Cholesterol metabolism in mouse models of atherosclerosis and adrenal steroidogenesis

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ELIMINATION OF ADRENOCORTICAL APOLIPOPROTEIN E PRODUCTION DOES NOT IMPACT GLUCOCORTICOID OUTPUT IN WILD-TYPE MICE

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ABSTRACT

Apolipoprotein E (ApoE) deficient mice exhibit unexplained hypercorticosteronemia. Given that ApoE is also produced locally within the adrenals, we evaluated the effect of adrenal-specific ApoE deficiency on the glucocorticoid function. Hereto, one adrenal containing or lacking ApoE was transplanted into adrenalectomized wild-type mice. Adrenal ApoE deficiency did not impact adrenal total cholesterol levels. Importantly, the ability of the two adrenal types to produce glucocorticoids was also not different as judged from the similar plasma corticosterone levels. Adrenal mRNA expression levels of HMG-CoA reductase and the LDL receptor were decreased by respectively 72 % ($P<0.01$) and 65 % ($P=0.07$), suggesting that cholesterol acquisition pathways were inhibited to possibly compensate the lack of ApoE. In support, a parallel increase in the expression level of the cholesterol accumulation-associated ER stress marker CHOP was detected (+117 %; $P<0.05$).

In conclusion, our studies show that elimination of adrenocortical ApoE production does not impact glucocorticoid output in wild-type mice.

INTRODUCTION

Apolipoprotein E (ApoE) is an important member of the apolipoprotein family of proteins. It provides stability to lipoproteins and contributes to binding of lipoprotein particles by cell surface receptors thereby facilitating their clearance from the circulation [1,2]. ApoE specifically interacts with the low-density lipoprotein receptor (LDLr) and the LDLr-related protein 1 (LRP1) [3,4]. High amounts of ApoE are found on chylomicron- and very-low-density lipoprotein (VLDL) remnant particles [5,6], while some high-density lipoproteins (HDL) species also contain ApoE [7,8]. Total body ApoE deficiency, i.e. in ApoE knockout mice, is therefore associated with an impaired clearance of VLDL/chylomicron remnants from the circulation and marked hypercholesterolemia. Given that hypercholesterolemia is an established risk factor for atherosclerosis, ApoE knockout mice spontaneously develop atherosclerotic lesions already when fed a regular chow diet and represent a commonly used atherosclerosis mouse model [9].

Interestingly, in addition to the observed hypercholesterolemic phenotype, ApoE deficient mice also exhibit an increased plasma level of the primary glucocorticoid species corticosterone [10]. Glucocorticoids are adrenal-derived steroid hormones that play an important role in the physiological response to stress by modulating the expression of genes involved in metabolism and inflammation [11]. Since the cholesterol substrate used for the production of glucocorticoids by the adrenals is primarily derived from lipoproteins, the rise in plasma glucocorticoid levels could theoretically be secondary to the systemic ApoE deficiency-associated hypercholesterolemia. However, it should be noted that in vitro studies have suggested that locally produced ApoE may impact directly on cell cholesterol homeostasis and glucocorticoid output. More specifically, Prack et al. showed that murine Y1 adrenocortical cells expressing human ApoE have an increased cellular cholesterol content and display, in contrast to other ApoE-secreting cell types (i.e. macrophages), a markedly reduced efflux of free cholesterol as compared to control Y1 cells that do not express ApoE [12]. This is paralleled by a significant reduction in the cellular steroid output [13–15]. From these in vitro findings it can be hypothesized that the lack of ApoE within the adrenals is the driving force in generating the hypercorticosteronemia present in total body ApoE knockout mice. In the current study, we validated a potential role of locally produced ApoE on adrenocortical cell cholesterol and steroid metabolism in an in vivo setting using adrenal transplantation.

MATERIALS AND METHODS

IN VIVO SETUP

Female total body ApoE knockout mice (ApoE-/-) on a C57BL/6 background and C57BL/6 mice (ApoE+/+) were bred in house. Throughout the experiment, all mice were group housed in a temperature and light-cycle (12 hour light / dark) controlled room. Bilateral adrenalectomy of the recipient mice and subsequent transplantation of the donor adrenal were carried out as previously described [16]. In brief, adrenal glands of young (10 days postnatal) female donor ApoE -/- (n=9) or C57BL/6 (ApoE+/+; n=10) pups were collected to serve as transplant donors [17]. Bilateral adrenalectomy was performed on 8 week-old female recipient C57BL/6 mice under isoflurane anesthesia through a dorsal midline skin incision and lateral retroperitoneal incisions though the abdominal muscle layer. Subsequently, one donor adrenal was placed per recipient under the renal capsule. The skin wounds were closed using Michel suture clips (size 7.5x1.75 mm, Aesculap, Tuttlingen, Germany). Throughout the experiment, all mice were group-housed and supplied with chow diet (RM3 (E) DU; Special Diet Services, Witham, England), normal water and a 0.9 % NaCl solution ad libitum. For an optimal post-surgery recovery, all cages were supplied with a heating matrass for the duration of one week. Based on our previous studies [16], adrenal transplants were given 8 weeks to become fully mature before subsequent transplant function and morphology analyses were executed.

After the recovery period blood was collected in EDTA coated tubes (Sarstedt, Numbrecht, Germany) at 9:00 AM for basal fed corticosterone measurement. At the end of the study, mice were subjected to overnight food deprivation from 05:00 PM to 09:00 AM to induce physiological stress. Blood was obtained for stressed corticosterone measurement and the mice were subsequently anesthetized with a mixture of Xylazine (70 mg/kg), Ketamine (350 mg/kg) and Atropine (1.8 mg/kg) prior to in situ perfusion with phosphate buffered saline (pressure 100 mmHg) for 10 min via a cannula in the left ventricular apex. Subsequently, organs were collected, weighed, and either snap–frozen (RNA measurements) or stored in 3.7 % formalin (tissue sectioning Formal-fixx®, Shandon Scientific Ltd., UK). Post mortem macroscopic inspection showed no signs of endogenous adrenal regeneration in any of the transplanted mice. All animal experiments were executed at the Gorlaeus Laboratories of Leiden University, where the Leiden Academic Centre for Drug Research is located. All animal work was approved by the Dutch Ethics Committee and regulatory authority at Leiden University and was carried out in compliance with Dutch government guidelines and the Directive

2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

PLASMA LIPID ANALYSIS

At sacrifice orbital whole blood from each individual mouse was collected in EDTA-coated tubes for quantification of plasma lipids. Free cholesterol (FC) was determined by using an enzymatic colorimetric assay with 0.048 U/mL cholesterol oxidase (Calbiochem, San Diego, CA, USA) and 0.065 U/mL peroxidase (Sigma-Aldrich, Steinheim, Germany) in reaction buffer (1.0 KPi buffer, pH=7.7 containing 0.01 M phenol, 1 mM 4-amino-antipyrine, 1 % polyoxyethylene-9-laurylether, and 7.5 % methanol). For the determination of total cholesterol (TC), 0.03 U/mL cholesteryl esterase (Calbiochem, San Diego, CA, USA) was added to the reaction solution. Absorbance was read at 490 nm on a Powerwave 340 from BioTek. Lipoproteins in pooled plasma were separated by means of fast protein liquid chromatography (FPLC; Superose 6 column; 3.2 x 30 mm; Smart-System, Pharmacia, Uppsala, Sweden) and the cholesterol content was analyzed as described above. Fractions 1-13 represented the non-HDL species VLDL and LDL and fractions 13-19 represented HDL.

ANALYSIS OF GENE EXPRESSION BY REAL-TIME QUANTITATIVE PCR

Quantitative expression analysis was performed on equal amounts of RNA as previously described [18]. In brief, total RNA was isolated using a standard phenol/chloroform extraction method and reverse transcribed using RevertAid Reverse Transcriptase. Expression analysis was performed using SYBR-Green technology (Eurogentec) on a 7500 Fast Real time PCR system (Applied Biosystems, Foster city, US). Primers were validated for an equal efficiency. Relative expression values were determined by subtracting the Ct of the target transcript from the average Ct of the housekeeping transcripts and raised by 2 to the power of this difference. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ribosomal protein lateral stalk subunit P0 (36B4) and beta-actin (β-actin) were used for normalization. General information of the primers used is summarized in Table 1.

Table 1: Primers used for Real Time Quantitative PCR.

Gene	Ref Seq (mRNA)	Forward sequence	Reverse sequence
36B4	NM_007475	5′-CTGAGTACACCTTCCCACTTACTGA-3′	5′-CGACTCTTCCTTTGCTTCAGCTTT-3′
3βHSD	NM_153193	5′-AGCCTTCCTGTGCCCCTACT-3′	5′-CAGGAGGAAGCTCACAGTTTCC-3′
ABCA1	NM_13454	5′-AGAGCAAAAAGCGACTCCACATAGAA-3′	5′-CGGCCACATCCACAACTGTCT-3′
ACAT1	NM_144784	5′-AGCTGTTTCTCTGGGCCATCCAAT-3′	5′-GAACTCTCCTGGCTTCAGGGCAT-3′
CHOP	NM_007837	5′-CTCTTGACCCTGCGTCCCTAG-3′	5′-TGGGATGTGCGTGTGACCT-3′
CYP11A1	NM_019779	5′-AGAACATCCAGGCCAACATTACCGAG-3′	5′-AGGACTTCAGCCCGCAGCATC-3′
CYP11B1	NM_001033229	5′-TGCCATCCAGGCTAACTCAATGGAACT-3′	5′-AGGGCCTGCTGAACATCTGGGT-3′
CYP21A1	NM_009995	5′-GGGAACTGCCCAGCAAGTT-3′	5′-AGGATGGTGTTCTGGGATTCTTC-3′
GAPDH	NM_008084	5′-ATCCTGCACCACCAACTGCTTA-3′	5′-CATCACGCCACAGCTTTCCAG-3′
HMGCR	NM_008255	5′-CGAGCCACGACCTAATGAAGAATG-3′	5′-TGCATCACTAAGGAACTTTGCACC-3′
HSL	NM_010719	5′-CTGACAATAAAGGACTTGAGCAACTC-3′	5′-AGGCCGCAGAAAAAAGTTGAC-3′
LDLR	NM_010700	5′-TGAGGTTCCTGTCCATCTTCTTCCC-3′	5′-TTGATGTTCTTCAGCCGCCAGTTC-3′
SR-BI	NM_016741	5′-AAACAGGGAAGATCGAGCCAGTAG-3′	5′-CGTAGTGAAGAACCTGGGGCAT-3′
β-actin	NM_007393	5′-AACCGTGAAAAGATGACCCAGAT-3′	5′-CACAGCCTGGATGGCTACGTA-3′

ADRENAL GLUCOCORTICOID FUNCTION TEST

One week prior to the sacrifice, tail blood was collected in EDTA-coated (Sarstedt, Numbrecht, Germany) tubes under low stress conditions to set the basal level. At the sacrifice day, tail blood was collected in EDTA-coated tubes for corticosterone determination under overnight fasting stress conditions. Corticosterone levels were measured in 1:200 diluted EDTA-plasma using a ^{125}I-corticosterone kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol.

BLOOD ACTH LEVELS

Plasma adrenocorticotropic hormone (ACTH) levels were determined in tail blood under overnight fasting stress conditions using a ^{125}I-radiolabeled kit following the manufacturer's protocol (Phoenix pharmaceuticals, Burlingame, CA, USA).

HISTOLOGICAL VISUALIZATION

Cellular lipid content was visualized within the transplants. Hereto, 10µm cryostat sections (Leica CM 350S cryostat) embedded in OCT compound (Tissue-Tek, Sakura Finetek, Alphen a/d Rijn, The Netherlands) were collected. Sections were stained for neutral lipids with Oil red O and counterstained with hematoxylin (Sigma-Aldrich, Steinheim, Germany). Free cellular cholesterol was visualized with Filipin III (Cayman chemicals, Ann Arbor, MI, USA) and counterstained with the fluorescent compound Sytox green (Life technologies/Thermo Fisher, Waltham, MA, USA).

STATISTICAL ANALYSES

Statistical analysis was performed using GraphPad Instat Software (San Diego, CA, USA, http://www.graphpad.com). Normality testing was performed using the method of Kolmogorov and Smirnov. Significance was calculated using a two-tailed Student's t-test or two-way ANOVA with Bonferroni's post-test where appropriate. $P<0.05$ was considered significant.

RESULTS

In the current study we evaluated a potential contribution of ApoE produced locally in adrenocortical cells to overall adrenal cholesterol homeostasis and glucocorticoid output. For this purpose we generated mice with a genetic lack of ApoE selectively within the adrenals. Hereto, we transplanted one adrenal from either ApoE knockout (ApoE -/-) mice or C57BL/6 wild-type (ApoE+/+) controls into bilaterally

adrenalectomized C57BL/6 wild-type recipient mice. Our previous studies using SR-BI knockout mice have already proven that this approach represents a valid method to uncover the in vivo role of individual gene products in adrenal cholesterol and steroid metabolism [19].

Adrenal ApoE deficiency does not impact on the plasma lipoprotein profile

Adrenalectomized C57BL/6 mice reconstituted with a wild-type adrenal exhibited plasma free and total cholesterol levels that are in the normal range for (normolipidemic) wild-type mice [20]. Given that the liver is regarded the primary site for the production of ApoE circulating in the plasma compartment, we anticipated that adrenal-specific deletion of ApoE would not impact significantly on overall lipoprotein clearance. In accordance, as evident from **Figure 1A**, plasma free and total cholesterol levels were virtually identical in ApoE knockout adrenal recipient mice. Furthermore, the distribution of cholesterol over the different lipoprotein subclasses was also not different in the two experimental groups. Both types of adrenal recipients carried the majority of their cholesterol in HDL (**Figure 1B**).

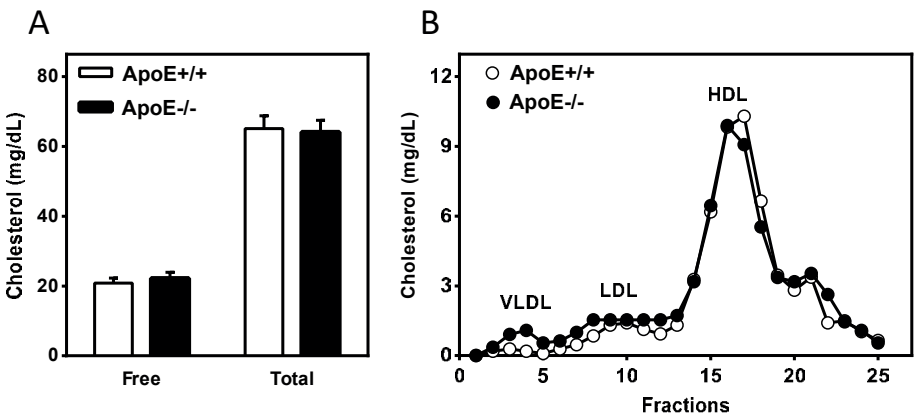

Figure 1: Adrenal-specific ApoE deficiency in C57BL/6 mice did not alter the plasma levels of free or total cholesterol (A). Data represent means+SEM with a group size of ApoE-/- n=9 and ApoE+/+ n=10. The absence of adrenal ApoE also did not change the distribution of cholesterol over the different lipoprotein fractions (B). VLDL, very-low-density lipoprotein; LDL; low-density lipoprotein; HDL, high-density lipoprotein.

Adrenocortical cell-derived ApoE does not modulate cellular cholesterol acquisition

Adrenal transplants were isolated at sacrifice to in detail investigate the effect of adrenocortical cell ApoE deficiency on adrenal cholesterol homeostasis. Our previous studies [19,21,22] have shown that, in mice, the HDL receptor SR-BI is crucial in the generation of the adrenal neutral lipid stores. Adrenal relative mRNA expression levels of SR-BI were not different in the two experimental groups, which suggests that equal amounts of cholesterol esters were acquired from HDL (**Figure 2A**). Gene expression levels of the cholesterol (de-)esterification enzymes ACAT-1 and HSL were also not significantly changed in response to adrenal ApoE deficiency (**Figure 2B & 2C**). This latter finding suggests that the cholesterol acquired from lipoproteins was distributed in

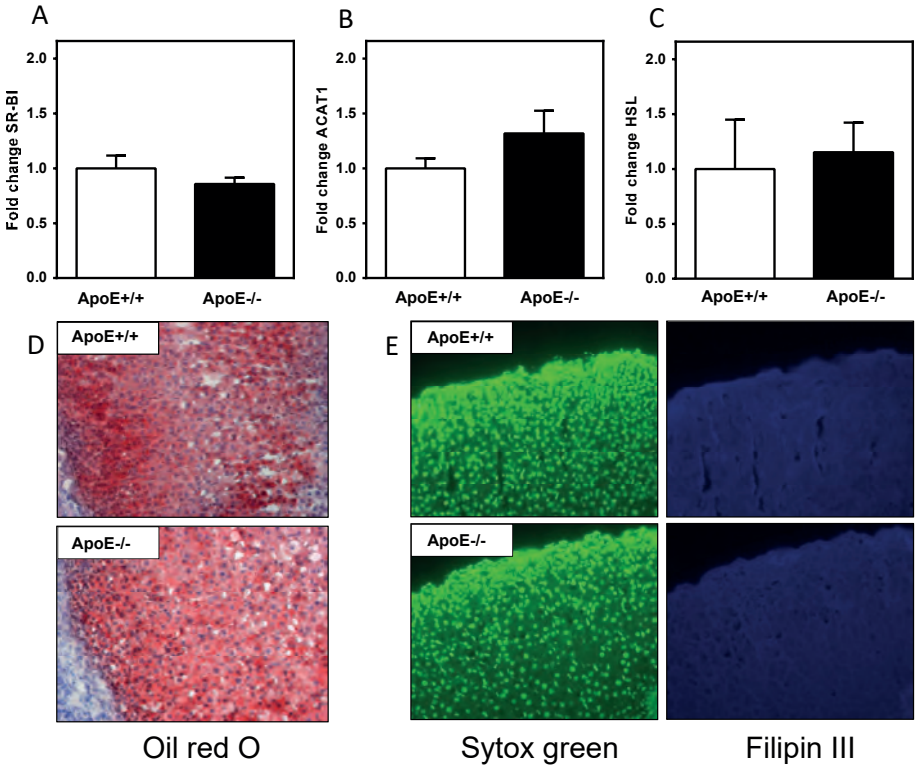

Figure 2: ApoE-/- adrenal transplants showed unaltered mRNA expression levels of SR-BI (A), ACAT1 (B) and HSL (C). Adrenal neutral lipid (Oil red O; D) and free cholesterol (Filipin III; E) content did not differ between ApoE+/+ or ApoE-/- transplant groups (original magnification 400X). All data represent means+SEM with a group size of ApoE-/- n=9 and ApoE+/+ n=10.

a similar manner over the cellular free cholesterol and cholesterol ester pools. Sections of the different adrenal transplants were prepared to visualize the adrenocortical cell cholesterol accumulation. No evident difference in the general morphology of the adrenal transplants could be observed in the two experimental groups. In addition, adrenal ApoE deficiency did not appear to execute a significant effect on the extent of cellular cholesterol ester or free cholesterol accumulation as judged from the stainings depicted in respectively **Figures 2D and 2E**. More specifically, the intensity and cellular distribution of the Oil red O and Filipin III staining was highly similar in the two types of adrenal transplants.

Adrenal-specific ApoE knockout mice exhibit a normal adrenal steroid function

In response to a stress trigger, pituitary-derived adrenocorticotropic hormone (ACTH) stimulates the transcription of a dedicated panel of genes to facilitate an optimal generation of corticosterone from lipoprotein-derived cholesterol by adrenocortical cells [23]. Plasma ACTH levels were similar in the two types of adrenal transplanted mice under stressed conditions (**Figure 3A**). Adrenal-specific ApoE deficiency was also not associated with a change in the mRNA expression levels of the ACTH-responsive genes STAR and steroidogenic enzyme mitochondrial cholesterol side-chain cleavage cytochrome (CYP11A1) that respectively mobilize cholesterol to the steroidogenic machinery within the mitochondria and mediate the conversion of cholesterol into pregnenolone (**Figure 3B**). These combined findings suggest an identical ability of

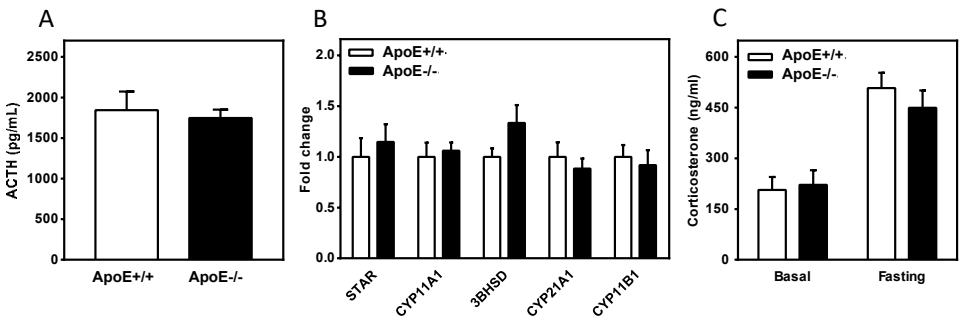

Figure 3: C57BL/6 mice with ApoE-/- and ApoE+/+ adrenal transplants exhibited similar plasma ACTH levels (A) and did not show a change in adrenal mRNA expression levels of the key regulators of steroidogenesis (B), resulting in an identical plasma corticosterone outcome under both basal and fasting stress conditions (C). All data represent means+SEM with a group size of ApoE-/- n=9 and ApoE+/+ n=10.

ACTH to stimulate glucocorticoid production in the two adrenal transplanted groups. In further support, the mRNA expression of the downstream steroidogenic genes 3β-hydroxysteroiddehydrogenase/Δ5-Δ4-isomerase type 2 (HSD3B2), steroid-21-hydroxylase (CYP21A1) and steroid-11β-hydroxylase (CYP11B1) was also unaltered (**Figure 3B**). Interestingly, the null effect of adrenal ApoE deficiency on adrenal steroidogenic gene expression was paralleled by the absence of an effect on plasma glucocorticoid levels. Fasting corticosterone levels were 254 ± 23 ng/ml for wild-type adrenal recipients, while ApoE knockout adrenal transplanted mice exhibited plasma corticosterone values of 224 ± 26 ng/ml (**Figure 3C**). It thus appears that a selective disruption of ApoE only within the adrenals does not recapitulate the stimulating effect on plasma corticosterone levels observed in response to total body ApoE deficiency.

Adrenocortical cell-derived ApoE protects against cholesterol-induced endoplasmic reticulum stress

In vitro overexpression studies have suggested that expression of ApoE in adrenocortical cells executes a negative impact on cellular cholesterol efflux 12,24. In our current in vivo study setup, deletion of ApoE from adrenocortical cells was associated with a 120 % increase (P=0.08; **Figure 4A**) in the expression of ATP-binding cassette transporter A1 (ABCA1) that mediates the efflux of cholesterol from cells to lipid-poor apoA1 to generate nascent HDL particles. Transcription of ABCA1 is generally stimulated by the oxysterol-sensing nuclear receptor liver X receptor (LXR) to overcome the buildup of free cholesterol in cells [25,26]. Notably, the increase in ABC transporter expression coincided with a marked 55 % decrease (P<0.01) in the gene expression of the cholesterol synthesis enzyme HMG-CoA reductase (HMGCR) (**Figure 4B**). Transcription of HMGCR and the LDL receptor is subject to negative feedback in response to cholesterol overload in the endoplasmic reticulum [27, 28]. A clear trend towards a 45 % decrease (P=0.07) was observed in LDL receptor gene expression levels (**Figure 4C**). These combined findings suggest an apparent accumulation of a regulatory free cholesterol pool specifically in the endoplasmic reticulum compartment in response to adrenal ApoE deficiency. Free cholesterol overload has previously been suggested to contribute to endoplasmic reticulum stress [29]. In further support of a potential effect of adrenal-specific ApoE deficiency on specifically ER cholesterol levels, ApoE knockout adrenal transplants exhibited a 117 % increase in the mRNA expression level of the cholesterol accumulation-associated ER stress marker gene CHOP compared to the ApoE producing transplants (P<0.05) (**Figure 4D**).

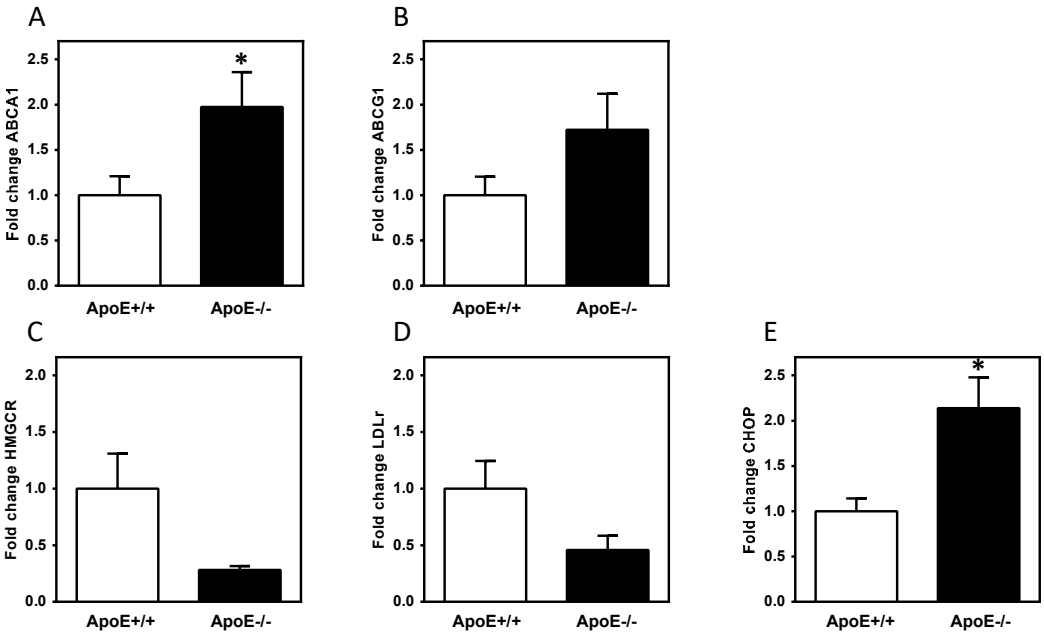

Figure 4: Adrenal-specific ApoE deficiency was associated with a trend towards higher adrenal mRNA expression levels of ABCA1 (A), while respective levels of HMGCR (B) and LDLr (C) were lower or tended to decrease. CHOP mRNA expression levels were significantly increased in ApoE-/- adrenal transplants (D). All data represent means+SEM with a group size of ApoE-/- n=9 and ApoE+/+ n=10. * P<0.05, ** P<0.01.

DISCUSSION

In the current study we determined the effect of adrenal-derived ApoE on cholesterol and steroid metabolism in vivo. Here we show that adrenal-specific ApoE deficiency in C57BL/6 wild-type mice is associated with a downregulation of cholesterol acquisition pathways, whilst the ability of adrenals to produce glucocorticoids is not affected.

This study was based upon the hypothesis, derived from previously published in vitro studies, that ApoE produced within the adrenal executes an inhibitory effect upon the synthesis of glucocorticoids through interfering with the ACTH-induced adrenal cyclic AMP (cAMP) [13, 30–32] response and that total body ApoE knockout mice therefore exhibit higher plasma levels of corticosterone. Our current findings provide clear support for the notion that, in vivo, adrenocortical-cell derived ApoE actually does not impact on the steroidogenic process. An important point to take into account when interpreting our null result on glucocorticoid output is that not only plasma cholesterol levels were similar but that plasma ApoE levels were probably also very similar in both groups of adrenal recipients. In contrast, total body ApoE knockout mice lack adrenal ApoE in the context of a parallel disappearance of ApoE from the plasma compartment when comparing them to wild-type mice. Theoretically a potential effect of the ApoE deficiency within the adrenals on steroid synthesis and output may therefore be nullified for by the fact that our adrenal transplanted mice do have ApoE circulating. However, it should be noted that Thorngate et al. have shown that plasma ACTH levels as well as the adrenal response to an ACTH challenge with respect to tissue cholesterol levels and growth are not different between total body ApoE knockout mice and wild-type controls [33]. In light of our finding that the adrenal expression of ACTH-sensitive genes was not different in mice transplanted with ApoE knockout adrenals as compared to those receiving a wild-type adrenal, we think it is fair to conclude that, as opposed to what was to be expected from the in vitro data, ApoE generally does not impact on the ability of the adrenals to be activated by ACTH in vivo. The fact that the current in vivo findings do not match those previously obtained in vitro provides further support for our working hypothesis - originally derived from studies regarding the role of the LDL receptor [34] - that the relative impact of individual gene products on adrenocortical cell-mediated steroidogenesis depends much on the complexity of the study system.

Given that ApoE apparently does not directly influence the adrenal steroidogenesis rate, it has to be concluded that the hypercorticosteronemia detected in ApoE knockout mice is a secondary effect of the global lack of ApoE. It is easy to speculate that the

ApoE deficiency-associated rise in VLDL/LDL-cholesterol levels generates a larger pool of substrate that can be acquired by the adrenals for use in steroidogenesis. Although we do not regard this option very likely given the already high flux of cholesterol through the VLDL/LDL compartment in wild-type mice, studies in mice that have a normal expression of ApoE in the adrenals but exhibit similar hypercholesterolemia as observed in total body ApoE knockout mice are warranted to exclude that an increased substrate availability is causally related to the overall higher steroid output.

Besides the local effect of ApoE on steroidogenesis, Raber et al. showed that, total body ApoE depletion affects the sensitivity of the negative feedback by glucocorticoids on the hypothalamus – pituitary – adrenal (HPA)-axis within the brain, thereby prolonging the ACTH activation [10]. This misbalance in the HPA-axis feedback mechanism could contribute to the increased glucocorticoids in ApoE knockout mice under basal conditions. In our setting we circumvented this phenotype by the use of C57Bl/6 recipient mice that displayed equal ACTH levels upon stress activation.

Grootendorst et al. have shown that the glucocorticoid phenotype in ApoE knockout mice becomes even more evident upon aging [35]. Pro-inflammatory cytokines such as interleukins 1 and 6 and tumor necrosis factor-alpha are potent activators of the HPA axis and stimulate the secretion of anti-inflammatory glucocorticoids to overcome, for instance, sepsis-related mortality [36-40]. ApoE knockout mice as compared to wild-type mice exhibit a higher basal inflammation status and susceptibility for infections [41], since ApoE stimulates the development of an anti-inflammatory macrophage phenotype [42]. Over time ApoE knockout mice develop atherosclerotic lesions that further stimulate the pro-inflammatory state in these animals, explaining why the hypercorticosteronemia also increases with age in these animals. From these combined findings it can be suggested that the hypercorticosteronemia in total body ApoE knockout mice is secondary to the general higher inflammation status and the fact that the mice develop atherosclerotic lesion that exacerbate the pro-inflammatory state.

An interesting finding of our studies is that adrenal ApoE deficiency is associated with a genetic response that is aimed to reduce cellular cholesterol levels. Based upon this observation it can be suggested that ApoE facilitates the removal of cholesterol from adrenocortical cells. Previous studies have already indicated that ApoE could be an important mediator of cholesterol efflux. Remaley et al. observed that exogenous ApoE can serve as an acceptor for cholesterol transported out of the cells by ABCA1

[43]. Similarly, Huang et al. found that depletion of ApoE from plasma disrupts the efflux of radiolabeled cholesterol from fibroblasts [44]. Our in vitro studies have shown that macrophage ApoE deficiency is associated with a marked reduction in the extent of passive cholesterol efflux to albumin and a mild decrease in the active (ABC transporter-mediated) transport of cholesterol from macrophages towards HDL [45]. In accordance with the notion that ApoE captures endogenous cholesterol for subsequent transport out of the cell, Heeren et al. have shown that cellular recycling of ApoE within cultured hepatocytes is associated with cholesterol efflux [46].

Our data also put forward the suggestion that adrenocortical cell-derived ApoE may be involved in the cellular protection against endoplasmic reticulum stress. The hepatotoxic drug cyclosporine-A is a known inducer of endoplasmic reticulum stress [47–49]. Importantly, Kockx et al. have shown that cyclosporin-A treatment is associated with an accumulation of ApoE specifically in vesicular structures in the endoplasmic reticulum [50]. Niculescu et al. noted that HDL-mediated protection against endoplasmic reticulum stress coincides with a significant increase in cellular ApoE secretion [51]. When taking these latter findings into account, it appears from our findings that ApoE could have a more general role in endoplasmic reticulum physiology as it – by actively mobilizing cholesterol from the endoplasmic reticulum for subsequent transport from the cell – may maintain organelle cholesterol levels within the normal range to overcome the development of ER stress.

CONCLUSIONS

We have shown that elimination of adrenocortical ApoE production does not impact glucocorticoid output in wild-type mice. Furthermore, our studies (1) imply that ApoE probably plays a role in the removal of cholesterol from the adrenals and (2) further highlight the need for proper in vivo testing of the relevance of individual gene products for adrenal steroidogenesis.

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