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# Apolipoprotein E*3-Leiden Transgenic Mice as a Test Model for Hypolipidaemic Drugs

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## Summary

Apolipoprotein (APO) E*3-Leiden mice with impaired chylomicron and VLDL (very low density lipoprotein) remnant metabolism display hyperlipidaemia and atherosclerosis. In the present study, these mice were used for testing the hypolipidaemic effect of two marketed agents, lovastatin (CAS 75330-75-5) and gemfibrozil (CAS 25812-30-0) as well as a novel compound, SB 204990 (the 5-ring lactone of ±(3R*,5S*) 3-carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic acid, CAS 154566-12-8), a potent inhibitor of cholesterol and fatty acid synthesis at the level of ATP-citrate lyase. APOE*3-Leiden mice were fed a saturated fat and cholesterol-rich diet supplemented with either 0.05 or 0.1 % w/w of lovastatin, 0.1 or 0.2 % w/w of gemfibrozil or 0.1 or 0.2 % w/w of SB 204990. Lovastatin showed a dose-related decrease in plasma cholesterol levels (up to −20 %) due to a lowering of LDL and HDL (low density resp. high density lipoprotein)-cholesterol (−20 and −18 %, respectively), while plasma triglyceride levels were unaffected. Gemfibrozil had no effect on plasma total cholesterol levels but gave significant dose-dependent decreases in plasma (VLDL) triglyceride levels (up to −53 %). SB 204990 resulted in a dose-dependent reduction of plasma cholesterol (up to −29 %) by lowering VLDL, LDL and HDL-cholesterol (−50, −20 and −20 %, respectively). In addition, a strong dose dependent reduction of plasma (VLDL) triglycerides up to −43 % was observed with this compound. Although the effects of gemfibrozil and SB 204990 were not simply explained by changes in a single determinant of VLDL metabolism – no effects of these drugs were seen on post-heparin plasma lipoprotein lipase activity, in vivo rate of VLDL synthesis or hepatic apoC-III mRNA levels – APOE*3-Leiden mice were found to give robust hypolipidaemic responses to these test compounds. The responsiveness to hypolipidaemic therapy combined with a clear relationship between aortic lesion size and plasma cholesterol exposure, as demonstrated previously, makes this mouse an attractive model for the testing of anti-atherosclerotic properties of hypolipidaemic drugs.

## Zusammenfassung

*Transgene Apolipoprotein-E*3-Leiden-Mäuse als Testmodell für hypolipidämische Arzneistoffe*

Apolipoprotein(APO)-E*3-Leiden-Mäuse mit beeinträchtigtem Chylomikron- und Remnant-Metabolismus weisen Hyperlipidämie und Atherosklerose auf. In der vorliegenden Studie wurde an diesen Mäusen die hypolipidämische Wirkung verschiedener Substanzen getestet, und zwar der beiden auf dem Markt befindlichen Arzneimittel Lovastatin (CAS 75330-75-5) und Gemfibrozil (CAS 25812-30-0) sowie der neuartigen Substanz SB 204990 (5-Ring-Lacton

von ±(3R*,5S*)3-Carboxy-11-(2,4-dichlorphenyl)-3,5-dihydroxy-undecansäure, CAS 154566-12-8), eines starken Hemmers der Synthese von Cholesterin und Fettsäuren auf der Stufe der ATP-Citratlyase. Die APOE*3-Leiden-Mäuse erhielten Nahrung mit einem hohen Anteil an gesättigten Fettsäuren und Cholesterin sowie einem Zusatz von 0,05 bzw. 0,1 Gew.% Lovastatin oder 0,1 bzw. 0,2 Gew.% Gemfibrozil oder 0,1 bzw. 0,2 Gew.% SB 204990. Lovastatin zeigte eine dosisabhängige Reduzierung des Plasma-Cholesterinspiegels (bis zu –20 %), d. h. eine Senkung der LDL- und HDL-Cholesterin-Konzentration (–20 bzw. –18 %), während der Plasma-Triglyceridspiegel unverändert blieb. Gemfibrozil beeinflußte nicht den Gesamt-Cholesterinspiegel im Plasma, induzierte jedoch eine signifikante dosisabhängige Abnahme des (VLDL-)Triglycerid-Plasmaspiegels (bis zu –53 %). SB 204990 erzeugte eine dosisabhängige Senkung des Plasma-Cholesterinspiegels (bis zu –29 %) durch Senkung von VLDL-, LDL- und HDL-Cholesterin (–50, –20 und –20 %). Bei dieser Substanz wurde außerdem eine starke dosisabhängige Reduzierung der Plasma-(VLDL)-Triglyceride um bis zu –43 % beobachtet. Zwar ließ sich die Wirkung von Gemfibrozil und SB 204990 nicht allein mit der Veränderung einer einzelnen Determinanten des VLDL-Metabolismus erklären – diese Arzneistoffe zeigten keine Wirkung auf die Post-Heparin-Plasma-Lipoproteinlipase-Aktivität, die In-vivo-Rate der VLDL-Synthese oder den hepatischen apoC-III-mRNA-Spiegel –, doch die APOE*3-Leiden-Mäuse zeigten eine deutliche hypolipidämische Reaktion auf diese Testsubstanzen. Auf Grund der damit demonstrierten guten Reaktion auf die hypolipidämische Behandlung sowie des klaren Kausalzusammenhangs zwischen Größe von Aorta-Läsionen und Plasma-Cholesterinspiegel stellt diese Maus ein attraktives Modell für die Prüfung der antiatherosklerotischen Eigenschaften hypolipidämischer Arzneistoffe dar.

**Key words** ±(3R*,5S*)3-Carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic acid, 5-ring lactone · CAS 154566-12-8 · Gemfibrozil · Hypolipidaemic drugs, testing in transgenic mice · Lovastatin · SB 204990, hypolipidaemic effect

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## 1. Introduction

For the development of novel hypolipidaemic and anti-atherosclerotic drugs, small animal models in which both the hypolipidaemic properties of candidate drugs and their potential to affect lesion formation could be tested, would be of great benefit. Normal rats and mice are not very suitable as plasma lipids in these animals are very low, even on high fat/cholesterol diets making rodents very resistant against the development of atherosclerosis [1]. Although hamsters become hyperlipidaemic on high fat/cholesterol diets and have a lipoprotein profile more similar to that seen in human also this species does not easily develop atherosclerotic lesions [2].

With the recent progress in techniques for gene insertion and gene silencing in murine germ- and embryonic cells this picture has changed. These developments have resulted in the production of a number of murine strains with defects in plasma lipid and lipoprotein metabolism. Some of these strains were shown to develop spontaneous or diet-induced hyperlipidaemia and advanced atherosclerotic lesions in the aorta and other main arteries. Recently, we described the generation of transgenic mice expressing the human apolipoprotein (APO) E*3-Leiden gene [3]. These mice have an impaired clearance of chylomicron and VLDL remnant lipoproteins from the blood circulation by the liver [3–5]. As a consequence, these mice have raised plasma cholesterol and triglyceride levels due to increases in VLDL-LDL lipoproteins. In addition, in these mice the plasma cholesterol and triglyceride levels are highly responsive to small changes in chylomicron and VLDL metabolism [4, 5]. In a subsequent study we have shown that APOE*3-Leiden mice are susceptible for diet-induced atherosclerosis and we demonstrated that the size of the aortic lesions correlates very well with the exposure of the vasculature to raised concentrations of plasma cholesterol [4, 6]. Their more human-like lipoprotein profile, the extreme sensitivity of plasma lipid levels to changes in lipoprotein metabolism and the clear relation between aortic lesion size and cholesterol exposure suggested to us that APOE*3-Leiden mice may serve as a suitable animal model for the testing of lipid lowering and anti-atherosclerotic effects of hypolipidaemic drugs.

In the present study we have evaluated the responsiveness of plasma lipids in these mice for hypolipidaemic drugs using two marketed agents, lovastatin (CAS 75330-075-5) and gemfibrozil (CAS 25812-30-0), as well as a novel experimental compound SB 204990[1] (the 5-ring lactone of ± (3R*, 5S*) 3-carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic acid, CAS 154566-12-8), an inhibitor of de novo cholesterol- and fatty acid synthesis at the level of hepatic ATP-citrate lyase [7].

## 2. Materials and methods

### 2.1. Animals

All studies were performed with male APOE*3-Leiden transgenic mice (line #2) [3]. Transgenic mice were obtained by mating male transgene carriers with C57BL/

[1] Manufacturer: SmithKline Beecham Pharmaceuticals, Harlow, Essex (UK).

6J females (The Broekman Institute bv, Someren, The Netherlands). Mice of the F7 generation were used for all studies. Transgenic mice are identified by sandwich ELISA for the presence of human apoE in the serum as described previously [5]. At the time of the study animals were 3–4 months of age.

## 2.2. Study design

The study had a parallel group design and included 6 experimental groups (9 animals per group and 2 dose levels of each of the drugs) and a control group of 18 mice. Animals were distributed over these groups, stratifying for a balanced age distribution. They were housed (3 per cage) in shoebox cages with hoppers that allowed feeding of a powdered diet and weighing of food consumption. Animals had free access to food and water. Two weeks prior to the start of drug administration, mice were switched from a chow diet to a powdered semi-synthetic sucrose-rich diet, containing cocoa butter (15 % w/w) and cholesterol (0.25 % w/w). The diet was composed essentially according to Nishina et al. [8], and described by us earlier [5] (Western or HFC diet, Hope Farms, Woerden, The Netherlands). During the subsequent testing period of two weeks, mice were fed the same powdered diet supplemented with 0.05 or 0.1 % w/w of lovastatin, 0.1 or 0.2 % w/w of gemfibrozil (gifts from the respective manufacturers) or 0.1 or 0.2 % w/w of the SB 204990 (SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK [7]).

At the beginning and end of the experimental period mice were fasted from 7 to 1 pm, weighed and approximately 50 µl of blood was obtained in an EDTA coated vial through tail-bleeding under light isofluothane anaesthesia. Plasma samples were stored on ice until lipid and lipoprotein analysis. Following the post-drug blood sampling, animals were sacrificed, livers were excised, frozen into liquid nitrogen and stored at −70 °C until RNA isolation.

For determination of post-heparin plasma lipoprotein lipase (LPL) activity (methods see below), indicated parts of the experiment were repeated under identical conditions using mice with comparable genetic background, sex and age. In vivo hepatic VLDL triglyceride production rate (methods see below) was determined in mice that were used for determining post-heparin plasma LPL activity. Therefore, these mice were fed the experimental diets for one additional week. 3 wk plasma lipid data were not different from the 2-week data (not shown).

## 2.3. Lipid and lipoprotein analysis

Total plasma cholesterol and triglyceride levels (without measuring free glycerol) were measured enzymatically using commercially available kits: #997-64909 (Wako Chemicals GmbH, Neuss, Germany) and #14149 (E. Merck, Darmstadt, Germany).

For size fractionation of lipoproteins, 30 µl of pooled serum (from 3 mice), containing 2.5 mmol/l EDTA, was injected onto a Superose 6B column (SMART system, Pharmacia, Uppsala, Sweden) eluted at a constant flow rate of 30 µl/min with 150 mmol/l NaCl, 1 mmol/l EDTA, pH 8.0. The effluent was collected in 30 µl fractions. Cholesterol and triglyceride concentrations in lipoprotein fractions were measured enzymatically, as described above.

## 2.4. Post-heparin plasma lipoprotein lipase activity

For measurements of post-heparin plasma lipoprotein lipase (LPL) activity fasted mice received an intravenous injection of heparin (Leo Pharmaceutical products bv, Weesp, The Netherlands, dose: 100 U/kg body weight) at 1 pm, following a 6 h fasting period. After 10 min 200 µl blood was drawn from the tail vein and stored on ice. Plasma was frozen in liquid nitrogen and shipped on dry ice to Umeå for LPL activity measurements. Plasma LPL activity was assayed as described by Bengtsson Olivecrona et al. [9]. In brief, postheparin plasma samples were incubated with a goat anti-hepatic lipase IgG antibody for 2 h at 4 °C [10]. 20 µl of the samples were incubated with substrate (Intralipid into which $^{3}$H-labelled triolein had been incorporated by sonication) in the presence of 10 µl of heat-activated rat serum (as source of apo CII) and 6 % (w/v) bovine serum albumin (BSA) in a total volume of 200 µl. The assay temperature was 25 °C. Enzyme activity is expressed in mU, corresponding to 1 nmol of fatty acid released per min.

## 2.5. In vivo hepatic triglyceride production

After a 6 h fasting period mice were injected intravenously via a tail vein with Triton WR1339 (500 mg/kg body weight) [11] using 15 % (wt/vol) Triton solution in 0.9 % by wt NaCl. At 0, 15, 30 and 45 min after injection blood samples were drawn from the tail and analyzed for triglycerides as described above. Production rate of hepatic triglyceride was calculated from the slope of the curve and expressed as mmol/h/kg body weight. Plasma volume was assumed to be 3.3 % of the body weight [12].

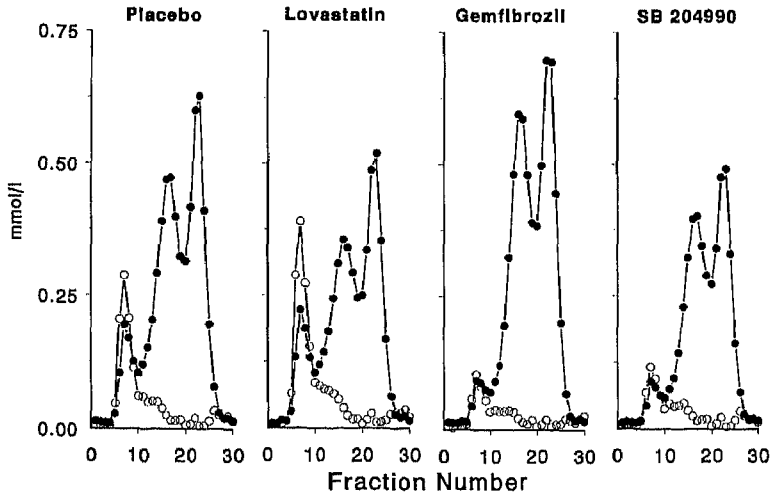
## 2.6. Hepatic apoC-III mRNA measurements in the liver

Mouse hepatic apoC-III mRNA were determined as described previously [13]. In brief, total cellular RNA was prepared from the liver using the guanidinium thiocyanate/phenol-chloroform method [14]. Northern blots of total cellular RNA were subsequently hybridized with a $^{32}$P-labelled probes of rat apoC-III and rat ribosomal 36B4 cDNA [15]. The intensity of the hybridization signal was quantified and the level of apoC-III mRNA was related to the level of 36B4 mRNA.

## 2.7. Statistical analysis

For analysis of treatment effects on serum cholesterol and triglyceride levels, analysis of variance was performed. The pre-study cholesterol levels, pre-study triglyceride level and the pre-study body weight were both found to be important factors, therefore included as covariates in the analysis of the cholesterol and triglyceride levels. A logarithmic scale was used in the analysis of the lipid data. The variability of these measurements tended to increase with the level of response. Using a logarithmic scale standardises the variability producing better analysis. Geometric means for each treatment and the ratio of treatment relative to the control mean for plasma lipid levels were presented, along with their respective 95 % confidence intervals. A 95 % confidence interval for the ratio not containing one indicates a statistically significant difference at 5 % level.

Analysis of VLDL production rate, postheparin plasma LPL activity and hepatic apoC-III mRNA levels were performed by analysis of variance including terms for treatment and cage. Means for each treatment and the difference of treatment means relative to the control mean are presented, along with their 95 % confidence intervals. A 95 % confidence interval of a difference not containing zero indicates a statistically significant difference at the 5 % level.




'he effect of lovastatin, gemfibrozil and SB 204990 on lipoprotein profile of APOE*3-Leiden mice fed a Western diet. Pool of three mice was applied to a Superose 6B column as described in the Methods section. Fractions were analyzed for rol (●) and triglycerides (○). Lipoprotein profiles are shown for untreated Western fed APOE*3-Leiden mice and Western )E*3-Leiden mice treated with lovastatin (0.1 % w/w), gemfibrozil (0.2 % w/w) and SB 204990 (0.2 % w/w). The profiles re the mean profiles of three separate pools (six for the control group). Fractions 4–10, 11–20 and 21–27 correspond with IDL/LDL and HDL, respectively.

## ults

### e effect of lovastatin, gemfibrozil and ·990 on plasma lipids and lipoproteins

ıg a Western-type diet markedly increased a cholesterol concentrations in APOE*3-Leiice as compared to animals fed a normal rohow (7.3 versus 2.1 mmol/l) while triglycer-'ere unaltered (1.7 versus 1.66 mmol/l, n = 'wo weeks after the switch to the Western-iet, animals were put on diet supplemented ɔvastatin, gemfibrozil or SB 204990 and the on plasma lipids were tested after a two treatment period. The effects of hypolipiddrug therapy on plasma lipids are given in 1. Compared with the control (no drug), , lovastatin showed a dose-related decrease in a cholesterol but the difference only reached ical significance in the 0.1 % w/w dose group %). SB 204990 also gave a dose-related hypoterolaemic response, both dosing levels re-ɡ statistical significance (up to 30 % decrease ared with the no drug group). No effect on plasma cholesterol was seen in the animals treated with gemfibozil. Interesting effects of the hypolipidaemic drugs were also seen on plasma triglyceride concentrations. Both gemfibrozil and SB 204990 decreased plasma triglycerides in a dose-related manner (up to −53 %) while no clear effects were seen in the groups treated with lovastatin. Although in all drug treatment groups statistically significant effects on body weight development were seen (weight increments compared with the control group were 0.4–1.2 g at the end of the 14 day dosing period) these differences were small in comparison with body weights (around 27 g). No significant differences were seen in food intake between any of the treatment groups and controls (average food intake around 12 g/d per cage of 3 mice).

To investigate which lipoprotein classes are affected by the drug treatment, plasmas of mice were analyzed by high-performance gel filtration chromatography and the results of these analyses are shown in Fig. 1. Profiles in this figure represent

: The effect of lovastatin, gemfibrozil and SB 204990 on plasma lipids of APOE*3-Leiden mice.

| Compound (dose) | n | Cholesterol (mmol/l) | | | Triglycerides (mmol/l) | | |
|---|---|---|---|---|---|---|---|
| | | Geometric mean | Ratio | 95 % C.I. | Geometric mean | Ratio | 95 % C.I. |
| l | 18 | 7.3 | | | 1.7 | | |
| ıtin (0.05 % w/w) | 9 | 6.8 | 0.93 | (0.84, 1.03) | 1.47 | 0.86 | (0.73, 1.02) |
| ıtin (0.1 % w/w) | 9 | *5.86* | *0.80* | *(0.73, 0.88)*[a] | 1.99 | 1.17 | (0.99, 1.38) |
| ›rozil (0.1 % w/w) | 9 | 7.3 | 1.00 | (0.92, 1.10) | *1.11* | *0.65* | *(0.55, 0.76)*[a] |
| ›rozil (0.2 % w/w) | 9 | 6.9 | 0.95 | (0.86, 1.04) | *0.79* | *0.47* | *(0.40, 0.55)*[a] |
| 990 (0.1 % w/w) | 9 | *5.68* | *0.78* | *(0.71, 0.86)*[a] | *1.19* | *0.70* | *(0.59, 0.83)*[a] |
| 990 (0.2 % w/w) | 9 | *5.18* | *0.71* | *(0.65, 0.78)*[a] | *0.97* | *0.57* | *(0.49, 0.68)*[a] |

ber of animals; C.I., confidence interval. Male APOE*3-Leiden mice were fed for two weeks a Western diet supplemented vastatin, gemfibrozil, or SB 204990 at indicated doses. After a fasting period, mice were bled and plasma cholesterol and rides were determined. Geometric means for plasma cholesterol and triglyceride levels for each treatment and the ratio of ›nt means to the control means are presented along with their 95 % confidence intervals. [a] 95 % confidence interval for the ɔt containing one, indicating a statistically difference at 5 % level (indicated in italics).

means for 9 (18 for the control group) animals, analyzed in 3 (6 for the control group) plasma pools, each composed of 3 animals. Statistical analysis of the differences in lipoprotein lipids in comparison with the control (no drug) group showed that lovastatin treatment significantly decreases LDL and HDL cholesterol at 0.1 % w/w dosing level (by −22 % and −18 %, respectively). SB 204990 significantly decreased VLDL cholesterol and triglycerides at the 0.1 % and 0.2 % w/w dosing levels (by up to −50 % and −55 %, respectively) and decreased LDL and HDL cholesterol at the highest (0.2 % w/w) dose (both by 20 %). Gemfibrozil at both dosing levels significantly decreased VLDL cholesterol and triglycerides (by up to −55 % and −60 %, respectively) but increased rather than decreased LDL and HDL cholesterol, although not statistically significant (+10 %). Although not entirely identical to what would be predicted from documented effects of lovastatin and gemfibrozil therapy in human, clear parallels can be seen in lipoprotein changes in APOE*3-Leiden mice and man (lovastatin's main effect on plasma (LDL) cholesterol and not on triglycerides; gemfibrozil's main effect on plasma (VLDL) triglycerides with a small but non-significant rise in HDL cholesterol but also LDL cholesterol). Responses of the mice to SB 204990, an inhibitor of hepatic cholesterol and fatty acid synthesis, were also as anticipated, with clear dose-related decreases in plasma (VLDL and LDL) cholesterol and plasma (VLDL) triglycerides.

### 3.2. Effect of hypolipidaemic drugs on post-heparin plasma LPL activity, hepatic VLDL triglyceride production rate and hepatic apoC-III expression

As gemfibrozil and SB 204990 were found to induce major changes in plasma VLDL concentrations, the underlying mechanism of these effects was investigated in more detail. The major determinants of the plasma concentration of VLDL are the rate of VLDL triglyceride degradation, mediated by endothelial lipoprotein lipase, and the rate of secretion of VLDL by the liver. To investigate whether the hypotriglyceridaemic properties of gemfibrozil and SB 204990 was due to increased VLDL triglyceride catabolism, groups of mice, exposed to the same dietary and drug regimen as described for the plasma lipid studies, were injected intravenously with heparin and post-heparin plasma LPL activity was determined as described in Methods. Results of these experiments are given in Table 2. Neither gemfibrozil nor SB 204990 administration resulted in a clear statistically significant change in post-heparin LPL activity, suggesting that the hypotriglyceridaemic properties of either drug are not well explained at the level of lipoprotein lipase activity.

To investigate whether the hypolipidaemic response to gemfibrozil and SB 204990 were related to changes in VLDL synthesis, hepatic VLDL triglyceride production rates were determined in vivo in animals exposed to a dietary and drug treatment regimen as described for the plasma lipid studies. For hepatic VLDL triglyceride synthesis measurements animals received i.v. Triton WR 1339 as described in Methods and from the increase in plasma triglyceride levels over a period of up to 45 min, production rate were calculated. The results of those experiments are given in Table 2. In APOE*3-Leiden control (no drug) mice on a Western-type diet the mean hepatic VLDL-triglyceride production rates was 0.125 mmol/h/kg body weight. However, no clear effects of gemfibrozil or SB 204990 on VLDL-triglyceride production rate were found (Table 2), suggesting again that the hypotriglyceridaemic properties of either drugs are not well explained at the level of hepatic VLDL synthesis.

As neither lipoprotein lipase activity nor VLDL production rate could give a satisfactory explanation for the observed hypotriglyceridaemic responses, differences in the composition of VLDL particles between the controls and the drug treated animals were considered. VLDL apolipoprotein C-III abundancy is known to affect the rate of lipolysis by lipoprotein lipase [16, 17]. As the hepatic apoC-III gene mRNA was found to be decreased in rats after fenofibrate treatment [13], this option was investigated using the livers of animals used

*Table 2:* The effect of gemfibrozil and SB 204990 on post-heparin plasma LPL activity and in vivo hepatic VLDL-triglyceride production rate in APOE*3-Leiden mice.

| Compound (dose) | n | Post-heparin plasma LPL activity (mU/ml) | | | Hepatic VLDL-triglyceride production rate (mmol/h/kg body weight) | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Difference | 95 % C.I. | Mean | Difference | 95 % C.I. |
| Control | 18 | 1141 | | | 0.125 | | |
| Gemfibrozil (0.1 % w/w) | 8 | 1056 | −85 | (−353, 183) | 0.112 | −0.014 | (−0.064, 0.037) |
| Gemfibrozil (0.2 % w/w) | 10 | 1278 | 137 | (−131, 405) | 0.150 | 0.025 | (−0.026, 0.075) |
| SB 204990 (0.1 % w/w) | 8 | 1112 | −29 | (−297, 239) | 0.126 | 0.001 | (−0.050, 0.050) |
| SB 204990 (0.2 % w/w) | 10 | 949 | −192 | (−460, 76) | 0.114 | −0.011 | (−0.062, 0.040) |

n, number of animals; C.I., confidence interval. Male APOE*3-Leiden mice were fed for two weeks a Western diet supplemented with gemfibrozil or SB 204990 at indicated doses. After a 6 h fasting period, mice received an intravenous injection of heparin and post-heparin plasma LPL activity was determined (see methods). After one additional week of feeding the experimental diets and following a 6 h fasting period, mice were intravenously injected with Triton WR1339 and hepatic VLDL triglyceride production rate was determined (see Methods). Means for each treatment and the difference of treatment means relative to control means, with 95 % confidence intervals are presented. A 95 % confidence interval of a difference not containing zero indicates a statistically significant difference at the 5 % level.

*Table 3:* The effect of gemfibrozil and SB 204990 on hepatic mouse apoCIII mRNA levels of APOE*3-Leiden mice.

| Compound (dose) | n | mRNA apoC-III/36B4 (arbitrary units) | | |
|---|---|---|---|---|
| | | Mean | Difference | 95 % C.I. |
| Control | 8 | 2.13 | | |
| Gemfibrozil (0.2 % w/w) | 8 | 2.15 | −0.02 | (−0.56, 0.60) |
| SB 204990 (0.2 % w/w) | 8 | 1.73 | −0.40 | (−0.98, 0.18) |

n, number of animals; C.I., confidence interval. Male APOE*3-Leiden mice were fed for two weeks a Western diet supplemented with gemfibrozil or SB 204990, at indicated doses. After a fasting period mice were bled for determination of plasma cholesterol and triglyceride levels (see Table 1). Thereafter, mice were killed and liver was excised. Total liver RNA was isolated and 10 µg was used for northern blot analysis followed by hybridization with a $^{32}$P-labeled probe of mouse apo-CIII (see Methods). RNA levels are relative to internal standard 36B4. Means for each treatment and the difference of treatment means relative to control means, with 95 % confidence intervals are presented. A 95 % confidence interval of a difference not containing zero indicates a statistically significant difference at the 5 % level.

for the study shown in Table 1 and Fig. 1. The results of these measurements are given in Table 3. Once again, differences between mice in the control (no drug) and high dose gemfibrozil or SB 204990 were small and not statistically significant.

Thus, APOE*3-Leiden transgenic mice exhibit significant hypolipidaemic responses in absence of a detectable effect on plasma LPL activity, hepatic VLDL production, or hepatic apoC-III mRNA expression.

## 4. Discussion

For the development of hypolipidaemic and anti-atherosclerotic drugs, a small animal model in which both efficacy endpoints could be tested would be of great advantage. The APOE*3-Leiden mouse overexpresses a human dysfunctional apo E variant and develops hyperlipidaemia and atherosclerosis when fed lipid/cholesterol-enriched diets [3–6]. In the present study, APOE*3-Leiden transgenic mice were evaluated as a testmodel for hypolipidaemic drugs by treating these mice with three types of hypolipidaemic compounds, lovastatin, gemfibrozil and SB 204990.

Lovastatin does not affect plasma cholesterol levels in normal mice and rats, due to a strong compensating upregulation of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene expression and the relative minor role of LDL receptors in the removal from plasma of apo B containing lipoproteins [18, 19]. Interestingly, this drug expressed a mild hypolipidaemic response in APOE*3-Leiden mice fed a Western-type diet. In the APOE*3-Leiden mice removal from plasma of remnants of triglyceride-rich lipoproteins is hampered due to the enrichment of dysfunctional apoE3-Leiden on these particle [3–5]. Consequently, the importance of the LDL receptor in the removal of IDL and LDL may be increased and it is speculated that an increase in LDL receptor activity, induced by lovastatin, may have greater impact on plasma IDL and LDL concentrations in apo E3-Leiden mice than in normal animals.

The hypotriglyceridemic effect of gemfibrozil in APOE*3-Leiden mice is in line with the observed hypotriglyceridemic response in normal mice [20] and rats [21]. The possible mechanism by which gemfibrozil, and fibrates in general, lower plasma triglycerides include upregulation of lipoprotein lipase activity [22, 23], reducing VLDL synthesis [24, 25] and down-regulation of hepatic apoC-III gene expression [13]. In the present study, a clear hypotriglyceridemic response of this drug in APOE*-3-Leiden mice was observed. However, this response could not well be explained by either one of these effects. Whether a combination of small changes in these parameters combined with others e.g. VLDL size and composition, are responsible for the hypotriglyceridaemic response remains to be seen. If so, plasma VLDL concentrations in APOE*3-Leiden mice may be very sensitive to small changes in triglyceride-rich lipoprotein metabolism, a conclusion supported by our earlier work with this model [4, 5].

This sensitivity of plasma VLDL concentrations in APOE*3-Leiden mice is also evident when mice were treated with SB 204990. SB 204990 inhibits de novo cholesterol and fatty acid synthesis in rat hepatocytes and Hep G2 cells and decreases plasma cholesterol, triglycerides and the rate of VLDL synthesis in rats by up to 50 % when mixed in the diet at levels of 0.05 %–0.25 % w/w [7]. APOE*3-Leiden mice exhibit a clear hypotriglyceridaemic response to SB 204990 treatment but surprisingly, this response was not clearly explained by a reduced hepatic VLDL production rate. Post-heparin lipoprotein lipase activity was also unaltered as was the level of mRNA for apoC-III. Again, whether a combination of small changes in these parameters are responsible for the hypotriglyceridaemic response remains to be seen. Notwithstanding the lack of hard data to underpin the underlying mechanisms, APOE*3-Leiden mice seem to be susceptible for treatment with statins and fibrates as well as SB 204990, the lacton precursor of a potent inhibitor of ATP-citrate lyase. This responsiveness to hypolipidaemic therapy combined with the demonstration elsewhere [6] of a clear relationship between aortic lesion size and plasma cholesterol exposure, makes this mouse an attractive model for the testing of anti-atherosclerotic properties of hypolipidaemic drugs.

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