Increased glucocorticoid activation during mouse skin wound healing

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Abstract
Glucocorticoid (GC) excess inhibits wound healing causing increased patient discomfort and infection risk. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activates GCs (converting 11-dehydrocorticosterone to corticosterone in rodents) in many tissues including skin, where de novo steroidogenesis from cholesterol has also been reported. To examine the regulation of 11β-HSD1 and steroidogenic enzyme expression during wound healing, 5 mm wounds were generated in female SKH1 mice and compared at days 0, 2, 4, 8, 14, and 21 relative to unwounded skin. 11β-HSD1 expression (mRNA and protein) and enzyme activity were elevated at 2 and 4 days post-wounding, with 11β-HSD1 localizing to infiltrating inflammatory cells. 11β-HSD2 (GC-deactivating) mRNA expression and activity were undetectable. Although several steroidogenic enzymes displayed variable expression during healing, expression of the final enzyme required for the conversion of 11-deoxycorticosterone to corticosterone, 11β-hydroxylase (CYP11B1), was lacking in unwounded skin and post-wounding. Consequently, 11-deoxycorticosterone was the principal progesterone metabolite in mouse skin before and after wounding. Our findings demonstrate that 11β-HSD1 activates considerably more corticosterone than is generated de novo from progesterone in mouse skin and drives GC exposure during healing, demonstrating the basis for 11β-HSD1 inhibitors to accelerate wound repair.

Key Words
- 11β-hydroxysteroid dehydrogenase
- steroidogenesis
- glucocorticoid
- skin
- wound healing

Introduction
Glucocorticoid (GC) excess, whether of endogenous (e.g., Cushing’s syndrome, stress) or exogenous (e.g., topical or systemic therapy) origin, adversely affects multiple elements of wound healing, causing prolonged discomfort and increased infection risk. Mechanisms include interference with epidermal growth factor signaling, epidermal cell migration and re-epithelialization (Lee et al. 2005), keratinocyte growth factor signaling and cross-talk between dermal fibroblasts and epidermal keratinocytes (Brauchle et al. 1995, Chedid et al. 1996), transforming growth factor-β signaling (Frank et al. 1996), and the IGF1 system (Bitar 2000), although the complex associations between these inter-related pathways are not fully understood.

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a bidirectional enzyme that, in intact cells, generates corticosterone from 11-dehydrocorticosterone (11-DHC) in rodents (and cortisol from cortisone in humans), regulating local GC availability. In contrast, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) mediates the opposing conversions and is expressed predominantly in mineralocorticoid-responsive tissues (e.g., kidney),
preventing inappropriate activation of the mineralocorticoid receptor by GC (Quinkler & Stewart 2003). In placenta, 11β-HSD2 also protects the developing embryo from excessive exposure to maternal circulating GC (Brown et al. 1996). 11β-HSD1 is ubiquitously expressed with well-defined physiological and pathological roles in the liver, brain, muscle, bone, and adipose tissue (Tomlinson et al. 2004, Cooper & Stewart 2009). Recently, 11β-HSD expression and activity were also characterized in human and rodent skin (Tiganescu et al. 2011), a tissue in which GC metabolism remains relatively unexplored.

Previous studies have also established a role for extra-adrenal GC generation from cholesterol in skin (reviewed by Slominski et al. 2012). Expression of steroidogenic enzymes in this pathway, including cytochromes CYP11A1, CYP17A1, and CYP21A2 has been detected in human skin (Slominski et al. 1996), with cortisol synthesis detected in hair follicles (Ito et al. 2005), keratinocytes (Cirillo & Prime 2011, Hannen et al. 2011, Vukelic et al. 2011), melanocytes (Slominski et al. 2005a), and fibroblasts (Slominski et al. 2005b, 2006). Corticosterone synthesis has also been detected in rodent skin (Slominski et al. 2000a).

Interestingly, early studies demonstrated GC metabolism during wound healing (Nabors & Berliner 1969) two decades before the purification and cloning of 11β-HSD (Lakshmi & Monder 1988, Agarwal et al. 1989). However, although a recent study has identified a possible role for de novo GC synthesis during wound repair (Vukelic et al. 2011), the regulation of 11β-HSDs during wound healing remains unexplored.

In this report, we examined 11β-HSD and steroidogenic enzyme expression and activity during mouse skin wound healing. Although corticosterone synthesis from progesterone was detectable at low levels, 11β-HSD1-mediated corticosterone generation was significantly greater. Furthermore, 11β-HSD1 levels increased during wound healing supporting the results of previous studies by us and others, demonstrating accelerated wound healing following local or global 11β-HSD1 blockade (Tiganesco et al. 2013). Our findings indicate that increased 11β-HSD1 activity originates from the infiltrating leukocytes during the inflammatory stage of wound healing. Therefore, 11β-HSD1 may also contribute to the pathology of chronic wounds characterized by persistent inflammation. Collectively, these findings demonstrate that elevated 11β-HSD1 may limit wound repair and support the use of local 11β-HSD1 blockade to promote wound healing.

Materials and methods

Declarations

Studies presented in this paper were approved by the Institutional Animal Care and Use Committee and San Francisco Veterans Affairs Medical Center Veterinary Medical Unit. Materials were obtained from Sigma-Aldrich unless otherwise stated.

Wounding studies

Female SKH1 hairless mice (8–10 weeks) were obtained from Charles River Labs (Wilmington, MA, USA) and acclimatized in-house for 2 weeks before initiation of experiments. Females were chosen due to their lower aggressiveness, which may have increased wound healing rate variability. Mice were group-housed (four per cage), supplied with a basal chow diet and allowed to feed ad libitum, and exposed to a standard 12h light:12h darkness cycle.

Mice were anesthetized under 2% isoflurane. Mid-line upper dorsal skin (a site with minimal risk for secondary trauma from scratching/grooming) was lifted from underlying fascia and wounded using a 5 mm biopsy punch (Acuderm, Fort Lauderdale, FL, USA) resulting in two symmetrical biopsies on the upper right and left flanks. Wounds received 20 μl bupivacaine 0.25% (analgesc) before recovery and were monitored daily throughout the experimental period.

At 2, 4, 8, 14, and 21 days post-wounding, mice were killed and wounds were photographed before excision by 5 mm punch biopsy. Unwounded skin was obtained from the lower dorsal region to control for systemic effects. Adrenal glands were harvested as positive controls for steroidogenic enzyme expression and activity assays. Wound areas were determined using ImageJ (NIH, Bethesda, MD, USA).

Quantitative PCR

Freshly isolated tissue (20–40 mg skin or whole adrenal) was immediately snap-frozen and stored at −80 °C. Tissues were homogenized in 1 ml Trizol reagent and RNA extracted using a Purelink RNA Mini Kit (Life Technologies): 1.2 μg RNA was used for RT-PCR using a Tetro cDNA Synthesis Kit (Bioline, Taunton, MA, USA). qPCR was conducted in 10 μl reactions containing SensiFAST Probe Kit mastermix (Bioline), TaqMan target cDNA primers (900 nM)/FAM probe (250 nM) mix, TaqMan 18S rRNA primers (50 nM), VIC probe (200 nM) mix (Applied Biosystems), and 10 ng cDNA. Duplicate PCRs were
performed on a 384-well plate format using a 7900 HT instrument (Applied Biosystems) with the following parameters: 5 min 95 °C followed by 40 cycles of 95 °C for 10 s and 60 °C for 50 s. Raw Ct values were normalized to internal control 18S rRNA expression (ΔCt) and averaged. Statistical analysis was performed on ΔCt values of biological replicates, although these values were transformed to arbitrary units (2^−ΔΔCt) for graphical purposes.

**Protein isolation and western blot**

Approximately, 20–40 mg snap-frozen tissue was homogenized in 300 µl RIPA buffer (Pierce, Rockford, IL, USA) supplemented with protease inhibitor cocktail (Roche). Proteins were quantified using a BCA Protein Assay Kit (Pierce) and denatured in 25 µl reactions as follows: 25 µg total protein lysate, NuPAGE LDS buffer (Life Technologies), and 5% β-mercaptoethanol heated to 95 °C for 5 min: 20 µg denatured protein was loaded into 1 mm, 12-well NuPAGE 4–12% Bis–Tris gels and run in NuPAGE MES SDS buffer at 100 V using the XCell SureLock Mini Platform (Life Technologies). Separated proteins were transferred to 0.2 µm Immun-Blot PVDF membrane (Bio-Rad) in NuPAGE Transfer buffer at 30 V for 2 h using XCell II Blot Module (Life Technologies).

The membranes were blocked for 1 h at room temperature (RT) under agitation in 5% milk in PBS with 1% Tween20 (PBST), rinsed briefly in PBST, and incubated under agitation overnight at 5 °C under agitation in 5% milk in PBS (step omitted for anti-β-HSD1 antibody detected a band of the predicted size (34 kDa) in mouse skin and liver (positive control tissue). These bands were neutralized by pre-incubating β-HSD1 antibody with 2 µg/ml immunizing peptide of matching sequence (Abcam) for 30 min at RT (Supplementary Fig. 1A and B, see section on supplementary data given at the end of this article).

**11β-HSD activity assays**

**11β-HSD1 (oxoreductase)** Freshly isolated tissue (20–40 mg) was incubated immediately in 1 ml high glucose, pyruvate, and DMEM (Life Technologies) containing 100 nM 11-DHC (Steroids, Newport, RI, USA) with ~1500 c.p.m. of [3H] 11-DHC-generated in-house as follows:

Five µCi [3H] corticosterone, specific activity 70–100 Ci/mmol (Perkin Elmer, Waltham, MA, USA), 450 µg homogenized mouse placenta protein (exhibiting high 11β-HSD2 activity), and 500 µM NAD^+ cofactor in 500 µl (total volume) 0.1 M potassium phosphate buffer pH 7.4 (Bio-World, Dublin, OH, USA) were incubated under agitation at 37 °C for 3 h. [3H] 11-DHC was extracted by vortexing in 5 ml dichloromethane, removal of aqueous/protein phase by aspiration, and concentration under air at 55 °C for 20 min. [3H] 11-DHC was resuspended in 100 µl dichloromethane, spotted onto foil-backed silica plates, and separated by TLC in a 186:14 ml chloroform:ethanol mobile phase for 90 min with 10 mM 11-DHC run in adjacent lanes. Plates were visualized under u.v. to localize 11-DHC and this area also containing [3H] 11-DHC was excised and eluted overnight in 500 µl ethanol at 4 °C. Ethanol concentration was adjusted to 1500 c.p.m./µl by liquid scintillation.

11β-HSD1 assays were incubated at 37 °C for 16 h (predetermined by time course to generate ~25% conversion in unwounded control samples). Subsequently, tissues were weighed and discarded and steroids were extracted and separated as described earlier (co-migrating with 10 mM 11-DHC/corticosterone standards). Plate regions containing 11-DHC and corticosterone were excised and percentage conversion of the latter determined by liquid scintillation.

**11β-HSD2 (dehydrogenase)** Freshly isolated tissue (20–40 mg) was assayed as described earlier, replacing 100 nM 11-DHC with corticosterone and [3H] 11-DHC with [3H] corticosterone diluted 1:500 in ethanol.

**Immunohistochemistry**

Freshly isolated tissue was stored in Formalde-Fresh (Fisher Scientific, Pittsburgh, PA, USA) and processed into paraffin blocks. The 5 µm sections were de-waxed, rehydrated, and
stained with hematoxylin and eosin (H&E) (Leica, Buffalo Grove, IL, USA and Thermo Scientific, Kalamazoo, MI, USA) or for 11β-HSD1 as follows: following rehydration and antigen unmasking for 20 min at 98 °C pH 6 (Vector Labs, Burlingame, CA, USA), the sections were treated with 1% H2O2 (diluted in methanol) for 15 min at RT. Following two 5 min Tris-buffered saline (TBS) washes, slides were blocked in 4% BSA/0.5% cold water fish gelatin in TBS for 1 h at RT. Slides were rinsed twice for 2 min in TBS and incubated with anti-11β-HSD1 primary antibody at 1 µg/ml in blocking solution for 30 min at RT. Primary antibody neutralized with immunizing peptide was used for negative control sections. Slides were rinsed twice for 2 min in TBS and incubated in biotinylated goat anti-rabbit IgG secondary antibody (1:500, Vector Labs) for 10 min at RT. Slides were rinsed twice for 2 min in TBS and incubated in avidin–biotin-complex (Vector Labs) for 15 min at RT. Following two 5 min TBS washes sections were incubated with DAB peroxidase (Vector Labs) for 15 min at RT. Following two 5 min Tris-buffered saline (TBS) washes, slides were blocked in 4% BSA/0.5% cold water fish gelatin in TBS for 1 h at RT. Following two 5 min Tris-buffered saline (TBS) washes, slides were blocked in 4% BSA/0.5% cold water fish gelatin in TBS for 1 h at RT. Following two 5 min Tris-buffered saline (TBS) washes, slides were blocked in 4% BSA/0.5% cold water fish gelatin in TBS for 1 h at RT. Following two 5 min Tris-buffered saline (TBS) washes, slides were blocked in 4% BSA/0.5% cold water fish gelatin in TBS for 1 h at RT. Following two 5 min Tris-buffered saline (TBS) washes, slides were blocked in 4% BSA/0.5% cold water fish gelatin in TBS for 1 h at RT.

### Steroidogenic enzyme assay

Freshly isolated tissue (20–40 mg skin or minced whole adrenal) was incubated immediately and processed for 11β-HSD activity assays (16 h at 37 °C), replacing 100 nM 11-DHC/corticosterone with 100 nM progesterone and ~1500 c.p.m. of [3H] progesterone, specific activity 90–115 Ci/mmol (Perkin Elmer). Following extraction, progesterone metabolites were separated by TLC and co-migrated with 10 mM progesterone, 11-deoxycorticosterone (11-DOC), 11-deoxycortisol (11-DOF), 11-DHC, corticosterone, and cortisol standards. Percent conversion of progesterone to these metabolites was determined by liquid scintillation.

### Statistical analysis

Following confirmation of data displaying a normal distribution, significance levels were determined by two-way ANOVA using GraphPad Prism (La Jolla, CA, USA).

### Results

#### Increased 11β-HSD1 expression and activity during wound healing

The regulation of 11β-HSD1 (Fig. 1A) during skin wound healing has not been previously investigated. Therefore, we examined changes in 11β-HSD1 (Hsd11b1) expression and activity following full-thickness dorsal punch biopsies in mice (wound closure occurred between day 8 and 10). Hsd11b1 expression was tenfold higher in day 2 wounds compared with unwounded control tissue (ΔCT ± S.E.M. 16.8 ± 0.4 vs 20.6 ± 0.5 respectively, P < 0.001) and threefold higher in day 4 wounds (P < 0.001), returning to levels similar to those of unwounded by day 8 (Fig. 1B). Correspondingly, 11β-HSD1 protein expression (normalized to β-actin) was 2.5-fold higher in day 2 wounds (P < 0.01) and twofold greater in day 4 wounds (P < 0.05) relative to unwounded control skin and was similar to that observed in unwounded skin at days 8, 14, and 21 (Fig. 1C). In contrast, 11β-HSD2 mRNA expression was low (ΔCT ± S.E.M. 23.3 ± 0.7) and unaffected by wound healing (Fig. 1B).

Although abundantly expressed, 11β-HSD1 nicotinamide adenine dinucleotide phosphate (NADPH) cofactor-supplying enzymes, hexose-6-phosphate dehydrogenase (ΔCT ± S.E.M. 16.9 ± 0.1) and GC receptor (ΔCT ± S.E.M. 15.8 ± 0.09) mRNA, were not significantly altered during wound healing (Supplementary Fig. 2A and B, see section on supplementary data given at the end of this article). However, mRNA expression for the mineralocorticoid receptor was decreased at days 2 (65%, P < 0.01) and 4 (63%, P < 0.001) post-wounding (Supplementary Fig. 2C), although expression was considerably lower than that of GC receptor in unwounded skin (ΔCT ± S.E.M. 20.6 ± 0.1).

Consistent with these expression data, 11β-HSD1 activity (11-DHC to corticosterone) was >2.5-fold higher in day 2 (pmol corticosterone/h 4.21 ± 0.2 vs 1.45 ± 0.3, P < 0.001) and day 4 (4.4 ± 0.3 vs 1.45 ± 0.5, P < 0.001) wounds (Fig. 1D). In contrast, 11β-HSD2 activity (corticosterone to 11-DHC) in unwounded skin was negligible (pmol 11-DHC/h < 0.2) and remained unchanged post-wounding (Fig. 1D).

In summary, 11β-HSD1 is the dominant 11β-HSD isozyme expressed in mouse skin and demonstrates increased expression and activity during early wound repair.

#### 11β-HSD1 localizes to wound inflammatory infiltrate during wound healing

Compared with unwounded skin (Fig. 2A), H&E histology revealed a large influx of inflammatory cells at day 2 (Fig. 2C) and 4 (Fig. 2F), which diminished by day 8 when fibroblasts became more abundant (Fig. 2H). These inflammatory cells were largely undetectable in day 14 (Fig. 2J) and day 21 (Fig. 2L) wounds. In unwounded skin, 11β-HSD1 was expressed in keratinocytes, fibroblasts, hair follicles, and sebaceous glands (Fig. 2B) as previously...
demonstrated (Tiganescu et al. 2011). In support of our expression and activity data, we observed increased 11\(\beta\)-HSD1 staining in day 2 wounds where 11\(\beta\)-HSD1 protein localized to infiltrating inflammatory cells accumulating in the wound gap (Fig. 2D arrowheads). Negligible 11\(\beta\)-HSD1 staining was observed in sections incubated with 11\(\beta\)-HSD1 antibody neutralized with immunizing peptide (Fig. 2E).

Positively stained inflammatory cells remained detectable 4 days post-wounding (Fig. 2G), but were greatly diminished at 8 days post-wounding (Fig. 2I), consistent with the resolution of inflammation during this timeframe. Day 14 wounds displayed fibroblasts, aligning along new collagen fibers during extracellular matrix remodeling with 11\(\beta\)-HSD1-positive staining of a similar intensity to unwounded skin (Fig. 2K). This remained the case at 21 days post-wounding (Fig. 2M), where further collagen remodeling was evident with the reappearance of dermal appendages such as hair follicles and sebaceous glands.

The H&E and 11\(\beta\)-HSD1 immunostaining are presented at a lower magnification as an additional Supplementary Fig. 3A, B, C, D, E, F, G, H, I, J, K, and L, see section on supplementary data given at the end of this article.

**Steroidogenic enzyme expression during wound healing**

Similar to 11\(\beta\)-HSDs, changes in steroidogenic enzyme expression during wound healing have not been
investigated in detail. Therefore, we analyzed mRNA levels for six key enzymes required for sequential conversion of cholesterol to corticosterone/cortisol (Fig. 3A). Expression of the mitochondrial cholesterol transporter StAR, regarded as the rate-limiting step in steroid biosynthesis, was modestly elevated in day 4 wounds ($P < 0.05$, Fig. 3B), but overall expression was considerably lower than that observed in adrenal gland (Supplementary Fig. 4A, see section on supplementary data given at the end of this article). Expression of cholesterol side-chain cleavage enzyme (CYP11A1), which converts cholesterol to pregnenolone, was variable, with trends toward decreased expression in day 2 wounds and increased expression in day 14 wounds (Fig. 3C).

Subsequent conversion of pregnenolone to progesterone is conducted by 3β-hydroxysteroid dehydrogenase type 6 (3β-HSD6), expression of which was also variable during wound healing (Fig. 3D). Alternatively, pregnenolone and progesterone conversion to the sex steroid and cortisol precursors 17α-hydroxypregnenolone/progesterone is catalyzed by 17α-hydroxylase (CYP17), which displayed $>50\%$ decreased expression in day 2 and 4 wounds ($P < 0.05$, Fig. 3E). This reaction is lacking in mouse adrenals due to the absence of appreciable Cyp17a1 expression (Supplementary Fig. 4A).

Interestingly, expression of the 21-hydroxylase (CYP21A1), which converts progesterone and 17α-progesterone to GC precursors 11-DOC and 11-DOF, was increased 25- and fivefold in day 2 and 4 wounds respectively ($P < 0.001$, Fig. 3F). However, expression of the final enzyme in the GC synthesis pathway, 11β-hydroxylase (CYP11B1), required for conversion of these precursors to corticosterone and cortisol respectively was undetectable (Supplementary Fig. 4A). Expression of CYP11B2, which converts 11-DOC to the mineralocorticoid receptor (MR) ligand aldosterone, was also undetectable in unwounded mouse skin or during wound healing (Supplementary Fig. 4A).

Although steroidogenic enzyme mRNA expression exhibited a mixed regulatory profile during wound healing, expression of all except Cyp17a1 was considerably lower in skin than in the adrenal glands. Importantly, the lack of Cyp11b1 expression indicates that this pathway is unable to synthesize GC de novo in murine skin.
Steroidogenic enzyme activity during wound healing

Steroidogenic enzyme activity was determined by incubation with radiolabeled progesterone and analysis of downstream steroid (corticosterone and cortisol) and steroid precursor (11-DOC, 11-DOF, and 11-DHC) metabolites. Corticosterone was the progesterone metabolite detectable at the lowest levels in skin and was largely unaffected by wound healing, supporting our findings indicating a lack of Cyp11b1 expression (Fig. 4A). Indeed, corticosterone levels appeared lower than the levels of cortisol used as a negative control. In skin, 11-DOC, 11-DOF, and 11-DHC were the dominant progesterone-derived metabolites in contrast to mouse adrenals where, as anticipated, corticosterone was the principal progesterone metabolite (Supplementary Fig. 4B). Moreover, wound healing appeared to decrease corticosteroidogenesis in skin with reduced 11-DOC and 11-DOF metabolites at days 2 and 4 (Fig. 4A), the former also correlating with lower Cyp17a1 expression.

Finally, comparison of corticosterone generation from progesterone (by steroidogenesis) or from 11-DHC (by 11β-HSD1) demonstrates that the latter generates considerably more corticosterone in unwounded skin and post-wounding (Fig. 4B). This difference is probably underestimated as our analysis assumed complete conversion of radiolabeled progesterone to the metabolites analyzed; in fact only ~25% was recovered (Supplementary Fig. 5A, see section on supplementary data given at the end of this article) while radiolabeled 11-DHC recovery was considerably greater (Supplementary Fig. 5B).

Discussion

Despite the adverse effects of GC on wound healing being well documented, changes in local GC synthesis and metabolism during this process remained, until recently, largely unexplored. Early studies demonstrated increased conversion of cortisone to cortisol during the inflammatory phase of wound healing in mouse skin (Nabors & Berliner 1969). Although these experiments preceded the current understanding of 11β-HSD enzyme functions, they proffered evidence for a possible role of GC metabolism in wound repair.

In this study, we demonstrate increased GC-activating 11β-HSD1 expression and activity during the initial phases of wound healing in mouse skin. This finding explains the early observations cited earlier and underpins recent observations by us and others demonstrating accelerated wound healing following topical 11β-HSD1 inhibitor treatment in young mice and in aged 11β-HSD1 KO mice, despite elevated circulating corticosterone levels (Terao et al. 2011, Tiganescu et al. 2013). The lack of

Figure 3
Steroidogenic enzyme expression during wound healing. (A) Schematic representation of the steroidogenic pathway in mouse skin. (B) mRNA expression during wound healing (normalized to 18S rRNA) for StAR, (C) cholesterol side-chain cleavage enzyme (CYP11A1), (D) 3β-hydroxysteroid dehydrogenase type 6 (3β-HSD6), (E) 17α-hydroxylase/17, 20 lyase (CYP17), and (F) 21-hydroxylase (CYP21A1, n=4–14). *P<0.05, **P<0.01, ***P<0.001.
regulate mouse macrophage phagocytosis (Gilmour et al. 2006), human neutrophil survival (Kardon et al. 2001). Although not previously investigated in the context of wound healing, corticosteroidogenic expression is consistent with results from a previous study (Tiganescu et al. 2011).

The immunohistochemistry data presented here indicate that the elevated 11β-HSD1 activity during wound healing derives from numerous inflammatory cells that infiltrate the tissue immediately following wounding. Although not previously investigated in the context of wound healing, 11β-HSD1 expression and activity in murine lymphocytes have been reported and postulated to prevent the development of excessive inflammation (Zhang et al. 2005). 11β-HSD1 has also been reported to regulate mouse macrophage phagocytosis (Gilmour et al. 2006), human neutrophil survival (Kardon et al. 2008), and murine mast cell degranulation (Coutinho et al. 2013), although the beneficial/adverse effects of 11β-HSD1 blockade in various models of inflammation are still under debate (Chapman et al. 2013, Hardy et al. 2013).

Interestingly, limiting GC levels accelerated skin wound healing despite increased pro-inflammatory IL1β expression (Vukelic et al. 2011), indicating that the adverse effects of GC activation during wound healing may outweigh beneficial anti-inflammatory functions.

Although significantly lower than glucocorticoid receptor (GR), MR expression was decreased during wound healing possibly as a negative-feedback mechanism in response to increased availability of GC (which also binds MR with high affinity). Similar abnormalities in skin barrier development have been reported for GR- and MR-overexpressing mouse models (Pérez et al. 2001, Sainte Marie et al. 2007); therefore, dissociating GR/MR-mediated effects of GC excess in skin would be of interest in future studies.

Several steroidogenic enzymes have previously been identified in rodent skin (Abbaszade et al. 1997, Slominski et al. 2004), supporting our finding that all except CYP11B1 are expressed at the mRNA level in mouse skin. Accordingly, we observed an accumulation of 11-DOC and 11-DOF progesterone metabolites consistent with their impaired metabolism to GC (due to lack of Cyp11b1), with other studies also reporting a lack of pregnenolone metabolism to corticosterone in rodent skin (Dalla Valle et al. 1992). In the absence of Cyp11b1, the low levels of corticosterone metabolized from progesterone could be derived from 11-DHC (11β-HSD1 substrate), also reported by others to be the dominant progesterone metabolite in rodent skin (Slominski et al. 2000a). Furthermore, although Cyp17a1 and Cyp21a1 were decreased and increased respectively during wound healing, Cyp11b2 expression was also lacking in mouse skin, precluding aldosterone synthesis from 11-DOC. However, as Cyp17a1 expression was readily detected in mouse skin, sex steroid metabolism from progesterone remains a possibility.

The data presented here compare de novo GC synthesis and GC activation by 11β-HSD1 as sources of GC generation in unwounded mouse skin and during wound healing. Recent findings have indicated that both mechanisms contribute to local GC levels and a large body of evidence supports the hypothesis that human skin is capable of generating appreciable levels of GC through steroidogenesis (reviewed in Slominski et al. 2012). However, here we demonstrate that the contribution made by steroidogenesis in mouse skin is negligible compared with GC activation by 11β-HSD1, which generates corticosterone at concentrations capable of GR activation (Dong et al. 2006). Furthermore, while 11β-HSD1 expression and activity increase during wound healing, corticosteroidogenic enzymes display variable mRNA regulation but decreased activity. The latter may be due to increased progesterone metabolism from 11-DOC. However, as Cyp17a1 expression was readily detected in mouse skin, sex steroid metabolism from progesterone remains a possibility.
clearance to steroid metabolites not analyzed (i.e., sex steroids) or to water-soluble metabolites discarded during the steroid extraction process.

Recently, a study using human and porcine models has indicated that steroidogenesis contributes directly to increased GC levels generated during wound healing (Vukelic et al. 2011). This study demonstrated that inhibition of cortisol synthesis and acceleration of wound healing following metyrapone and IGF1 treatment suggested to act through inhibition of CYP11B1 activity and expression respectively. However, both treatments are also known 11β-HSD1 inhibitors (Stewart et al. 2001, Hellmich et al. 2013), indeed metyrapone-mediated inhibition of 11β-HSD1 is postulated to contribute to improved memory consolidation and retrieval in rodent studies (Hellmich et al. 2013). Furthermore, GH-deficient (and subsequently IGF1-deficient) patients exhibit increased global 11β-HSD1 activity (Weaver et al. 1994) and a skin phenotype characterized by epidermal thinning (Lange et al. 2001). This is also a common phenotype of aged skin, which we found to display elevated 11β-HSD1 activity in both humans and mice (Tiganescu et al. 2013) while circulating GH/IGF1 levels are also known to decline with advancing age. It is therefore possible that the accelerated healing induced by IGF1 and metyrapone treatment (Vukelic et al. 2011) was due to inhibition of 11β-HSD1 rather than CYP11B1.

It is important to emphasize that differences in skin endocrine pathways do exist between mice and man, including for CRH and proopiomelanocortin (Slominski et al. 2000b) in addition to 11β-HSD2 and CYP11B1 which are expressed in human skin (Brown et al. 1996, Hannen et al. 2011). Although our studies were conducted in mice, the ability of human skin to synthesize cortisol de novo also remains debatable, with studies reporting both negative (Slominski et al. 2002) and positive findings (Cirillo & Prime 2011, Hannen et al. 2011, Vukelic et al. 2011). Moreover, the quantities of cortisol generated de novo in these studies appear to be considerably lower than those reported to be generated by 11β-HSD1 in human skin (Tiganescu et al. 2011, 2013).

Several clinical trials using selective 11β-HSD1 inhibitors as therapies for obesity-related diabetes mellitus have reported positive outcomes (Feig et al. 2011, Gibbs et al. 2011, Hollis & Huber 2011, Liu et al. 2011, Park et al. 2012). Local 11β-HSD1 blockade could be particularly effective in accelerating wound repair in older patients, whereas elevated 11β-HSD1 activity in skin (Tiganescu et al. 2011, 2013) is coupled to slower healing and delayed but prolonged inflammation (Ashcroft et al. 1997, Brubaker et al. 2013). 11β-HSD1 levels were also found to be elevated in the lymphocytes of aged mice (Zhang et al. 2005). In the elderly, a vicious cycle of increased local GC activation and decreased GC sensitivity (Wilkinson et al. 1997, Rohleder et al. 2002) may contribute to blunted diurnal cortisol secretion patterns (Van Cauter et al. 1996) and ‘inflamm-aging’ (Shaw et al. 2010). Given the restoration of normal wound healing capacities by GR-inhibitor (RU486) treatment in GC-impaired and diabetic wound models (Bitar 2011), the involvement of 11β-HSD1 in the etiology of chronic diabetic wounds also remains an exciting prospect.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-13-0420.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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