Glucose added to a fat load suppresses the postprandial triglyceridemia response in carriers of the -1131C and 56G variants of the *APOA5* gene

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Short title:
Postprandial triglyceridemia in carriers of *APOA5* variants

Summary

Apolipoprotein A-V plays an important role in the determination of plasma triglyceride (TG) concentration. We aimed to determine whether polymorphisms -1131T>C (rs662799) and 56C>G (rs3135506) of the *APOA5* gene have an impact on the course of postprandial lipemia induced by a fat load and a fat load with added glucose.

Thirty healthy male volunteers, seven heterozygous for the -1131C variant and three for the 56G variant (HT) carriers, and 20 wild-type (WT) carriers underwent two 8-hour tests of postprandial lipemia – one after an experimental breakfast consisting of 75 g of fat and second after a breakfast consisting of 75 g of fat and 25 g of glucose.

HT carriers had a higher postprandial response after fat load than WT carriers (AUC TG: 14.01 ± 4.27 vs 9.84 ± 3.32 mmol*h/l, respectively, $p=0.016$). Glucose added to the test meal suppressed such a difference.

Heterozygous carriers of the variants of *APOA5* (-1131C and 56G) display more pronounced postprandial lipemia after pure fat load than WT carriers. This statistically significant difference disappears when glucose is added to a fat load, suggesting that meal composition modulates the effect of these polymorphisms on the magnitude of postprandial lipemia.

Key words

Apolipoprotein A-V, triglycerides, postprandial lipemia, glucose, genetics

1. Introduction

Apolipoprotein A-V (apoA-V) has been shown to have a pronounced impact on triglyceride concentration in circulation (van der Vliet *et al.* 2001).

Interestingly, the mechanism by which it affects triglyceridemia is not fully clarified yet. It has been shown that apoA-V enhances triglyceride-rich lipoproteins (TRL) clearance from circulation by stimulating lipoprotein lipase (LPL) activity and/or binding TRL to the endothelium (Fruchart-Najib *et al.* 2004, Merkel *et al.* 2005, Shu *et al.* 2010). Alternatively, apoA-V may reduce VLDL secretion (Goto *et al.* 2010, Schaap *et al.* 2004, Weinberg *et al.* 2003). Three common haplotypes of the apoA-V-encoding gene (*APOA5*) in humans have been described – haplotype 1 (wild-type), haplotype 2 (-1131T>C), a complex promoter haplotype that includes four single nucleotide polymorphisms (rs662799: -1131T>C, rs651821: -3A>G, rs2072560: 751A>G, and rs2266788: 891T>C) and haplotype 3 (rs3135506: 56C>G), which encodes the S19W variant. Carriers of the -1131C and 56G variants have been repeatedly shown to have increased triglyceridemia (Hubacek *et al.* 2014, Pennacchio *et al.* 2001, Pennacchio *et al.* 2002).

Elevated fasting triglyceridemia is recognized as an independent risk factor of cardiovascular disease (Chapman *et al.* 2011, Talmud *et al.* 2006). However, humans spend most of the day in a postprandial state and it has been suggested that non-fasting triglyceride (TG) concentration is more closely associated with cardiovascular disease risk (Mora *et al.* 2008, Nordestgaard *et al.* 2007, Nordestgaard *et al.* 2016). The magnitude of postprandial triglyceridemia and thus also non-fasting TG concentration is determined by a number of factors, including the quantity and quality of fat in a meal, dietary habits, physical activity, age, gender and, last but not least, genetic factors.

Interestingly, the studies that have analyzed the impact of TG-raising alleles of the *APOA5* gene (19W and -1131C) on postprandial lipemia have not come to an unequivocal conclusion

– some have found more pronounced postprandial triglyceridemia (Jang *et al.* 2004, Moreno *et al.* 2006), whereas others have not observed any effect (Martin *et al.* 2003, Masana *et al.* 2003). Such discrepancies can be explained by differences in experimental design, meal composition or characteristics of subjects included.

In a recent study of ours, we tested how the addition of glucose to a fat load affects selected parameters of postprandial lipemia in young healthy men with normal lipid concentrations (Zemankova *et al.* 2015). To better understand the role of *APOA5* in the regulation of PPL, we decided to genotype the subjects in that study and analyze the interaction between the effect of meal composition (glucose addition) and the *APOA5* genotype on postprandial lipemia.

2. Methods

1.1. Study Design

The study was carried out in 30 healthy male volunteers as described earlier (Zemankova *et al.* 2015). Briefly, two tests of postprandial lipemia were carried out. In the first experiment, volunteers consumed an experimental breakfast consisting of 75 g of fat and 25 g of glucose (F+G meal). In the control experiment, they consumed just 75 g of fat (F meal). The blood was collected before breakfast (time: 0 h) and 0.5, 1, 1.5, 2, 4, 6 and 8 hours after the breakfast. At the end of the tests, heparin (100 IU/kg of weight) was injected and 10 min later post-heparin plasma was collected for determination of lipoprotein lipase (LPL) concentration. All participants gave their written informed consent and the study was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital in Prague.

1.2. Genotyping

We genotyped the rs662799 (-1131T>C) and rs3135506 (56C>G) *APOA5* variants in all 30 subjects in the study as described previously (Hubacek *et al.* 2004).

1.3. Biochemical Measurements

Blood samples for plasma TG, cholesterol, free fatty acid (FFA), glucose and insulin concentrations were collected in EDTA vacutainer tubes. Post-heparin plasma was collected into heparinized vacutainer tubes. Aliquots of plasma acquired at all time points were stored at - 80°C until analyzed. The triglyceride-rich lipoproteins (TRL) were separated from plasma collected at times 0, 1, 2, 4, 6, and 8 hours. The concentrations of TG, cholesterol, FFA, glucose, insulin, TRL-TG, TRL-C, TRL-apoB-48 and LPL in post-heparin plasma were measured as described earlier (Zemankova *et al.* 2015).

1.4. Statistical analysis

The differences between changes of parameters under study were evaluated using ANOVA for repeated measures with one grouping factor (genotype) and, where appropriate, corresponding post hoc tests were carried out (JMP® 10.0.0 program, SAS Institute, Inc.). Differences in AUC and AUIC were evaluated using the t-test or its non-parametric analogue on GraphPad Instat® 3.1 (GraphPad Software, Inc.). The power of the study to detect a 40 % difference in the magnitude of postprandial lipemia between 10 heterozygous subjects and 20 wild-type carriers at p = 0.05 was 86 %.

3. Results

Three out of total 30 subjects were heterozygous carriers of 56C>G and seven heterozygous carriers of -1131T>C variants. All heterozygous carriers (HT) were then pooled for further analyses. They did not differ from homozygous carriers of wild-type variants (WT) in age, BMI, plasma lipids, glucose, and insulin (Table 1).

The addition of 25 g of glucose to a 75 g fat load induced a 4.5-fold increase in insulinemia, peaking 30 min after the meal (Table 2). After the pure fat load, a relatively small increment in insulin concentration was observed. Importantly, there were no differences in the course of glucose and FFA concentrations between HT and WT (data not shown).

When fat alone was used as the experimental meal, the *APOA5* heterozygous carriers exhibited more pronounced postprandial triglyceridemia – the area under the curve of TG (AUC TG) in HT was 42% higher than that in WT (Fig. 1 A, Table 2). The difference was even more pronounced when the incremental areas under curve (AUICs) were compared – AUIC TG in HT was 2.25 times higher than that in WT (Fig. 1 C+D, Table 2). Consistent data were obtained when the TRL-TG concentration was compared – AUC TRL-TG and AUIC TRL-TG in HT were 48% and 107% higher than those in WT (Table 2). The courses of TG and TRL-TG concentrations differed between HT and WT subjects when analyzed by ANOVA for repeated measurements with genotype as a grouping factor. A similar but statistically non-significant trend was also observed for TRL-C (Table 2).

No statistically significant difference between HT and WT was observed when 25 g of glucose was added to fat (Fig. 1 B, Table 2). Therefore, the addition of glucose to the test meal suppressed the difference between HT and WT subjects.

Importantly, no differences between the response of apoB-48 in chylomicrons and their remnants (TRL-apoB-48) between F and F+G load were noted in both HT and WT subjects (Fig. 1 E+F, Table 2).

There were no differences in the concentration of LPL in post-heparin plasma collected 8 hours after the experimental breakfast (HT: F+G meal 498 ± 93 ng/ml; HT: F meal 446 ± 56 ng/ml; WT: F+G meal 505 ± 108 ng/ml; WT: F meal LPL = 490 ± 108 ng/ml).

Table 1.

Characteristics of study participants.

	Heterozygous (HT)	Wild-type (WT)
n	10	20
Age [years]	33.5 ± 8.0	34.5 ± 8.3
BMI [kg/m^2]	26.9 ± 3.8	25. 6 ± 2.0
TG [mmol/l]	1.22 ± 0.53	1.05 ± 0.46
Cholesterol [mmol/l]	4.41 ± 0.85	4.41 ± 0.71
Glucose [mmol/l]	5.50 ± 0.48	5.43 ± 0.43
Insulin [mIU/l]	7.51 ± 3.37	6.67 ± 2.83
FFA [mmol/l]	0.42 ± 0.18	0.45 ± 0.18
TRL-TG [mmol/l]	0.88 ± 0.50	0.68 ± 0.38
TRL-C [mmol/l]	0.34 ± 0.20	0.27 ± 0.17
TRL-apoB-48 [mg/l]	7.32 ± 6.57	4.94 ± 3.65

Data are presented as mean ± SD. There were no statistically significant differences between HT and WT subjects.

Table 2.

AUCs and AUICs of selected parameters after F and F+G meals

		8h AUC		**p (pair. t-test)**	**8h AUIC**		**p (pair. t-test)**
	Genotype	**F**	**F+G**	**p**	**F**	**F+G**	**P**
TG [mmol*l/h]	WT	9.84	11.08	0.184	2.03	2.16	0.674§
		±3.32	±4.50		±2.39	±1.68	
	HT	14.01	13.28	0.151	4.58	3.16	0.084§
		±4.27	±4.94		±2.13	±2.18	
	t-test	0.016	0.253		0.005§§	0.222	
TRL -TG [mmol*l/h]	WT	7.29	7.65	0.282	1.96	2.09	0.834
		±3.01	±3.83		±2.16	±1.43	
	HT	10.80	10.31	0.577	4.05	3.05	0.282
		±4.25	±4.39		±1.70	±2.0	
	t-test	0.035	0.122		0.008	0.192	
TRL -C [mmol*l/h]	WT	2.17	2.39	0.287	0.03	0.17	0.559
		±1.01	±1.49		±0.65	±0.60	
	HT	3.20	3.26	0.800	0.60	0.36	0.222
		±1.4	±1.84		±0.75	±0.77	
	t-test	0.063	0.217		0.056	0.496	
TRL-apoB-48 [mg*l/h]	WT	45.46	46.82	0.820	7.75	5.53	0.708
		± 24.98	± 28.04		± 24.86	± 16.40	
	HT	70.99	73.16	0.716	20.13	6.95	0.298
		± 48.83	± 50.34		± 31.02	± 30.87	
	t-test	0.147	0.150		0.289	0.894	

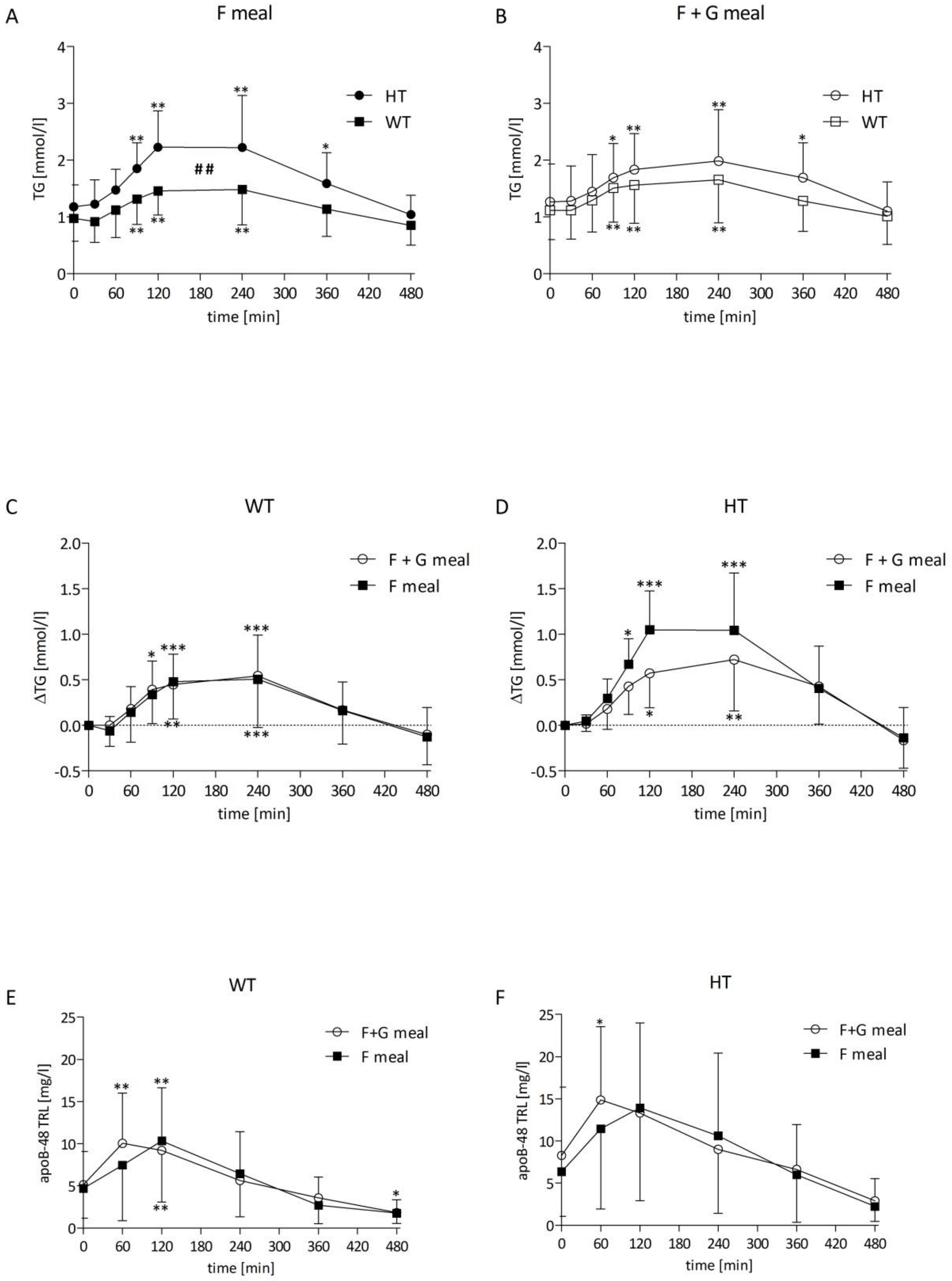
		2h AUC		**p (pair. t-test)**	**2h AUIC**		**p (pair. t-test)**
	Genotype	**F**	**F+G**	**p**	**F**	**F+G**	**P**
Glucose [mmol*l/h]	WT	10.31	10.80	0.108	-0.54	- 0.08	0.042
		± 1.01	± 1.54		± 0.53	± 1.07	
	HT	10.40	11.07	0.067	-0.71	0.20	0.040
		± 0.95	± 1.31		±0.61	± 1.32	
	t-test	0.817	0.621		0.461	0.570	
Insulin [mIU*l/h]	WT	18.50	37.40	< 0.001	5.82	23.39	< 0.001
		± 6.46	± 18.4		± 4.79	± 15.02	
	HT	20.55	38.76	< 0.001	6.42	22.86	0.001
		± 7.45	± 15.8		±4.20	± 13.1	
	t-test	0.469	0.835		0.731	0.921	
FFA	WT	0.81	0.70	0.118	-0.09	-0.21	0.032

[mmol*l/h]		± 0.24	±0.19		±0.25	±0.29	
	HT	0.77	0.65	0.052	0.01	-0.29	0.005
		± 0.18	± 0.15		± 0.22	±0.30	
	t-test	0.568	0.424		0.252	0.481	

Data are presented as mean ± SD. Areas under 8-hour curve (AUCs) and areas under 8-hour incremental curve (AUICs) for TG, TRL-TG, TRL-C and TRL-apoB-48 concentrations, and areas under 2-hour curve (AUCs)(0-2 hours) and areas under 2-hour incremental curve (AUICs) for glucose, insulin and NEFA concentrations. HT …heterozygous carriers of -1131T>C and 56C>G *APOA5* variants, WT … *APOA5* wild-type carriers. The p-values were obtained from unpaired and paired t-tests except for § and §§, where the Wilcoxon matched pairs test and the Mann-Whitney test were used, respectively.

Figure 1.

Changes of TG concentrations and TRL-apoB-48 after the experimental breakfast.



Data are presented as mean ± SD.

A ... concentration of TG after 75 g of fat load (F) in heterozygous carriers of *APOA5* variants (HT) and homozygous carriers of wild-type *APOA5* (WT);

B ... concentration of TG after 75 g of fat load + 25 g of glucose (F+G) in HT and WT;

C ... increment of TG concentration after F or F+G meal in WT subjects;

D ... increment of TG concentration after F or F+G meal in HT subjects;

E ... concentration of TRL-apoB-48 after F or F+G meal in WT subjects;

F ... concentration of TRL-apoB-48 after F or F+G meal in HT subjects;

... $p = 0.01$ (ANOVA for repeated measures)

* ... $p < 0.05$, ** ... $p < 0.01$, *** ... $p < 0.001$ vs time 0 (TG: ANOVA for repeated measures with Dunnett´s test; ΔTG, TRL-apoB-48: ANOVA for repeated measures with Dunn´s test)

4. Discussion

In this study of 30 healthy volunteers we found that postprandial lipemia is increased in subjects heterozygous for the -1131C or 56G variants of the *APOA5* gene (HT) compared to wild-type allele carriers (WT) after consumption of 75 g of fat. Such a difference between HT and WT subjects was not observed when 25 g of glucose was added to the test meal.

It has been demonstrated in *in vitro* experiments that the secretion of apoA-V should be lower due to the lower transcription rate in carriers of the -1131C variant (Palmen *et al.* 2008) and due to diminished translocation of apoA-V into the secretory pathway in 56G variant carriers (Talmud *et al.* 2005). It should be pointed out that tryptophan in position 19 in 56G variant carriers is a part of the signal protein that is removed before secretion. The carriers of both *APOA5* variants should then secrete from the liver the same mature protein as wild-type carriers. That allows carriers of both variants to be pooled for the analysis. It may be, therefore, expected that HT subjects should have a lower apoA-V concentration in circulation. This indeed has been demonstrated in some studies (Ishihara *et al.* 2005, Kim *et al.* 2013); however, other studies have not confirmed such findings (Hahne *et al.* 2008, Henneman *et al.* 2007). It cannot be excluded that the inverse relationship between the presence of these variants and apoA-V concentrations is lost or even reversed when carriers of these *APOA5* variants have increased triglyceridemia, or when they are diabetic or obese. Importantly, HT participants in our study were young healthy men that had low TG concentrations not different from those in WT subjects (triglyceridemia above 2.5 mmol/l was among the exclusion criteria of our study) (Zemankova *et al.* 2015). We can therefore assume that HT subjects in our study had lower apoA-V concentration than WT subjects.

Heterozygotes for *APOA5* variants have a higher plasma TG concentration then carriers of wild-type variants (Pennacchio *et al.* 2001, Pennacchio *et al.* 2002, Wang *et al.* 2008). However, there is no unambiguous explanation for how *APOA5* variants can augment triglyceridemia. It has been repeatedly demonstrated that apoA-V enhances TRL clearance from circulation by stimulating LPL activity and/or binding TRL to the endothelium (Fruchart-Najib *et al.* 2004, Merkel *et al.* 2005, Shu *et al.* 2010). However, the studies that have brought such evidence have been carried out with transgenic animals or have used apoA-V concentrations higher than physiological concentrations. Up to now there is no evidence that apoA-V at physiological concentration (that is more than 10 times lower than VLDL concentration and more than 300 times lower than the concentration of apolipoprotein C-II, a principal cofactor of LPL) can affect LPL activity *in vivo*. It is very unlikely that changes in apoA-V concentration in the physiological range (due to its genetic variability) could significantly affect the rate of TRL lipolysis in circulation.

Alternatively, it has been demonstrated that apoA-V reduces VLDL-TG secretion (Schaap *et al.* 2004). Such findings are supported by experiments which indicate that apoA-V can redirect “budding” lipid droplets from an association with nascent VLDL to storage in cytoplasma in hepatocytes (Goto *et al.* 2010). If this is the case, it can be expected that *APOA5* variant carriers that secrete less apoA-V should produce more VLDL-TG. That could explain the increased triglyceridemia in subjects carrying *APOA5* variants and accord with our observation that postprandial triglyceridemia is increased in HT subjects when given a pure fat load. Because baseline triglyceridemia does not differ between HT and WT subjects, the difference can be attributed to increased VLDL production in HT subjects.

Last but not least, the possibility that the differences in TG response to fat load between HT and WT subjects are due to differences in chylomicron production should not be left out of consideration. However, there was no statistically significant difference in the response of apoB-48 in chylomicrons and their remnants to F and F+G load in both WT and HT subjects (Fig. 1 E+F, Tab. 2). Moreover, it has been documented that *APOA5* is expressed mainly in the liver and its expression in the intestine is three orders of magnitude lower (Guardiola *et al.*

2012). It is then unlikely that intestinal apoA-V can have any pronounced impact on lipoprotein metabolism in postprandial phase.

It remains to be clarified though why such a difference is diminished by the addition of a relatively small amount of glucose to a fat load. Although glucose only represents a 15% increase in energy intake, it induces a reasonable increase in glycemia and an expected physiological response of insulin. Insulin was shown to downregulate *APOA5* expression and even to decrease apoA-V concentration in plasma in a hyperinsulinemic euglycemic clamp study (Nowak *et al.* 2005), but it is unlikely that it should have any profound impact on postprandial lipemia in its early phase. On the contrary, glucose per se has been shown to activate *APOA5* expression (Nowak *et al.* 2008), but it is not entirely clear whether it may significantly affect apoA-V secretion in our study design. On the other hand, insulin has been shown to suppress VLDL secretion due to the suppression of lipolysis in adipose tissue and thus the lower influx of FFA (as a principal substrate for synthesis of TG) into the liver, and due to its direct effect on apoB and VLDL secretion in hepatocytes (Weinberg *et al.* 2003, Xiao *et al.* 2014). Our data may then suggest that the suppressive effect of insulin on VLDL secretion from the liver may outweigh the role of apoA-V in the regulation of VLDL secretion in the postprandial phase and therefore diminish the differences in the magnitude of postprandial lipemia between HT and WT subjects.

Our observation clearly highlights an interaction between the *APOA5* genotype and the composition of experimental meals used to induce postprandial lipemia and may contribute to an explanation for some inconsistencies between the results of studies that have analyzed the effect of -1131C and 56G variants on postprandial lipemia. However, it should be pointed out that most of the studies that detected differences between carriers of these alleles and control subjects used a mixed meal that should induce a regular insulin response (Jang *et al.* 2004, Moreno *et al.* 2006). Even in our study the magnitude of postprandial lipemia was 20 %

higher in HT subjects than in WT subjects when the fat load was given with glucose, even though the difference was not statistically significant. Therefore, it cannot be excluded that the effect of *APOA5* variants on the magnitude of postprandial lipemia in these studies should have been more profound if only the fat load was used instead of the mixed meal.

A certain limitation of our study is that it was not originally designed to test the effect of the *APOA5* polymorphism on the magnitude of postprandial lipemia (although it should be stressed that it provided us with enough statistical power to detect the observed differences between HT and WT subjects after F load).

We can conclude that postprandial lipemia is increased in carriers of the -1131C and 56G variants of the *APOA5* gene when given 75 g of fat. The addition of 25 g of glucose, which elicits a physiological response of insulin, diminishes the differences in the magnitude of postprandial lipemia between carriers of *APOA5* variants and control subjects and reveals an important interaction between the *APOA5* genotype and the composition of the experimental meal. In the context of recently discussed role of postprandial lipids, especially triglycerides in pathogenesis of cardiovascular disease (Nordestgaard *et al.* 2007, Nordestgaard *et al.* 2016), we think that our results could add valuable information regarding particular genetic factors which could modify the response of circulating lipids to well-defined prandial burden.

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Conflict of interest

The authors have no conflict of interest and have nothing to disclose.

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