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Investigation of plasma vitamin A levels in hyperlipidemic subjects

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Objective: The aim of the study was to investigate the relationships between vitamin A and plasma lipid and apolipoprotein levels.

Method: We measured levels of plasma vitamin A, retinol-binding protein, apolipoproteins AI and B, insulin, free testosterone and some plasma lipids including total cholesterol, high density lipoprotein (HDL)-cholesterol, very low density lipoprotein (VLDL)-cholesterol and low density lipoprotein (LDL)-cholesterol, in health male subjects aged 25-50 years having similar body mass indices. The subjects were divided into four groups: (A) normal (n=13); (B) high plasma triglyceride with normal plasma total cholesterol (n=12); (C) high plasma total cholesterol with normal plasma triglyceride (n=13) and (D) high plasma total cholesterol with high plasma triglyceride (n=16).

Results There was positive correlation between vitamin A and triglyceride concentrations in both hypertriglyceridemic and normolipidemic groups (respectively r=0.625, r=0.563, p<0.05). However, there was no significant correlation between the measured values in the groups in which cholesterol levels were high.

Conclusion: We concluded that, in the hyperlipidemic subjects, the levels of plasma vitamin A tend to be elevated above normal and consequently it is important to pay attention to vitamin A levels if retinoid treatment is contemplated in such subjects.

Key words Vitamin A. retinol-binding protein, lipoproteins, insulin, free testosterone.

Introduction

It is known for many years that vitamin A (or retinoids) is particularly effective on dermatological diseases. At present, the effects of synthetic retinoids are being searched and with this aim, more than 1500 different retinoid compounds have been developed and the biological effectiveness of them are being tested (1).

As a result of the increase in number and the success of synthetic retinoids in the treatment of dermatological diseases, these compounds have been started to be used commonly. Particularly, in the developed Western populations due to increase in the consumption of vitamin A with a recipe or by self-medication, has produced the vitamin A hypervitaminosis risk (2).

A characteristic and frequent side effect of retinoids is that it causes an increase in the level of plasma lipids (particularly triglycerides) (3). Therefore as a result of the increase of consumption of retinoids, an increase in the incidence of hyperlipidemias, can also be expected. However, the exact mechanism how retinoids cause hyperlipidemia has not been explained, yet (4).

In this work, by examining the correlations of vitamin A with plasma lipids, investigation of the probable mechanisms between them has been aimed. In three different hyperlipidemic and in one normolipidemic, totally in four groups; serum vitamin A, retinol-binding protein (RBP), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), lipoprotein (Lp) electrophoresis fractions (alpha, pre beta, beta), apolipoprotein A-I (apo A-I), apolipoprotein B (apo B) and some hormones (free testosterone, insulin) related with lipoprotein metabolism have been determined. In addition to comparisons of the above parameters, their correlations with vitamin A have been analysed and evaluated, also.

Material and Method

Subjects: Male subjects aged between 25-50 and body mass index <35 weight/m^2 were included only, into the work. Subjects who had hypertension and any of the metabolic-endocrine diseases and the heavy drinkers were excluded. Study was done in three cases and one control group. These groups were determined according to the serum TG and TC levels of the subjects (5).

Group 1 (hypertriglyceridemic group): TG> 200mg/dl, TC<220mg/dl,

Group 2 (mixed hyperlipidemic group): TG> 200mg/dl, TC>220mg/dl,

Group 3 (hypercholesterolemic group): TG< 200mg/dl, TC>220mg/dl,

Group 4 (normolipidemic group): TG<200mg/dl, TC<220mg/dl. This was the control group.

Blood was taken from all the subjects following about a 12 hour fasting period, at sitting position, into the vacutainer tubes (Becton-Dickenson, France) and sera were separated. An aliquot of the each serum sample in which vitamin A and retinol binding protein (RBP) analysis would be made were put into the tubes covered with aluminium sheets, to protect the samples from light.

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Methods: Serum vitamin A concentration was measured by using the spectrophotometric method described by Neeld and Pearson (6). The principle of this method is that, double-bond compounds which are extracted into an organic solvent phase, reacts with the colour reagent trifluoroacetic acid and forms a blue coloured complex, which gives absorbency at 620 nm. RBP was measured by using radial immune diffusion plaques (Behring/Germany). Insulin, free and total testosterone were determined by radioimmunoassay (Coat A count/UK). Apolipoprotein A-I, B and lipoprotein (a) were measured nephelometrically (Behring/Germany). Agarose gel electrophoresis was performed according to the REP lipoprotein procedure (Helena/UK). Total cholesterol and triglycerides were determined enzymatically (Biosystems/Spain). HDL-C was quantified by the same enzymatic method after precipitation of VLDL and LDL with magnesium chloride-dextrane sulphate. LDL-C was calculated according to the Friedwald equation (7) and in the cases who had TG values >400 mg/dl according to the Wielend-Seidel equation (8).

Statistical Analysis: Mann-Whitney U test was used to determine the significance of difference between means. A p value of <0.05 was considered as significant. Pearson's linear correlation was adopted for correlation analysis.

Results

Plasma lipids, apolipoproteins, vitamin A and RBP concentrations are shown in Table I. Vitamin A levels were found to be significantly higher in all the hyperlipidemic groups compared to controls. Vitamin A was highest in the mixed type hyperlipidemic group (Group 2), while it was least high in the hypercholesterolemic group (Group 3). In Table 2, correlations of vitamin A with other parameters and linear correlation coefficient (r), and statistical significance (p) of the r, and regression equations are given. In addition, the relationships between vitamin A and the hormones (free testosterone, insulin) were evaluated. There were no significant correlations between vitamin A and them (Not shown data).

Discussion

After the ingestion of vitamin A, it is transported to liver in Lp particles. Therefore when plasma Lp levels increase, it is normal to expect an increase in vitamin A levels also, paralleling the increase of Lp's. In a work made in hypercholesterolemic subjects, it is reported that vitamin A levels are also found to be increased compared to controls (9). In other works positive correlations have been found between the fasting TG levels and retinyl esters' increase in plasma ($r=0.708$, $p<0.05$) and plasma vitamin A and TGs, made both in test animals and in humans (10-12). In our work, vitamin A value of the hypertriglyceridemic group (Group 1) was found as 44.5±5.3 (X±SD) and it was significantly higher compared to control group value of 36.8+6.5 μg/dl (Table I).

Table I. Mean values and the comparison of the parameters between the groups.

Parameters	Group I (n=12)	Group II (n=16)	Group III (n=13)	Group IV (n=13)
Retinol (μg/dl)	44.5±5.3**	48.3±8.8**	43.4±7.6*	36.8±6.5
RBP (g/L)	51.6±9.8*	53.2±9.2**	52.7±5.7**	43.1±6.8
TC (mg/dl)	200±16*	264±32***	251±23***	181±27
TG (mg/dl)	317±97***	283±84***	130±45**	96±34
HDL-C (mg/dl)	43.3±8.2	38.3±10.4	44.8±9.9	39.0±6.7
LDL-C (mg/dl)	109±15	154±24*	190±26***	129±27
Alpha-Lp. (%)	21.6±2.4***	23.2±6.7**	28.8±6.3	31.2±5.8
Prebeta-Lp. (%)	40.4±5.8***	35.2±7.9***	26.6±10.1	22.8±6.2
Beta-Lp. (%)	37.8±4.3**	41.4±6.9*	44.5±6.8	45.8±6.2
Apo B (mg/dl)	134±17*	175±28***	163±24***	115±23
Apo AI (mg/dl)	139±19	143±21	166±31	148±19
Lp (a) (mg/dl)	31.8±24.9*	25.1±18.4	29.0±22.8*	14.3±4.7
Apo B/Apo AI	0.98±0.2*	1.22±0.1***	0.99±0.1**	0.78±0.2
TC/HDL-C	4.7±0.8	7.2±1.7***	5.7±1.1*	4.6±0.9
TC/LDL-C	1.85±0.2***	1.72±0.2***	1.32±0.1	1.41±0.1
Prebeta/Alpha	1.90±0.4***	1.67±0.7***	1.09±0.5	0.77±0.4
Beta/Alpha	1.76±0.2	1.93±0.6	1.60±0.3	1.53±0.3
Prebeta/Beta	1.10±0.3***	0.88±0.2**	0.64±0.3	0.59±0.2

TC: Total cholesterol, TG: Triglyceride, Apo B: Apolipoprotein B, Apo AI: Apolipoprotein, Lp (a): Lipoprotein (a), RBP: Retinol binding protein
*p<0.05, **p<0.01, ***p<0.001

Table II. In the groups, linear regression equations and correlation coefficients of retinol with various lipid parameters and their statistical significance.

Parameters	Group I (n=12)	Group II (n=16)	Group III (n=13)	Group IV (n=13)
	r=0.671*	r=0.538*	r=0.819*	r=0.899*
RBP	y=-3.3+1.2x	y=24.6+0.5x	y=24.8+0.6x	y=8.6+0.9x
	r=-0.081	r=-0.154	r=0.454	r=0.020
TC	y=212-0.2x	y=292-0.5x	y=113+1.4x	y=177+0.08x
	r=0.625*	r=0.017	r=0.152	r=0.563*
TG	y=-191+11x	y=276+0.1x	y=77+1.2x	y=-12+2.9x
	r=0.029	r=0.058	r=-0.190	r=0.133
HDL-C	y=41+0.4x	y=35+0.06x	y=55-0.2x	y=34+0.1x
	r=-0.339	r=0.004	r=-0.385	r=-0.100
LDL-C	y=152-0.9x	y=154+0.01x	y=248-1.3x	y=145-0.4x
	r=-0.664*	r=-0.213	r=-0.043	r=-0.005
Alpha-Lp.	y=34-0.2x	y=31-0.1x	y=30-0.03x	y=31-0.004x
	r=0.569	r=0.020	r=0.124	r=0.398
Prebeta-Lp.	y=8.5+0.6x	y=34+0.01x	y=19+0.1x	y=8.9+0.3x
	r=-0.393	r=0.176	r=-0.147	r=-0.393
Beta-Lp.	y=52-0.3x	y=34+0.1x	y=50-0.1x	y=57-0.3x
	r=0.146	r=-0.436	r=-0.246	r=-0.155
Apo B	y=113+0.4x	y=244-1.4x	y=197-0.7x	y=135+0.5x
	r=0.113	r=-0.546*	r=-0.038	r=0.354
Apo AI	y=121+0.4x	y=207-1.3x	y=172-0.1x	y=110+1.03x
	r=0.068	r=-0.121	r=0.127	r=-0.059
Lp (a)	y=17+0.3x	y=37-0.2x	y=12+0.3x	y=15-0.04x
	r=0.132	r=0.003	r=-0.167	r=-0.285
Apo B/Apo AI	y=0.74+5.3x	y=1.2+0.01x	y=1.1-0.001x	y=1.1-0.007x
	r=-0.060	r=-0.136	r=0.005	r=0.003
TC/HDL-C	y=5.2-1.8x	y=8.5-0.02x	y=5.7+0.01x	y=4.4+0.01x
	r=0.397	r=-0.170	r=0.007	r=0.312
TC/LDL-C	y=1.1+0.1x	y=1.8-0.01x	y=1.2+0.01x	y=1.1+0.01x
	r=0.626*	r=0.234	r=0.118	r=0.312
Prebeta/Alpha	y=-0.2+0.1x	y=0.8+0.01x	y=0.7+0.01x	y=1.1+0.01x
	r=0.168	r=0.314	r=0.071	r=-0.127
Beta/Alpha	y=1.4+0.01x	y=0.8+0.01x	y=1.4+0.01x	y=1.8-0.001x
	r=0.450	r=0.069	r=0.073	r=0.485
Prebeta/Beta	y=-0.7+0.1x	y=0.8+0.01x	y=0.5+0.01x	y=0.01+0.1x

*$p<0.05$, (Statistical non-significant p values are not given in the table) See Table I for abbreviations.

In humans who have mild and severe hypertriglyceridemia, plasma vitamin A and retinyl ester levels are generally observed to be increased paralleling the increase of TG levels and a significant correlation has been found between the plasma retinyl ester and TG levels ($r=0.721$, $p<0.01$) (13). We have also found a similar significant correlation between the same parameters ($r=0.625$, $p<0.05$), (Table II). In another work it is said that hypertriglyceridemic cases are prone to vitamin A hypervitaminosis (14).

According to the relation between vitamin A and TG, we can expect a relation also between vitamin A and chylomicron and VLDL metabolisms. Already retinyl esters are used as a marker in the metabolism of chylomicrons (15). In our work we have observed that there was a positive correlation ($r=0.569$) between vitamin A and VLDL in the hypertriglyceridemic group, although it was statistically not significant. But in the same group VLDL levels were found to be statistically higher compared to controls ($p<0.001$) (Table I). Vitamin A can increase VLDL levels by decreasing the catabolism of VLDL (4). In a work made in rats; when TG clearance was blocked with Triton WR-1339, plasma TG levels have been observed to increase and this increase has been more in rats given a natural derivative of vitamin A, retinoic acid (16). Lp catabolism may decrease as a result of a decrease in LPL activity also. In rats when heparin is given, it has been seen that hypertriglyceridemia induced by vitamin A is reversed (17). Although heparin is known classically as an activator of LPL, recently, it is said that it provides the stability of LPL and functions as a component of it (18). Although the biochemical mechanism(s) by which vitamin A decreases LPL activity is not still exactly explained, there are some probable mechanisms, offered. For

example vitamin A may decrease the activity of LPL by increasing the Apo C-III synthesis at the DNA-gene level. In another explanatory mechanism, it is suggested that it may concern the enzyme kinetics, because in recent years it is reported that LPL hydrolyses the retinyl esters at the same time also (19). In subjects with hypervitaminosis of vitamin A, plasma retinyl ester levels may increase to 2-8 fold of normal (20). In these cases, as a result of retinyl ester hydrolysis, a lot of retinol (or probably free fatty acids) may cause feedback inhibition on LPL.

We have found also, a statistically significant and positive correlation between vitamin A and TGs ($r=0.563$, $p<0.05$), in normolipidemic group (Table II), while there was not any correlation in the hypercholesterolemic group (Group 3). This gave us the idea that total cholesterol being high, somehow affected and changed the correlation of vitamin A with TG, in Group 3. When all the results are evaluated, consequently, we can say that vitamin A affects lipid metabolism primarily by increasing the plasma TG levels and it has no any marked effect on total cholesterol, HDL-C and apolipoprotein levels. In the end we can say that, in patients having retinoid medication, whatever the reason, care must be taken, particularly if the subject is hypertriglyceridemic and intermittently plasma TG levels should be measured.

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