



# Simvastatin treatment aggravates the glucocorticoid insufficiency associated with hypocholesterolemia in mice



Amber B. Ouweneel, Ronald J. van der Sluis, Joya E. Nahon, Miranda Van Eck, Menno Hoekstra\*

Division of Biopharmaceutics, Cluster BioTherapeutics, Leiden Academic Centre for Drug Research, Gorlaeus Laboratories, Leiden, The Netherlands

## ARTICLE INFO

### Article history:

Received 13 October 2016  
Received in revised form  
30 January 2017  
Accepted 17 February 2017  
Available online 20 February 2017

### Keywords:

Cholesterol synthesis  
Simvastatin  
Adrenal  
Glucocorticoid  
Steroidogenesis  
Mice

## ABSTRACT

**Background and aims:** Statin treatment disrupts HMG-CoA reductase-mediated endogenous cholesterol synthesis and lowers plasma LDL-cholesterol levels. Although statin treatment can theoretically impair adrenal steroid hormone synthesis, thus far, no effect on glucocorticoid output has been described, as LDL-cholesterol levels usually remain within the physiological range. However, novel statin-based treatment regimens that dramatically decrease LDL-cholesterol levels are currently employed. Here, we assessed whether inhibition of cholesterol synthesis under these relatively hypocholesterolemic conditions may alter adrenal glucocorticoid output.

**Methods:** Hypocholesterolemic apolipoprotein A1 (*apoA1*) knockout mice were administered high dose simvastatin twice daily for 3 days.

**Results:** Simvastatin treatment did not change plasma cholesterol levels or modify the adrenal expression levels of genes involved in cholesterol metabolism. However, simvastatin treatment lowered basal plasma levels of the primary glucocorticoid corticosterone (−62%;  $p < 0.05$ ). Upon injection with adrenocorticotrophic hormone, control-treated *apoA1* knockout mice already showed only a mild increase in plasma corticosterone levels, indicative of relative glucocorticoid insufficiency. Importantly, simvastatin treatment further diminished the adrenal glucocorticoid response to adrenocorticotrophic hormone exposure (two-way ANOVA  $p < 0.05$  for treatment). Peak corticosterone levels were 49% lower ( $p < 0.01$ ) upon simvastatin treatment.

**Conclusions:** We have shown that simvastatin treatment aggravates the glucocorticoid insufficiency associated with hypocholesterolemia in mice. Our data suggest that (1) HMG-CoA reductase activity controls the adrenal steroidogenic capacity under hypocholesterolemic conditions and (2) imply that it might be important to monitor adrenal function in humans subjected to statin-based treatments aimed at achieving sub-physiological LDL-cholesterol levels, as these may potentially execute a negative impact on the glucocorticoid function in humans.

© 2017 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Glucocorticoids, through an interaction with their cognate nuclear glucocorticoid receptor, modulate a great variety of physiological processes, including glucose metabolism and inflammation. High levels of glucocorticoids are secreted by adrenals in response to activation of the hypothalamus-pituitary-adrenal axis to

facilitate the physiological response to stress [1]. In line with the importance of glucocorticoids in normal physiology, subjects suffering from glucocorticoid insufficiency, i.e. Addison's disease patients, display an overall increased risk for mortality [2].

Glucocorticoids, such as cortisol in humans and corticosterone in mice, are synthesized from their common precursor cholesterol. Several lines of evidence have suggested that adrenal *de novo* cholesterol synthesis does not contribute to the generation of the steroidogenic cholesterol pool under normolipidemic conditions. More specifically, studies in rats have shown that activity of the HMG-CoA reductase, the rate-limiting enzyme in *de novo* cholesterol synthesis, within the adrenals is relatively low as long as cholesterol can be obtained from lipoproteins in the plasma

\* Corresponding author. Division of Biopharmaceutics, Cluster BioTherapeutics, Leiden Academic Centre for Drug Research, Gorlaeus Laboratories, Einsteinweg 55, 2333CC Leiden, The Netherlands.

E-mail address: [Hoekstra@lacr.leidenuniv.nl](mailto:Hoekstra@lacr.leidenuniv.nl) (M. Hoekstra).

compartment [4]. In addition, little to no activity of HMG-CoA reductase can be detected in adrenals of wild-type mice [3]. As such, lipoproteins should be regarded as the primary source of cholesterol used for glucocorticoid production by the adrenals.

From *in vivo* studies, it has become apparent that low-density lipoprotein (LDL) as compared to high-density lipoprotein (HDL) is the preferred lipoprotein substrate for the adrenals in humans. Human HDL deficiency or lack of a functional HDL receptor is associated with a decreased urinary cortisol concentration [7,8]. In addition, plasma HDL-cholesterol levels correlate with the synacthen-induced cortisol response in critically ill patients [9]. HDL may therefore also contribute, at least a bit, to adrenal glucocorticoid synthesis in humans. However, HDL-cholesterol is not able to compensate for a lack of cholesterol associated with LDL particles in humans. As a result, LDL deficient abetalipoproteinemic patients, that contain relatively normal amounts of HDL-cholesterol, suffer from glucocorticoid insufficiency [11–13]. In further support of a limited relevance of HDL in the supply of cholesterol to the adrenal steroidogenic machinery, HDL is redundant for adrenal steroidogenesis in mice with a human-like, LDL-rich, lipoprotein phenotype [10].

LDL-cholesterol lowering, i.e. through statin therapy, is the first treatment goal in high-risk cardiovascular disease patients, given that the presence of relatively high plasma levels of cholesterol associated with LDL is an established risk factor for the development of atherosclerotic lesions [14]. A ~30% cardiovascular disease risk reduction is generally achieved through statin treatment [15], which shows the success of the application of statins as LDL-cholesterol lowering drugs in clinical practice. Despite the fact that the adrenals acquire the majority of the cholesterol used for glucocorticoid synthesis from LDL particles, thus far, statin treatment has not shown to execute a major effect on the adrenal steroid output in humans [16–22]. It should, however, be acknowledged that LDL-cholesterol levels usually remain within the normal physiological range in response to classical statin therapies, as they mostly have been applied to reverse (excessive) hypercholesterolemia. In contrast, novel cardiovascular therapeutic approaches, e.g., anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody/statin co-treatment and ezetimibe/statin co-treatment, aim to create a treatment condition of relative hypocholesterolemia, i.e., in which the amount of LDL-cholesterol in the blood circulation is actually lower than that of normolipidemic subjects [23,24].

Hypocholesterolemic mice as compared to normolipidemic wild-type mice display a marked increase in adrenal relative mRNA expression levels of HMG-CoA reductase, which translates into a significant rise in adrenal HMG-CoA reductase activity [3,5]. It thus appears that when relatively low amounts of cholesterol can be acquired from lipoproteins, adrenal *de novo* cholesterol synthesis is activated in an attempt to overcome cholesterol insufficiency. Based upon this notion, it can be anticipated that *de novo* cholesterol synthesis also makes a significant contribution to the generation of the steroidogenic cholesterol pool in humans subjected to the aggressive LDL-cholesterol lowering therapies, such as high dose statin/PCSK9 inhibitor (combination) treatment. Statins execute their cholesterol-lowering effect by inhibiting HMG-CoA reductase activity, which leads to an upregulation of LDL receptors and a concomitant increase in the clearance of LDL particles from the blood circulation by the liver. Importantly, studies in mice have suggested that statin treatment not only diminishes hepatic cholesterol synthesis, but also significantly inhibits the incorporation of acetate into lipids within the adrenals [28]. When applied in aggressive LDL-cholesterol lowering combination therapies, statins may, therefore, also disrupt the (compensatory) increase in adrenal cholesterol synthesis induced by relative hypocholesterolemia,

theoretically resulting in an impairment of the adrenal glucocorticoid function.

Here we aimed to provide experimental proof for our working hypothesis that statin-mediated suppression of adrenal *de novo* cholesterol synthesis will affect glucocorticoid output under hypocholesterolemic conditions. To this purpose, we subjected genetically hypocholesterolemic mice to simvastatin treatment and investigated the adrenal glucocorticoid function.

## 2. Materials and methods

### 2.1. Experimental mice and treatment

Apolipoprotein A1 (*apoA1*) x LDL receptor (*ldlr*) double knockout mice were obtained from Dr. Jan Albert Kuivenhoven from the Department of Experimental Vascular Medicine, Academic Medical Centre, Amsterdam. Male double knockout mice were mated with female C57BL/6 mice to generate double heterozygous offspring, which was intercrossed to ultimately derive *apoA1* single knockout mice that were inbred for further expansion of the colony. Animal experiments were performed in a temperature and light cycle (12 h light/12 h dark) controlled room at the Gorlaeus Laboratories of the Leiden Academic Centre for Drug Research, in accordance with the National Laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Male *apoA1* knockout mice (13–18 weeks old; ~30 g) were single housed for 1.5 weeks precluding as well as during the experiment. All mice had continuous free access to food and water. Simvastatin was obtained in tablets from Pharmachemie (Haarlem, The Netherlands). Tablets were crushed with a mortar and pestle and subsequently dissolved in tap water. Age-matched groups of *apoA1* knockout mice (N = 9 per group) were orally administered twice a day 250 µl of water either with or without 100 mg/kg body weight simvastatin supplementation for 3 days, with the final dose given at 9:00 AM [25]. Two hours after the final dosing, all mice were bled via tail tip cut for plasma cholesterol and basal corticosterone measurements. Subsequently, mice were administered 200 µg of adrenocorticotrophic hormone (ACTH; MT-Diagnostics, Etten-Leur, The Netherlands) intraperitoneally to stimulate the adrenal glucocorticoid output [26]. One and two hours after ACTH injection, tail blood samples were obtained for corticosterone measurement and/or hematological analysis. During blood draws, mice were handled identically and restrained for a maximum of 30 s to exclude an impact on blood corticosterone levels. Mice were sacrificed through cervical dislocation and organs were harvested. Adrenals and livers of all mice were isolated within 30 min, i.e., maximally 2.5 h and 4.5 h after the administration of ACTH and the final dose of simvastatin, respectively, and stored at –20 °C until further use.

### 2.2. Plasma cholesterol analysis

Concentrations of free and total cholesterol were determined in plasma obtained from tail blood using enzymatic colorimetric assays (Roche Diagnostics).

### 2.3. Plasma corticosterone analysis

Corticosterone levels were determined in tail blood plasma using the [<sup>3</sup>H] radioactive kit from MP Biomedicals (Irvine, CA).

### 2.4. Analysis of gene expression by real-time quantitative PCR

Quantitative gene expression analysis on livers and adrenals was performed as described [27]. In short, total RNA was isolated

using a standard phenol/chloroform extraction method and reverse transcribed with RevertAid™ reverse transcriptase. Gene expression analysis was performed using real-time SYBR Green technology (Eurogentec). Primers were validated for identical efficiencies. Primer sequences are available upon request. Beta-actin, glyceraldehyde-3-phosphate dehydrogenase, ribosomal protein L27, and peptidylprolyl isomerase A were used as standard housekeeping genes in both liver and adrenal gene expression analyses. Relative expression levels were calculated by subtracting the cycle threshold number (Ct) from the gene of interest from the average housekeeping Ct and raising 2 to the power of this difference. No significant difference in the housekeeping Ct values was observed between the two experimental groups.

### 2.5. Data analysis

Statistical analysis was performed using Graphpad InStat software (San Diego, USA, <http://www.graphpad.com>). Normality testing of the experimental groups was performed using the method of Kolmogorov and Smirnov. Significance was calculated using a two-tailed Student's t-test or two-way analysis of variance (ANOVA) with Bonferroni post-test where appropriate. Probability values less than 0.05 were considered significant.

## 3. Results

Previous studies using gene knockout mice have indicated that, probably since HDL is the predominant lipoprotein species circulating in murine plasma, HDL represents the major source of cholesterol utilized for glucocorticoid synthesis in mice [3,5,6]. Genetic deletion of apolipoprotein A1 (*apoA1*) expression in mice is associated with marked hypocholesterolemia due to HDL deficiency [3]. To identify a potential effect on steroidogenesis of statin-mediated disruption of adrenal *de novo* cholesterol synthesis under hypocholesterolemic conditions, we determined the impact of simvastatin treatment on the glucocorticoid function of *apoA1* deficient mice. Simvastatin was chosen for our studies, since this statin species appears to be most effective in targeting adrenals *in vivo* [28]. Koga et al. previously observed that treatment of mice with a single oral dose of 20 mg/kg simvastatin reduces adrenal HMG-CoA reductase activity by 40–50% [28]. With the aim to achieve a maximal extent of adrenal *de novo* cholesterol synthesis inhibition, we applied an oral dosing regimen of simvastatin 100 mg/kg twice daily for 3 days [25] in our *apoA1* knockout mouse model.

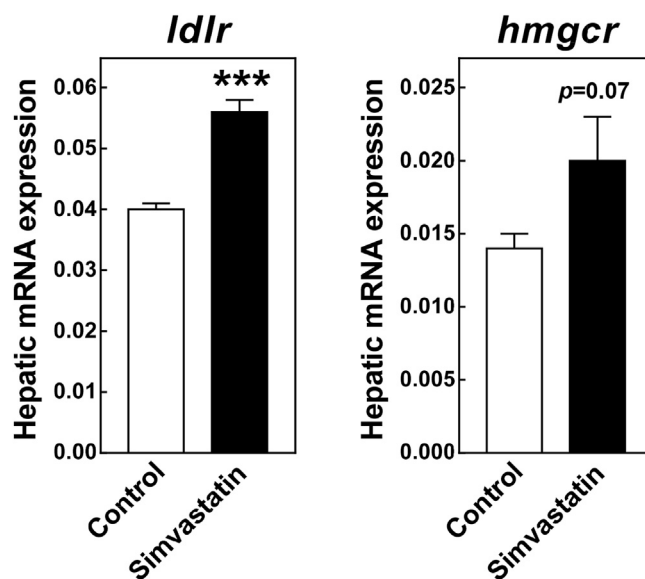
Schonewelle et al. have previously shown that, in C57Bl/6 wild-type mice, statin-induced inhibition of HMG-CoA reductase activity is associated with a compensatory increase in the hepatic mRNA expression of genes involved in cholesterol acquisition and synthesis [29]. As can be appreciated from Fig. 1, we also observed a significant rise in LDL receptor mRNA expression (+39%;  $p < 0.001$ ) in livers of our *apoA1* knockout mice upon simvastatin treatment. Furthermore, a 45% higher mRNA expression of HMG-CoA reductase was detected in livers from simvastatin-treated *apoA1* knockout mice as compared to those from water devoid of simvastatin control-treated *apoA1* knockout mice (Fig. 1). Although this latter effect did not reach statistical significance due to the large intra-group variation ( $p = 0.07$ ), it can be anticipated that in our current experimental setup simvastatin, indeed, effectively inhibited HMG-CoA reductase activity.

In contrast to the potent lipid-lowering effect of simvastatin observed in humans [30], simvastatin treatment did not impact on the extent of hypocholesterolemia. Simvastatin-treated *apoA1* knockout mice exhibited free and total cholesterol levels of  $11 \pm 1$  mg/dl and  $28 \pm 2$  mg/dl, while these respective levels were

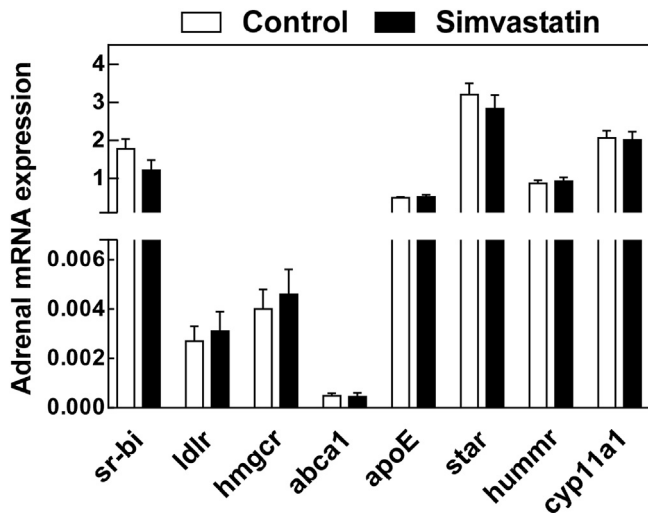
$10 \pm 1$  mg/dl and  $30 \pm 3$  mg/dl in control *apoA1* knockout mice orally administered the solvent only. A potential difference in the steroidogenic capacity of the adrenals in response to simvastatin exposure cannot be attributed to a change in exogenous substrate availability.

Since an efficient (intra)cellular mobilization of cholesterol by the adrenals is essential for glucocorticoid synthesis [6,31,32], we verified whether simvastatin treatment affected the adrenal expression levels of genes involved in cholesterol homeostasis. As evident from Fig. 2, no significant change in the relative transcript abundance of the scavenger receptor BI (*sr-bi*), the LDL receptor (*ldlr*), or HMG-CoA reductase (*hmgcr*) was observed in response to simvastatin treatment. A direct effect of simvastatin on adrenal lipoprotein-cholesterol uptake and *de novo* cholesterol synthesis can thus be considered highly unlikely. Relative mRNA expression levels of the ATP-binding cassette transporter A1 (*abca1*) and apolipoprotein E (*apoE*) were equal within the two groups, suggesting a similar rate of cholesterol efflux from adrenocortical cells to *apoE*-containing HDL-like particles. Notably, as judged from the unchanged expression levels of steroidogenic acute regulatory protein (*star*), mitochondria localized glutamic acid rich protein (*hummr/mgarp*), and cytochrome P450 family 11 subfamily A member 1 (*cyp11a1*), simvastatin treatment did not impact on the intracellular trafficking of free cholesterol between the endoplasmic reticulum and mitochondria or the actual conversion of cholesterol into steroids.

Plasma levels of the primary glucocorticoid species corticosterone are normally ~100 ng/ml in the basal state and increase rapidly to levels >250 ng/ml in response to stress in normolipidemic wild-type mice [3,5,6,31]. In agreement with the findings of Plump et al. that *apoA1* deficient mice as compared to wild-type mice show a reduced (basal) steroidogenesis rate [3], basal levels of corticosterone were  $31 \pm 9$  ng/ml in our water-treated *apoA1* knockout mice (Fig. 3A). Strikingly, despite the already low levels in the control condition, simvastatin treatment was able to further decrease baseline corticosterone values (–62% vs. control-treated mice;  $p < 0.05$ ; Fig. 3A).



**Fig. 1.** The effect of simvastatin treatment on hepatic relative expression levels of the LDL receptor (*ldlr*) and HMG-CoA reductase (*hmgcr*) in *apoA1* knockout mice. Beta-actin, glyceraldehyde-3-phosphate dehydrogenase, ribosomal protein L27, and peptidylprolyl isomerase A (PPIA) were used as housekeeping genes for normalization. Data represent means  $\pm$  SEM of 8–9 mice per group. \*\*\* $p < 0.001$  versus Control.



**Fig. 2.** The effect of simvastatin treatment on relative expression levels of genes involved in cholesterol uptake (*sr-bi/ldlr*) and synthesis (*hmgcr*), intracellular trafficking (*star/hummr*), and efflux (*abca1/apoE*) and metabolism of cholesterol to steroids (*cyp11a1*) in adrenals from *apoA1* knockout mice. Beta-actin, glyceraldehyde-3-phosphate dehydrogenase, ribosomal protein L27, and peptidylprolyl isomerase A (PPIA) were used as housekeeping genes for normalization. Data represent means  $\pm$ SEM of 8–9 mice per group.

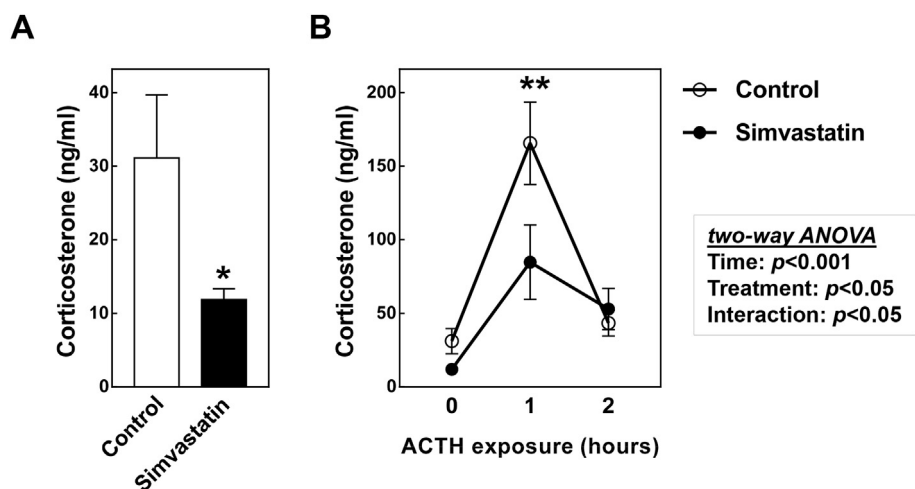
Pituitary-derived adrenocorticotrophic hormone (ACTH) is a potent endogenous activator of adrenal glucocorticoid production and an ACTH challenge is, therefore, an appropriate method to measure the maximal adrenal glucocorticoid output in mice [5,26]. Control *apoA1* knockout mice displayed a marked rise in plasma corticosterone levels as compared to baseline, at one hour after ACTH injection, which was, however, completely abrogated one hour later. In light of the fact that wild-type mice display a maximal plasma corticosterone level over a period of at least three hours in response to an ACTH challenge [5], this finding provides additional proof of a generally low maximal steroidogenic capacity of *apoA1* knockout adrenals [3]. Importantly, as evident from Fig. 3B, the one-hour plasma peak value of corticosterone was 49% lower in simvastatin-treated mice as compared to controls (two-way ANOVA Bonferroni post-test:  $p < 0.01$ ).

#### 4. Discussion

In the current study, we evaluated the effect of statin treatment on the adrenal glucocorticoid output under hypocholesterolemic conditions in mice. Our data confirm previous observations that hypocholesterolemia, i.e., HDL deficiency, in mice is associated with an impaired adrenal glucocorticoid response to stress [3,5,33]. Given that ACTH treatment was able to induce only a short-term (<2 h) and rather limited rise in plasma corticosterone levels in our HDL deficient *apoA1* knockout mice, lipoprotein-associated cholesterol should definitely be regarded as the primary steroidogenic cholesterol source.

Here we have shown that simvastatin-induced inhibition of HMG-CoA reductase activity in *apoA1* knockout mice translates into a >60% decrease in basal plasma corticosterone levels and a marked impairment to respond to an established steroidogenic trigger, e.g., ACTH exposure. We have not been able to measure the actual extent of inhibition of adrenal HMG-CoA reductase activity in response to simvastatin treatment. Although, based on previously published studies [28], we are confident that we achieved at least a 50% reduction in adrenal HMG-CoA reductase activity through simvastatin treatment, it is fair to anticipate that we did not completely block enzyme activity in the adrenals. This can explain why our simvastatin-treated animals were still able to increase their plasma corticosterone level, albeit to a relatively very low extent, upon ACTH exposure. Nevertheless, our data clearly indicate that intra-adrenal cholesterol synthesis contributes significantly to the generation of the steroidogenic cholesterol pool in a lipoprotein deficiency context as simvastatin treatment markedly reduced both the basal and maximal adrenal glucocorticoid output in *apoA1* knockout mice.

The decrease in glucocorticoid output upon simvastatin treatment was not due to a decrease in exogenous substrate availability as the extent of hypocholesterolemia was identical in the two groups of *apoA1* knockout mice. The absence of an effect on plasma cholesterol levels was to be expected, since statin treatment generally does not impact on plasma total cholesterol levels in mice [34]. Since statin treatment does lower LDL-cholesterol levels in humans, one could argue that the absence of a lipid-lowering effect in response to simvastatin treatment makes it hard to translate our findings from mice to the human situation. However, the main conclusion of our study is that under conditions where the amount



**Fig. 3.** The effect of simvastatin treatment on the adrenal glucocorticoid function in *apoA1* knockout mice.

(A) Basal plasma corticosterone levels and (B) the corticosterone response to an ACTH challenge. Data represent means  $\pm$ SEM of 8–9 mice per group. \*  $p < 0.05$ , \*\*  $p < 0.01$  versus Control.

of cholesterol substrate in the plasma compartment is limited, e.g., under low HDL conditions in mice and low LDL conditions in humans, adrenal HMG-CoA reductase is of importance to generate sufficient cholesterol to maintain a proper steroidogenesis rate. Furthermore, given the high similarity in the glucocorticoid insufficiency phenotype of LDL deficient humans [11–13] and HDL deficient mice [3,5], we do value that our findings have a high translational power. As such, we regard it important to determine the adrenal function in patients treated with aggressive statin (combination) treatments aimed at inducing relatively low LDL-cholesterol levels to identify a potential risk of developing glucocorticoid insufficiency.

In conclusion, we have shown that simvastatin treatment aggravates the glucocorticoid insufficiency associated with hypocholesterolemia. Our data (1) supply novel proof for the hypothesis, originally formulated by Plump et al. [3], that HMG-CoA reductase activity is increased under hypocholesterolemic conditions to compensate for the loss of lipoproteins as external cholesterol source and maintain the adrenal steroidogenic capacity and (2) imply that it might be important to monitor the adrenal function in humans subjected to high dose statins in (combination) therapies aimed at achieving sub-physiological LDL-cholesterol levels, as these may potentially execute a negative impact on the glucocorticoid function in humans.

#### Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

#### Financial support

These studies were supported by the Dutch Heart Foundation (Grants 2008T070 and 2012T080 awarded to Menno Hoekstra) and the Netherlands Organization for Scientific Research (VICI Grant 91813603 awarded to Miranda Van Eck).

#### Author contributions

Menno Hoekstra designed, executed and supervised the study and drafted the manuscript. Amber B. Ouweneel, Ronald J. van der Sluis and Joya E. Nahon were essential in the execution of the experiments and the analysis and interpretation of the data and critically read the manuscript. Miranda Van Eck is head of the lipid group within the Division of Biopharmaceutics, Cluster Bio-Therapeutics, of the Leiden Academic Centre for Drug Research and did critical revisions to the manuscript.

#### Acknowledgements

The authors wish to thank Juliet Price, Laura A. Bosmans, and Marie A.C. Depuydt for technical assistance.

#### References

- [1] N.C. Nicolaides, E. Kyrtzi, A. Lamprokostopoulou, G.P. Chrousos, E. Charmandari, Stress, the stress system and the role of glucocorticoids, *Neuroimmunomodulation* 22 (2015) 6–19.
- [2] R. Bergthorsdottir, M. Leonsson-Zachrisson, A. Odén, G. Johannsson, Premature mortality in patients with Addison's disease: a population-based study, *J. Clin. Endocrinol. Metab.* 91 (2006) 4849–4853.
- [3] A.S. Plump, S.K. Erickson, W. Weng, J.S. Partin, J.L. Breslow, D.L. Williams, Apolipoprotein A-I is required for cholesteryl ester accumulation in steroidogenic cells and for normal adrenal steroid production, *J. Clin. Invest.* 97 (1996) 2660–2671.
- [4] S. Balasubramaniam, J.L. Goldstein, J.R. Faust, G.Y. Brunschede, M.S. Brown, Lipoprotein-mediated regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesteryl ester metabolism in the adrenal gland of the rat, *J. Biol. Chem.* 252 (1977) 1771–1779.
- [5] M. Hoekstra, S.J. Korpelaar, R.J. van der Sluis, V. Hirsch-Reinshagen, A.E. Bochem, C.L. Wellington, T.J. Van Berkel, J.A. Kuivenhoven, M. Van Eck, LCAT deficiency in mice is associated with a diminished adrenal glucocorticoid function, *J. Lipid Res.* 54 (2013) 358–364.
- [6] M. Hoekstra, R.J. van der Sluis, M. Van Eck, T.J. Van Berkel, Adrenal-specific scavenger receptor BI deficiency induces glucocorticoid insufficiency and lowers plasma very-low-density and low-density lipoprotein levels in mice, *Arterioscler. Thromb. Vasc. Biol.* 33 (2013) e39–e46.
- [7] M. Vergeer, S.J. Korpelaar, R. Franssen, I. Meurs, R. Out, G.K. Hovingh, M. Hoekstra, J.A. Sierts, G.M. Dallinga-Thie, M.M. Motazacker, A.G. Holleboom, T.J. Van Berkel, J.J. Kastelein, M. Van Eck, J.A. Kuivenhoven, Genetic variant of the scavenger receptor BI in humans, *N. Engl. J. Med.* 364 (2011) 136–145.
- [8] A.E. Bochem, A.G. Holleboom, J.A. Romijn, M. Hoekstra, G.M. Dallinga-Thie, M.M. Motazacker, G.K. Hovingh, J.A. Kuivenhoven, E.S. Stroes, High density lipoprotein as a source of cholesterol for adrenal steroidogenesis: a study in individuals with low plasma HDL-C, *J. Lipid Res.* 54 (2013) 1698–1704.
- [9] P.H. van der Voort, R.T. Gerritsen, A.J. Bakker, E.C. Boerma, M.A. Kuiper, L. de Heide, HDL-cholesterol level and cortisol response to synacthen in critically ill patients, *Intensive Care Med.* 29 (2003) 2199–2203.
- [10] M. Hoekstra, M. Van Eck, HDL is redundant for adrenal steroidogenesis in LDLR knockout mice with a human-like lipoprotein profile, *J. Lipid Res.* 57 (2016) 631–637.
- [11] D.R. Illingworth, T.A. Kenny, E.S. Orwoll, Adrenal function in heterozygous and homozygous hypobetalipoproteinemia, *J. Clin. Endocrinol. Metab.* 54 (1982) 27–33.
- [12] D.R. Illingworth, T.A. Kenny, W.E. Connor, E.S. Orwoll, Corticosteroid production in abetalipoproteinemia: evidence for an impaired response ACTH, *J. Lab. Clin. Med.* 100 (1982) 115–126.
- [13] D.R. Illingworth, E.S. Orwoll, W.E. Connor, Impaired cortisol secretion in abetalipoproteinemia, *J. Clin. Endocrinol. Metab.* 50 (1980) 977–979.
- [14] W.P. Castelli, J.T. Doyle, T. Gordon, C.G. Hames, M.C. Hjortland, S.B. Hulley, A. Kagan, W.J. Zukel, HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study, *Circulation* 55 (1977) 767–772.
- [15] Cholesterol Treatment Trialists' (CTT) Collaborators, B. Mihaylova, J. Emberson, L. Blackwell, A. Keech, J. Simes, E.H. Barnes, M. Voysey, A. Gray, R. Collins, C. Baigent, The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials, *Lancet* 380 (2012) 581–590.
- [16] A.S. Dobs, H. Schrott, M.H. Davidson, H. Bays, E.A. Stein, D. Kush, M. Wu, Y. Mitchel, R.D. Illingworth, Effects of high-dose simvastatin on adrenal and gonadal steroidogenesis in men with hypercholesterolemia, *Metabolism* 49 (2000) 1234–1238.
- [17] S.S. Fojo, J.M. Hoegg, K.J. Lackner, J.M. Anchors, K.R. Bailey, H.B. Brewer Jr., Adrenocortical function in type II hyperlipoproteinemic patients treated with lovastatin (mevinolin), *Horm. Metab. Res.* 19 (1987) 648–652.
- [18] D.R. Illingworth, D. Corbin, The influence of mevinolin on the adrenal cortical response to corticotropin in heterozygous familial hypercholesterolemia, *Proc. Natl. Acad. Sci. U. S. A.* 82 (1985) 6291–6294.
- [19] R.H. Jay, R.H. Sturley, C. Stirling, H.H. McGarrigle, M. Katz, J.P. Reckless, D.J. Betteridge, Effects of pravastatin and cholestyramine on gonadal and adrenal steroid production in familial hypercholesterolaemia, *Br. J. Clin. Pharmacol.* 32 (1991) 417–422.
- [20] J.S. Prihoda, A.S. Pappu, F.E. Smith, D.R. Illingworth, The influence of simvastatin on adrenal corticosteroid production and urinary mevalonate during adrenocorticotropin stimulation in patients with heterozygous familial hypercholesterolemia, *J. Clin. Endocrinol. Metab.* 72 (1991) 567–574.
- [21] Y. Takeda, I. Miyamori, U. Karayalçin, R. Takeda, Influence of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, pravastatin, on corticosteroid metabolism in patients with heterozygous familial hypercholesterolemia, *Horm. Res.* 36 (1991) 75–77.
- [22] D. Travia, F. Tosi, C. Negri, G. Faccini, P. Moghetti, M. Muggeo, Sustained therapy with 3-hydroxy-3-methylglutaryl-coenzyme-A reductase inhibitors does not impair steroidogenesis by adrenals and gonads, *J. Clin. Endocrinol. Metab.* 80 (1995) 836–840.
- [23] A. Kiyosue, N. Honarpour, C. Kurtz, A. Xue, S.M. Wasserman, A. Hirayama, A phase 3 study of evolocumab (AMG 145) in statin-treated Japanese patients at high cardiovascular risk, *Am. J. Cardiol.* 117 (2016) 40–47.
- [24] D.P. Mikhailidis, G.C. Sibbring, C.M. Ballantyne, G.M. Davies, A.L. Catapano, Meta-analysis of the cholesterol-lowering effect of ezetimibe added to ongoing statin therapy, *Curr. Med. Res. Opin.* 23 (2007) 2009–2026.
- [25] K.M. Thelen, K.M. Rentsch, U. Gutteck, M. Heverin, M. Olin, U. Andersson, A. von Eckardstein, I. Björkhem, D. Lütjohann, Brain cholesterol synthesis in mice is affected by high dose of simvastatin but not of pravastatin, *J. Pharmacol. Exp. Ther.* 316 (2006) 1146–1152.
- [26] R.J. van der Sluis, M. Van Eck, M. Hoekstra, Adrenocortical LDL receptor function negatively influences glucocorticoid output, *J. Endocrinol.* 226 (2015) 145–154.
- [27] M. Hoekstra, J.K. Kruijt, M. Van Eck, T.J. Van Berkel, Specific gene expression of ATP-binding cassette transporters and nuclear hormone receptors in rat liver parenchymal, endothelial, and Kupffer cells, *J. Biol. Chem.* 278 (2003) 25448–25453.
- [28] T. Koga, Y. Shimada, M. Kuroda, Y. Tsujita, K. Hasegawa, M. Yamazaki, Tissue-selective inhibition of cholesterol synthesis in vivo by pravastatin sodium, a 3-

- hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, *Biochim. Biophys. Acta* 1045 (1990) 115–120.
- [29] M. Schonewille, J.F. de Boer, L. Mele, H. Wolters, V.W. Bloks, J.C. Wolters, J.A. Kuivenhoven, U.J. Tietge, G. Brufau, A.K. Groen, Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice, *J. Lipid Res.* 57 (2016) 1455–1464.
- [30] S.J. Nicholls, G. Brandrup-Wognsen, M. Palmer, P.J. Barter, Meta-analysis of comparative efficacy of increasing dose of Atorvastatin versus Rosuvastatin versus Simvastatin on lowering levels of atherogenic lipids (from VOYAGER), *Am. J. Cardiol.* 105 (2010) 69–76.
- [31] M. Hoekstra, I. Meurs, M. Koenders, R. Out, R.B. Hildebrand, J.K. Kruijt, M. Van Eck, T.J. Van Berkel, Absence of HDL cholesteryl ester uptake in mice via SR-BI impairs an adequate adrenal glucocorticoid-mediated stress response to fasting, *J. Lipid Res.* 49 (2008) 738–745.
- [32] M. Hoekstra, D. Ye, R.B. Hildebrand, Y. Zhao, B. Lammers, M. Stitzinger, J. Kuiper, T.J. Van Berkel, M. Van Eck, Scavenger receptor class B type I-mediated uptake of serum cholesterol is essential for optimal adrenal glucocorticoid production, *J. Lipid Res.* 50 (2009) 1039–1046.
- [33] M. Hoekstra, S.J. Korporaal, Z. Li, Y. Zhao, M. Van Eck, T.J. Van Berkel, Plasma lipoproteins are required for both basal and stress-induced adrenal glucocorticoid synthesis and protection against endotoxemia in mice, *Am. J. Physiol. Endocrinol. Metab.* 299 (2010) E1038–E1043.
- [34] V. Pecoraro, L. Moja, L. Dall'Olmo, G. Cappellini, S. Garattini, Most appropriate animal models to study the efficacy of statins: a systematic review, *Eur. J. Clin. Invest.* 44 (2014) 848–871.