mm) (HPLC1290; Agilent Technologies, Palo Alto, CA). Briefly, 200 μL of serum was mixed with 400 μL mixture of acetonitrile and ethanol (50:50) by 1-minute vortex oscillation. Then normal hexane containing 10% dichloromethane was added to the solution above, centrifuged for 3 minutes under 3000 rpm. Normal hexane containing retinol was transferred to a new tube. After drying under nitrogen, the extracts were redissolved by a mixture of methanol and water (92:8) for HPLC analysis. The elution solution was mixture of methanol and water (92:8) with a flow rate of 1 mL/minute. Retinol was detected under 292 nm and 325 nm with a UV-detector. RBP₄ was analyzed by enzyme-linked immunosorbent assay kit (SEA929Hu, Cloud-clone Corp., Wuhan, China).

Statistical analysis

For continuous variables, normal distribution was tested by Kolmogorov-Smirnov test. Considering that most continuous variables were skewed, they were expressed as median and interquartile range (IQR), and group differ-

ence was compared by nonparametric Kruskal-Wallis test. Categorical data were expressed as number (percentage), and chi-square test was used to test for independence of the groups. Correlation between different variables was tested by Spearman's correlation coefficient. Logistic regression was used to calculate the odds ratio (OR) of diabetic retinopathy in T2DM subjects before and after adjusting for potential confounding factors, including sex, age, BMI, smoking and alcohol consumption. All these data analyses were conducted by SPSS 20. The sample size calculation was conducted using G*Power 3.1.9.2, with an effect size of Cohen $d=0.8$, significance $\alpha=0.05$, an expected power $(1-\beta)=0.95$. This means at least 37 subjects in each group are needed.

RESULTS

Characteristics of subjects

The fasting blood glucose levels in DG, DRG and DNRG all significantly higher than in HCG, and the medians (IQR) (mmol/L) were 7.43 (5.84, 10.60), 7.02 (5.52, 10.50), 7.85 (6.05, 11.59) and 5.16 (4.86, 5.56), respectively. There was no significant difference in fasting blood glucose between DRG and DNRG. No significant difference was observed in age, sex, BMI, smoking or alcohol consumption between these groups (Table 1).

Dietary intake

Dietary intake of energy and carbohydrate was significantly lower in DRG than in DNRG and HCG. There was no significant difference in dietary intake of energy, protein, fat and carbohydrate between DG, DRG, DNRG and HCG (Table 2).

Dietary retinol and retinol equivalent levels were significantly higher in DNRG than in DRG and HCG. No significant difference was observed between DRG and HCG in dietary retinol and retinol equivalent. The medians (IQR) of dietary retinol ($\mu\text{g}/\text{day}$) in DRG, DNRG and HCG were 289.00 (83.99, 450.02), 423.53 (245.11, 639.26) and 198.93 (41.52, 404.11), respectively. The medians (IQR) of dietary retinol equivalent ($\mu\text{g}/\text{day}$) in DRG, DNRG and HCG were 919.74 (604.23, 1244.88), 1166.19 (823.46, 1603.07), 925.67 (547.05, 1373.90), respectively (Figure 1). There was no significant difference in dietary carotene among the three groups.

Serum retinol, RBP₄ and prealbumin

Serum retinol and RBP₄ was significantly lower in DNRG than in DRG and HCG (Figure 2). There was no significant difference in serum retinol and RBP₄ between DRG and HCG. The medians (IQR) of serum retinol (mg/L) in DRG, DNRG and HCG were 0.46 (0.38, 0.50), 0.37 (0.32, 0.44) and 0.47 (0.39, 0.55), respectively. The medians (IQR) of serum RBP₄ (mg/dL) in DRG, DNRG and HCG were 1.65 (1.49, 1.78), 1.39 (1.28, 1.65) and 1.61 (1.45, 1.84), respectively. There was no significant difference in serum prealbumin between DRG, DNRG and HCG (Figure 2).

Serum retinol was positively correlated with RBP₄ and prealbumin, and the correlation coefficient was 0.391 ($p<0.001$) and 0.555 ($p<0.001$). However, no significant correlation was observed between serum retinol and dietary retinol ($r=-0.087$; $p=0.357$), dietary carotene

Table 1. Characteristics of included subjects

Characteristics	DG (n=86)	DRG (n=43)	DNRG (n=43)	HCG (n=40)	<i>p</i> 1 [†]	<i>p</i> 2 [‡]
Sex					0.903	0.684
Male	44 (51.2%)	24 (55.8%)	20 (46.5%)	20 (50.0%)		
Female	42 (48.8%)	19 (44.2%)	23 (53.5%)	20 (50.0%)		
Age [†]	57 (46, 65)	59 (49, 66)	53 (44, 65)	54 (47, 67)	0.444	0.162
BMI [†]	25.4 (23.2, 27.3)	25.4 (23.8, 27.8)	24.7 (23.1, 27.1)	24.8 (23.2, 27.6)	0.571	0.715
Glucose [†]	7.43 (5.84, 10.60) [‡]	7.02 (5.53, 10.50) [§]	7.85 (6.05, 11.59) [§]	5.16 (4.86, 5.56) [¶]	<0.001	<0.001
Smoking					0.092	0.226
Yes	32 (37.2%)	15 (34.9%)	17 (39.5%)	9 (22.5%)		
No	53 (61.6%)	27 (62.8%)	26 (60.5%)	31 (77.5%)		
Alcohol					0.588	0.568
Yes	30 (34.9%)	13 (30.2%)	17 (39.5%)	12 (30.0%)		
No	56 (65.1%)	30 (69.8%)	26 (60.5%)	28 (70.0%)		

DG: diabetes group (including all diabetic subjects); DRG: diabetic retinopathy group; DNRG: diabetes with no retinopathy group; HCG: healthy control group.

*p*1, DG vs HCG; *p*2, DRG vs DNRG vs HCG.

[†]Data were expressed as median (IQR); other data were expressed as number (percentage)

[‡]Data were significantly different from HCG.

^{§,¶}Data marked with different letter indicating significant group difference (multiple comparison)

Table 2. Dietary energy and macronutrients intake of included subjects[†]

Dietary intake	DG	DRG	DNRG	HCG	<i>p</i> 1	<i>p</i> 2
Energy (kcal)	1715 (1314, 2167)	1528.69 (1287, 1908)	1913 (1304, 2603)	1885 (1540, 2276)	0.125	0.064
Protein (g/day)	82.9 (61.9, 103)	74.41 (59.7, 93.8)	87.01 (66.2, 121)	95.6 (74.8, 109)	0.140	0.106
Fat (g/day)	46.8 (29.2, 67.9)	37.55 (25.3, 60.9)	50.0 (33.0, 76.1)	55.5 (37.8, 87.9)	0.052	0.059
Carbohydrate	262 (182, 344)	251 (179, 284)	283 (185, 380)	273 (226, 338)	0.240	0.074

DG: diabetes group (including all diabetic subjects); DRG: diabetic retinopathy group; DNRG: diabetes with no retinopathy group; HCG: healthy control group.

[†]All data were expressed as median (IQR).

*p*1, DG vs HCG; *p*2, DRG vs DNRG vs HCG.

($r=0.099$; $p=0.281$), or dietary retinol equivalent ($r=-0.075$; $p=0.426$).

Association of retinol, carotene, retinol equivalent, RBP₄ and prealbumin with risk of diabetic retinopathy in T2DM subjects

The risk of diabetic retinopathy was significantly higher in T2DM subjects with a lower dietary intake of retinol or retinol equivalent than T2DM subjects with a higher dietary intake of retinol or retinol equivalent: per 100 µg/day higher dietary retinol intake was associated with 17% lower risk of diabetic retinopathy (OR, 0.83; 95% CI, 0.70 to 0.98; $p=0.032$), and after adjusting for potential confounding factors, the OR was 0.82 (95% CI, 0.69 to 0.99; $p=0.036$); per 100 µg/day higher dietary retinol equivalent intake was associated with 12% lower risk of diabetic retinopathy (OR, 0.88; 95% CI, 0.79 to 0.97; $p=0.010$), and after adjusting for potential confounding factors, the OR was 0.88 (95% CI, 0.79 to 0.98; $p=0.025$) (Table 3). There was no significant association between dietary carotene intake and risk of diabetic retinopathy before adjusting for confounding factors.

Serum retinol and RBP₄ was positively associated with the risk of diabetic retinopathy, and the OR for per 0.1 mg/L higher serum retinol and per 0.1 mg/dL higher

RBP₄ was 2.47 (95% CI, 1.41 to 4.32; $p=0.002$) and 1.27 (95% CI, 1.06 to 1.53; $p=0.011$), respectively; after adjusting for potential confounding factors, the OR was 2.76 (95% CI, 1.40 to 5.44; $p=0.003$) and 1.23 (95% CI, 1.00 to 1.50; $p=0.048$), respectively. There was no significant association between serum prealbumin and risk of diabetic retinopathy before adjusting for confounding factors.

DISCUSSION

In the present study, serum retinol is positively correlated with RBP₄ and prealbumin. This result was consistent with one previous study in type 1 diabetic patients.¹⁴ Retinol is stored in the form of retinol esters in the liver. Before release, these esters are hydrolysed, and the free alcohol is linked to RBP.¹⁰ The retinol-RBP compound is then secreted into the blood flow, where it is bonded to prealbumin by means of a noncovalent link.¹⁰ RBP₄ is the only specific transport protein for retinol in the circulation.^{8,9} These evidence well explained the positive correlation of serum retinol with RBP₄ and prealbumin, which also in turn demonstrated the reliability of our data concerning serum retinol, RBP₄ and prealbumin.

We observed that serum retinol was significantly lower but dietary intake of retinol and retinol equivalent was

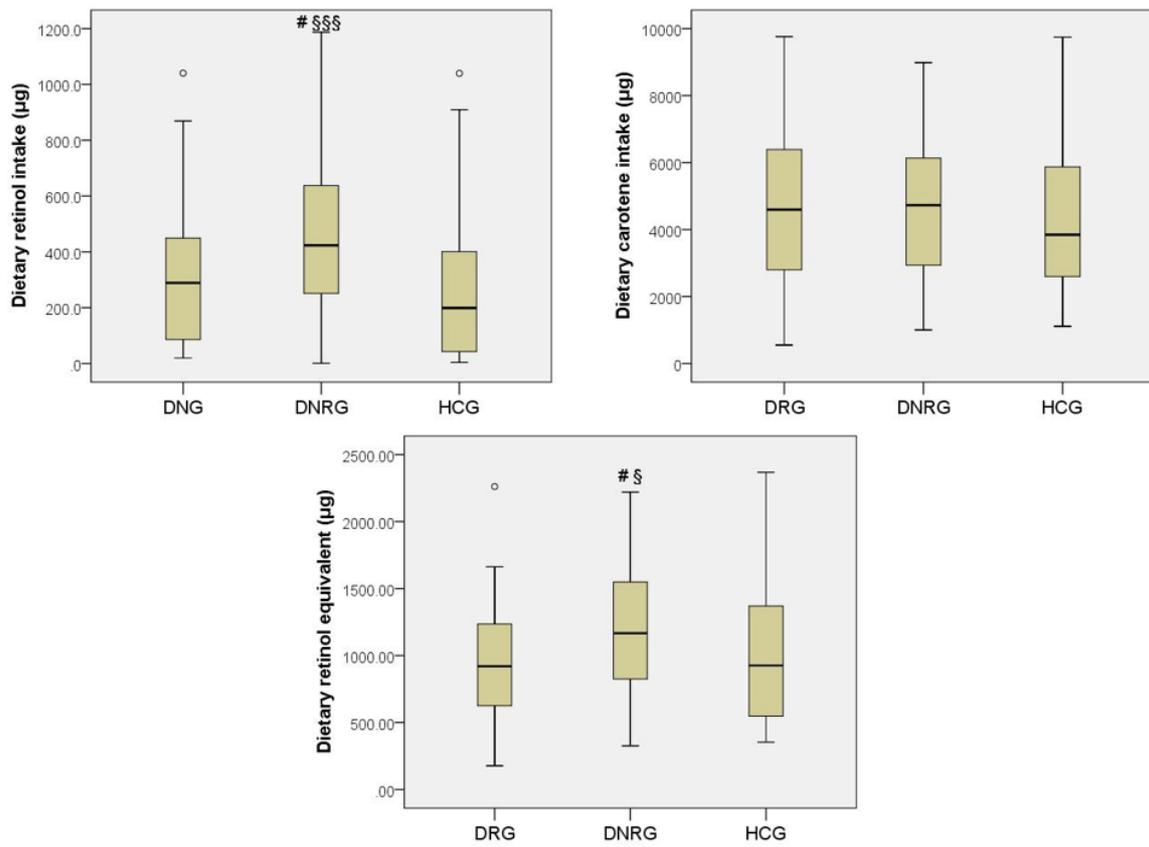


Figure 1. Box plot for dietary retinol, carotene and retinol equivalent intake. DRG, diabetic retinopathy group; DNRG, diabetes with no retinopathy group; HCG, healthy control group. #, $p < 0.05$ (compared with DRG); \$ and \$\$\$, $p < 0.05$ and < 0.001 , respectively (compared with HCG). No significant difference was observed between DRG and HCG.

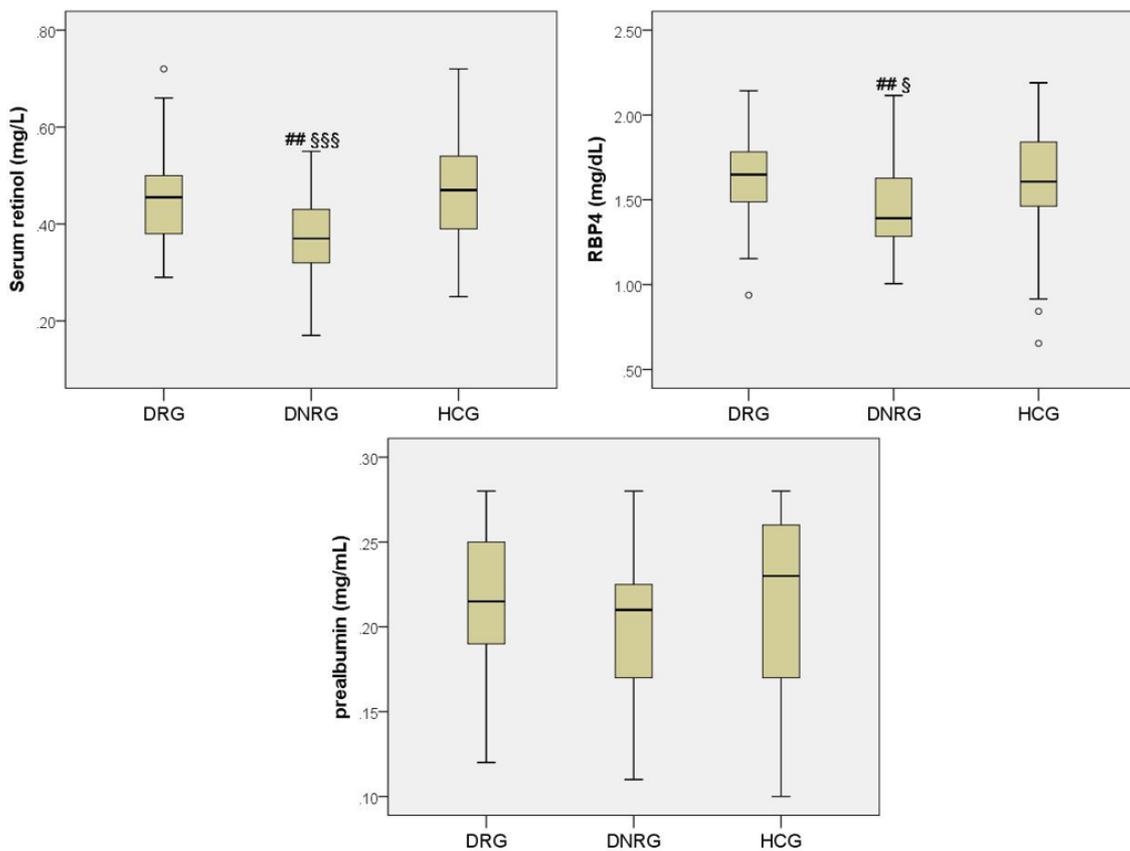


Figure 2. Box plot for serum retinol, RBP4 and prealbumin. DRG, diabetic retinopathy group; DNRG, diabetes with no retinopathy group; HCG, healthy control group. ##, $p < 0.01$ (compared with DRG); \$ and \$\$\$, $p < 0.05$ and < 0.001 , respectively (compared with HCG). No significant difference was observed between DRG and HCG.

Table 3. Association of retinol, carotene, RBP₄ and prealbumin with risk of diabetic retinopathy in T2DM subjects

	Crude			Adjusted [†]		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
Serum retinol (per 0.1 mg/L increase)	2.47	(1.41, 4.32)	0.002	2.76	(1.40, 5.44)	0.003
Serum RBP ₄ (per 0.1 mg/dL increase)	1.27	(1.06, 1.53)	0.011	1.23	(1.00, 1.50)	0.048
Serum prealbumin (per 0.1 mg/mL increase)	2.40	(0.70, 8.32)	0.166	2.70	(0.65, 11.28)	0.173
Dietary retinol equivalent (per 100 µg/day increase)	0.88	(0.79, 0.97)	0.01	0.88	(0.79, 0.98)	0.025
Dietary retinol (per 100 µg/day increase)	0.83	(0.70, 0.98)	0.032	0.82	(0.69, 0.99)	0.036
Dietary carotene (per 100 µg/day increase)	1.00	(0.99, 1.02)	0.783	1.00	(0.98, 1.03)	0.749

OR: odds ratio.

[†]Adjusted for sex, age, BMI, smoking and alcohol consumption.

significantly higher in diabetic subjects free of diabetic retinopathy than healthy ones. This finding reflects the regulatory effect of liver on serum retinol level. In human body, retinol is stored in liver as retinol esters, and the serum retinol level is homeostatically maintained by the liver for normal physiological functions.¹⁵ In diabetic rats, serum retinol was significantly lower but hepatic free retinol was significantly higher than healthy ones, and this result remained unchanged after vitamin A supplementation.⁴ These data indicated that the hepatic mobilization of retinol is inhibited in diabetes.

Serum RBP was significantly lower in insulin-dependent diabetic children than healthy ones, and glycemic control by insulin use and diet intervention significantly increased serum RBP level, indicating that hyperglycemia could inhibit RBP synthesis.¹⁶ In the present study, we observed that RBP₄ was significantly lower in diabetic subjects with no retinopathy than healthy ones. As previously mentioned, retinol is secreted into circulation by binding to RBP, and RBP₄ was the only specific transport protein for retinol in the circulation. Therefore, we hypothesized that the lower serum retinol observed in diabetic subjects without retinopathy than healthy ones may be attributed to inhibited hepatic mobilization of retinol induced by hyperglycemia, and decreased hepatic RBP₄ synthesis may be one crucial mechanism. In addition, decreased level of circulating RBP₄ may also limit the transport of retinol from intestinal absorption and hyperglycemia may also influence the intestinal conversion of carotene to retinol, which may be another reason for the lower serum retinol observed in diabetic subjects without retinopathy than healthy ones.

However, serum retinol and RBP₄ were all significantly higher but dietary intake of retinol and retinol equivalent was significantly lower in subjects with diabetic retinopathy compared with diabetic subjects with no retinopathy, and were all not significantly different from healthy ones. We also observed that serum retinol and RBP₄ levels were positively associated with the risk of diabetic retinopathy in T2DM subjects, whereas dietary intake of retinol and retinol equivalent showed negative associations. Retinol plays an important role in visual cycle by transformation between all-trans-retinal and 11-cis-retinal (in RPE, retinol is reisolomerized by means of cis-trans isomerase to 11-cis-retinal).³ The 11-cis-retinal in retina was significantly lower in diabetic rats than healthy ones.⁴ The mRNA and protein level of interphotoreceptor retinoid-binding protein (IRBP) was significantly lower in eyes of patients with diabetic retinopathy than

healthy controls.¹⁷ IRBP is a protein that functions to solubilize retinal and retinol, which are otherwise insoluble in water, and mediates the targeting of these compounds and defines transport direction.³ The lack of 11-cis-retinal and inhibited transport of retinal and retinol in retina caused by diabetes also demonstrated the important role of retinol in the development of diabetic retinopathy.

As previously discussed, diabetes can decrease circulating retinol level, possibly by inhibiting hepatic RBP₄ synthesis and intestinal conversion of carotene to retinol. Therefore, T2DM subjects may need higher dietary intake of retinol or retinol equivalent to maintain normal retinol level in blood and tissue (including retina) in T2DM subjects than healthy ones. This may explain why despite dietary intake of retinol and retinol equivalent of subjects with diabetic retinopathy were comparable with those of the healthy subjects, the intake levels were still insufficient for T2DM patients. When diabetic retinopathy occurs, we hypothesize that the body may increase the hepatic mobilization of retinol by increasing RBP₄ synthesis and perhaps also increase intestinal conversion from carotene to retinol to try to reverse the retinal deficiency in retina caused by diabetes. This may explain why serum retinol and RBP₄ levels were significantly higher in subjects with diabetic retinopathy than diabetic subjects without retinopathy, and why they were positively associated with the risk of diabetic retinopathy in T2DM subjects. The higher RBP₄ level in subjects with diabetic retinopathy compared with diabetic subjects without retinopathy observed in the present study was consistent with two previous studies.^{12,18} Overexpressed RBP₄ in mice could lead to progressive retinal degeneration,¹⁹ indicating that the increased RBP₄ may in turn aggravate diabetic retinopathy. But this seems not the case in the present study because no significant difference in RBP₄ level was observed between subjects with diabetic retinopathy and healthy ones, and the two previous studies only compared the level of RBP₄ between diabetic subjects with diabetic retinopathy or no diabetic retinopathy without a healthy comparison group.¹² The regulatory effect of liver and intestine on circulating retinol level discussed above can also help explain why serum retinol was not significantly correlated with dietary intake of retinol, carotene or retinol equivalent in the present study.

The association between RBP₄ and diabetes was controversial in previous human studies. A previous study indicated that serum RBP₄ was significantly higher in T2DM subjects compared with healthy subjects.²⁰ However, another study indicated that serum total RBP (the

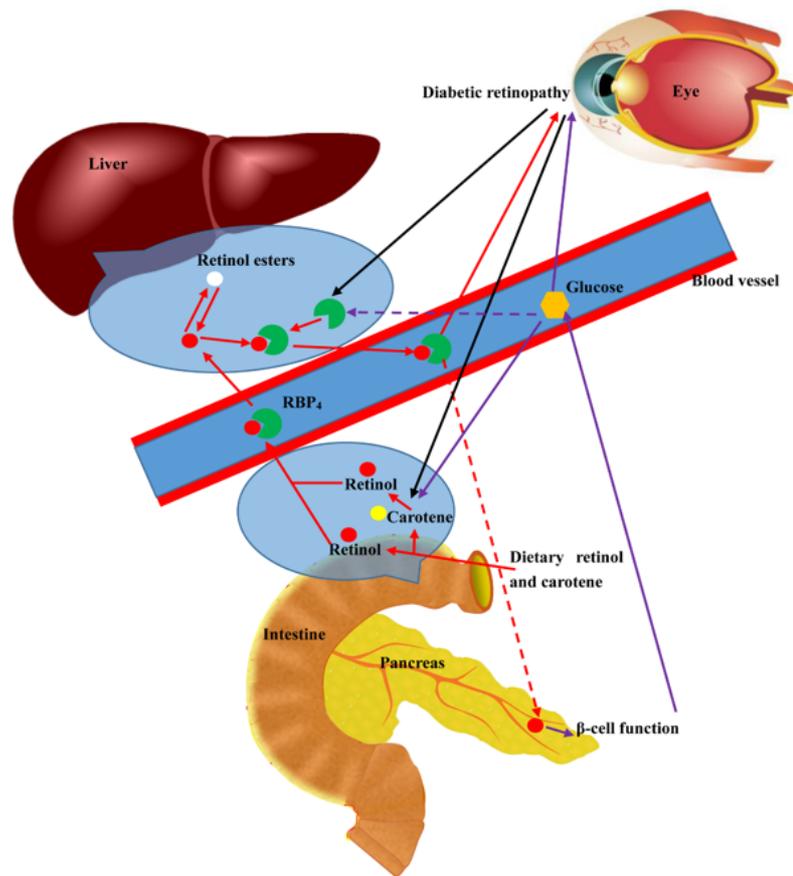


Figure 3. The hypothesized mechanism for the association of dietary retinol, serum retinol and RBP4 with diabetic retinopathy. Red arrows indicated the process of retinol transport from intestine to circulation, liver, pancreas and retina. Purple arrows indicated the relationship between retinol, glycometabolism and diabetic retinopathy. Chronic hyperglycemia can damage the retinal blood vessels, leading to involution of the vascular changes in microcirculation and loss of normal capillary exchange and promoting leakage of endovascular products. Hyperglycemia may inhibit hepatic RBP4 synthesis, hepatic mobilization of retinol and intestinal conversion of carotene to retinol, leading to a lower serum level of RBP4 and retinol (as we observed in diabetic subjects free of diabetic retinopathy) and further a lower retinol content in retina as well as β -cell. Retinol deficiency in β -cell leads to β -cell dysfunction and exacerbates hyperglycemia. Retinol deficiency in retina exacerbates diabetic retinopathy. Dark arrows indicated the effect of diabetic retinopathy on liver mobilization of retinol and intestinal conversion of carotene to retinol. When diabetic retinopathy happens, we hypothesize that the body may increase the hepatic mobilization of retinol by increasing RBP4 synthesis and perhaps also increase intestinal conversion from carotene to retinol to try to reverse the retinal deficiency in retina caused by diabetes.

major subtype in blood is RBP₄) was significantly lower in diabetic subjects compared with healthy ones, and glycemic control by insulin use and diet intervention significantly increased serum total RBP level.¹⁶ All these studies did not take into consideration the effect of diabetic retinopathy on RBP₄ level. Reversal of the inhibiting effect of diabetes on RBP₄ level by diabetic retinopathy was observed in the present study. Therefore, difference in the proportion of subjects with diabetic retinopathy in total diabetes subjects may be one important reason for the inconsistent result concerning the association between RBP₄ and diabetes in previous studies.

Based on all available data, we developed a graph to show the potential mechanism for the association of dietary retinol, serum retinol and RBP₄ with diabetic retinopathy (Figure 3).

Conclusions

T2DM is associated with lower serum level of retinol. Higher dietary intake of retinol or retinol equivalent is associated with lower risk of diabetic retinopathy.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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