Lipoprotein lipase activity determined *in vivo* is lower in carriers of apolipoprotein A-V gene variants 19W and -1131C

Jan Kovář[1,2], Věra Adámková[1]

[1] Institute for Clinical and Experimental Medicine, Prague, Czech Republic

[2] Centre for Cardiovascular Research, Prague, Czech Republic

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Apolipoprotein A-V variants and lipoprotein lipase activity

Corresponding author:

Jan Kovář, PhD

Institute for Clinical and Experimental Medicine

Laboratory for Atherosclerosis Research

Vídeňská 1958/9

140 21 Prague 4

Czech Republic

Tel: +420 261363369

Fax: +420 241721666

Email: jan.kovar@medicon.cz

Abstract

The apolipoprotein A-V (apo A-V) plays an important role in regulation of triglyceride (TG) concentration in serum. To better understand how apo A-V affects triglyceridemia and glucoregulation, the lipoprotein lipase (LPL) activity was determined using intravenous fat tolerance test (IVFTT) and oral glucose tolerance test (oGTT) was performed in carriers of apolipoprotein A-V gene (*APOAV*) variants known to be associated with increased triglyceridemia.

Twelve carriers of 19W variant, 16 carriers of -1131C variant, 1 combined heterozygote and 16 control subjects homozygous for wild type variants (19S/-1131T) were selected from a population sample and matched with respect to body mass index and age. The *APOAV* variants carriers had increased TG, very low density lipoprotein-TG, and apo B concentrations ($p < 0.05$). The LPL activity evaluated as k_2 rate constant for clearance of Intralipid® was 14% lower in *APOAV* variants carriers. The depression of nonesterified fatty acids (NEFA) concentration after glucose load was delayed in *APOAV* variants carriers in spite of the same insulinemia and glycemia.

Our results suggest that variants of *APOAV* associated with increased triglyceridemia are associated with lower LPL activity *in vivo* and with disturbances of regulation of NEFA concentration after glucose load.

Key words

apolipoprotein A-V – genetics – lipoprotein lipase – nonesterified fatty acids

Introduction

Apolipoprotein A-V (apo A-V), a recently discovered member of apolipoprotein family (Pennacchio et al. 2001, van der Vliet 2001, for review Šeda and Šedová 2003), is a new important player in a regulation of lipoprotein metabolism. It has been suggested that this apolipoprotein is involved in the lipoprotein lipase (LPL) activation (Fruchart-Najib et al. 2004, Merkel et al. 2005) or in the control of hepatic very low density lipoprotein (VLDL) assembly and/or secretion (Weinberg et al. 2003); however, the exact mechanism of apo A-V action has not been clarified yet. Importantly, two variants of human apolipoprotein A-V gene (*APOAV*), -1131T/C and 19S/W, were found to be associated with increased triglyceridemia in population studies (Pennacchio et al. 2002, Hubáček et al. 2004). The same variants are more frequent among the patients with extreme hypertriglyceridemia (Hořínek et al. 2003, Vrablík et al. 2003) and it can be assumed that -1131C and 19W variants are dysfunctional with respect to regulation of triglyceridemia. Therefore, to better understand the role that these variants play in the regulation of lipoprotein lipase activity, the intravenous fat tolerance test (IVFTT) was carried out in both -1131C and 19W variant carriers and in control subjects to determine the LPL activity *in vivo*. Moreover, it has been shown recently that heterozygous LPL deficiency, another genetic defect associated with the development of milder forms of hypertriglyceridemia, can contribute to development of insulin resistance (Holzl et al. 2002). To determine whether these *APOAV* variants can affect the glucoregulation, the oral glucose tolerance test (oGTT) was carried out in the same subjects.

Methods

Subjects and design of the study

Subjects, carriers of *APOAV* variants, were selected from a 1% population sample of two districts in Czech Republic originally examined in Czech MONICA follow-up study (Cífková

and Škodová 2004) in 1997 and 1998. They were invited for another examination 7 or 8 years later. The control subjects (homozygous for 19S and -1131T variants) were selected from the same population sample. They were matched to *APOAV* variants carriers with respect to age and body mass index (BMI) determined at the time of their examination during the population study and they did not differ in these parameters at the entry into the study (Table 1). The 19S/W and -1131T/C polymorphisms of *APOAV* gene and apolipoprotein E gene (*APOE*) polymorphism were determined in the above mentioned population study (Hubáček et al. 2004). Twelve carriers of 19W allele (all of them heterozygous), 16 carriers of -1131C allele, 1 subject heterozygous for 19W/-1131C and 16 control subjects (homozygous for both 19S and -1131T variants) were recruited. Five subjects were homozygous for -1131C allele. They did not differ from heterozygous carriers in age, anthropometric parameters, lipids and lipoproteins and, therefore, homo- and heterozygous carriers of -1131C allele were pooled for statistical analysis.

All subjects included into the study were examined twice. On the first occasion, they underwent clinical examination and the fasting blood for determination of lipids in serum was obtained. Oral glucose tolerance test was then carried out. At the second visit, fasting blood was obtained for isolation of lipoproteins by ultracentrifugation and intravenous fat tolerance test was carried out to determine LPL activity *in vivo*. The study protocol was approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine and all the participants gave their informed consent.

Methods

The standard oral glucose tolerance test (oGTT) (75 g of glucose) was carried out and blood for determination of glucose, insulin and nonesterified fatty acids (NEFA) in serum was collected before and 30, 60 and 120 minutes after glucose load.

Intravenous fat tolerance test (IVFTT) was carried out as previously described (Rössner 1974) with slight modifications (Jindřichová et al. 2007). Intralipid® (0.1 g of fat/kg of body

weight) was injected to subjects and blood for nephelometric determination of k_2 rate constant was collected before application and then for 40 min after application in 5min intervals. The turbidity of diluted serum was measured on MARK IV nephelometer (Scientific Furnishings LTd, England). The k_2 rate constant is considered to be the measure of LPL activity *in vivo*.

Very low density lipoproteins (VLDL) were isolated by ultracentrifugation (Havel et al. 1955).

Cholesterol and triglycerides in serum and VLDL were determined using enzymatic kits from Roche Diagnostics, Switzerland. High density lipoprotein-cholesterol (HDL-C) was measured after precipitation of lipoproteins containing apolipoprotein B (apo B) using enzymatic kits from the same provider and low density lipoprotein-cholesterol (LDL-C) was then calculated from Friedewald formula. Glucose concentration was determined using enzymatic kits from LACHEMA, Czech Republic, nonesterified fatty acids (NEFA) using enzymatic kits from Wako Chemicals GmbH, Germany, and apo B was determined using kits from Orion Diagnostica, Finland.

Statistics

Depending on the data distribution, the differences between control subjects and all *APOAV* variants carriers were evaluated using t-test or Mann-Whitney test. To detect differences between controls, 19W and -1131C variant carriers, the ANOVA or Kruskall-Wallis statistic were used. When needed, multiple comparisons were carried out using t-test with Bonferroni correction or Dunn's test, respectively (Glantz 1992).

Results

The *APOAV* variants carriers had increased concentration of triglycerides, VLDL-TG, apolipoprotein B and had lower ratio VLDL-C/VLDL-TG than control subjects (Table 2). The statistical significance of differences in TG concentration disappeared when *APOAV* variants

carriers were further subdivided likely due to a smaller sample size. Also, the variability of triglyceridemia was more pronounced in *APOAV* variants carriers - 12 out of 29 *APOAV* variant carriers had TG concentration higher than 2 mmol/l (compared to 2 out of 16 controls), pronounced hypertriglyceridemia (TG > 4 mmol/l) was detected in 4 carriers and none of the controls. Importantly, there were no differences between 5 homozygous and 11 heterozygous -1131C allele carriers in any of analyzed parameters and therefore they were pooled for the analysis.

Remarkably, there were 4 diabetics and 13 subjects with impaired glucose tolerance among the subjects included into the study, 1 and 5 among 19W variant carriers, 2 and 4 among -1131C variant carriers and 1 and 4 among controls, respectively. However, none of the subjects was treated for diabetes and/or glucose intolerance at the entry into the study.

The k_2 rate constant, the measure of LPL activity determined *in vivo* in intravenous fat tolerance test, was decreased in carriers of *APOAV* variants (Table 3). The difference did not remain statistically significant when the carriers were subdivided into subgroups (19W and -1131C) ($p < 0.10$) likely because of the small size of the subgroups and due to the fact that highest k_2 value in the study ($k_2 = 10.2\%/min$) was found in one of the 19W allele carriers (Table 3).

There was no difference either in the course of glycemia and insulinemia or in the HOMA index between *APOAV* variants carriers and controls (Table 3). However, the depression of serum NEFA concentration after oral glucose load was markedly delayed in both 19W and -1131C allele carriers (Table 3).

Discussion

To summarize, we have found that LPL activity determined *in vivo* using intravenous fat tolerance test is lower in subjects carrying 19W or -1131C allele of *APOAV* gene than in carriers of wild type *APOAV* variants. Moreover, although we did not detect any differences in insulin

resistance between control subjects and carriers of 19W and -1131C *APOAV* variants, the suppression of serum nonesterified fatty acid concentration after oral glucose administration was markedly delayed in *APOAV* variants carriers.

Up to now, the evidence that apo A-V can activate LPL has been growing up. However, the role of apo A-V in the process is quite different than that of apo C-II. Although the first papers on the topic were not very convincing because of supraphysiological concentration of apo A-V (Fruchart-Najib et al. 2004, Schaap et al. 2004), there are recent data suggesting that apo A-V can facilitate interaction between VLDL, LPL and heparan sulfate at the endothelium (Merkel et al. 2005). The different role of apo A-V in LPL activation is underlined by the fact that it cannot prevent development of hyperchylomicronemia in apo C-II deficient subjects. However, up to now there has been no evidence that LPL activity is affected by apo A-V in humans. Our data obtained using IVFTT show that LPL activity is indeed lower in those carrying *APOAV* variants known to be associated with hypertriglyceridemia. It should be stressed that results of IVFTT reflect the LPL activity *in vivo* contrary to the measurement of LPL *in vitro*, which corresponds to the amount of active form of enzyme released into circulation by heparin. *In vitro* measurement cannot reflect the factors affecting LPL activity *in situ* such as blood flow rate and the complex interactions between all the components of the lipolytic system including LPL, VLDL, heparan sulphate, availability of apo C-II, the rate of transfer of fatty acids released from the site of hydrolysis and others.

It has been suggested that 19W variant is not secreted efficiently from the liver and, therefore, may have a lower concentration in circulation (Talmud et al. 2005). Although the same authors were unable to identify any exact role for -1131C variant, they pointed out that this variant is a part of more complex haplotype *APOAV*2*. This haplotype is in linkage disequilibrium with apo C-III variant defective with respect to downregulation by insulin (Waterworth et al. 2003) – the inhibition of LPL may result from the increased apo C-III concentration in circulation. In spite of possible differences in the effect of -1131C and 19W

APOAV variants on metabolism of triglyceride-rich lipoproteins, we were unable to detect any differences between these *APOAV* variants in their effect on lipoprotein metabolism in our study.

Although there are no differences in the insulinemia and glycemia after oral glucose load between carriers of *APOAV* variants and control subjects, there are profound differences in the nonesterified fatty acids concentration. Concerning the mechanism of delayed suppression of NEFA concentration after oral glucose load, we do not have any data to provide an explanation. It is generally accepted that NEFA in circulation originate from lipolysis in adipose tissue, which is tightly inhibited by insulin - that was actually observed in control subjects in the study. The findings of delayed suppression of NEFA concentration after oral glucose load in *APOAV* variants carriers might suggest that the regulation of lipolysis in adipose tissue by insulin is defective in these subjects. Nevertheless, the mechanism of that is puzzling because we have not seen any differences in insulinemia and glycemia. If insulin action on lipolysis in adipose tissue is the same in all the subjects, it could be then speculated that higher concentration of NEFA in circulation after glucose load may rather reflect the increased leakage of fatty acids released by LPL into circulation. If this is a case, apo A-V might be involved in some way in directing fatty acids from the site of lipolysis at the endothelial surface to the tissue. It could be also speculated that, if the concentration of NEFA is higher in subjects with *APOAV* variants throughout the day, the VLDL production should be also increased and contribute to hypertriglyceridemia.

It should be also noted that the lower ratio VLDL-C/VLDL-TG along with pronounced triglyceridemia suggest that *APOAV* variants carriers have on average larger VLDL particles than control subjects. Moreover, the fact that *APOAV* variants carriers have the same LDL-C together with increased apo B concentration implies that they have higher proportion of small dense LDL particles in circulation than carriers of wild type alleles of *APOAV*. Such lipoprotein profile could contribute to a higher risk of cardiovascular disease in *APOAV* variants carriers, however, our findings need to be confirmed in other studies.

In conclusion, for the first time it has been documented that subjects carrying 19W and -1131C variant of *APOAV* gene have lower LPL activity *in vivo* than those having wild type *APOAV*. Also, the subjects carrying 19W and -1131C allels were found to have defective regulation of nonesterified fatty acid concentration after glucose load.

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Table 1

The age, body mass index (BMI) and waist-hip ratio (WHP) in control subjects and *APOAV* variants carriers included into the study.

	Subjects					p
	Controls	Carriers of *APOAV* variants				
		All	19W	-1131C	19W/-1131C	
n	16	29	12	16	1	
age [years]	52.8±10.2	52.5±9.1	53.7±9.4	51.0±9.0	61.0	n.s.
BMI [kg/m^2]	28.7±2.6	29.0±3.5	29.8±3.2	28.1±3.5	33.6	n.s.
waist [cm]	98±8	96±8	98±9	95±8	108	n.s.
WHR	0.93±0.05	0.92±0.05	0.92±0.05	0.92±0.04	0.95	n.s.

Data are means ± SD. The differences between control subjects and all *APOAV* variants carriers were evaluated using t test, the differences between three subgroups (controls, 19W, and -1131C carriers) using ANOVA.

p - probability of differences between groups detected using ANOVA or Kruskall-Wallis statistic.

Table 2

Concentration of lipids, lipoproteins and apo B in control subjects and *APOAV* variants carriers included into the study.

	Subjects					p
	Controls	Carriers of *APOAV* variants				
		All	19W	-1131C	19W/-1131C	
n	16	29	12	16	1	
TG [mmol/l]	1.57±0.68 [1.45]	3.29±4.09* [1.93]	3.84±3.85 [2.88]	2.98±4.46 [1.76]	1.89	< 0.10
cholesterol [mmol/l]	5.62±1.01	6.10±1.32	6.02±1.40	6.21±1.32	5.29	n.s.
HDL-C [mmol/l]	1.31±0.24	1.28±0.39	1.28±0.48	1.31±0.31	0.81	n.s.
LDL-C [mmol/l]	3.55±0.86	3.60±0.91	3.33±0.89	3.80±0.94	3.62	n.s.
apo B [g/l]	1.09±0.25	1.33±0.45*	1.45±0.64	1.24±0.22	1.23	n.s.
VLDL-TG [mmol/l]	1.01±0.50 [1.02]	2.68±5.24* [1.52]	1.73±0.96 [1.76]	3.20±6.53 [1.52]	1.89	< 0.05
VLDL-C [mmol/l]	0.43±0.20 [0.41]	0.79±1.11 [0.64]	0.49±0.33 [0.35]	0.95±1.36 [0.64]	0.67	< 0.10
VLDL-C/ VLDL-TG	0.45±0.09 [a]	0.36±0.10*	0.30±0.12 [b]	0.39±0.08 [a,b]	0.35	< 0.01

Data are means ± SD [medians]. The medians are shown for variables with non Gaussian distribution.The differences between control subjects and all *APOAV* variants carriers were evaluated using t test except of TG, VLDL-TG, and VLDL-C concentrations when Mann-Whitney test was used. The differences between three subgroups (controls, 19W, and -1131C carriers) were evaluated using ANOVA or Kruskall-Wallis statistic (TG, VLDL-TG, VLDL-C). The t test with Bonferroni correction or the Dunn's test, respectively, were used for multiple comparisons if appropriate.

* - $p < 0.05$ - controls vs all *APOAV* variants carriers.

p - probability of differences between groups detected using ANOVA or Kruskall-Wallis statistic,

[a,b] - the same letters belongs to groups (controls, 19W carriers, -1131C carriers) that do not differ.

Table 3

Lipoprotein lipase activity determined as k_2 rate constant in intravenous fat tolerance test, changes of glucose, insulin and NEFA concentrations during oral glucose tolerance test and HOMA index in control subjects and carriers of *APOAV* variants.

		Subjects					p
		Controls	Carriers of *APOAV* variants				
			All	19W	-1131C	19W/-1131C	
Intravenous fat tolerance test (IVFTT)							
k2 [%/min]		4.69±1.15	3.93±1.73*	4.40±2.46	3.60±1.19	5.02	< 0.10
Oral glucose tolerance test (oGTT)							
	Time [min]						
glucose	0	5.9±1.1	6.0±2.7	6.7±3.8	5.5±1.4	5.7	n.s.
[mmol/l]	30	9.6±2.0	10.4±3.1	11.5±3.9	9.7±2.3	9.1	n.s.
	60	9.0±2.0	10.9±4.5	12.6±4.9	9.7±4.0	10.7	n.s.
	120	6.1±2.0	7.3±5.1	8.7±7.3	6.4±2.7	5.5	n.s.
insulin	0	10±8	9±5	11±6	7±4	18	n.s.
[mIU/l]	30	62±51	52±33	58±41	45±26	77	n.s.
	60	65±37	69±53	89±67	49±29	138	< 0.10
	120	33±34	32±27	45±36	22±13	40	n.s.
NEFA	0	0.45±0.27	0.53±0.28	0.52±0.27	0.54±0.30	0.47	n.s.
[mmol/l]	30	0.20±0.09[a]	0.42±0.34**	0.44±0.42[b]	0.41±0.29[b]	0.47	< 0.01
	60	0.10±0.07[a]	0.22±0.23**	0.24±0.23[b]	0.20±0.24[a,b]	0.11	< 0.05
	120	0.06±0.03	0.13±0.19	0.14±0.20	0.13±0.20	0.06	n.s.
HOMA index		2.72±2.48	2.67±2.51	3.68±3.24	1.75±1.39	4.48	n.s.

Data are means ± SD. The differences between control subjects and all *APOAV* variants carriers were evaluated using Mann-Whitney test. Kruskall-Wallis statistic was used to detect differences

between controls, 19W and -1131C carriers and the Dunn's test was used for multiple comparisons when appropriate.

*, ** - $p < 0.05$, $p < 0.01$, respectively - controls vs all *APOAV* variants carriers (Mann-Whitney test)

p - probability of differences between groups detected using Kruskall-Wallis statistic, [a,b] - the same letters belongs to groups (controls, 19W carriers, -1131C carriers) that do not differ.