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Hormone-Sensitive Lipase Deficiency in Mice Causes Lipid Storage in the Adrenal Cortex and Impaired Corticosterone Response to Corticotropin Stimulation

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Hormone-sensitive lipase (HSL, E.C.3.1.1.3, gene designation Lipe) is reportedly the major cholesteryl esterase of adrenal cortex. Because of the potential importance of cholesteryl ester hydrolysis in steroidogenesis, gene-targeted HSL-deficient mice were assessed for adrenal cortical morphology and function. Compared with control animals, HSL deficiency results in a marked accumulation of lipid droplets both in zona glomerulosa and zona fasciculata. In the zona fasciculata, lipid accumulation was observed progressively from the outer to the inner regions, culminating near the corticomedullary junction with the formation of syncytial-lipoid structures having the appearance of degenerative cells. These morphological changes did not significantly alter the basal levels of circulating corticosterone, but following ACTH stimulation, corticosterone levels were decreased ($P < 0.001$). The observation of normal basal corticosterone and aldosterone levels demonstrates that some free cholesterol for steroid synthesis can be produced independently of HSL. Taken together, these results indicate that HSL-deficient mice accumulate lipid droplets in such a way as to impair acute ACTH stimulation of corticosterone secretion. Such observations are also found in some forms of congenital adrenal hyperplasia. By extension, HSL deficiency may be a cause of hereditary adrenocortical hypofunction in humans. (*Endocrinology* 143: 3333–3340, 2002)

THE RATE-LIMITING STEP in steroidogenesis is the transport of free cholesterol from the outer to the inner mitochondrial membrane, mediated by the steroidogenic acute regulatory protein (StAR). This is followed by the conversion of cholesterol into pregnenolone, cleavage of the cholesterol side chain by cytochrome P450scc and its cofactors, and a series of reactions in the endoplasmic reticulum and mitochondria, allowing for rapid production and release of aldosterone and cortisol/corticosterone following stimulation (1, 2). In all animal species, the most potent stimulus of steroidogenesis is the hormone ACTH (3–5).

In the adrenal, free cholesterol is derived from two sources, *de novo* synthesis from acetyl-coenzyme A (6) or, to a greater extent, hydrolysis of cholesteryl esters stored in lipid droplets (7). The conversion of cholesteryl esters into free cholesterol may play an important role in controlling cholesterol availability for steroidogenesis. Free cholesterol is obtained by receptor-mediated endocytosis of low-density lipoprotein (LDL) (8) and cleavage of cholesteryl esters by lysosomal acid lipase and transport to the cytoplasm by a mechanism involving the Nieman Pick-C1 and Pick-C2 proteins (NPC 1 and NPC 2) (9), followed by reesterification in the cytoplasm by acetyl-coenzyme A/cholesterol acyl transferase (ACAT) (10–14). Cholesteryl esters derived from high-density lipoproteins (HDLs) can also be transferred to the cell interior via scavenger receptor B1. There is accumulating evidence indicating that lipid droplets are an important morphological characteristic reflecting the rate of steroid hormone production (15).

Hormone-sensitive lipase (HSL, E.C.3.1.1.3) (16–19) is a fatty acyl hydrolase that cleaves fatty acyl esters of cholesterol, steroid hormones, and glycerol (20). Until recently, research on HSL focused primarily on its role in adipose tissue, in which HSL mediates the release of fatty acids from triglycerides in response to various stimuli, including β-adrenergic stimulation. Following protein kinase A-dependent phosphorylation, HSL is translocated from the cytoplasm to the surface of lipid droplets from which it exerts its catalytic function (18).

HSL is also expressed in nonadipose tissues including skeletal muscle, myocardium, pancreatic β-cells, testicle, and adrenal gland (21, 22). In bovine adrenal, evidence suggests that HSL is the major cholesteryl esterase, consistent with a role for HSL in adrenal steroidogenesis (10, 18). Furthermore, steroid hormones can also be formed from fatty acyl esters, the hydrolysis of which has been hypothesized to yield a presynthesized, immediately available pool of steroid hormones (20, 23).

The adrenal gland consists of two endocrine tissues with distinct functions. The cortex synthesizes and secretes steroids, and the medulla produces catecholamines and several neuropeptides (4). The adult cortex is composed of three well-identified layers. The external zona glomerulosa is composed of four to five layers of cells arranged in clusters surrounding a central capillary. Adjacent is the zona fasciculata, which consists of parallel and radial columns of

Abbreviations: ACAT, Acetyl-coenzyme A/cholesterol acyl transferase; ACTH, adrenocorticotropin; HDL, high-density lipoprotein; HSL, hormone-sensitive lipase; LDL, low-density lipoprotein; NPC, Nieman Pick-C; SLS, syncytial lipoid structures; StAR, steroidogenic acute regulatory protein.

large cells separated by sinusoids; finally, adjacent to the medulla, the zona reticularis, forming an irregular network of small cells. Although all adrenocortical cells have the capacity to produce corticosterone in rodents, only the zona glomerulosa produces aldosterone (24).

It has previously been difficult to directly study the importance of cholesteryl and steroid ester hydrolysis in the normal structure and function of the adrenal gland. We recently reported the creation of a line of gene-targeted mice with complete deficiency of HSL (25). The aim of the present study was to investigate whether HSL deficiency affects adrenal gland morphology and function under basal conditions and following ACTH stimulation.

Materials and Methods

HSL-deficient mice

HSL-deficient mice were created by gene targeting (25). Homozygous HSL-deficient mice have no detectable HSL activity or protein. Mice described in this article were derived by breeding F1 mice (25) onto a C57BL/6 background for five generations and then crossing heterozygotes. Except where indicated, 6-month-old mice were used in this study. Mice were exposed to a 12-h light-dark cycle and fed *ad libitum* with Purina mouse chow 5051 (Agribrands Purina Canada, Woodstock, Ontario, Canada). Studies were approved by the Hôpital Ste-Justine animal care committee, which is accredited by the Canadian Council on Animal Care.

Previous studies have shown that the homozygous $HSL^{-/-}$ mice have no detectable HSL peptide or cholesteryl esterase activity in adipose tissue. $HSL^{-/-}$ mice have normal body weight but reduced abdominal fat mass, compared with normal littermates (25). In addition, the $HSL^{-/-}$ mice were normoglycemic and normoinsulinemic under basal conditions but showed a 30% reduction of circulating free fatty acids with respect to control and heterozygous animals after an overnight fast. HSL-null mice were also glucose intolerant and displayed a lack of a rise in plasma insulin after a glucose challenge, suggesting that they are insulin resistant (26).

ACTH stimulation test

ACTH (α-1-24 corticotropin) (Cortrosyn, Organon Canada Ltd., Scarborough, Ontario, Canada), 0.8 μg/g body weight (25 μg/30 g per 0.2 ml) was injected ip to conscious fed mice. Then 100 μl blood were collected from the tail vein in heparinized capillary tubes before and 1 h after ACTH injection, centrifuged at 5100 $\times$ *g* for 20 min at 4 C, and plasma was stored at −20 C until steroid measurements by RIA.

Metabolite and hormone measurements

Corticosterone and aldosterone were measured directly in plasma with commercial RIA kit (corticosterone kit, Medicorp, Montréal, Québec, Canada) and aldosterone (Intermedico, Montréal, Québec, Canada). Glycemia was measured with a portable blood glucose meter (Medisense, Inc., Bedford, MA).

Histological procedures

For phase-contrast microscopy, adrenal glands were immersed immediately after removal in 4% paraformaldehyde for 24 h, embedded in paraffin, cut into 3-μm sections, and dehydrated through xylene and graded alcohol series for standard hematoxylin-eosin staining. Slides were mounted in nonaqueous mounting media (VectaMount, Vector Laboratories, Burlingame, CA). Images were observed using an Eclipse 300 microscope (Nikon, Montréal, Québec, Canada) equipped with a CoolSnap color digital camera. Acquired images were processed and analyzed using Adobe Photoshop 4.0 (Delray Beach, FL).

For electron microscopy, mice were anesthetized with sodium pentobarbital (Somnitol, MTC Pharmaceuticals, Hamilton, Canada). Mice were perfused-fixed through the heart with 5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After the perfusion, adrenal glands were removed, cut into 1-mm^3 pieces, processed, embedded in Epon, and prepared for electron microscopy as described previously (3).

The number and volume of lipid droplets were calculated as described by Nussdorfer *et al.* (27). The estimation of the average volume of lipid droplets was based on the assumption of a spherical shape because phase-contrast or electron microscopy shows circular structures. The volume was calculated by the formula $4/3\ \pi\ (D_{LD}/2)$ (3). The diameter distribution (D_{LD}) was determined on the electron micrographs as indicated in Table 1.

Data analysis

The data are presented as means ± SEM from the number of animals or cells studied. Control and $HSL^{-/-}$ groups were compared by the unpaired two tailed *t* test.

Results

Adrenal gland morphology in HSL-deficient mice

Histological examination of 6-month-old $HSL^{-/-}$ mice using phase-contrast microscopy indicated major modifications in the adrenal cortex. Cells from the zona glomerulosa and zona reticularis of $HSL^{-/-}$ mice exhibited minor modifications, compared with control mice (Fig. 1, A and B). In contrast, fasciculata cells from $HSL^{-/-}$ mice appeared hypertrophied because of an accumulation of intracellular lipid droplets (Fig. 1B). Larger aggregates of lipid droplets, which we termed syncytial lipoid structures (SLS), occurred at the junction between the zona reticularis and medulla (Fig. 1C), sometimes invading the medulla. Of note, chromaffin cells themselves were not modified (Fig. 1C, *black arrow).* When compared with the outer portion of adrenals from control mice (Fig. 2, A and B), cells adjacent to zona glomerulosa of $HSL^{-/-}$ mice display an abundance of small lipid droplets, which engorge the cell, displacing and compressing the nucleus against the cell membrane, a phenotype found in all cells (Fig. 2C, *arrow).* Deeper in the cortex, lipid droplets were larger (Fig. 2D, *black arrows)* and small SLS appeared (Fig. 2D, *white arrows).*

Electron microscopy examination extended these observations and indicated that glomerulosa cells were also affected by HSL deficiency. The size of lipid droplets in glomerulosa cells of $HSL^{-/-}$ mice was increased (5.2-fold increase, compared with controls, Table 1), but their number was decreased (3.5-fold decrease, Table 1). Moreover, lipid

TABLE 1. Effect of HSL deficiency on the number and volume of lipid droplets in the adrenal cortex of male mice

	$HSL^{+/+}$ mice		$HSL^{-/-}$ mice	
	No. of lipid droplets per cell	Volume of lipid droplets (μm^3)	No. of lipid droplets per cell	Volume of lipid droplets (μm^3)
Zona glomerulosa	54.5 ± 2.6 (4)	0.373 ± 0.052 (78)	15.3 ± 1.98 (7)	11.93 ± 0.47 (60)
Zona fasciculata	69.7 ± 4.5 (4)	0.535 ± 0.072 (92)	36.0 ± 4.83* (8)	1.67 ± 0.173* (110)

Cells from the zona fasciculata used for measurements were from the adjacent portion of zona glomerulosa, where the lipid droplets have similar sizes. Analyses were performed on pictures similar to that illustrated in Figs. 3 and 4, A and B. [a] $P < 0.001$, compared with its own $HSL^{+/+}$ control.

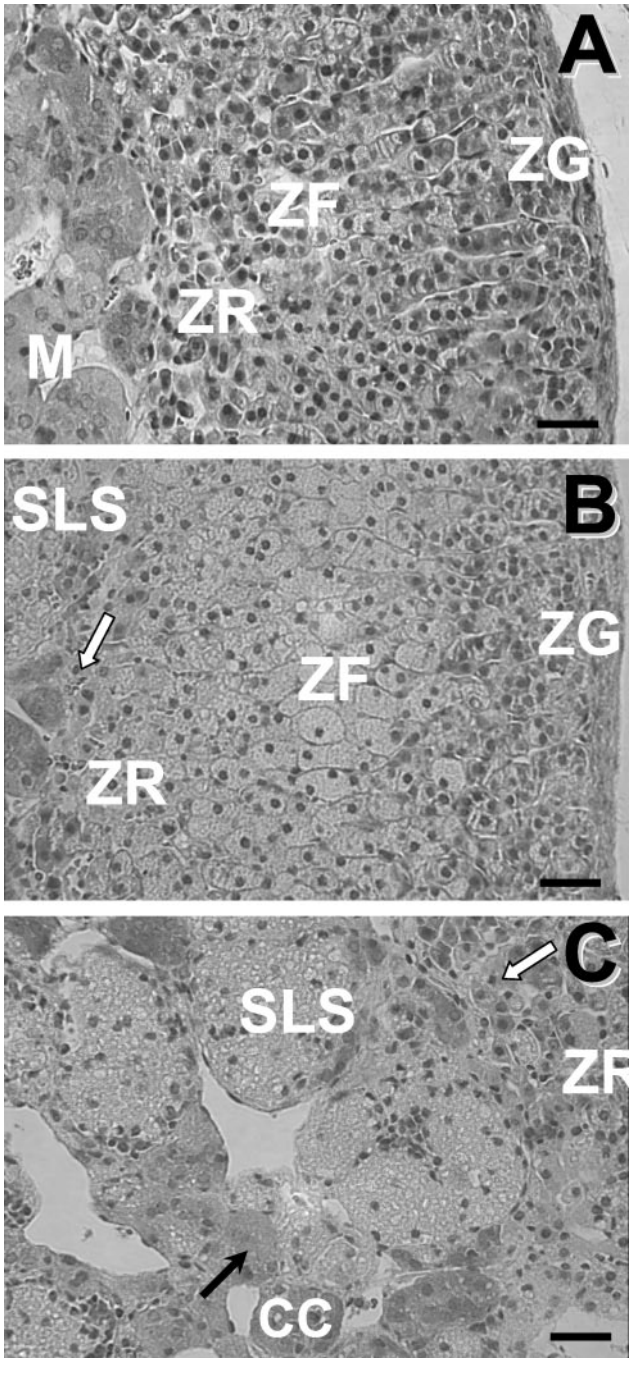

FIG. 1. Histological comparisons of adrenal glands from male mice control and hormone-sensitive lipase deficiency $HSL^{-/-}$ mice. Whole gland sections from 6-month old mice were fixed in 4% paraformaldehyde, embedded in paraffin, and sliced into 3-μm sections and processed by hematoxylin-eosin staining as described in *Materials and Methods*. A, Adrenal cortex of control $HSL^{+/+}$ mice adrenal gland. B, Adrenal cortex from hormone-sensitive lipase-deficient ($HSL^{-/-}$) mice, exhibiting enlarged fasciculata cells and the presence of SLS in the inner part of the cortex. C, Inner part of the adrenal cortex from hormone-sensitive lipase-deficient mice, characterized by the presence of syncytium lipoid structures, surrounding cells from zona reticularis (*white arrow*) and chromaffin *cells* (*black arrow*), which retain their control morphological appearance. ZG, Zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis; M, medulla; CC, chromaffin cells. Images were taken with an objective at ×40 and are representative illustrations of six different adrenal glands. *Scale bars*, 100 μm.

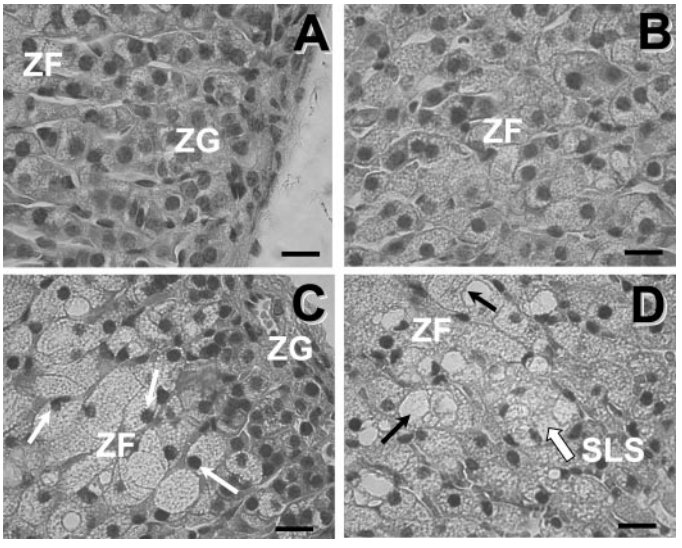

FIG. 2. Histological comparisons of fasciculata cells between adrenal glands from male control mice and hormone-sensitive lipase-deficient $HSL^{-/-}$ mice. Whole gland sections from 6-month-old mice were fixed in 4% paraformaldehyde, embedded in paraffin, and sliced into 3-μm sections, and processed by hematoxylin-eosin staining as described in *Materials and Methods*. A, Zona glomerulosa from control adrenal gland, B, Zona fasciculata cells from control adrenal gland. C, External portion of the adrenal cortex from hormone-sensitive lipase-deficient mice, exhibiting large fasciculata cells, containing homogenous population of small lipid droplets; *arrow* indicates the location of nucleus near the cell membrane. D, Internal portion of zona fasciculata in which cells are characterized by a heterogeneous population of lipid droplets of various sizes (*black arrows*) and formation of SLS (*white arrow*). ZG, Zona glomerulosa; ZF, zona fasciculata. Images were taken ×100 and are representative illustrations of six different adrenal glands. *Scale bars*, 26 μm.

droplets were often irregular, with a tendency to fuse (Fig. 3, A and B). The zona fasciculata was characterized by a progressive enlargement of the lipid droplets from the outer to the inner portions (Fig. 4). Near the zona glomerulosa, several cells, but not all (one of four in Fig. 4B), had fewer (Table 1) and larger (3.1-fold increase, Table 1) lipid droplets than cells from similar regions of control $HSL^{+/+}$ mice (Fig. 4A). Deeper in the gland, lipid droplet size was increasingly heterogeneous, with some cells being occupied by fused lipid droplets (Fig. 4D) and a small number of large vacuoles near zona reticularis (Fig. 4E). Moreover, cell membranes appeared irregular; the pericapillary spaces were enlarged, with the presence of microvilli whose number and length increased from the periphery to the central portion of the cortex *(arrows,* Fig. 4, C–E). Finally, at the junction between zona reticularis and medulla, the syncytial lipoid structures were revealed to be aggregates of damaged cells in which lipids accumulated as rectangular crystalloid bodies and cytoplasmic structures were profoundly altered (Fig. 4, F and G).

Examination under higher magnification revealed changes in mitochondria and lipid droplets. Figure 5A, from zona glomerulosa of control mice, illustrated normal mitochondria with elongated cristae and lipid droplets surrounded by dense material. In the $HSL^{-/-}$ mice (Fig. 5B), the number of mitochondria cristae increased, with a mean value of 8.22 ± 0.81 (n = 56) for the $HSL^{-/-}$ adrenals, compared

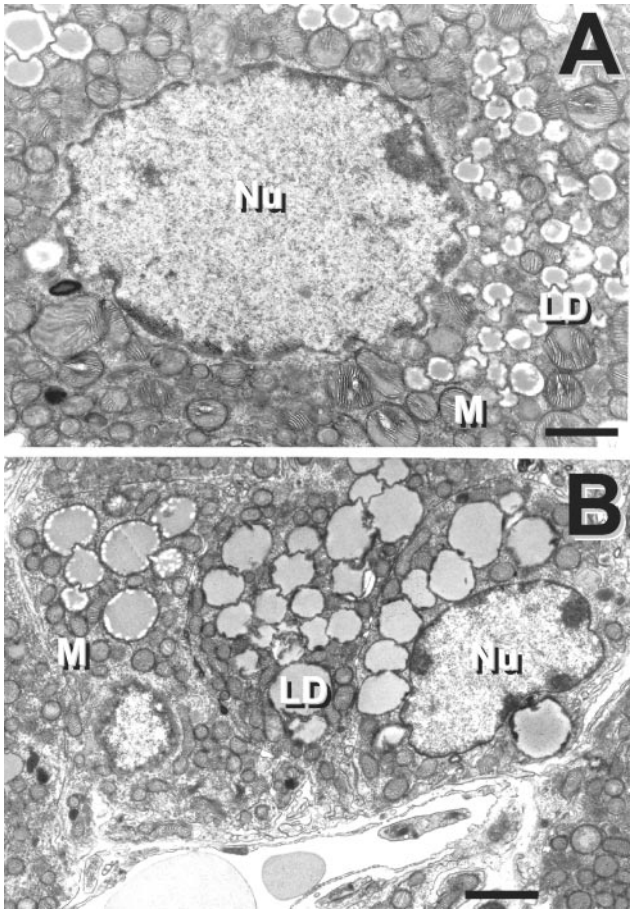

FIG. 3. Electron microscopy of glomerulosa cells from control male mice and from hormone-sensitive lipase-deficient $HSL^{-/-}$ mice. Glomerulosa cell from control $HSL^{+/+}$ mice (A) was taken at a higher magnification (×10,250) than the glomerulosa cell from HSL-deficient mice (B) (×5,000). In spite of such differences, lipid droplets in B appeared 3-fold larger than on the micrographs in A. *Scale bars,* 1 μm; M, mitochondria; LD, lipid droplets; Ny, nucleus.

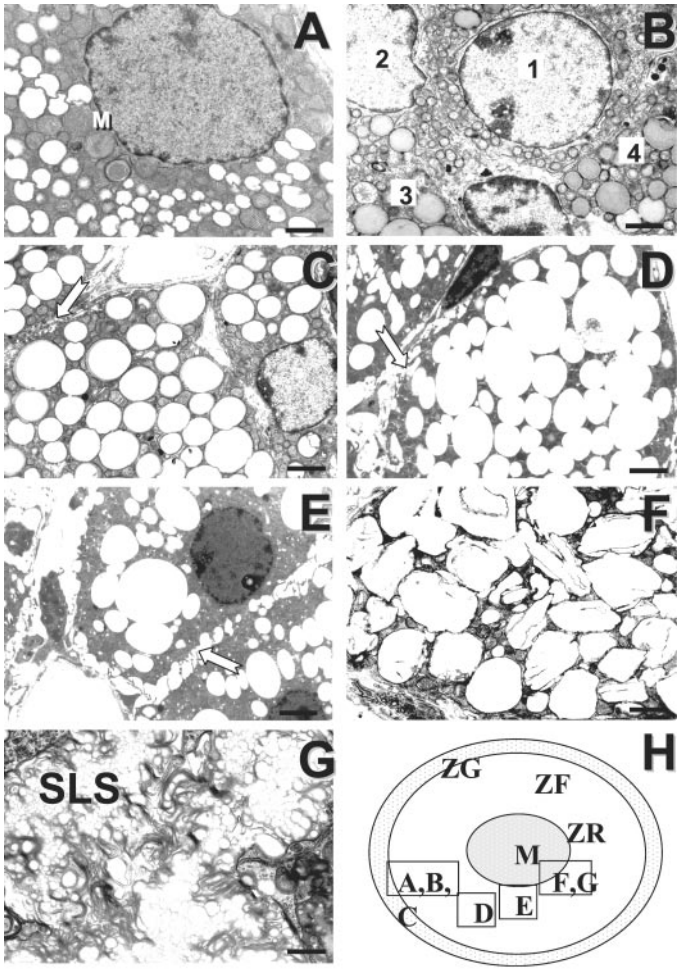

FIG. 4. Electron microscopy of fasciculata cells from control male $HSL^{+/+}$ mice (A) and hormone-sensitive lipase-deficient $HSL^{-/-}$ mice (B–G). A, A control fasciculata cell from a control $HSL^{+/+}$ mice (×10,250). B–G, Fasciculata cells from HSL-deficient mice (B and F, ×5,000; C, D, E, and G, ×10,250). Images illustrate cells from the outer to the inner portion of adrenal cortex, as shown in the illustrated diagram in H. In B, the cell numbered 1 is not altered, but cells numbered 2, 3, and 4 have fewer but larger lipid droplets. A progressive increase in size of the lipid droplets was observed from B and C to F. Near the medulla, SLS were revealed to be aggregates of damaged cells in which lipids accumulate as crystal-rectangular shaped. *Arrows* (C–E) indicate the progressive enlargement of the pericapillary spaces. M (A) indicates typical mitochondria from mouse fasciculata cells showing circular collection of cisternae. *Scale bars*, 1 μm.

with 5.54 ± 0.4 (n = 56) per mitochondria in the control adrenal glands ($P < 0.001$), and they appeared dilated. In addition, the surface of the lipid droplets in $HSL^{-}/^{-}$ cells was devoid of dense material.

Those effects were more pronounced in females, in which zona fasciculata were more hypertrophied with syncytial-lipoid structures greater in size and number (data not shown), consistent with previous observations that in females, adrenal cholesterol ester accumulation is higher than in males (28).

Plasma corticosterone and aldosterone levels under basal conditions and following ACTH stimulation

Steroid hormone levels were measured before and 1 h after ACTH administration in male and female animals to evaluate the capacity of the $HSL^{-/-}$ adrenal glands to respond to acute ACTH stimulation. In 6-month-old male animals, the mean basal levels of corticosterone were slightly lower, but not statistically significant, in $HSL^{-/-}$ mice, compared with $HSL^{+/+}$ mice (0.34 ± 0.05 *vs.* 0.47 ± 0.08 nmol/ml, respectively, n = 9, $P = 0.10$). After ACTH stimulation, these levels were significantly lower in $HSL^{-/-}$ mice, compared with controls (0.70 ± 0.04 *vs.* 1.02 ± 0.04 nmol/ml, respectively, n = 9, $P < 0.001$) (Fig. 6A). Similar observations were found in $HSL^{-/-}$ females, in which basal levels of corticosterone were not significantly different but following ACTH stimulation were significantly lower than levels in normal controls. This was observed both in mice aged 6 months (Fig. 6B) and 2 months (post-ACTH corticosterone levels, $HSL^{+/+}$, 1.43 ± 0.06 nmol/ml; $HSL^{-/-}$, 1.03 ± 0.07 nmol/ml; $P = 0.001$). Aldosterone levels were assessed in females. Levels did not differ under basal conditions between the two strains of animals (1.02 ± 0.14 *vs.* 1.00 ± 0.18 pmol/ml). Lower mean aldosterone levels were observed in $HSL^{-/-}$ mice with re-

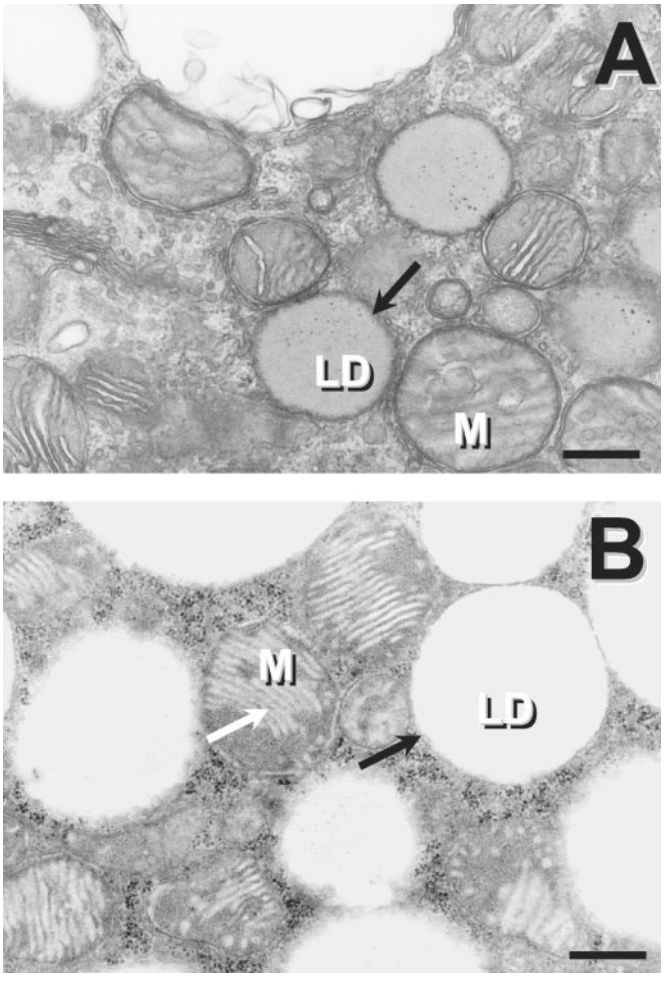

FIG. 5. Comparative electron microscopy of mitochondria and lipid droplets of glomerulosa cells from control male $HSL^{+/+}$ mice (A) and hormone-sensitive lipase-deficient $HSL^{-/-}$ mice (B). Micrographs were taken at a magnification ×100,000. In contrast to the control mice, the mitochondria from HSL-deficient mice exhibited dilated cristae (*white arrow*). In addition, the surface of the lipid droplets were devoid of dense material (*black arrow*). *Scale bars*, 100 nm.

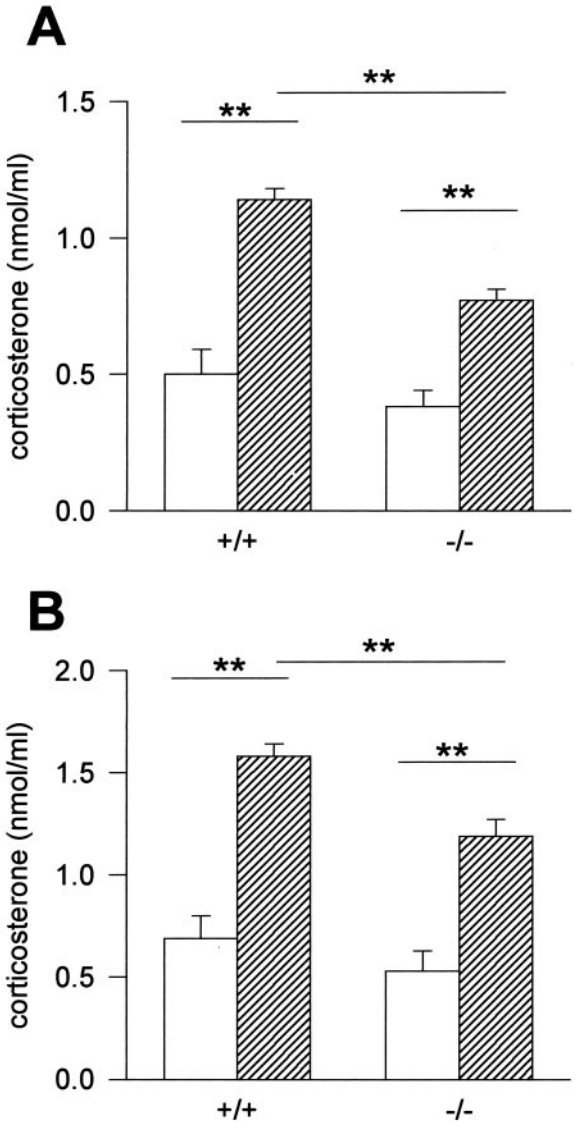

FIG. 6. Plasma corticosterone levels from control $HSL^{+/+}$ mice and hormone-sensitive lipase-deficient mice. Plasma corticosterone secretion were measured before (□) and after 1-h ACTH administration (0.8 μg/g) (■) in 6-month-old male (A) and female (B) animals. Results are a mean ± SE of nine animals. **, $P < 0.001$, the statistical significance between control and ACTH-stimulated mice or between ACTH-stimulated $HSL^{+/+}$ and $HSL^{-/-}$ mice.

spect to $HSL^{+/+}$ animals, but this was not significant (data not shown).

Blood glucose levels before and after ACTH stimulation did not differ significantly between $HSL^{-/-}$ mice (10.1 ± 1.1 *vs.* 10.2 ± 0.9 mmol/liter before and after ACTH stimulation, respectively) and controls (9.7 ± 0.5 *vs.* 11.0 ± 0.8 mmol/liter, respectively).

Discussion

The profound morphologic alterations in HSL-deficient mice demonstrate the physiological importance of HSL in adrenals and are consistent with previous evidence, suggesting that HSL is the major or only cholesteryl esterase of the adrenal cortex (18). HSL-deficient adrenals showed a marked accumulation of lipid droplets in both zona glomerulosa and zona fasciculata. Glomerulosa cells and fasciculata cells adjacent to the zona glomerulosa have similar ultrastructural modifications, characterized by a reduced number of large-sized lipid droplets. The zona fasciculata demonstrates a gradient of increasing size of cells and lipid droplets, proceeding from the outer to the inner regions of the zona fasciculata and culminating near or within the medulla in the degenerative-appearing multicellular formations that we termed syncytial lipoid structures. In addition, although $HSL^{-/-}$ deficiency had no significant effect on basal corticosterone levels, the plasma levels following ACTH stimulation are lower in $HSL^{-/-}$ than in control $HSL^{+/+}$ mice.

Lipid accumulation in the zona fasciculata and formation of cholesterol-laden lipoid structures in zona reticularis also occurs in other states of decreased cholesterol utilization. Examples include chronic treatment with aminoglutethimide, an inhibitor of the cholesterol enzyme P450scc (29) and

congenital lipoid adrenal hyperplasia in humans and mice, a disorder caused by hereditary deficiency of the StAR protein (30). However, in contrast to these conditions, HSL-deficient mice do not accumulate cholesterol or other lipids in lysosomes (15, 29). The observations in HSL deficiency are consistent with predictions of the metabolic role of HSL, suggesting that in HSL deficiency, cholesterol esters accumulate in lipid droplets and are not delivered into the cytoplasm, as is the case when the deficiency is due to a metabolic block. The observation of increased lipid accumulation and necrotic lipoid cells in the perimedullar region and medulla supports the migration theory of adrenocortical cell renewal and differentiation (31), which posits that young zona fasciculata cells arise at the zona glomerulosa junction and then migrate through zona fasciculata and zona reticularis. The finding of degenerative cellular aggregates suggests that intracellular lipid accumulation became toxic for steroidogenic cells. However, syncytial lipoid structures invade the medulla without affecting the morphology of adjacent chromaffin cells, suggesting that the syncytial lipoid structures and their contents are not themselves toxic for neighboring cells.

Our observations permit several predictions to be made of the metabolic consequences of HSL deficiency, many of which can now be tested directly. The finding of dilated pericapillary spaces and an increased number and length of microvilli suggest a compensatory change to increase HDL and/or LDL uptake, as observed under ACTH stimulation (15, 29). However, the modest decrease in steroidogenesis in $HSL^{-/-}$ mice contrasts with the severe histological changes. The persistent steroidogenesis can be explained by an increased steroidogenic activity of the intact cells, as suspected by the ultrastructural modifications shown in mitochondria of the $HSL^{-/-}$ zona glomerulosa cells in which cristae appeared dilated and increased in number. It differs strikingly from StAR-deficient mice, which die during the first days of life because of markedly deficient adrenal and gonadal steroidogenesis (32, 33). In mice, HDL normally provide most of the free cholesterol for steroid production, with lesser contributions from LDL and *de novo* synthesis (28, 34–36). It is plausible that the lack of cholesteryl ester hydrolysis may stimulate a compensatory decrease in ACAT activity or increases in LDL uptake or in endogenous cholesterol synthesis (Fig. 7). Of note, the high level of circulating cholesterol in HSL-deficient mice (25, 37) could further enhance lipid accumulation in adrenocortical cells. In HSL deficiency, ACTH stimulation, which produces a coordinated increase of lipoprotein uptake and processing (38) and cholesterol synthesis (39–41), may increase lipid accumulation and hence be deleterious in HSL-deficient mice.

There are at least two mechanisms by which adrenocortical hypofunction may arise in HSL deficiency. In the first, deficient steroidogenesis may arise directly from the metabolic block, *i.e.* from failure of cholesteryl ester hydrolysis to provide sufficient free cholesterol for steroidogenesis. By the second mechanism, lipid accumulation may disrupt adrenocortical cells, causing a secondary decrease in their function. Of note, the one significant functional consequence documented in HSL deficiency, *i.e.* subnormal corticosterone levels following ACTH challenge, coincides with the anatomical site of the most severe lipid accumulation (zona fasciculata). We speculate that membrane lipid composition may be changed to increase membrane fragility and/or that mechanical factors arising from the marked increase in lipid content and cell size, directly disrupting the cell.

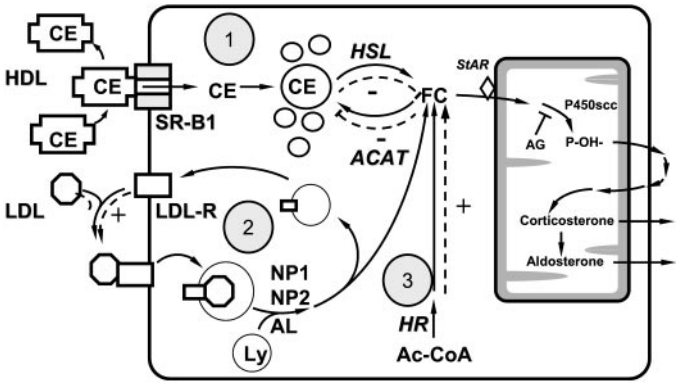

FIG. 7. Cholesteryl ester metabolism in mouse adrenal cortex. Three pathways lead to free cholesterol: 1) cholesteryl ester internalization from HDL particles, followed by cleavage by HSL; 2) LDL receptor-mediated cycling, involving endocytosis of the LDL particle, cleavage of cholesteryl esters by acid lipase and transport of free cholesterol to the cytoplasm involving the NPC1 and NPC2 proteins, and recycling of the LDL receptor to the cell surface; and 3) *de novo* synthesis of cholesterol from acetyl-coenzyme A. If esterified by ACAT, free cholesterol from any source must be hydrolyzed by HSL before use for steroidogenesis. The lack of cholesteryl ester hydrolysis in $HSL^{-/-}$ mice may stimulate a compensatory decrease in ACAT activity or stimulate an increase in LDL uptake or endogenous cholesterol synthesis. In addition, endogenous synthesis of cholesterol might bypass the lipid droplet pathway and be used directly for steroidogenesis. All these proposed events are represented by *dashed lines*. Ac-CoA, Acetyl-coenzyme A; AG, aminoglutethimide; AL, acid lipase; CE, cholesteryl esters; FC, free cholesterol; HR, HMG-Coenzyme A reductase; LDL-R; LDL receptor; Ly, lysosome; P450scc, cholesterol side chain cleavage enzyme; SR-B1, scavenger receptor B1.

During preparation of this manuscript, Kraemer *et al.* (42) reported that neutral cholesteryl esterase activity was severely deficient in an independently derived line of HSL-deficient mice, directly showing HSL to be the major adrenocortical neutral cholesteryl esterase. In contrast with their earlier report (37), they also found a statistically significant decrease in post-ACTH corticosterone levels in HSL-deficient mice, compared with controls, although this was present only in females. This article also provided further evidence for an increase in cholesteryl ester content in female adrenals of normal rats, with respect to normal male controls. Although the morphological changes in HSL-deficient adrenals were less severe in males than in females, we found highly significant differences between post-ACTH levels of corticosterone levels in both sexes. This discrepancy between the results of Osuga *et al.* (37) and Kraemer *et al.* (42) and our results is difficult to explain. It may involve the age of mice (we studied 6-month-old males), whereas age was not specified in the most recent publication of Kraemer *et al.* (42) and was less than 3 months in the previous report (37). However, in 2-month-old HSL-deficient female mice, we observed a similar low level of post-ACTH corticosterone increase as in older mice. The differences may also be due to parental background effects retained in each strain during crosses to the C57BL background. In any case, the recent report of

Kraemer *et al.* (42) provides independent confirmation of the importance of the morphological and functional observations presented here.

To date, HSL deficiency has not been described in humans. The adrenal manifestations of human HSL deficiency are expected to be qualitatively similar to those of mice but possibly less severe. Endogenous synthesis, by which cholesterol might bypass the lipid droplet and be used directly for steroidogenesis, accounts for only about 4% of adrenal steroidogenesis in mice, a species in which uptake of cholesterol esters from HDL predominates (34–36, 43). In mice, recent studies confirm the importance of HDL as the main plasma lipoprotein because HDL-deficient mice with mutant apolipoprotein A1 have a reduced steroidogenic response to stress (28). By comparison, experiments conducted with cultured human adrenal cells indicated that LDL uptake and endogenous cholesterol synthesis account for a larger fraction of steroidogenesis (44).

In addition to progressive lipoid degeneration and possible insufficiency of the adrenal cortex, HSL-deficient humans may have similar abnormalities in others tissues as $HSL^{-/-}$ mice, including abnormal adipose tissue histology and function (25), reduced glucose-stimulated insulin secretion (26), and male infertility caused by azoospermia (45). Of note, infertility in HSL-deficient male mice does not result from abnormal steroid hormone synthesis (37, 45) but rather from a direct role of HSL in spermatogenesis (45). HSL deficiency could reasonably be considered in patients suspected of congenital lipoid adrenal hyperplasia in whom StAR deficiency cannot be documented, particularly if this is associated with abnormalities in other tissues similar to those of murine HSL deficiency.

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