



Universiteit
Leiden
The Netherlands

Plasma lipoproteins are required for both basal and stress-induced adrenal glucocorticoid synthesis and protection against endotoxemia in mice

Hoekstra, M.; Korpelaar, S.J.; Li, Z.; Zhao, Y.; Eck, M. van; Berkel, T.J. van

Citation

Hoekstra, M., Korpelaar, S. J., Li, Z., Zhao, Y., Eck, M. van, & Berkel, T. J. van. (2010). Plasma lipoproteins are required for both basal and stress-induced adrenal glucocorticoid synthesis and protection against endotoxemia in mice. *American Journal Of Physiology: Endocrinology And Metabolism*, 299(6), E1038-1043. doi:10.1152/ajpendo.00431.2010

Version: Not Applicable (or Unknown)

License: [Leiden University Non-exclusive license](#)

Downloaded from: <https://hdl.handle.net/1887/62010>

Note: To cite this publication please use the final published version (if applicable).

Plasma lipoproteins are required for both basal and stress-induced adrenal glucocorticoid synthesis and protection against endotoxemia in mice

Menno Hoekstra, Suzanne J. A. Korpelaar, Zhaosha Li, Ying Zhao, Miranda Van Eck, and Theo J. C. Van Berkel

Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, Leiden, The Netherlands

Submitted 21 July 2010; accepted in final form 14 September 2010

Hoekstra M, Korpelaar SJ, Li Z, Zhao Y, Van Eck M, Van Berkel TJ. 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: E1038–E1043, 2010. First published September 21, 2010; doi:10.1152/ajpendo.00431.2010.—Lipoprotein-associated cholesterol has been suggested to make a significant contribution to adrenal steroidogenesis *in vivo*. To determine whether lipoproteins indeed contribute to optimal adrenal steroidogenesis in mice, in the current study we have determined the effect of relative lipoprotein deficiency on adrenal steroidogenesis in C57BL/6 wild-type mice. Feeding C57BL/6 mice the lipid-lowering drug probucol (0.25% wt/wt) for 2 wk induced a 90% decrease in plasma high-density lipoprotein (HDL) cholesterol levels and a 77% reduction in low-density lipoprotein (LDL) cholesterol levels. Neutral lipid stores were depleted upon probucol treatment specifically in the glucocorticoid-producing zona fasciculata of the adrenal, leading to a 44% decreased plasma corticosterone level under basal conditions. Exposure to lipopolysaccharide (LPS) induced a 37% increase in the adrenal uptake of HDL cholesteryl esters. Probucol-treated mice could induce only a relatively minor corticosterone response upon a LPS challenge compared with controls, which coincided with an approximately twofold increased hepatic expression level of interleukin-6 and tumor necrosis factor (TNF) α and an 89% higher TNF α response in plasma. Furthermore, a compensatory two- to fivefold upregulation of LDL receptor (cholesterol uptake) and HMG-CoA reductase (cholesterol synthesis) expression was noticed in the adrenals of probucol-treated mice. In conclusion, we have shown that lipoprotein deficiency in mice as a result of probucol feeding is associated with decreased adrenal cortex cholesterol levels, a lower basal and stress-induced plasma glucocorticoid level, and an increased susceptibility to LPS-induced inflammation. Therefore, it is suggested that plasma lipoproteins are required for optimal adrenal steroidogenesis and protection against endotoxemia in mice.

probucol; scavenger receptor BI; low-density lipoprotein receptor; hydroxylmethylglutaryl-coenzyme A reductase; lipopolysaccharide

STEROID HORMONES CONSTITUTE A CLASS OF HORMONES that are derived from cholesterol. They regulate a vast number of physiological processes such as growth and metabolism. Steroid hormones include the sex hormones testosterone and estrogen as well as glucocorticoids and mineralocorticoids that are secreted by the adrenals. A constant supply of cholesterol is required within adrenal cells to serve as a precursor for the conversion to glucocorticoids and mineralocorticoids. The rate-limiting step for corticoid synthesis is the transport of unesterified cholesterol from the outer mitochondrial membrane to the inner membrane by steroidogenic acute regulatory

protein (StAR) (6, 7, 18). However, in response to a stressful lifestyle, subjects can exhibit a sustained increase in plasma glucocorticoid (i.e., cortisol) levels, which requires relatively large amounts of cholesterol to be used for steroidogenesis within the adrenals. Theoretically, the unesterified cholesterol needed for optimal adrenal steroidogenesis under stress conditions can be derived from several different sources: 1) *de novo* synthesis of cholesterol from acetyl-CoA, 2) intracellular catabolism of stored cholesteryl esters, and 3) uptake of extracellular cholesterol from lipoproteins [reviewed by Kraemer (13)]. The relative contribution of these three pathways to basal and stress-induced steroidogenesis has been studied extensively in adrenocortical cells *in vitro*. The production of glucocorticoids in cultured bovine adrenocortical cells is dependent on the *de novo* synthesis of cholesterol, since pharmacological inhibition of hydroxylmethylglutaryl (HMG)-CoA reductase activity significantly impairs both basal and adrenocorticotrophic hormone (ACTH)-stimulated cortisol secretion (24). In addition, the breakdown of cholesteryl esters to free cholesterol by hormone-sensitive lipase (HSL) is also of quantitative importance for adrenal glucocorticoid synthesis since HSL deficiency is associated with a significantly lower basal and stimulated corticosterone production in mouse adrenocortical cells *in vitro* (14). Importantly, already in the 1950s it was shown that >95% of the adrenal cholesterol pool in rats does not originate from local sources (i.e., *de novo* synthesis or cellular catabolism) but rather from the plasma compartment (21). Although cholesterol is also an important building block for cell membranes, this finding does clearly suggest that lipoprotein-associated cholesterol may also make a significant contribution to adrenal steroidogenesis *in vivo*. To determine whether lipoproteins indeed contribute to optimal adrenal steroidogenesis in mice, in the current study we have determined the effect of relative lipoprotein (i.e., LDL and HDL) deficiency on basal and stress-induced glucocorticoid secretion by the adrenals in C57BL/6 wild-type mice.

MATERIALS AND METHODS

Animals. Female C57BL/6 wild-type mice were obtained from the Jackson Laboratory and fed a sterilized regular chow powder diet (RM3; Special Diet Services, Witham, UK) with or without 0.25% (wt/wt) probucol (Sigma) supplementation. Mice were maintained on a standardized light-dark cycle and were handled regularly and in an identical manner prior to and during the experiment. Animal experiments were performed at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with the national laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of the Leiden University.

Lipid analyses. The distribution of cholesterol over the different lipoproteins in plasma was analyzed by fractionation of 30 μ l of plasma of each mouse using a Superose 6 column (3.2 $\times$ 300 mm,

Address for reprint requests and other correspondence: M. Hoekstra, Div. of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, P. O. Box 9502, 2300RA Leiden, The Netherlands (e-mail: hoekstra@lacdr.leidenuniv.nl).

Table 1. Plasma and adrenal lipid levels in probucol-treated and control mice

Parameter	Control	Probucol	P Value
Body weight, g	17.8 ± 0.5	17.8 ± 0.7	1.00
Plasma			
Total cholesterol, mg/dl	58 ± 2	6.5 ± 0.3	<0.001
Phospholipid, mg/dl	136 ± 6	39 ± 1	<0.001
Triglyceride, mg/dl	57 ± 9	39 ± 3	0.11
Adrenals			
Weight, mg	6.4 ± 1.1	6.2 ± 0.8	0.90
Weight, %BW	0.036 ± 0.006	0.035 ± 0.005	0.94
Total cholesterol, µg/mg protein	17 ± 1	8 ± 1	0.0015

Data represent means ± SE of 5 mice. %BW, percentage of body weight.

Smart-System; Pharmacia). Total cholesterol content of the effluent was determined using enzymatic colorimetric assays (Roche Diagnostics). Seven-micrometer cryosections of snap-frozen adrenals were prepared on a Leica CM3050-S cryostat. Cryosections were routinely stained with hematoxylin (Sigma) for nuclei and Oil Red O (Sigma) for lipid visualization. Images were obtained with a Leica image analysis system consisting of a Leica DMRE microscope coupled to a camera and Leica Qwin Imaging software (Leica, Cambridge, UK).

Analysis of gene expression by real-time quantitative PCR. Quantitative gene expression was performed using real-time SYBR Green technology (Eurogentec). Primers were validated for identical efficiencies [slope = −3.3 for a plot of threshold cycle (C_T) vs. log ng cDNA]. Primer sequences are available on request. Hypoxanthine guanine phosphoribosyl transferase (HPRT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β -actin, and acidic ribosomal phosphoprotein P0 (36B4) were used as the standard housekeeping genes. Relative gene expression numbers were calculated by subtracting the C_T number of the target gene from the average C_T of HPRT, GAPDH, β -actin, and 36B4 (C_T housekeeping) and raising 2 to the power of this difference. The average C_T of four housekeeping genes was used to exclude that changes in the relative expression were caused by variations in the expression of the separate housekeeping genes.

Corticosterone and TNF α response upon a lipopolysaccharide challenge. Blood was drawn between 0800 and 0900 via tail cut using a mouse restrainer for basal “nonstressed” plasma values of corticosterone and TNF α levels. Subsequently, mice were intravenously injected at 0900 with a sublethal dose of 50 µg/kg lipopolysaccharide (LPS) from *Salmonella* Minnesota R595 (List Biological Laboratories, Hornby, ON, Canada) into the tail vein. At the indicated times following LPS injection, tail cut blood samples were collected and plasma corticosterone and TNF α levels determined by [125 I]radioimmunoassay (RIA) from MP Biomedicals (Irvine, CA) and ELISA (OptEIA kit; BD Biosciences Pharmingen, San Diego, CA), respectively.

Tissue uptake of [3 H]cholesteryl ether HDL. Human HDL was isolated from blood of healthy subjects by differential ultracentrifugation and dialyzed against PBS with 1 mM EDTA. HDL ($1.063 < d < 1.21$) was labeled with [3 H]cholesteryl ether (Cet) via exchange from donor particles. A dose of 200 µg of apolipoprotein ($\pm 1.2 \times 10^6$ dpm) of [3 H]HDL-Cet (total volume 100 µl) was injected into the tail vein. For analysis of tissue cholesteryl ether uptake, 2 h after tracer injection tissues were excised, weighed, solubilized, and counted for [3 H]radioactivity in a Packard liquid scintillation unit. A correction was made for the radioactivity in the blood present in the tissues at the time of sampling. Values were expressed as percent of the injected dose per microgram of tissue. To determine the effect of LPS on the tissue uptake of [3 H]HDL-Cet, mice were preinjected with 50 µg/kg LPS 1 h before injection of the [3 H]HDL-Cet.

Data analysis. Statistical analyses were performed using two-tailed unpaired *t*-test or two-way ANOVA, using Graphpad Prism Software

(<http://www.graphpad.com>; Graphpad Software, San Diego, CA). Normality testing of the experimental groups was performed using the method of Kolmogorov and Smirnov (Graphpad Instat Software). $P < 0.05$ was considered significant.

RESULTS

To induce an acute and short-term depletion of plasma lipoproteins, C57BL/6 mice were fed a regular chow control diet or a chow diet supplemented with the lipid-lowering drug probucol for 2 wk. As anticipated, treatment with probucol induced a marked decrease in plasma total cholesterol (−89%, $P < 0.001$) and phospholipid (−71%, $P < 0.001$) levels in ad libitum-fed mice (Table 1). Lipoprotein distribution analysis revealed that the decrease in the plasma total cholesterol level upon probucol treatment could be attributed to an 87% decrease in HDL cholesterol levels and a 77% decrease in LDL cholesterol levels (Fig. 1). VLDL/chylomicron cholesterol levels were unchanged after probucol treatment (Fig. 1). In parallel, no significant difference was detected in plasma triglyceride levels between probucol-fed and control-fed mice (Table 1).

Strikingly, the gross appearance of the adrenals was markedly different between probucol-treated mice and control mice. Although we did not observe a difference in weight of the adrenals (Table 1), adrenals of probucol-treated mice appeared darker (more red) compared with those of controls (Fig. 2A). In parallel, a significant 54% decrease ($P = 0.0015$) in total adrenal cholesterol stores was observed upon probucol treatment (Table 1). Oil Red O staining revealed that the neutral lipid (i.e., cholesteryl ester) content of the adrenal cortex was clearly decreased in probucol-fed mice compared with control-fed mice. In particular, a decrease in Oil Red O-positive neutral lipid stores was detected in the zona fasciculata, the area of the adrenal cortex that contains the glucocorticoid-producing cells, whereas the lipid content of mineralocorticoid-producing zona glomerulosa seemed to be essentially unaffected (Fig. 2B).

Importantly, a significant decrease in the plasma corticosterone levels was noted in probucol-treated mice under basal nonstressed conditions ($P = 0.016$; Fig. 3A). In the adrenals, probucol did not affect the relative gene expression level of

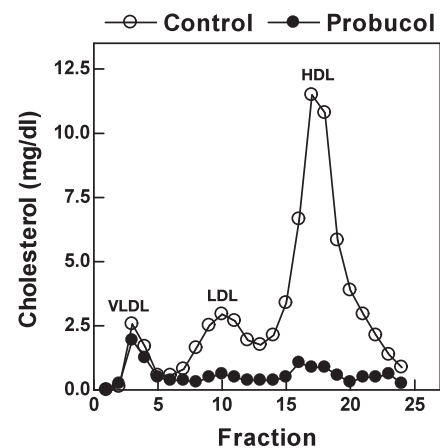
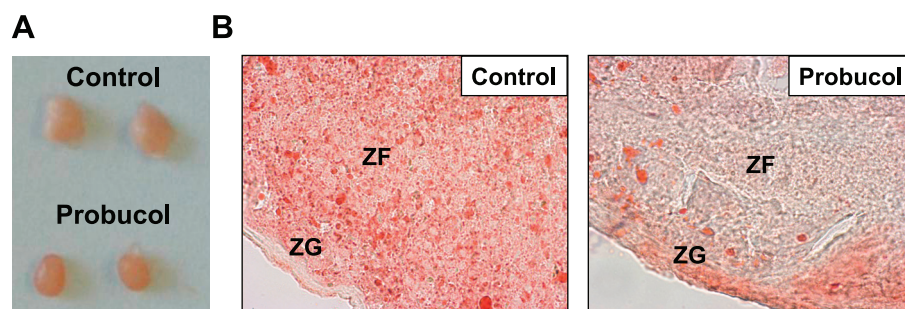


Fig. 1. The effect of probucol on the plasma lipoprotein cholesterol distribution. Blood samples were drawn from mice fed a regular chow diet (○) or a diet supplemented with 0.25% probucol (●) for 2 wk. Pooled plasma from 5 mice was loaded onto a Superose 6 column, and fractions were collected. Fractions 3–7 represent VLDL, fractions 8–13 represent LDL, and fractions 14–20 represent HDL.

Fig. 2. The effect of probucol treatment on the gross morphology of the adrenals (A) and adrenal cortex lipid levels (B). Note the clear darkening of the adrenals in probucol-fed mice. Cryosections of adrenals were stained with Oil Red O for neutral lipids and counterstained with hematoxylin for nuclei. ZG, zona glomerulosa; ZF, zona fasciculata.



StAR (Fig. 3B), the rate-limiting factor for acute adrenal steroidogenesis. In addition, no significant change was observed in the mRNA expression level of cytochrome P450_{scc} (CYP11A1; Fig. 3B) that catalyzes the first and rate-limiting step in the biosynthesis of hormones in all steroidogenic tissues, namely the conversion of cholesterol to pregnenolone. Furthermore, no significant change in the relative mRNA expression level of the HDL receptor scavenger receptor BI (SR-BI), the LDL receptor, HSL, or acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1) was observed (Fig. 3B), suggesting that the activity of lipoprotein uptake as well as intracellular cholesterol ester synthesis and catabolism systems was unaltered by probucol treatment. In contrast, probucol treatment significantly increased the adrenal relative expression level of HMG-CoA reductase (+47%, $P = 0.036$; Fig. 3B). This suggests a compensatory stimulation of the rate of cholesterol synthesis from acetyl-CoA in response to probucol treatment. Thus it seems that the decrease in the basal adrenal glucocorticoid synthesis rate after probucol feeding was not due to a change in the expression level of essential steroidogenic enzymes but more likely due to impaired substrate (unesterified cholesterol) availability.

The liver is a key target organ of glucocorticoids, since via the action of the nuclear glucocorticoid receptor they are able to modulate the hepatic expression of genes involved in glucose, cholesterol, and bile acid metabolism (5, 27). Probucol did not change the expression of the glucocorticoid-responsive

genes apolipoprotein A4 and cholesterol 7 α -hydroxylase under basal feeding (nonstressed) conditions (Fig. 3C). This suggests that the decrease in plasma corticosterone levels upon probucol treatment was not associated with a concomitant change in hepatic glucocorticoid receptor activity under basal conditions.

As a first-line defense protective “stress” response to overcome inflammation-associated morbidity (i.e., sepsis) and mortality, adrenals readily secrete high levels of anti-inflammatory glucocorticoids upon exposure to LPS. To investigate the effect of probucol on the adrenal response to stress, probucol-fed and chow-fed mice were therefore exposed to a sublethal dose of LPS. As anticipated, exposure to LPS induced a rapid time-dependent increase in plasma corticosterone levels in control-treated mice (Fig. 4A). In probucol-fed mice, an acute rise in plasma corticosterone levels also occurred within the first hour after the injection with LPS. Subsequently, however, the plasma corticosterone concentration did not increase further and actually declined gradually in the probucol-treated mice, resulting in a fourfold lower ($P < 0.001$) plasma corticosterone level 3 h after the LPS injection and a significantly decreased overall corticosterone response compared with control-treated mice ($P < 0.001$; Fig. 4A). Thus it seems that probucol has no major effect on the “acute” (<1 h) steroidogenesis but does impair the “sustained” adrenal corticosterone response to stress.

The initial response of adrenocortical cells to stress (i.e., ACTH stimulation) is an increase in cholesterol esterase activ-

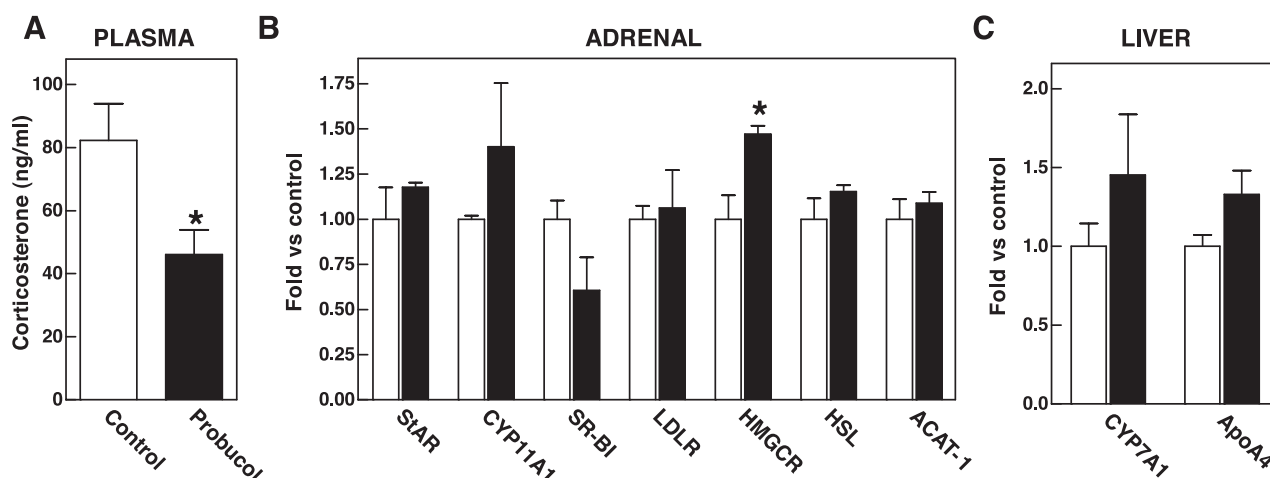


Fig. 3. The effect of probucol treatment on the plasma corticosterone level (A), the adrenal relative mRNA expression levels of steroidogenic acute regulatory protein (StAR), cytochrome P450_{scc} (CYP11A1), scavenger receptor BI (SR-BI), the LDL receptor (LDLR), hydroxymethylglutaryl-CoA reductase (HMGCR), hormone-sensitive lipase (HSL), and acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1) (B), and the hepatic relative mRNA expression levels of glucocorticoid-responsive genes cholesterol 7 α -hydroxylase (CYP7A1) and apolipoprotein A4 (apoA4) under basal “nonstressed” conditions (C). Data represent means $\pm$ SE of 5–10 mice. * $P < 0.05$ compared with controls.

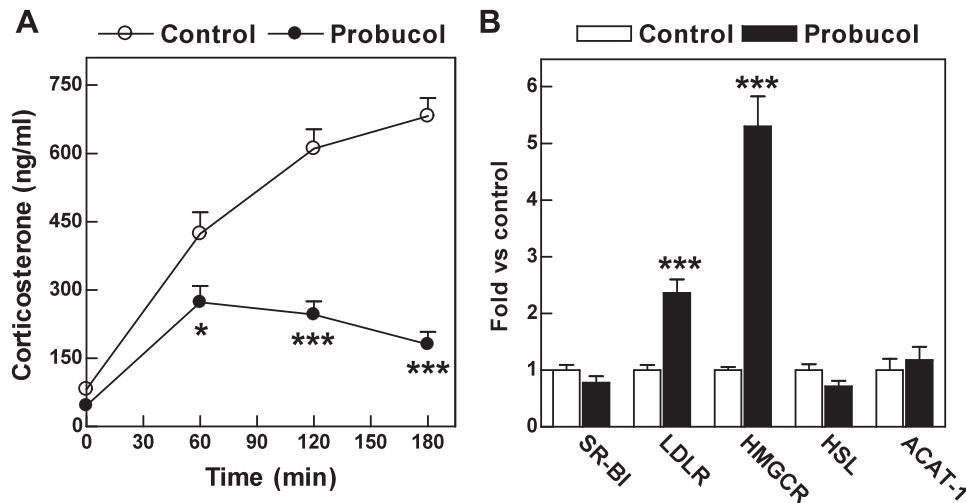


Fig. 4. *A*: the effect of probucol treatment on the corticosterone response to a lipopolysaccharide (LPS) challenge. Mice that were fed a regular chow diet supplemented with (●) or without 0.25% probucol (○) were injected with 50 μ g/kg LPS, and at the indicated times blood was drawn for analysis of plasma corticosterone levels. *B*: adrenal relative mRNA expression levels of SR-BI, the LDLR, HMGCR, HSL, and ACAT1 under LPS-induced “stress” conditions. Gene expression levels were determined in adrenals from probucol-fed (filled bars) and control mice (open bars) 4 h after a LPS (50 μ g/kg) challenge. Data represent means $\pm$ SE of 5 mice. * P < 0.05 and *** P < 0.001 compared with controls.

ity, presumably reflecting the physiological purpose of having a readily accessible store of substrate for steroidogenesis (25). Also, upon LPS exposure, no change in the mRNA expression level of cholesteryl ester synthesis (ACAT1) or catabolism (HSL) genes was observed between probucol-fed and control mice (Fig. 4*B*). However, we detected a 2.4- and 5.3-fold increase (P < 0.001 for both) in the adrenal relative mRNA expression of the LDL receptor and HMG-CoA reductase from probucol-fed mice compared with control-fed mice under high steroidogenic pressure conditions (Fig. 4*B*). This suggests a compensatory upregulation of a cholesterol delivery route by the LDL receptor and an increased cholesterol synthesis by HMG-CoA reductase in mice that exhibit relative lipoprotein deficiency in response to probucol treatment.

To determine whether the impaired response in glucocorticoid secretion to LPS due to probucol feeding was physiologically relevant in terms of the downstream glucocorticoid effects, the relative expression level of glucocorticoid receptor-responsive genes involved in inflammation was examined. Four hours after LPS exposure, proinflammatory cytokine IL-6 and TNF α mRNA expression was significantly higher in livers of probucol-treated mice compared

with control mice (P < 0.05 for both), whereas that of the activated macrophage marker MARCO (macrophage receptor with collagenous domain) did not differ between the two groups of mice (Fig. 5*A*). In parallel with the observed higher hepatic TNF α mRNA expression level, the LPS-induced TNF α level in plasma was significantly increased in probucol-treated mice (area under the curve: 498 $\pm$ 68 vs. 263 $\pm$ 28, P = 0.012; Fig. 5*B*). Combined, these findings suggest that a probucol-mediated reduction in plasma lipoprotein levels is associated with adrenal glucocorticoid insufficiency due to a lack of substrate availability, which is paralleled by a lower hepatic glucocorticoid receptor activity and an enhanced susceptibility to endotoxemia.

To prove that cholesterol is actually acquired from plasma lipoproteins by the adrenals under conditions of high steroidogenic pressure, the uptake of 3 H-labeled HDL cholesteryl ether (3 H]HDL-CET) was determined under “basal” and LPS-induced “stress” conditions. As shown in Fig. 6, a relatively high uptake of 3 H]HDL-CET per microgram of tissue was detected in the adrenals and liver of wild-type mice under basal conditions. This was to be expected since both the liver and adrenals express high mRNA levels of the HDL receptor SR-BI (1). As

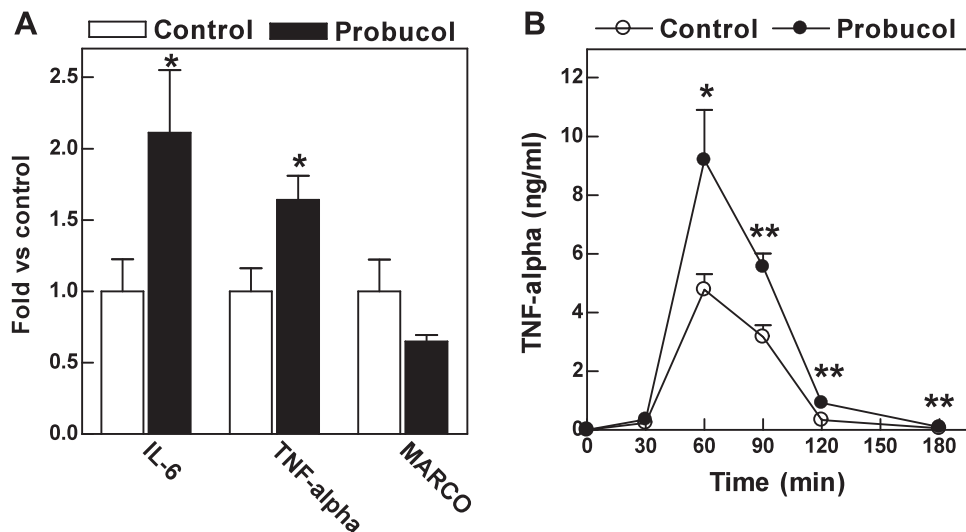


Fig. 5. *A*: the effect of probucol treatment on the hepatic relative mRNA expression level of IL-6, TNF α , and the activated macrophage marker MARCO (macrophage receptor with collagenous domain) under LPS-induced stress conditions. Gene expression levels were determined in livers from probucol-fed (filled bars) and control mice (open bars) 4 h after a LPS (50 μ g/kg) challenge. *B*: the plasma TNF α response to a LPS challenge in mice that were fed a regular chow diet supplemented with (●) or without 0.25% probucol (○). Data represent means $\pm$ SE of 5 mice. * P < 0.05 and ** P < 0.01 compared with controls.

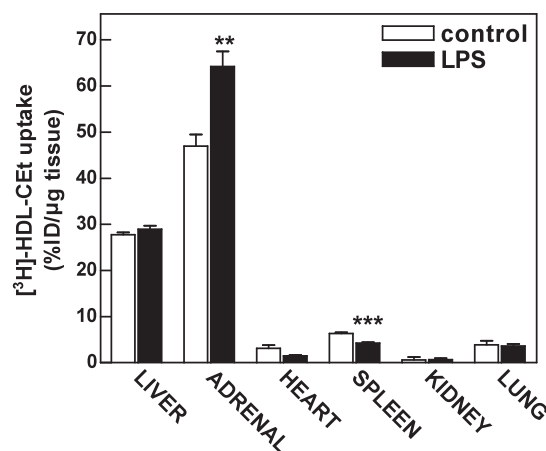


Fig. 6. The effect of LPS on the tissue uptake of ^3H -labeled HDL cholesteryl ether (^3H -HDL-CEt). One hour after a LPS (50 $\mu\text{g}/\text{kg}$) challenge, mice were given a bolus injection of ^3H -HDL-CEt. After 2 h, the radioactivity present in the indicated organs was determined in LPS-injected (filled bars) and control-injected mice (open bars) and expressed as a percent of the injected dose (%ID)/ μg tissue. Data represent means $\pm$ SE of 5 mice. ** $P < 0.01$ and *** $P < 0.001$ compared with controls.

anticipated from *in vitro* studies showing that LPS decreases the expression of SR-BI and the uptake of HDL in human and mouse macrophages (3), the uptake of ^3H -HDL-CEt was decreased by LPS in the macrophage-rich tissue spleen (-33% , $P < 0.001$; Fig. 6). Importantly, in accordance with a prominent role for lipoproteins in adrenal steroidogenesis, the uptake of ^3H -HDL-CEt was stimulated specifically in the adrenals ($+37\%$, $P = 0.006$; Fig. 6) in response to an increase in steroidogenic pressure upon LPS injection. In contrast, no change in the uptake of ^3H -HDL-CEt by the liver was detected, which argues against a nonspecific effect of LPS on the cellular uptake of HDL-associated cholesteryl esters via SR-BI.

DISCUSSION

Although *in vitro* studies have indicated that optimal adrenal steroidogenesis is dependent on both *de novo* synthesis of cholesterol and the catabolism of intracellular stored cholesteryl esters, it is conceivable that cholesterol derived from plasma lipoproteins makes a large contribution to the pool of cholesterol used for steroid hormone synthesis. In the current study, we have studied the effect of plasma lipoprotein depletion by the lipid-lowering drug probucol on adrenal steroidogenesis in mice. The transport of unesterified cholesterol from the outer mitochondrial membrane to the inner membrane by StAR is considered to be the rate-limiting step for glucocorticoid synthesis under basal conditions. Strikingly, we detected a 44% lower plasma corticosterone level in probucol-treated mice already in the basal setting. This suggests that low plasma lipoprotein levels impair the basal adrenal glucocorticoid synthesis rate probably because of a depletion of the intracellular cholesterol pool used by the StAR protein. In accordance, we observed that probucol-induced relative lipoprotein deficiency was associated with a marked depletion of neutral lipids in the zona fasciculata of the adrenal cortex, where glucocorticoid synthesis is executed. Importantly, under these conditions the adrenal mRNA expression of HMG-CoA reductase was increased. *De novo* adrenal cholesterol synthesis was apparently

not able to compensate for a loss of lipoprotein-derived cholesterol. Thus it can be suggested that plasma lipoproteins are required to maintain optimal steroidogenesis in mice and probably are the major contributor to glucocorticoid synthesis under basal conditions. Apolipoprotein A1 (apoA1)-knockout mice that lack the main apolipoprotein constituent of HDL, apoA1, exhibit a similarly lowered plasma HDL level, as detected in the current study. In contrast, basal plasma corticosterone levels are not different in apoA1-knockout mice compared with their wild-type littermates (23). In addition, mice that are deficient in the HDL receptor SR-BI and have an impaired adrenal uptake of HDL-associated cholesteryl esters also do not suffer from adrenal glucocorticoid insufficiency in the basal state (9, 10). Thus it seems that a combination of increased endogenous cholesterol synthesis and receptor-mediated uptake of lipoproteins other than HDL (i.e., LDL) can overcome glucocorticoid insufficiency in these animals. Importantly, a defect in uptake of LDL via the LDL receptor alone also does not affect adrenal steroidogenesis (15). *In vitro* studies have indicated that both HDL and LDL can be utilized as substrate for steroid synthesis in adrenocortical cells (2, 7, 8, 12). Combined, these findings suggest that both HDL and LDL may be substrates actually used for basal steroidogenesis in mice *in vivo*. However, the relative contribution of the two different lipoproteins for steroid hormone synthesis under basal conditions in wild-type mice remains to be established. Our current studies also show that probucol-induced relative lipoprotein deficiency is associated with a hampered protective adrenal corticosterone response to an inflammatory stimulus and an enhanced susceptibility to LPS-induced endotoxemia. From our previous findings in SR-BI-knockout mice with and without expression of the cholesteryl ester transfer protein (10), it can be expected that the diminished glucocorticoid stress response to LPS in probucol-treated mice can contribute to the lowered plasma HDL but not LDL level. We now also show that the uptake of HDL cholesteryl esters is increased specifically in the adrenals but not other SR-BI-expressing organs such as liver, indicating that cholesterol associated with HDL is indeed used efficiently for adrenal steroidogenesis under high steroidogenic pressure conditions (i.e., upon LPS exposure) in mice. Probuco has potent antioxidant and anti-inflammatory properties (16). However, probucol-treated mice do still exhibit a higher $\text{TNF}\alpha$ response upon exposure to a sublethal dose of LPS. Glucocorticoids, via the action of the nuclear glucocorticoid receptor, inhibit the expression of proinflammatory cytokines in immune cells such as dendritic cells and macrophages (17, 22). Glucocorticoid insufficiency as a result of adrenal removal (adrenalectomy) is associated with an enhanced susceptibility to endotoxemia in mice (22). Therefore, it is anticipated that the anti-inflammatory effect of probucol is overruled by the proinflammatory effect of adrenal glucocorticoid insufficiency induced by probucol in mice.

In a multivariate analysis in human patients with liver failure and patients post-liver transplantation, low HDL cholesterol levels were the only variable predictor of adrenal insufficiency (19). Furthermore, in critically ill patients, in whom the need for anti-inflammatory steroid hormone production is high, a low plasma HDL cholesterol level was associated with an impaired glucocorticoid (cortisol) response to synacthen stimulation (26). This suggests that HDL is also required for stress-induced adrenal glucocorticoid secretion in the human

situation, similar to what was observed for mice in the current study. In humans, partial HDL deficiency or hypo- α -lipoproteinemia, defined as an age- and sex-adjusted plasma HDL cholesterol concentration below the 10th percentile, can be caused by a defect in several genes essentially involved in HDL metabolism, including ATP-binding cassette transporter A1, apoA1, lecithin-cholesterol acyltransferase, and phospholipid transfer protein (11, 20). To further establish the importance of HDL for stress-induced adrenal glucocorticoid synthesis in the human situation, it will be of interest to measure the cortisol response to synacthen/ACTH stimulation in carriers of functional mutations in these gene products.

In conclusion, we have shown that relative lipoprotein deficiency in mice as a result of probucol feeding is associated with decreased adrenal cortex cholesterol levels, a lower basal and stress-induced plasma glucocorticoid level, and an increased susceptibility to LPS-induced inflammation. Therefore, it is suggested that plasma lipoproteins are required for optimal adrenal steroidogenesis and protection against endotoxemia in mice.

GRANTS

This research was supported by Top Institute Pharma (TIPharma project T2-110; M. Hoekstra, Z. Li, and T. J. C. Van Berkel), by Grants 2008T070 (M. Hoekstra), 2006B107 (S. J. A. Korpelaar), and 2001T4101 (Y. Zhao) from the Netherlands Heart Foundation, and by VIDI Grant 917.66.301 from the Netherlands Organization for Scientific Research (M. Van Eck). M. Van Eck is an established investigator for the Netherlands Heart Foundation (Grant 2007T056).

DISCLOSURES

All authors have nothing to disclose.

REFERENCES

- Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 271: 518–520, 1996.
- Brown MS, Kovanen PT, Goldstein JL. Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. *Recent Prog Horm Res* 35: 215–257, 1979.
- Buechler C, Ritter M, Quoc CD, Agildere A, Schmitz G. Lipopolysaccharide inhibits the expression of the scavenger receptor Cla-1 in human monocytes and macrophages. *Biochem Biophys Res Commun* 262: 251–254, 1999.
- Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipid adrenal hyperplasia. *Proc Natl Acad Sci USA* 94: 11540–11545, 1997.
- Cassuto H, Kochan K, Chakravarty K, Cohen H, Blum B, Olswang Y, Hakimi P, Xu C, Massillon D, Hanson RW, Reshef L. Glucocorticoids regulate transcription of the gene for phosphoenolpyruvate carboxykinase in the liver via an extended glucocorticoid regulatory unit. *J Biol Chem* 280: 33873–33884, 2005.
- Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). *J Biol Chem* 269: 28314–28322, 1994.
- Gwynne JT, Hess B. The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis. *J Biol Chem* 255: 10875–10883, 1980.
- Higashijima M, Nawata H, Kato K, Ibayashi H. Studies on lipoprotein and adrenal steroidogenesis: I. Roles of low density lipoprotein- and high density lipoprotein-cholesterol in steroid production in cultured human adrenocortical cells. *Endocrinol Jpn* 34: 635–645, 1987.
- Hoekstra M, Meurs I, Koenders M, Out R, Hildebrand RB, Kruijt JK, Van Eck M, Van Berkel TJ. 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: 738–745, 2008.
- Hoekstra M, Ye D, Hildebrand RB, Zhao Y, Lammers B, Stitzinger M, Kuiper J, Van Berkel TJ, Van Eck M. Scavenger receptor class B type I-mediated uptake of serum cholesterol is essential for optimal adrenal glucocorticoid production. *J Lipid Res* 50: 1039–1046, 2009.
- Kiss RS, Kavaslar N, Okuhira K, Freeman MW, Walter S, Milne RW, McPherson R, Marcel YL. Genetic etiology of isolated low HDL syndrome: incidence and heterogeneity of efflux defects. *Arterioscler Thromb Vasc Biol* 27: 1139–1145, 2007.
- Kovanen PT, Faust JR, Brown MS, Goldstein JL. Low density lipoprotein receptors in bovine adrenal cortex. I. Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured adrenocortical cells. *Endocrinology* 104: 599–609, 1979.
- Kraemer FB. Adrenal cholesterol utilization. *Mol Cell Endocrinol* 265–266: 42–45, 2007.
- Kraemer FB, Shen WJ, Harada K, Patel S, Osuga J, Ishibashi S, Azhar S. Hormone-sensitive lipase is required for high-density lipoprotein cholesteryl ester-supported adrenal steroidogenesis. *Mol Endocrinol* 18: 549–557, 2004.
- Kraemer FB, Shen WJ, Patel S, Osuga J, Ishibashi S, Azhar S. The LDL receptor is not necessary for acute adrenal steroidogenesis in mouse adrenocortical cells. *Am J Physiol Endocrinol Metab* 292: E408–E412, 2007.
- Kuzuya M, Kuzuya F. Probucol as an antioxidant and antiatherogenic drug. *Free Radic Biol Med* 14: 67–77, 1993.
- Li YH, Brauner A, Jonsson B, Van der Ploeg I, Söder O, Holst M, Jensen JS, Lagercrantz H, Tullus K. Inhibition of macrophage proinflammatory cytokine expression by steroids and recombinant IL-10. *Biol Neonate* 80: 124–132, 2001.
- Lin D, Sugawara T, Strauss JF 3rd, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science* 267: 1828–1831, 1995.
- Marik PE, Gayowski T, Starzl TE; Hepatic Cortisol Research and Adrenal Pathophysiology Study Group. The hepatoadrenal syndrome: a common yet unrecognized clinical condition. *Crit Care Med* 33: 1254–1259, 2005.
- Miller M, Rhyne J, Hamlette S, Birnbaum J, Rodriguez A. Genetics of HDL regulation in humans. *Curr Opin Lipidol* 14: 273–279, 2003.
- Morris MD, Chaikoff IL. The origin of cholesterol in liver, small intestine, adrenal gland, and testis of the rat: dietary versus endogenous contributions. *J Biol Chem* 234: 1095–1097, 1959.
- Pettipher ER, Labasi JM, Salter ED, Stam EJ, Cheng JB, Griffiths RJ. Regulation of tumour necrosis factor production by adrenal hormones in vivo: insights into the antiinflammatory activity of rolipram. *Br J Pharmacol* 117: 1530–1534, 1996.
- Plump AS, Erickson SK, Weng W, Partin JS, Breslow JL, Williams DL. Apolipoprotein A-I is required for cholesteryl ester accumulation in steroidogenic cells and for normal adrenal steroid production. *J Clin Invest* 97: 2660–2671, 1996.
- Rainey WE, Rodgers RJ, Mason JL. The role of bovine lipoproteins in the regulation of steroidogenesis and HMG-CoA reductase in bovine adrenocortical cells. *Steroids* 57: 167–173, 1992.
- Vahouny GV, Chanderbhan R, Hinds R, Hodges VA, Treadwell CR. ACTH-induced hydrolysis of cholesteryl esters in rat adrenal cells. *J Lipid Res* 19: 570–577, 1978.
- van der Voort PH, Gerritsen RT, Bakker AJ, Boerma EC, Kuiper MA, de Heide L. HDL-cholesterol level and cortisol response to synacthen in critically ill patients. *Intensive Care Med* 29: 2199–2203, 2003.
- Wang DP, Stroup D, Marrapodi M, Crestani M, Galli G, Chiang JY. Transcriptional regulation of the human cholesterol 7 α -hydroxylase gene (CYP7A) in HepG2 cells. *J Lipid Res* 37: 1831–1841, 1996.