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Apolipoprotein AV does not contribute to hypertriglyceridaemia or triglyceride lowering by dietary fish oil and rosiglitazone in obese Zucker rats

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Abstract *Aims/hypothesis:* Apolipoprotein AV (apoAV) is a recently discovered apolipoprotein with a triglyceride-lowering effect in genetically modified mice. Transcription of the human gene encoding apoAV (*APOA5*) is suppressed by insulin and stimulated by fibrates. Our goal was to study the expression of *Apoa5*, in comparison with *Apoa4* and *Apoc3,* in hypertriglyceridaemic, obese and insulin-resistant Zucker rats receiving the insulin sensitiser rosiglitazone and/or a fish oil diet to lower triglycerides. *Methods:* Hepatic *Apoa5, Apoa4* and *Apo3* mRNA and liver and plasma apoAV were measured in lean and obese Zucker rats receiving rosiglitazone while on a coconut oil or fish oil diet. *Results:* Basal hepatic *Apoa5* expression was similar in obese and lean Zucker rats. Unexpectedly, obese Zucker rats tended to have higher plasma apoAV levels despite their hypertriglyceridaemic state. Both rosiglitazone and the fish oil diet significantly increased *Apoa5* mRNA, by about 70%, but tended to lower liver and plasma apoAV. Rosiglitazone had no effect on *Apoa5* mRNA in cultured rat hepatocytes. No intact PPAR (peroxisome proliferator-activated receptor) response element was identified in the rat *Apoa5* promoter. *Conclusions/interpretation:* Our data indicate that apoAV does not contribute to the hypertriglyceridaemia of obese Zucker rats or to the hypolipidaemic effect of rosiglitazone or a fish oil diet. The divergent changes of *Apoa5* mRNA and apoAV levels suggest co- or post-translational regulation. The increase in *Apoa5* mRNA induced by rosiglitazone is not directly mediated by peroxisome proliferator-activated receptor γ.

Keywords Apolipoprotein AV · Apolipoprotein A-IV · Apolipoprotein C-III · Rosiglitazone · Fish oil diet · Zucker rats · Hypertriglyceridaemia

Abbreviations apo: apolipoprotein · PPAR: peroxisome proliferator-activated receptor · PPRE: PPAR response element · PUFA: polyunsaturated fatty acids

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Introduction

Apolipoprotein AV (apoAV), a recently discovered member of the apolipoprotein family, is considered to play an important role in plasma triglyceride transport [1, 2]. The gene for apoAV (*APOA5*) is part of the *APOA1*/*A4*/*C3*/*A5* gene cluster on chromosome 11 in man, and *APOA5* is predominantly expressed in the liver. Human *APOA5* polymorphisms are associated with elevated plasma triglyceride levels [2–4]. Studies in genetically modified mice and adenovirus overexpression experiments revealed a strong triglyceride-lowering effect of apoAV [2, 5–7]. This effect involves the stimulation of lipoprotein lipase activity and possibly inhibition of hepatic VLDL-triglyceride secretion [5–8]. ApoAV is considered to have effects opposite to those of apoC-III in triglyceride metabolism, as shown by studies in genetically modified mice (reviewed in

[9]). Agonists of peroxisome proliferator-activated receptor α (PPARα) stimulate the transcription of human *APOA5* and increase serum apoAV levels in cynomolgus monkeys, suggesting a contribution of apoAV to the hypotriglyceridaemic effect of these drugs [10–12]. Human *APOA5* transcription is repressed by liver X receptor agonists and insulin [13, 14]. ApoA-IV, the apolipoprotein most closely related to apoAV [1, 2], participates in reverse cholesterol transport as an acceptor of cellular cholesterol and activator of LCAT (lecithin:cholesterol acyltransferase) [15, 16]. ApoA-IV exerts a strong anti-atherogenic effect [17] and has also been suggested to act as a satiety factor. Plasma apoA-IV is elevated in human type 2 diabetes and obesity and decreases during weight reduction [18, 19].

fa/fa Zucker rats develop severe obesity due to the missense mutation fatty (Glu269Pro) in the extracellular domain of the leptin receptor. Obese Zucker rats are widely used as a model for the hypertriglyceridaemia and insulin resistance associated with human obesity [20]. The hypertriglyceridaemia of obese Zucker rats results from increased hepatic VLDL production [21]. Although subject to controversy [6, 8], this step in lipoprotein metabolism has been shown to be influenced by apoAV [5]. The severe insulin resistance in obese Zucker rats can be alleviated by rosiglitazone and other PPARγ agonists, which are used to treat human type 2 diabetes [22]. The resulting improvement in insulin sensitivity is associated with a marked decrease in plasma triglycerides. In addition to pharmacological interventions, the increased plasma triglycerides in obese Zucker rats can be reduced by fish oil diets rich in ω-3 polyunsaturated fatty acids (PUFA) [23–25]. These diets reduce hepatic lipogenesis and VLDL synthesis by incompletely understood mechanisms and increase fatty acid oxidation via activation of PPARα (reviewed in [26]). Fish oil diets markedly reduce the transcription of *Apoa1* and *Apoa4* in rat liver without affecting *Apoc3* mRNA abundance [24, 25, 27]. It is unknown whether the expression of *Apoa5* is altered by dietary ω-3 PUFA and whether apoAV may contribute to their triglyceride-lowering effect.

In the liver of obese Zucker rats and other rat models of obesity, *Apoa4* mRNA is increased [24, 25, 28, 29], whereas *Apoc3* mRNA levels are similar in lean and obese Zucker rats [25]. Dose-dependent inhibition of apoA-IV production in rat hepatocyte cultures by insulin has been described [30], suggesting that the overexpression of *Apoa4* in the liver of obese rats may be related to insulin resistance. Rosiglitazone does not alter hepatic *Apoa4* or *Apoc3* expression in lean rats [31], but its effect on the increased *Apoa4* mRNA in the liver of obese rats is not known.

The present study was designed to (1) determine the basal expression of *Apoa5* in hypertriglyceridaemic, insulin-resistant obese Zucker rats; (2) determine whether *Apoa5* expression in these animals is altered when insulin resistance is improved and/or triglycerides are lowered by rosiglitazone or fish oil diet; and (3) study the effect of rosiglitazone on the increased expression of *Apoa4* in the liver of obese Zucker rats.

Materials and methods

Experimental animals

Male (*Fa*/−) lean and (*fa*/*fa*) obese Zucker rats, 8–10 weeks of age, were purchased from Harlan Winkelmann (Borchen, Germany) and housed in a room with a 12-h light 12-h dark cycle (lights on from 06.00 to 18.00 h). Animal experiments, performed in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 52-23 revised 1985) and with the Austrian Law on the Protection of Animals, were approved by the Animal Research Committee of the Medical University of Vienna.

Experimental diets and rosiglitazone treatment

The control diet (High Saturated Fat Diet; ICN, Cleveland, OH, USA) contained 20% (wt/wt) coconut oil (main fatty acids, C12:0, lauric, 45.7%; C14:0, myristic, 17.3%; C16:0, palmitic, 8.8%; C18:0, stearic, 12.0%). The fish oil diet (High Menhaden Oil Diet; ICN) contained 20% (wt/wt) Menhaden oil (main fatty acids, C16:0, palmitic, 15.2%; C16:1, palmitoleic, 11.6%; C18:1, oleic, 9.5%; C18:2, linoleic: 1.8%, C18:3, linolenic, 1.8%, C20:4, arachidonic, 2.3%, C20:5, eicosapentaenoic, 16.0%, docosahexaenoic, C22:6, 10.8%). The fish oil diet was stored in aliquots under nitrogen at −80°C to avoid autooxidation. Rats received fresh aliquots daily. Rosiglitazone tablets (Avandia; GlaxoSmithKline, Uxbridge, UK) were pulverised and admixed to the diet at a concentration of 0.003% wt/wt, resulting in a rosiglitazone intake in the range of 3–4 mg kg^{-1} day^{-1}.

Experimental design

To study the effects of dietary ω-3 PUFA, rosiglitazone and a combination of them, lean and obese Zucker rats were fed a control diet or fish oil diet ad libitum and/or received rosiglitazone as indicated in Table 1. Food was removed at 07.00 h. Animals were killed 2–5 h later by sodium pentobarbital injection (5 mg/100 g i.p.), and liver and blood samples were collected.

Tissue culture

Primary rat hepatocyte cultures were prepared from adult male Sprague–Dawley rats using the collagenase perfusion technique [32]. Cells were plated at a density of 5×10^6 cells/30 mm dish on a fibronectin matrix in DMEM, 4 mmol/l glutamine, 10 mmol/l HEPES, pH 7.6, 10% FCS, and

Table 1 Experimental design

Phenotype	*n*	Week 1	Week 2	Week 3
Lean	5	Control diet →		
Obese	6	Control diet →		
Lean	5	Control diet + rosiglitazone →		
Obese	6	Control diet + rosiglitazone →		
Lean	5	Control diet →		Fish oil diet →
Obese	6	Control diet →		Fish oil diet →
Lean	5	Control diet + rosiglitazone →		Fish oil diet + rosiglitazone
Obese	6	Control diet + rosiglitazone →		Fish oil diet + rosiglitazone

incubated with the same medium containing rosiglitazone or fenofibrate dissolved in DMSO (final concentration 0.2%) at the concentrations indicated for 24 h.

Laboratory methods

Plasma cholesterol and triglycerides were measured by enzymatic methods. HDL cholesterol was determined using polyanion precipitation. Plasma NEFA were quantitated by an enzymatic assay (Wako Chemicals, Neuss, Germany). Rat insulin was determined by RIA (Sensitive Rat Insulin RIA Kit; Linco Research, St Charles, MO, USA). RNA was isolated using the RNeasy Midi Kit (Qiagen, Hilden, Germany).

Northern and slot blotting

Total RNA (10 µg) was separated on 1% agarose-formaldehyde gels. Northern blotting and hybridisation to full-length rat *Apoa4*, *Apoa5*, *Apoc3* and *Acox1* cDNA probes [1, 33] or to a rat 28S rRNA oligonucleotide (5′-AAT CCT GCT CAG TAC GAG AGG AAC CGC AGG 3′) labelled with ^{32}P were carried out as described [25]. *Apoa4, Apoa5*, and *Apoc3* mRNA abundance was determined by quantitative slot blotting [34] using 1, 2 and 4 µg of RNA/sample and was standardised to 28S rRNA.

The sequence of rat *Apoa5* was retrieved from the *Rattus norvegicus* chromosome 8 BAC CH230-416D7 (GenBank accession number AC135409). The promoter regions of human *APOA5* [11] and rat *Apoa5* were aligned using the AlignX program of the Vector NTI 9.1 Advance package (Invitrogen, Carlsbad, CA, USA).

Western blotting

Recently, ELISA methods have been developed for determination of apoAV levels in human plasma ([35–37] and F. G. Schaap et al., unpublished results); Western blotting [1] is the only technique currently available for measuring apoAV in rats. Cross-reactivity of antibodies employed in our own ELISA with rat apoAV was insufficient to allow quantification of apoAV in rat samples. Therefore, rat apoAV was assessed semiquantitatively by immunoblot analysis. Minced liver tissue was homogenised in PBS containing Protease Inhibitor cocktail (Roche, Mannheim, Germany) using a Potter–Elvehjem homogeniser. For apoAV detection, 5 µl pooled serum or 250 µg pooled total liver protein was separated on 1.5-mm thick 10% polyacrylamide gels under denaturing and reducing conditions. Immunodetection of apoAV was performed as described previously [1]. Chemiluminescent signals were captured using a LumiImager-F1 workstation (Roche) and quantified with LumiAnalyst software (Roche). Immunoblot analysis of apoAV in pooled plasma samples and liver homogenates was performed at least in triplicate. To confirm the results, samples from lean controls and all groups of obese rats were also analysed individually.

Statistical analysis

All results are presented as mean±SD. The effects of obesity, rosiglitazone treatment and fish oil diet and their interactions were analysed by three-way ANOVA, followed by post hoc comparisons by Newman–Keul tests using the Statistica program package (Statsoft, Tulsa, OK, USA); p values <0.05 were considered significant.

Results

Basal expression of *Apoa5*, *Apoa4* and *Apoc3*

Plasma triglycerides were increased almost two-fold in obese Zucker rats when compared with lean littermates (Table 2, Fig. 2), but basal hepatic *Apoa5* mRNA abundance was not altered, as determined by Northern

Table 2 Effects of fish oil diet and rosiglitazone on weight gain, plasma lipids, insulin and NEFA in lean and obese Zucker rats

Rosiglitazone	Obese Zucker rats (6 rats/group)				Lean Zucker rats (5 rats/group)			
	Without rosiglitazone		With rosiglitazone		Without rosiglitazone		With rosiglitazone	
	Control diet	Fish oil diet	Control diet	Fish oil diet	Control diet	Fish oil diet	Control diet	Fish oil diet
Body weight (g)	518±5[c]	516±19[c]	552±11[c]	542±47[c]	370±28	363±13	372±24	384±47
Weight gain (g/day)	4.8±0.6	6.5±1.3	7.4±0.8[c,f]	7.7±2[b,f]	3.9±3.2	4.2±1.4	4.2±1.1	4.3±0.6
Triglycerides (mmol/l)	4.99±2.41[a]	1.82±0.56[f]	2.25±0.59[f]	0.90±0.14[f]	2.69±0.51	0.42±0.07[c]	1.12±0.41	0.46±0.12[c]
Cholesterol (mmol/l)	5.19±0.98[a]	4.08±0.8[f]	4.44±1.09	3.98±0.28[f]	2.30±0.21	1.73±0.13	2.17±0.18	2.02 ±0.39
HDL cholesterol (mmol/l)	1.76±0.88	2.17±0.28[c]	2.28±0.10[c]	2.09±4 [c]	1.27±0.08	1.06±0.05	1.29±0.13	1.24±0.13
Insulin (mU/l)	630±212[a]	743±331[a]	288±100[d]	208±44[d]	152±57	98±15	72±17	75±16
NEFA (μmol/l)	814±202[a]	521±122[e]	372±141[e]	207±18[e]	389±95	205±37[c]	165±31[b]	156±28[b]

Significant differences from lean control: [a]$p<0.001$; [b]$p<0.01$; [c]$p<0.05$
Significant differences from obese control: [d]$p<0.001$, [e]$p<0.01$; [f]$p<0.05$

blotting (Fig. 1) and quantitative slot blotting (Fig. 2). Liver apoAV protein content was unaltered. Plasma apoAV levels appeared increased on Western blots of pooled samples (Figs. 1 and 2). Using samples from individual rats, an increase of 56% was observed but this was not statistically significant (p=0.083, data not shown). As in earlier experiments [24, 25], basal hepatic *Apoa4* mRNA abundance was significantly higher in obese Zucker rats compared with lean controls, while *Apoc3* mRNA was unchanged (Figs. 1, 3).

Effects of rosiglitazone

Rosiglitazone significantly reduced plasma triglycerides in obese Zucker rats and induced an increase of about 70% in hepatic *Apoa5* mRNA in livers of lean and obese Zucker rats (Figs. 1 and 2, Table 2). Liver and plasma apoAV protein levels, however, tended to decrease, as judged by Western blot analysis of pooled samples (Figs. 1 and 2). Similar changes were observed using samples of individual obese rats, but only the decline of plasma apoAV to 33±17% of control in obese rats receiving rosiglitazone+fish oil diet was significant (p=0.038, data not shown). The ratio of plasma apoAV:*Apoa5* mRNA decreased significantly from 1.00±0.41 in obese controls to 0.42±0.29 (p=0.03) in obese rats treated with rosiglitazone. The ratio of liver apoAV:*Apoa5* mRNA decreased to 0.62±0.48 (not significant). Rosiglitazone markedly improved insulin sensitivity in obese Zucker rats, as shown by major decreases in plasma insulin and NEFA (Table 2), but had no significant effects on plasma total cholesterol and HDL cholesterol in obese Zucker rats (Table 2).

To determine whether the increase in *Apoa5* mRNA was due to a direct effect of rosiglitazone on liver cells, primary rat hepatocytes were incubated with 2 or 4 μmol/l rosiglitazone (Fig. 4). Incubation with fenofibrate, which has been reported to enhance *APOA5* transcription and mRNA levels several-fold in human hepatocytes [10, 11],

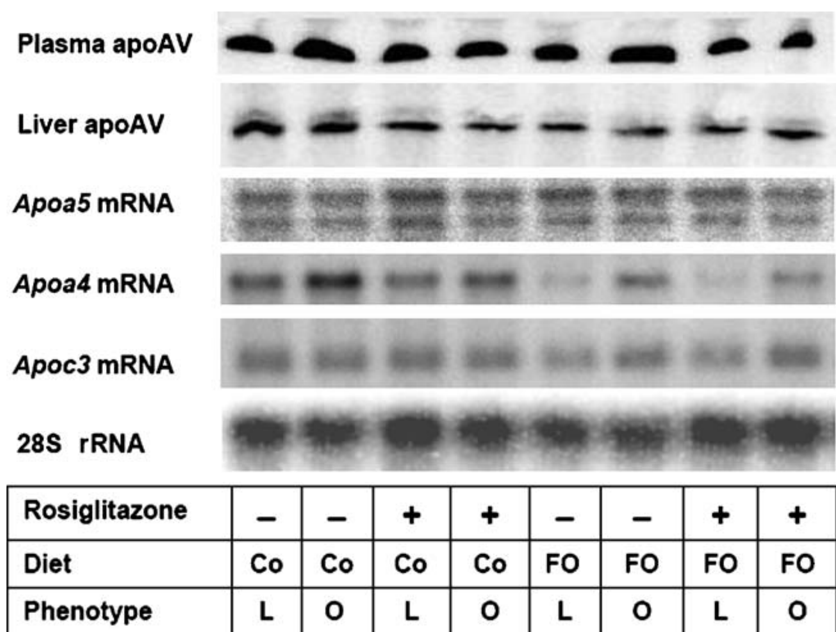

Fig. 1 Effects of rosiglitazone and fish oil diet on plasma and liver apoAV and on hepatic *Apoa5*, *Apoa4* and *Apoc3* mRNA in lean and obese Zucker rats. Pooled plasma and liver homogenate samples were analysed by Western blotting. Northern blots of pooled liver RNA were hybridised with rat *Apoa5*, *Apoa4* and *Apoc3* cDNA, and with a rat 28S rRNA oligonucleotide (five or six animals per group). *Co* Control diet, *FO* fish oil diet, *L* lean Zucker rats, *O* obese Zucker rats

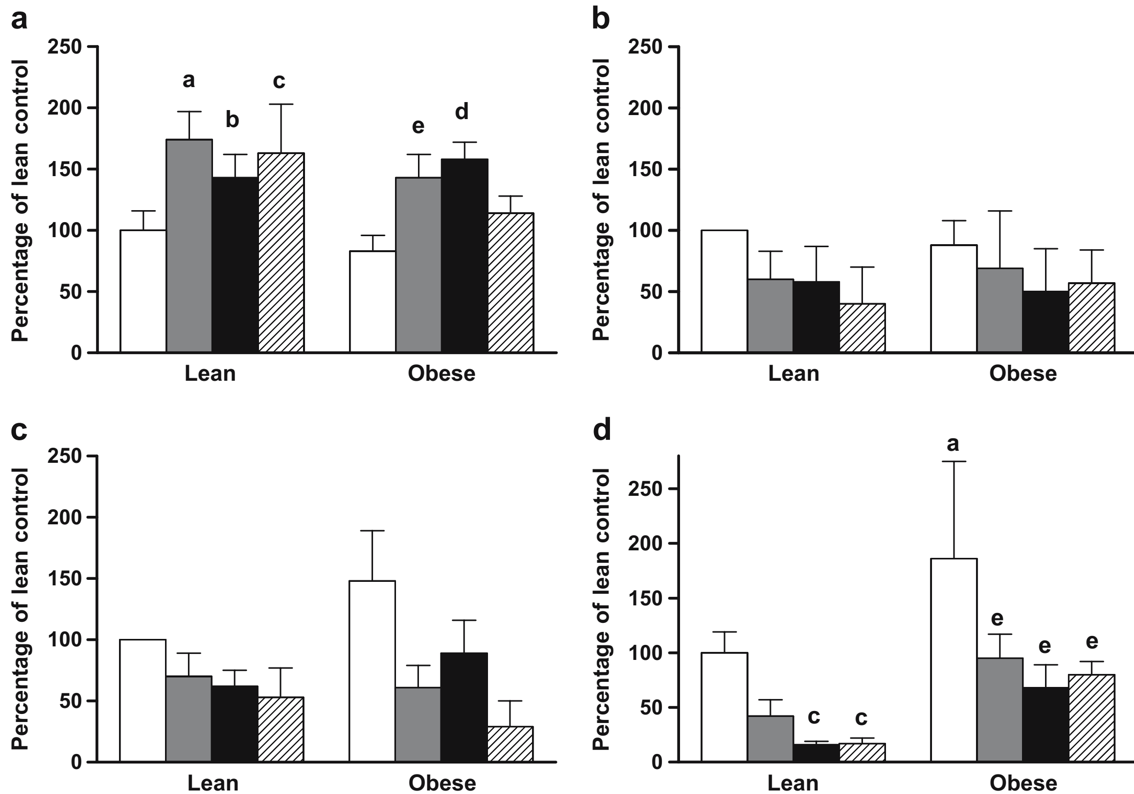

Fig. 2 Effect of rosiglitazone and fish oil diet on (**a**) hepatic *Apoa5* mRNA, (**b**) liver apoAV protein, (**c**) plasma apoAV protein, and (**d**) plasma triglycerides in lean and obese Zucker rats. Lean or obese Zucker rats (five or six rats per group) received the control diet (*white bars*), control diet+rosiglitazone (*grey bars*), fish oil diet (*black bars*) or rosiglitazone+fish oil diet (*hatched bars*) as indicated in Table 1. Liver *Apoa5* mRNA was quantitated in triplicate in individual samples from five or six rats per group by slot blotting standardised to rat 28S rRNA. *Apoa5* mRNA and plasma triglyceride levels were analysed by three-way ANOVA and Newman–Keul tests and are given as mean±SD. Significant differences from lean control: $^{a}p<0.001$; $^{b}p<0.01$; $^{c}p<0.05$; significant differences from obese control: $^{d}p<0.01$; $^{e}p<0.05$. Liver and plasma apoAV protein were determined in pooled samples from each group by Western blotting (three or four independent analyses). Data are means±SD

was used as a control. Like human hepatocytes [10, 11], primary rat hepatocytes expressed little *Apoa5* mRNA compared with liver tissue. Rosiglitazone had no effect on *Apoa5* mRNA levels, suggesting that the upregulation of *Apoa5* mRNA observed in livers of rats receiving the drug occurred via indirect mechanisms. Unlike in human hepatocytes, in rat hepatocytes fenofibrate failed to increase *Apoa5* mRNA, whereas induction of the PPARα marker gene *Acox1* was seen at a fenofibrate concentration of 100 μmol/l, as described previously [38]. This result suggested that rat *Apoa5* may be insensitive to stimulation by PPAR agonists, and prompted us to examine the rat

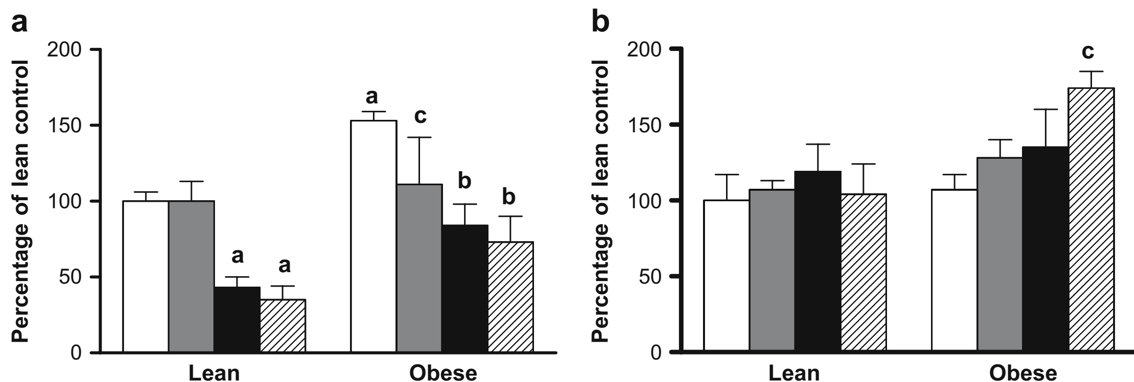

Fig. 3 Effects of rosiglitazone and fish oil diet on **a** hepatic *Apoa4* and **b** *Apoc3* mRNA in lean and obese Zucker rats. Lean or obese Zucker rats (five or six per group) received the control diet (*white bars*), control diet+rosiglitazone (*grey bars*), fish oil diet (*black bars*) or rosiglitazone+fish oil diet (*hatched bars*) as indicated in Table 1. Liver *Apoa4* and *Apoc3* mRNA was quantitated in triplicate by slot blotting standardised to rat 28S rRNA in individual samples from five or six rats per group. Data are means±SD. Significant differences from lean control: $^{a}p<0.001$; significant differences from obese control: $^{b}p<0.001$; $^{b}p<0.01$ (three-way ANOVA, Newman–Keul tests)

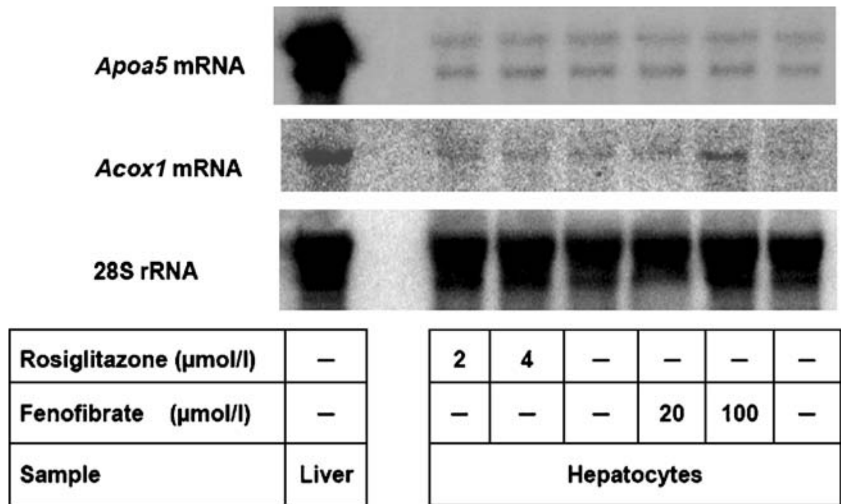

Fig. 4 Effects of rosiglitazone and fenofibrate on *Apoa5* mRNA in rat primary hepatocyte cultures. Hepatocyte cultures were incubated with rosiglitazone or fenofibrate for 24 h. Total RNA was analysed by Northern blotting (10 µg/lane) using rat *Apoa5* or *Acox1* cDNA and a 28S rRNA. Rat liver RNA served as a control (*first lane*)

Apoa5 promoter for the presence of PPAR response elements (PPREs). Alignment of the rat *Apoa5* promoter region with its human homologue revealed that the functional DR1 response element for PPAR contained in the human *APOA5* promoter at position −257 to −271 harbours an insertion and is not fully conserved in the rat *Apoa5* promoter (Fig. 5). No intact DR1 element was found within 1,000 nucleotides upstream of the transcription site of rat *Apoa5*. By contrast, the E-box element mediating the effect of insulin on the human *APOA5* promoter [13] is conserved in the rat gene.

Regulation of hepatic *Apoa4* expression by rosiglitazone differed clearly from that of *Apoa5* (Figs. 1 and 3). Rosiglitazone normalised the increased basal *Apoa4* mRNA levels in obese Zucker rats, but had no effect in lean rats. Hepatic *Apoc3* mRNA levels were similar in lean and obese Zucker rats and were not affected by rosiglitazone administration in animals receiving the control diet.

Effects of fish oil diet

The fish oil diet decreased plasma triglycerides and significantly increased hepatic *Apoa5* mRNA in both lean and obese Zucker rats (Figs. 1 and 2, Table 2). Again, changes in liver and plasma apoAV protein concentrations did not follow changes in mRNA levels: plasma and liver apoAV in lean and obese Zucker rats (Figs. 1 and 2) and the ratios of plasma apoAV:*Apoa5* mRNA and liver apoAV:*Apoa5* mRNA tended to decrease in obese rats (data not shown).

Apoa4 mRNA was significantly decreased, by about 50%, in lean and obese Zucker rats receiving fish oil (Figs. 1 and 3). The fish oil diet did not alter *Apoc3* mRNA significantly, as described previously (Figs. 1 and 3) [25]. Rosiglitazone treatment did not alter the response of hepatic *Apoa4* and *Apoa5* mRNA to the fish oil diet. Obese

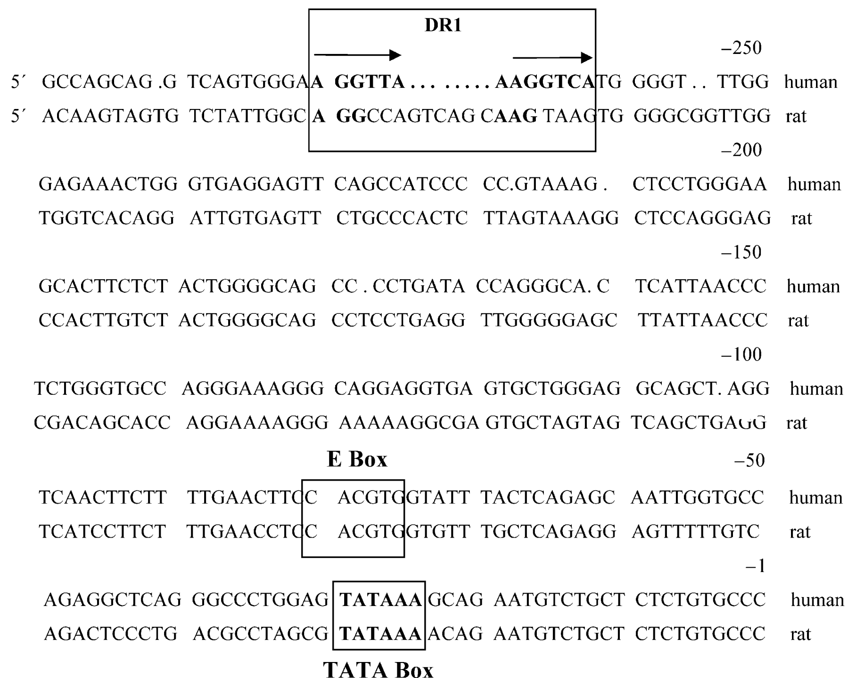

Fig. 5 Alignment of the promoter region of rat *Apoa5* and human *APOA5*. Relevant binding elements are boxed. Numbering refers to the position relative to the transcription start site

Zucker rats on rosiglitazone and the fish oil diet had higher *Apoc3* mRNA levels than all other groups (Figs. 1 and 3).

Discussion

Basal expression of *Apoa5*, *Apoa4* and *Apoc3*

Our study shows that the basal hepatic expression of *Apoa5* is not altered in hypertriglyceridaemic obese Zucker rats, whereas plasma apoAV may be moderately elevated. Obese Zucker rats develop hypertriglyceridaemia as a result of overproduction of triglyceride-rich VLDL enriched in apoE, apoC-III and apoA-IV by the liver [21, 29]. ApoAV may be secreted in association with VLDL, thus accounting for the elevated plasma apoAV levels in obese Zucker rats overproducing VLDL. From a report that apoAV can lower the secretion rate of VLDL triglycerides [5], it appeared conceivable that reduced expression of *Apoa5* in obese Zucker rats could contribute to the enhanced VLDL production. Our findings, however, suggest that apoAV is not involved in the pathogenesis of the secondary hypertriglyceridaemia associated with obesity and insulin resistance in these rats. In humans with hypertriglyceridaemia or familial combined hyperlipidaemia, polymorphisms in *APOA5* show a strong association with plasma triglyceride levels across several ethnic groups [2–4, 39]. ApoAV deficiency leads to severe hypertriglyceridaemia [40, 41]. Recent measurements of plasma apoAV in normolipaemic subjects revealed a weak negative correlation [35, 36] or a tendency towards a positive correlation of plasma apoAV and triglycerides (F. G. Schaap et al., unpublished results). On the other hand, in hypertriglyceridaemic subjects a significant positive correlation between plasma apoAV and triglyceride levels was observed (F. G. Schaap et al., unpublished results). Hypertriglyceridaemic patients with type 2 diabetes were reported to have fasting plasma apoAV levels similar to those of controls [37]. During postprandial hypertriglyceridaemia, plasma apoAV increased in these patients and remained unchanged in controls. These data and our findings in a naturally occurring animal model of hypertriglyceridaemia indicate that the relation of apoAV to plasma triglyceride levels is more complex than anticipated from the experiments in genetically modified mice.

Recently, transcriptional suppression of the human *APOA5* and the mouse *Apoa5* gene by insulin was reported, and insulin was shown to reduce plasma apoAV in euglycaemic clamp studies [13]. The E-box element mediating the effect of insulin on human *APOA5* transcription via upstream stimulating factors is conserved in the rat gene. The liver of obese Zucker rats is severely insulin-resistant [42]. Thus, one might expect an increase in *Apoa5* mRNA and plasma apoAV in obese compared with lean Zucker rats. Our results of unaltered *Apoa5* mRNA and liver protein in obese Zucker rats suggest that the effect of insulin on *Apoa5* mRNA may be blunted by other endocrine or metabolic changes in these animals.

In contrast to *Apoa5*, basal hepatic *Apoa4* mRNA abundance was significantly higher in obese Zucker rats than in lean controls, as in earlier experiments [24, 25]. The mechanism of this increase, which is also seen in Wistar fatty rats and in rats made obese by a ventromedial lesion of the hypothalamus, is post-transcriptional and probably occurs at an early step of mRNA maturation [24, 25, 28]. The unchanged *Apoc3* mRNA abundance in the liver of obese Zucker rats confirms the results of earlier studies [24, 25].

Effects of rosiglitazone

Administration of rosiglitazone increased hepatic *Apoa5* mRNA by about 70% but tended to decrease liver and plasma apoAV protein levels. Thus, neither liver nor plasma apoAV increased, whereas plasma triglyceride levels were lowered by more than 50%. Therefore, apoAV does not appear to contribute to the marked decrease in plasma triglycerides induced by rosiglitazone in obese Zucker rats. In line with our observations are human data showing that pioglitazone significantly lowered plasma triglycerides in patients with type 2 diabetes, while plasma apoAV levels tended to decrease rather than to increase [43]. In transgenic mice overexpressing human *APOA5*, lipolysis of triglyceride-rich lipoproteins is enhanced and apoB and apoC-III are removed from plasma together with triglyceride-rich lipoprotein particles [6]. If apoAV is also cleared from the circulation during lipolysis, this may account for the discrepancy between plasma triglyceride levels and plasma apoAV during rosiglitazone treatment.

The upregulation of hepatic *Apoa5* mRNA by rosiglitazone is not mediated by a direct interaction of PPARγ with *Apoa5* because, in contrast to human *APOA5*, no intact PPRE was found in the rat gene. Thus, the regulation of *APOA5* by PPARα appears to be species-specific, like the regulation by retinoic acid receptor α-related orphan receptor (ROR-α) [44]. Despite the apparent lack of a PPRE in the rat *Apoa5* promoter, fenofibrate effectively lowers triglycerides in Sprague–Dawley rats and in obese Zucker rats [22, 31]. This casts some doubt on the postulated importance of direct transactivation of *APOA5* transcription by PPARα for the hypolipidaemic effect of fenofibrate [10, 11].

The upregulation of *Apoa5* mRNA by rosiglitazone appears to require signalling from other cells as it could not be reproduced in isolated hepatocytes. It cannot be explained by the improvement of insulin sensitivity or by anti-inflammatory effects of rosiglitazone, either of which should lead to a decrease in *Apoa5* mRNA [13, 45]. Thiazolidinediones can alter lipid metabolism via molecules released from adipocytes, such as leptin, TNF-α, resistin, adiponectin and NEFA [46]. Although there is no information on the regulation of apoAV by adipokines, our results show that *Apoa5* is subject to regulation by fatty acids. Thus, the marked changes in plasma NEFA levels during rosiglitazone treatment may have contributed to the upregulation of *ApoA5* mRNA.

Whereas rosiglitazone significantly induced hepatic *Apoa5* mRNA abundance, the apoAV protein levels in liver and

plasma failed to increase. This suggests that, in addition to its well-documented transcriptional regulation, *Apoa5* may be subject to regulation at a co- or post-translational level. Tight control of apoAV production at or after translation is also suggested by the contrast of the relatively high abundance of *Apoa5* mRNA in rat liver, resulting in hybridisation signals on Northern blots approaching the strength of *Apoa4* signals in spite of very low hepatic apoAV protein levels. Translational regulation has been shown for *Apoa1*, *Apob* and *Apoe* [47–49]. For apoB, co- and post-translational degradation is the main determinant of gene expression and has been studied extensively (reviewed in [49]).

In our study rosiglitazone reduced the increased *Apoa4* mRNA abundance found in obese Zucker rats to a level indistinguishable from that in lean controls. Thus, the enhanced expression of *Apoa4* in the liver of several rat models of obesity [24, 25] may be a consequence of insulin resistance. The absence of an effect of rosiglitazone on hepatic *Apoc3* mRNA in lean and obese rats and on *Apoa4* mRNA in lean rats is confirmed by earlier studies [22, 28, 31].

Effects of fish oil diet

The effects of the triglyceride-lowering fish oil diet on *Apoa5* expression in Zucker rats resembled the effects induced by rosiglitazone: the abundance of hepatic *Apoa5* mRNA increased, whereas liver and plasma apoAV failed to increase. This argues against the involvement of apoAV in the lipid-lowering effect of ω-3 PUFA. Similarly, liver apoAV failed to increase when Sprague–Dawley rats were fed a single meal per day of a fish oil diet for 4 days, while plasma triglycerides had declined more than two-fold already after the first meal (B. Hagerty and W. Strobl, unpublished results). The mechanisms underlying the hypolipidaemic actions of ω-3 PUFA are incompletely understood at present. ω-3 PUFA decrease the concentration and nuclear translocation of sterol regulatory element binding protein 1 (SREBP 1) (reviewed in [26]). As SREBP 1 represses *APOA5* transcription [14], this may result in an increase in *Apoa5* mRNA. The apparently divergent regulation of liver *Apoa5* mRNA and protein by dietary ω-3 PUFA provides a second example pointing to the possibility that co- or post-translational regulation of *Apoa5* may occur. ω-3 PUFA are known to reduce hepatic apoB production by stimulating the post-endoplasmic reticulum presecretory pathway of apoB degradation [50].

In contrast to our earlier finding that the increased *Apoa4* mRNA abundance in the liver of obese Zucker and Wistar rats was insensitive to transcriptional downregulation by dietary fish oil [24, 25], such a diet did reduce liver *Apoa4* mRNA abundance in both lean and obese Zucker rats in the present study. In our earlier experiments, rat chow rather than a high-fat coconut oil diet served as a control, which may account for this difference.

In conclusion, this study shows that, at least in Zucker rats, neither the basal expression of *Apoa5* nor changes in *Apoa5* expression induced by a hypolipidaemic drug or a lipid-lowering diet are necessarily inversely related to plasma triglyceride levels. Moreover, *Apoa5* appears to be regulated not solely at the transcriptional level, but also during later stages of gene expression. This may be of importance for attempts to design lipid-lowering strategies exploiting the triglyceride-lowering effects of apoAV.

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