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Determinants of Plasma Apolipoprotein A-V and *APOA5* Gene Transcripts in Humans

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Abstract

Objective—Apolipoprotein A-V (apoAV) contributes to the regulation of triglyceride metabolism, which plays a role in the pathogenesis of atherosclerotic diseases. We therefore ascertained determinants of hepatic *APOA5* transcript and apoAV plasma levels in humans.

Design—We determined influences of anthropometric variables, biochemical factors related to lipid and glucose metabolism, hepatic mRNA levels transcribed from the *APOA1/C3/A4/A5* cluster and transcription factor genes implicated in the regulation of *APOA5* as well as common SNPs at the *APOA5* locus on *APOA5* expression in 89 obese patients and 22 non-obese controls.

Results—Mean, age and sex adjusted, hepatic *APOA5* mRNA or apoAV plasma levels did not differ by obesity status, HOMA-IR or inflammatory markers. In multivariate regression models, the c56C>G SNP, plasma apoCIII, plasma non-esterified fatty acids, hepatic *APOA5* transcripts, sex and a weak association with obesity status explained 61% of the variance in apoAV plasma levels. Hepatic transcript levels of *CPT1A1* and *PPARA*, plasma non-esterified fatty acids and the c56C>G SNP explained 48% of the variance in hepatic *APOA5* transcript levels.

Conclusion—ApoAV plasma levels are independently associated with plasma free fatty acid and hepatic *APOA5* mRNA levels. Associations of *APOA5* transcripts with *PPARA* and *CPT1A1* transcripts suggest that *APOA5* expression is intimately linked to hepatic lipid metabolism.

Keywords

Apolipoprotein A-V; *APOA5*; triglycerides; *PPARA*; *CPT1A*; SNP

Introduction

The role of fasting triglycerides (TG) in predicting atherosclerotic disease remains controversial, but postprandial TG have been established as an independent risk factor [1-4]. The recently identified apolipoprotein (apo) AV is a key player in TG metabolism [5,6]. Studies in genetically modified mice showed a TG-lowering effect of apoAV [7,8]. Although the underlying mechanism(s) are not fully understood, experimental data strongly suggest that apoAV directly enhances the catabolism of TG-rich lipoproteins by stimulating LPL-mediated lipolysis or indirectly by facilitating the binding of TG-rich lipoproteins to endothelial proteoglycans [9-13]. Consistent with observations showing that apoAV is a ligand for LDL receptor family members [14,15], apoAV also may enhance the removal of

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remnant particles by the liver [10]. In addition, apoAV may influence hepatic lipoprotein production by affecting the maturation of TG-rich lipoproteins [13,16]. Since apoCIII inhibits LPL-mediated lipolysis of TG-rich lipoproteins, apoAV and apoCIII would be expected to have opposite effects on the catabolism of TG-rich lipoproteins [17]. Indeed, deficiency or transgenic overexpression of both apoAV and apoCIII did not affect plasma TG levels in mice [18].

APOA5 is part of the *APOA1/A4/C3/A5* gene cluster located on human chromosome 11 and is mainly expressed in the liver [5]. Studies in cell culture and animal models identified several transcription factors including PPARα, LXRα, HNF4α, and USF1 that contribute to the regulation of *APOA5* expression [19-23]. Insulin was shown to repress *APOA5* transcription and to lower plasma apoAV in euglycaemic clamp studies. However, plasma apoAV levels were found to be unaltered or even decreased in patients with type 2 diabetes [24,25].

ApoAV circulates at very low plasma concentrations in association with TG-rich lipoproteins and HDL [26] and has been implicated in the magnitude of postprandial lipaemia and disorders associated with elevated TG such as familial combined hyperlipaemia and the metabolic syndrome [27-30]. ApoAV deficiency resulting from truncation-causing mutations (Q148X and Q139X) predisposes to severe hypertriglyceridaemia and reduced activation and/or mass of LPL [31-34]. In addition, two major *APOA5* haplotypes, termed *APOA5*2* and *APOA5*3* and tagged by the minor alleles of variants −1131T>C or c56C>G, respectively, are associated with elevated plasma TG in several populations [18,35-37]. However, the mechanisms underlying these associations are not clear. Contrary to two early reports [24,26] and expectations from studies in transgenic mice, several recent studies reported positive correlations between apoAV and TG in plasma [38-42]. Taken together, these studies in humans and in animal and cell culture models suggest complex mechanisms whereby apoAV may influence plasma TG.

The clinical relevance of TG metabolism for CAD emphasizes the need for identifying the factors determining *APOA5* expression in humans. We therefore measured hepatic mRNA levels transcribed from the *APOA1/A4/C3/A5* cluster in obese patients with varying degrees of insulin resistance or type II diabetes and control subjects and determined their relationship to mRNA levels of transcription factors implicated in the regulation of *APOA5* expression. In addition, we measured plasma levels of lipids and apolipoproteins and ascertained associations of these variables with −1131T>C and c56C>G SNPs.

Subjects and Methods

Study Subjects

Our study subjects included 89 obese patients and 22 non-obese controls who underwent weight reducing surgery or elective surgical procedures such as cholecystectomy. Tissue biopsies obtained from liver during surgery were collected in RNA-later (Ambion, Austin, U.S.A.). All study subjects provided informed consent and study protocols were approved by the local ethics committee. Type 2 diabetes was diagnosed by fasting plasma glucose concentrations of 7.0 mmol/L and/or use of hypoglycaemic medications. Homeostasis model assessment insulin resistance (HOMA-IR) was used to estimate insulin sensitivity/resistance [43]. Body fat mass was determined by bioelectric impedance analysis using the phase-sensitive, multifrequence analyzer BIA-2000M (Data input, Hofheim, Germany). Abdominal diameters of subcutaneous and visceral adipose tissues were determined by ultrasonography [44].

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Laboratory Analyses

Fasting plasma glucose, insulin, cholesterol, TG, HDL cholesterol, apolipoproteins AI and B and c-reactive protein (CRP) were determined as described [45]. Plasma non-esterified fatty acids (NEFA) were determined using the Free Fatty Acids Colorimetric Assay Kit (Roche). Plasma apoAV and apoCIII were determined by sandwich ELISA as described [40].

DNA was isolated from peripheral white blood cells using the Generation Capture Column Kit (Gentra Systems, Minneapolis, USA). *APOA5* SNPs -1131T>C (rs662799) and c56C>G (rs3135506) were determined using TaqMan Genotyping Assays (Applied Biosystems, Warrington, UK) C_2310403_10 and C__25638153_10, respectively. Typing was verified in 10% of subjects by restriction enzyme digestion.

Total RNA was isolated from liver biopsies using the RNeasy Mini Kit (Qiagen, Hilden Germany). RNA was digested with DNAse I (Promega, Mannheim, Germany) to eliminate any contaminating DNA. Equal amounts of RNA were reverse transcribed as described [46]. Levels of the following mRNAs were quantified using TaqMan gene expression assays (Applied Biosystems, Warrington, UK) listed in parenthesis and an iCycler iQ Multicolour Real-Time PCR Detector (Bio-Rad, Hercules, USA): transcripts of apolipoprotein genes *APOA5* (Hs00364830_m1), *APOA4* (Hs00166636_m1), *APOC3* (Hs00163644_m1), and *APOA1* (Hs00163641_m1), hepatocyte nuclear factor 4α (*HNF4A*, Hs00230853_m1), forkhead box 01A (*FOXO1A*, Hs00231106_m1), upstream stimulatory factor 1 (*USF1*, Hs00273038_m1), Peroxisome proliferator-activated receptor-alpha (*PPARA*, Hs00231882_m1), carbohydrate response element binding protein 1 (*CHREBP1*, Hs00263027_m1), liver X receptor alpha (*LXRA*, Hs00172885_m1), sterol regulatory element binding protein 1 (*SREBF-1*, custom made for quantification of transcripts encoding SREBP-1c), carnitine palmitoyltransferase 1 (*CPT1A*, Hs00157079_m1), fatty acid synthase (*FASN*, Hs00188012_m1), adipose triglyceride lipase (*ATGL*, 003386101_m1) and phosphoenolpyruvate carboxykinase 1 (*PCK 1*, Hs00159918_m1). Transcript levels were measured in duplicate, and constitutively expressed acidic ribosomal protein p0 (*ARP0*) mRNA was used as an internal standard for normalisation of mRNA abundance. Relative mRNA levels were calculated using the comparative threshold cycle method (ΔC_T) and the iCycler iQ Real-Time Detection System Software (Bio-Rad). The average inter-assay CV of mRNA measurements ascertained by overlapping cDNA samples was 12%. For data presentation, the average ratio of target mRNA to *ARP0* mRNA from all available measurements was assigned a value of 1. To compare transcript levels of *APOA5*, *APOA1, APOC3* and *ARP0*, gene segments containing the sequences targeted by the respective Taqman assays were cloned into the pGL4 vector (Promega). Increasing concentrations of plasmids were used to construct standard curves for the estimation of the respective transcripts in liver.

Statistical Analyses

Differences between groups were ascertained by analysis of variance. Logarithmic transformations were used, if the equal variance and normality assumptions of analysis of variance were rejected. We adjusted measurements, by multiple regression, for concomitant effects of sex, age and other variables as indicated. Allele frequencies were determined by gene counting. Agreement of genotype distributions with Hardy-Weinberg expectations was tested using a χ^2 goodness-of-fit test. Standardized pairwise linkage disequilibrium (LD) expressed in terms of D' and r^2 parameters and haplotype frequencies were estimated using the THESIAS software (www.genecanvas.org). The THESIAS program was also used for testing haplotype-phenotype associations. Covariate adjusted haplotype-phenotype parameters, expressed as mean effect for continuous phenotypes, were estimated for each haplotype by comparison to the most frequent haplotype. Results were compared for

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consistency to those obtained using the haplo.score software (http:/www.mayo.edu/statgen/). We used multivariate regression analysis to determine predictor variables of apoAV plasma and *APOA5* hepatic mRNA levels. Variable selection was carried out in an ordered manner using a priori considerations. For predictors of plasma levels, the set of significant anthropometric variables was identified; to this list the set of biochemical measures was added; finally, effects of SNPs and hepatic transcript levels were included. To determine predictors of hepatic transcript levels, mRNA levels of transcription factors followed by mRNA levels of genes indicative of metabolic pathways were included prior to the addition of genetic information. Reported *P* values are two-tailed and are not adjusted for multiple testing.

Results

As expected, obese subjects displayed higher average values for visceral to subcutaneous fat diameter, waist/hip ratio, plasma insulin, HOMA-IR, CRP and TG in comparison to control subjects, but lower average values for HDL cholesterol and apoAI. Plasma levels of apoCIII and apoAV did not differ between obese and control subjects (Table 1). Average plasma levels of apoAV adjusted for age and obesity status were higher in women than in men (465 vs. 331 μg/L, P=0.0024). In women, no associations of apoAV plasma levels with menopause, hormone replacement therapy or use of contraceptive hormones were observed (data not shown). Regression analyses adjusted for age, sex, use of lipid lowering medication and study group showed strong associations of apoAV with TG (P=0.0009) and apoCIII (P=0.0001) and weaker associations with plasma NEFA and apoAI (both P<0.05). No associations were observed with age, plasma cholesterol, CRP or HOMA-IR (data not shown). In a model containing both apoCIII and apoAV as independent variables, apoCIII, but not apoAV remained a significant predictor of TG (P<0.0001). Moreover, we noted a borderline association between the molar apoCIII/apoAV ratio with plasma TG (P=0.0210).

The genotype distributions at SNPs −1131T>C and c56C>G fulfilled Hardy-Weinberg expectations in the combined study groups and were in perfect negative linkage disequilibrium (D'=−1.0). The frequencies of the −1131C and the c56G alleles were 0.119 and 0.114, respectively, and tended to be higher than in a larger overlapping population comprising subjects without apoAV and apoCIII measurements (−1131C and c56G frequencies 0.078 and 0.083, respectively). No association of either SNP with obesity status was noted. Carriers of −1131C showed lower apoAV plasma levels than subjects homozygous for the −1131T allele (340 + 231 vs 442 + 281 μg/L, P=0.0336, Table 2). Average plasma levels of apoAV were twice as high in c56G carriers in comparison to subjects homozygous for the c56C allele (755 + 326 vs. 318 + 164 μg/l, P<0.0001). Plasma levels of apoCIII and TG tended to be higher and HDL cholesterol tended to be lower in carriers of variant alleles at either polymorphic site, but these differences were not significant. Consistent with the apoAV associations, a higher molar apoCIII/apoAV ratio was observed in carriers of −1131C, while this ratio was much lower in carriers of c56G in comparison to c56C/C subjects. Analyses stratified by obesity status showed significant effects of the c56C>G SNP on plasma apoAV and the apoCIII/apoAV ratio in both obese and control subjects. Differences in plasma apoCIII/apoAV ratios between carriers of the -1131C allele and -1131T/T subjects were significant and the trends of plasma apoCIII in the obese and the control group were similar and consistent with the combined analysis. As noted in the combined analysis, TG tended to be higher and HDL cholesterol tended to be lower in carriers of variant alleles at either polymorphic site in both obese and control subjects (Table I, supplementary information). Sex-specific analyses were also consistent with the combined analysis (Table II, supplementary information). In addition, multivariate regression models showed no interaction of either SNP with obesity status or sex regarding the variables shown in Table 2. Haplotype analyses showed only a significant effect of

*APOA5*3* (tagged by c56G) on apoAV plasma levels and apoCIII/apoAV molar ratios (*P*<0.0001), while effects of *APOA5*2* (tagged by −1131C) did not reach significance. After adjustment for haplotypes or for the c56C>G SNP, the strength of the association between the molar apoCIII/apoAV ratio with plasma TG increased (*P*=0.0004).

Hepatic *APOA5* and *APOC3* mRNA levels were similar between obese and control subjects (Table III, supplementary material). *APOA5* mRNA levels correlated most strongly with *APOA4* mRNA levels and, to a lesser extent, with *APOC3* and *APOA1* mRNA levels (Table IV, supplementary material). Average (SD) *APOC3/APOA5* and *APOA1/APOA5* transcript ratios were 295 (249) and 144 (114) in our study subjects. Average molar apoCIII/apoAV and apoAI/apoAV ratios in plasma were 1725 (987) and 7273 (3793), respectively. Thus, plasma levels, relative to hepatic transcript abundance, of apoCIII and apoAI were six- and fifty-fold higher than the respective level of apoAV. As judged from C_t values, *APOA4* mRNA levels were an order of magnitude lower than *APOA5* mRNA levels. *APOA5* transcript levels correlated with mRNA levels of *USF1*, *HNF4A and PPARA* (Table **3**). After consideration of all three transcription factor mRNAs in a single model, only *PPARA* mRNA levels remained as a significant predictor of *APOA5* mRNA (*P*=0.0036). No correlations were observed with mRNA levels of *SREBF-1*, *LXRA*, *CHREB1* or *FOXO1A*. Among genes involved in key metabolic pathways, correlations were observed with mRNA levels of *CPT1A* (*P*<0.0001), *ATGL* (*P*=0.0041) and *MTP* (P=0.0288), but not with *FASN* or *PCK1* mRNA (Table V, supplementary material). No significant interactions with sex were observed in these analyses.

The average level of *APOA5* mRNA was reduced in c56G carriers in comparison to subjects homozygous for c56C, whereas the −1131T>C SNP was not related to *APOA5* mRNA (Table **4**). No associations of either SNP with *APOC3* or *APOA1* mRNA levels were observed. Consistent results were obtained by haplotype analysis (not shown). In carriers of the c56G variant allele, the apoAV plasma/*APOA5* transcript ratio was three-fold higher than in wild-type subjects (*P*<0.0001). After adjustment for c56C>G, moderate associations of hepatic *APOA5* mRNA were observed with plasma apoAV levels and levels of NEFA (*P*<0.05), but not with glucose or insulin levels, HOMA-IR or CRP.

Multivariate regression analysis using a stepwise approach based on a priori considerations (see "Methods") revealed that the c56C>G SNP, plasma apoCIII and fatty acids, sex and hepatic *APOA5* transcript levels were independently associated with apoAV plasma levels (Table 5). Moreover, multivariate regression uncovered a weak relation of plasma apoAV with obesity status, which was not apparent in the comparison of mean age and sex adjusted plasma apoAV levels. Transcript levels of *CPT1A* and *PPARA*, plasma NEFA and the c56C>G SNP predicted *APOA5* transcript levels (Table 6). In both analyses, these variables remained significant after exclusion of subjects with type 2 diabetes and/or on lipid lowering medication.

Discussion

The main purpose of this study was to gain insight into the in vivo regulation of hepatic *APOA5* transcription and its influence on plasma apoAV levels. Previous studies identified sex, plasma TG and apoCIII, diabetes as well as the functional c56C>G SNP as predictors of apoAV plasma levels [24,38-42]. Our study confirms and extends these associations as plasma NEFA independently predicted both plasma apoAV and hepatic *APOA5* transcript levels. To our knowledge, this is the first report on hepatic *APOA5* as well as *APOA1* and *APOC3* expression in a large population. We also observed independent relations of hepatic *APOA5* mRNA with *PPARA* and *CPT1A* mRNA, suggesting associations of *APOA5*

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expression with fatty acid metabolism. Moreover, plasma apoAV was independently associated with hepatic *APOA5* mRNA.

Similar to previous studies [38,40], apoCIII was a much stronger predictor of plasma TG than apoAV. In our population with TG levels <7.0 mmol/L, we noted a positive correlation between the molar apoCIII/apoAV ratio and plasma TG supporting opposing roles of apoAV and apoCIII in the metabolism of TG-rich lipoproteins. Such an association was not detected in hypertriglyceridaemic patients [40]. Possible explanations relate to the higher prevalence of the functional *APOA5* variant and/other rare mutations that may confound the association between apoCIII and apoAV [47].

A link between sex and plasma apoAV, as observed in our study, has previously been reported by others [24,42]. Consistent with large epidemiologic studies [48], plasma TG, adjusted for obesity status, age and lipid lowering medication, were lower in women than in men (1.51±0.99 vs. 1.83±0.80 mmol/L; *P*=0.0203), while HDL cholesterol was higher (1.55±0.38 vs. 1.34±0.38 mmol/L, *P*=0.0042). Such a constellation points to a more effective clearance of TG-rich lipoproteins [49]. Thus, the relation of increased apoAV plasma level with high HDL cholesterol and low TG would be consistent with a role of apoAV in the catabolism of TG-rich lipoproteins.

Our data indicate that hepatic *APOA5* mRNA abundance is independently associated with plasma apoAV levels in humans. Even though hepatic *APOA5* mRNA explained only 3.1% of the total variance of plasma apoAV, the finding is of interest because the kinetics of plasma apoAV have not yet been studied in turnover experiments. In contrast, mRNA levels of *APOC3*, which is mainly expressed in the liver, showed no relationship to plasma apoCIII levels. *APOA5* transcript levels were two orders of magnitude lower than *APOA1* and *APOC3* transcript levels, but higher than transcript levels of *APOA4*, known to be mainly expressed in the intestine [50]. In plasma, the molar apoCIII/apoAV ratio was several-fold higher than the respective transcript ratio. Hence, compared to apoCIII, apoAV must be more rapidly cleared from the circulation and/or less efficiently produced or secreted by the liver. Sequestration of apoAV by endothelial proteoglycans [11,12] and/or binding to members of the LDL receptor family [14,15] may reduce the residence time of apoAV in the circulation. Partial retention of apoAV in the endoplasmic reticulum resulting in incomplete secretion has been demonstrated in COS cells transfected with *APOA5* expression constructs [51]. However, COS cells do not express MTP, required for VLDL biosynthesis. In human hepatocytes and hepatoma cells, up-regulation of *APOA5* expression by a thyroid hormone analogue resulted in increased apoAV levels in both cells and media [52].

The strong correlation of *APOA5* and *PPARA* mRNA suggests an important role of *PPARA* in the regulation of *APOA5* expression in vivo. This conclusion is consistent with transfection studies in human hepatoma cells [19,23] and with the observation that PPARα agonists increased serum apoAV in cynomolgus monkeys [53]. PPARα binds to and is activated by fatty acids and regulates numerous target genes involved in fatty acid oxidation. The correlation of *APOA5* with *CPT1A* and *ATGL* transcripts further supports the link of *APOA5* expression with hepatic lipid catabolism. In contrast, no correlations of *APOA5* mRNA with either *PCK1* or *FASN* transcripts, involved in gluconeogenesis or fatty acid synthesis, were observed.

Hepatic *APOA5* mRNA correlated with transcript levels of *USF1* and *HNF4*α, both of which have been implicated in the transcriptional activation of *APOA5* [20,21]. Insulin was reported to down-regulate *APOA5* by phosphorylation of USF1, thereby preventing its binding to a response element in the *APOA5* promoter. Insulin injection in mice decreased *Apoa5* mRNA and experimentally induced acute hyperinsulinaemia lowered the plasma

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level of apoAV in man [21]. However, in insulin resistant obese Zucker rats, liver *Apoa5* mRNA was not altered [54]. Similarly, we did not observe an association of plasma apoAV or hepatic *APOA5* mRNA with plasma insulin levels or HOMA-IR in our population. Previous studies suggested down-regulation of *APOA5* transcription by SREBP-1c through activation by LXRα [22]. Since SREBP-1c is also induced by insulin and stimulates hepatic de novo lipogenesis through activation of lipogenic genes [55], a negative association between *SREBF-1* and *APOA5* mRNA might have been expected. It is possible that such an association, if it does exist in human liver, was not detected, as hepatic lipogenesis is very low in the fasting state. Moreover, LXR-ligand availability might have played a role, and post-transcriptional regulation of SREBP-1c could not be ascertained because of the limited amounts of tissues available for this study.

Several studies reported associations of the c56G allele, which results in the substitution of Trp for Ser at residue 19 of the signal peptide and discriminates the *APOA5**3 from the *APOA5*1* haplotype, with increased apoAV and TG plasma levels [38,41,42]. These observations are difficult to reconcile with the TG-lowering effect of apoAV observed in animal studies. Furthermore, transfection studies of HepG2 and Huh7 cells with fusion protein constructs showed reduced functional activity of the variant signal peptide in comparison to the wild-type signal peptide [56]. Moreover, in mice engineered to express single *APOA5* haplotype copies, introduction of the *APOA5*3* defining c56G allele resulted in three-fold reduction of human apoAV plasma levels, while *APOA5* mRNA levels were not affected [57]. In contrast to these functional studies, but consistent with other human studies, we observed increased apoAV plasma levels in carriers of the c56G allele. In fact, the c56C>G SNP was the strongest predictor of apoAV plasma levels, explaining 37% of its variance. Since the *APOA5*-containing haplotype block does not extend into adjacent genes [35], the c56G allele must have caused the increase in plasma apoAV. Assuming the validity of the functional studies for the in vivo situation in humans and complete cleavage of the signal peptide, the association of apoAV with nascent hepatic lipoproteins may have been altered. As a result, apoAV-mediated targeting of TG-rich lipoproteins to endothelial proteoglycans or members of the LDL receptor family in the liver may have become less effective. On the other hand, an increased apoAV (and perhaps TG) secretion in c56G carriers can not be completely excluded, since the functional studies in mice and cell cultures may not have captured all aspects of apoAV secretion by the human liver. For instance, differences in the translocation of the Ser-19 and Trp-19 signal peptides, predicted by molecular modelling [56], might have influenced hydrophobic interactions of apoAV domains with the endoplasmic reticulum and/or lipid droplets destined for secretion. We have currently no explanation for the reduced *APOA5* mRNA level associated with c56G carrier status. As the c56C>G SNP is unlikely to alter a transcription factor binding site, feedback mechanisms resulting from apoAV-induced alteration in hepatic lipid processing might play a role.

We did not observe associations of the *APOA5*2* haplotype with plasma apoAV, apoCIII or their hepatic transcript levels. In Caucasian populations, the *APOA5*2* haplotype has been associated with three minor *APOC3* alleles that have been linked with plasma TG [58-62], even though *APOC3* and *APOA5* are located in distinct haplotype blocks. Thus, additional studies will be required to determine the effects of *APOC3* alleles on *APOC3* mRNA and apoCIII and TG plasma levels in the *APOA5**2 haplotype background.

In conclusion, our studies identified key determinants of plasma apoAV and hepatic *APOA5* mRNA levels in humans. Our data strongly support in vitro and animal experiments on the importance of PPARα for *APOA5* gene expression, suggest a link of *APOA5* expression with fatty acid metabolism and highlight complex effects of the c56C>G SNP on apoAV plasma levels.

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Supplementary Material

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Acknowledgments

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Abbreviations

Apo	apolipoprotein
ARP0	acidic ribosomal protein p0
NEFA	non-esterified fatty acids
HOMA-IR	homeostasis model assessment insulin resistance
TG	triglycerides

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TABLE 1

Characteristics of the Study Population

Trait	Controls	Obese Subjects	*P*
N (m/f)	12/10	29/60	n.s..
Age, years	46 (12)	42 (11)	n.s.
BMI, kg/m^2	26.6 (3.2)	43.8 (7.4)	n.d.
Fat mass, kg	20.3 (5.5)	56.4 (17.2)	n.d.
Visceral/subcutaneous fat	2.87 (2.02)	6.53 (2.82)	0.0001
Waist/Hip ratio	0.87 (0.09)	0.97 (0.10)	0.0001
Diabetes	2/20	14/75	n.s.
Lipid lowering medication *	1/21	3/86	n.s.
Glucose, mmol/L †	5.08 (0.36)	5.27 (0.57)	n.s.
Insulin, pmol/L †	39.7 (19.0)	102.8 (73.1)	0.0004
HOMA-IR †	1.50 (0.77)	4.28 (3.18)	0.0001
CRP, mg/L	3.9 (5.4)	9.3 (10.4)	0.0001
Cholesterol, mmol/L §	5.47 (0.96)	5.17 (0.91)	n.s.
Triglyceride, mmol/L §	1.33 (0.71)	1.73 (0.96)	0.0297
HDL cholesterol, mmol/L §	1.73 (0.44)	1.39 (0.36)	0.0005
ApoAI, mg/dL §	169 (19)	149 (29)	0.0088
ApoB, mg/dL §	82 (18)	82 (22)	n.s.
ApoCIII, mg/dL §	11.6 (4.4)	12.1 (4.6)	n.s.
ApoAV, μg/L §	400 (153)	402 (290)	n.s.

Data are numbers of observations or untransformed means (SD). Variables are adjusted for age and sex; Apo, apolipoprotein;

* only statins were used as lipid lowering medications;

† Subjects with type 2 diabetes mellitus excluded;

§ Subjects on lipid-lowering medication excluded.

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TABLE 2

APOA5 Polymorphisms and Apolipoprotein and Lipid Plasma Levels

Trait	−1131T>C			c56C>G		
	T/T (n=84)	T/C + CC (n=25)	*P*	CC (n=85)	C/G + GG (n=24)	*P*
ApoAV, μg/L	442 (281)	340 (231)	0.0336	318 (164)	755 (326)	0.0001
ApoCIII, mg/dL	11.6 (3.9)	12.5 (6.2)	n.s.	11.8 (3.8)	12.4 (3.4)	n.s.
ApoCIII/apoAV (mol/mol)	1568 (855)	2051 (1251)	0.0257	1957 (953)	865 (454)	0.0001
ApoAI, mg/dL	156 (25)	153 (36)	n.s.	155 (29)	155 (26)	n.s.
Triglyceride, mmol/L	1.51 (0.67)	1.87 (1.51)	n.s.	1.59 (0.97)	1.75 (0.77)	n.s.
HDL cholesterol, mmol/L	1.52 (0.38)	1.39 (0.38)	n.s.	1.50 (0.41)	1.42 (0.36)	n.s.

Adjusted for age, sex, obesity status and lipid lowering medication. One subject was homozygous for the C allele and one subject was homozygous for the G allele at SNPs −1131 and c56, respectively.

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TABLE 3

Associations of Hepatic *APOA5* and Transcription Factor mRNA Levels

Transcription factor	**Beta (SE)**	***P***
USF1	0.278 (0.104)	0.0095
HNF4A	0.367 (0.108)	0.0012
PPARA	0.322 (0.095)	0.0009
SREBF-1	0.084 (0.116)	n.s.
LXRA	0.112 (0.115)	n.s.
FOXO1	0.044 (0.118)	n.s.
CHREBP1	–0.019 (0.098)	n.s.

Adjusted for obesity status, sex, age, lipid lowering.

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TABLE 4

APOA5 SNPs and mRNA levels

Trait	−1131T>C			c56C>G		
	T/T (n=76)	**T/C + CC (n=21)**	***P***	**CC (n=76)**	**C/G + GG (n=21)**	***P***
APOA5 mRNA, A.U.*	1.04 (0.60)	0.91 (0.40)	n.s.	1.05 (0.56)	0.84 (0.54)	0.0162
APOC3 mRNA, A.U.	1.02 (0.54)	0.90 (0.42)	n.s.	0.96 (0.44)	1.11 (0.69)	n.s.
APOA1 mRNA, A.U.	1.06 (0.58)	0.91 (0.44)	n.s.	0.97 (0.55)	1.12 (0.60)	n.s.
APOA4 mRNA, A.U.	1.20 (2.02)	0.61 (0.64)	n.s.	1.00 (2.01)	1.06 (0.89)	n.s.

Adjusted for age, sex, obesity status and lipid lowering medication. One subject was homozygous for the C allele and one subject was homozygous for the G allele at SNPs −1131 and c56, respectively;

*arbitrary units.

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TABLE 5

Predictors of ApoAV Plasma Levels

Trait	Beta (SE)	*P*
Obesity status	−0.152 (0.074)	0.0438.
Age	−0.075 (0.077)	n.s.
Sex	0.197 (0.072)	0.0075
Lipid lowering medication	−0.008 (0.072)	n.s.
ApoCIII	0.328 (0.070)	0.0001
NEFA	0.224 (0.077)	0.0043
c56C>G	0.629 (0.069)	0.0001
APOA5 mRNA	0.199 (0.074)	0.0086

N=98, R^2= 0.6078

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TABLE 6

Predictors of Hepatic *APOA5* mRNA Levels

Trait	Beta (SE)	*P*
Obesity status	0.008 (0.092)	n.s.
Age	−0.024 (0.084)	n.s.
Sex	0.147 (0.083)	0.0827
Lipid lowering medication	0.111 (0.084)	n.s.
NEFA	0.273 (0.090)	0.0032
CPT1A1 mRNA	0.363 (0.086)	0.0001
PPARA mRNA	0.272 (0.082)	0.0014
c56C>G	−0.241 (0.080)	0.0032

N=91, R^2 =0.4864.