Increased glucocorticoid activation during mouse skin wound healing

Ana Tiganescu, Melanie Hupe, Yoshikazu Uchida, Theodora Mauro, Peter M Elias and Walter M Holleran

Department of Dermatology, University of California San Francisco, 1700 Owens Street, San Francisco, California 94158, USA

Correspondence should be addressed to A Tiganescu
Email
ana.tiganescu@ncire.org

Abstract

Glucocorticoid (GC) excess inhibits wound healing causing increased patient discomfort and infection risk. 11 β -hydroxysteroid dehydrogenase type 1 (11 β -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 11 β -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. 11 β -HSD1 expression (mRNA and protein) and enzyme activity were elevated at 2 and 4 days post-wounding, with 11 β -HSD1 localizing to infiltrating inflammatory cells. 11 β -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, 11 β -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 11 β -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 11 β -HSD1 inhibitors to accelerate wound repair.

Key Words

- ▶ 11 β -hydroxysteroid dehydrogenase
- ▶ steroidogenesis
- ▶ glucocorticoid
- ▶ skin
- ▶ wound healing

Journal of Endocrinology
(2014) 221, 51–61

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.

11 β -hydroxysteroid dehydrogenase type 1 (11 β -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, 11 β -hydroxysteroid dehydrogenase type 2 (11 β -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, 11 β -HSD2 also protects the developing embryo from excessive exposure to maternal circulating GC (Brown *et al.* 1996). 11 β -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, 11 β -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 11 β -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 11 β -HSDs during wound healing remains unexplored.

In this report, we examined 11 β -HSD and steroidogenic enzyme expression and activity during mouse skin wound healing. Although corticosterone synthesis from progesterone was detectable at low levels, 11 β -HSD1-mediated corticosterone generation was significantly greater. Furthermore, 11 β -HSD1 levels increased during wound healing supporting the results of previous studies by us and others, demonstrating accelerated wound healing following local or global 11 β -HSD1 blockade (Terao *et al.* 2011, Tiganescu *et al.* 2013). Our findings indicate that increased 11 β -HSD1 activity originates from the infiltrating leukocytes during the inflammatory stage of wound healing. Therefore, 11 β -HSD1 may also contribute to the pathology of chronic wounds characterized by persistent inflammation. Collectively, these findings demonstrate that elevated 11 β -HSD1 may limit wound repair and support the use of local 11 β -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 μ l bupivacaine 0.25% (analgesic) 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 μ g RNA was used for RT-PCR using a Tetro cDNA Synthesis Kit (Bioline, Taunton, MA, USA). qPCR was conducted in 10 μ l 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 (Δ Ct) and averaged. Statistical analysis was performed on Δ Ct values of biological replicates, although these values were transformed to arbitrary units ($2^{-\Delta\Delta C_t}$) for graphical purposes.

Protein isolation and western blot

Approximately, 20–40 mg snap-frozen tissue was homogenized in 300 μ l 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 μ l reactions as follows: 25 μ g total protein lysate, NuPAGE LDS buffer (Life Technologies), and 5% β -mercaptoethanol heated to 95 °C for 5 min: 20 μ g 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 μ m 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 0.05% Tween-20 (PBST), rinsed briefly in PBST, and incubated under agitation overnight at 5 °C in blocking buffer with 1 μ g/ml mouse anti-11 β -HSD1 (ab83522, Abcam, Cambridge, MA, USA), or 0.4 μ g/ml mouse anti- β -actin-peroxidase-conjugated primary antibody. The membranes were washed three times for 15 min under agitation and incubated under agitation in blocking buffer with 1:5000 sheep anti-mouse IgG-peroxidase antibody (GE Healthcare, Pittsburgh, PA, USA) for 1 h at room temperature (step omitted for anti- β -actin-peroxidase-treated membranes). Following a further three 15 min PBST washes, the membranes were incubated with ECL Plus western blotting substrate for anti-human 11 β -HSD1-treated membranes (Pierce), according to the manufacturer's guidelines. Proteins were visualized using a las-3000 luminescent image analyzer (FujiFilm, Stamford, CT, USA) and quantified by densitometry after normalization to β -actin (Multi Gauge v2.3). The anti-11 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 11 β -HSD1 antibody with 2 μ g/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).

11 β -HSD activity assays

11 β -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 (Steraloids, Newport, RI, USA) with $\sim$ 1500 c.p.m. of [3 H] 11-DHC-generated in-house as follows:

Five μ Ci [3 H] corticosterone, specific activity 70–100 Ci/mmol (Perkin Elmer, Waltham, MA, USA), 450 μ g homogenized mouse placenta protein (exhibiting high 11 β -HSD2 activity), and 500 μ M NAD $^+$ cofactor in 500 μ l (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. [3 H] 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. [3 H] 11-DHC was resuspended in 100 μ l 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 [3 H] 11-DHC was excised and eluted overnight in 500 μ l ethanol at 4 °C. Ethanol concentration was adjusted to 1500 c.p.m./ μ l by liquid scintillation.

11 β -HSD1 assays were incubated at 37 °C for 16 h (predetermined by time course to generate $\sim$ 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.

11 β -HSD2 (dehydrogenase) Freshly isolated tissue (20–40 mg) was assayed as described earlier, replacing 100 nM 11-DHC with corticosterone and [3 H] 11-DHC with [3 H] 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 μ m 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 11 β -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-11 β -HSD1 primary antibody at 1 μ g/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 substrate (Vector Labs), dehydrated, fitted with cover slips, and visualized on a Zeiss microscope (Jena, Germany) using AxioVision (Carl Zeiss Vision, Munich, Germany).

Steroidogenic enzyme assay

Freshly isolated tissue (20–40 mg skin or minced whole adrenal) was incubated immediately and processed for 11 β -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 11 β -HSD1 expression and activity during wound healing

The regulation of 11 β -HSD1 (Fig. 1A) during skin wound healing has not been previously investigated. Therefore, we

examined changes in 11 β -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 (Δ Ct $\pm$ 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, 11 β -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, 11 β -HSD2 mRNA expression was low (Δ Ct $\pm$ S.E.M. 23.3 ± 0.7) and unaffected by wound healing (Fig. 1B).

Although abundantly expressed, 11 β -HSD1 nicotinamide adenine dinucleotide phosphate (NADPH) cofactor-supplying enzymes, hexose-6-phosphate dehydrogenase (Δ Ct $\pm$ S.E.M. 16.9 ± 0.1) and GC receptor (Δ Ct $\pm$ 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 (Δ Ct $\pm$ S.E.M. 20.6 ± 0.1).

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

11 β -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, 11 β -HSD1 was expressed in keratinocytes, fibroblasts, hair follicles, and sebaceous glands (Fig. 2B) as previously

Figure 1

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) expression and activity increase during early wound healing. (A) Schematic representation of glucocorticoid activation in skin. In the endoplasmic reticulum (ER) lumen, 11β-HSD1 generates corticosterone from inactive 11-dehydrocorticosterone (11-DHC) in mouse or cortisol from cortisone in human skin. NADPH co-factor is supplied by hexose 6-phosphate dehydrogenase.

demonstrated (Tiganescu *et al.* 2011). In support of our expression and activity data, we observed increased 11β-HSD1 staining in day 2 wounds where 11β-HSD1 protein localized to infiltrating inflammatory cells accumulating in the wound gap (Fig. 2D arrowheads). Negligible 11β-HSD1 staining was observed in sections incubated with 11β-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

(B) 11β-HSD (*Hsd11b1* and *Hsd11b2*) mRNA expression during wound healing (WH; normalized to 18S rRNA) compared with expression in control (unwounded) skin ($n=4-14$). (C) 11β-HSD1 protein expression (34 kDa) normalized to β-actin (42 kDa) during wound healing compared with control (C, $n=5$). (D) 11β-HSD activity during wound healing compared with control ($n=4$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

with 11β-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 11β-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 11β-HSDs, changes in steroidogenic enzyme expression during wound healing have not been

Figure 2

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) protein localizes to infiltrating inflammatory cells during wound healing. H&E staining of unwounded mouse skin (A) and showing inflammatory (C and F), granulation (F and H) and remodeling (J and L) stages of wound healing. 11 β -HSD1-positive staining localized to epidermal keratinocytes (E), fibroblasts (long arrows), hair follicles (HF), and sebaceous glands (SG) in unwounded skin (B). 11 β -HSD1 staining was greatest in day 2 wounds

localizing largely to the inflammatory cell infiltrate (D, arrowheads) in the wound gap (WG). Staining was negligible in sections incubated with primary antibody pre-adsorbed with immunizing peptide (E). Positively staining cells were reduced in granulation tissue (GT) of day 4 wounds (G) and still further by day 8 (I). Staining remained low during later stages of dermal remodeling (K) and similar to that for unwounded skin at day 21 (M). D, dermis, representative images ($n=4$), scale bars 50 μ m.

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 3 β -hydroxysteroid dehydrogenase type 6 (3 β -HSD6), expression of which was also variable during wound healing (Fig. 3D). Alternatively, pregnenolone and progesterone conversion to the sex steroid and cortisol precursors 17 α -hydroxypregnenolone/progesterone is catalyzed by 17 α -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 17 α -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, 11 β -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.

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) 3 β -hydroxysteroid dehydrogenase type 6 (3 β -HSD6), (E) 17 α -hydroxylase/17, 20 lyase (CYP17), and (F) 21-hydroxylase (CYP21A1, $n=4-14$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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 11 β -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 11 β -HSD enzyme functions, they proffered evidence for a possible role of GC metabolism in wound repair.

In this study, we demonstrate increased GC-activating 11 β -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 11 β -HSD1 inhibitor treatment in young mice and in aged 11 β -HSD1 KO mice, despite elevated circulating corticosterone levels (Terao *et al.* 2011, Tiganeşcu *et al.* 2013). The lack of

Figure 4

Steroidogenic synthesis of corticosterone compared with activation by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) during wound healing. (A) Steroidogenic conversion of 100 nM progesterone to 11-deoxycorticosterone (11-DOC), 11-deoxycortisol (11-DOF), 11-dehydrocorticosterone (11-DHC), corticosterone (B), and cortisol (F, negative control) during wound healing (WH, $n=4$). (B) Corticosterone generation from 11-DHC (11 β -HSD1 substrate) compared with generation from progesterone (P4) in wounded (wh) and control (c) skin ($n=4$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Hsd11b2 expression is consistent with results from a previous study (Tiganescu *et al.* 2011).

The immunohistochemistry data presented here indicate that the elevated 11 β -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, 11 β -HSD1 expression and activity in murine lymphocytes have been reported and postulated to prevent the development of excessive inflammation (Zhang *et al.* 2005). 11 β -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 11 β -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 IL1 β 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-over-expressing 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 (Abbazade *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 (11 β -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 11 β -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 11 β -HSD1, which generates corticosterone at concentrations capable of GR activation (Dong *et al.* 2006). Furthermore, while 11 β -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

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 11 β -HSD1 inhibitors (Stewart *et al.* 2001, Hellmich *et al.* 2013), indeed metyrapone-mediated inhibition of 11 β -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 11 β -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 11 β -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 11 β -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 11 β -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 11 β -HSD1 in human skin (Tiganescu *et al.* 2011, 2013).

Several clinical trials using selective 11 β -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 11 β -HSD1 blockade could be particularly effective in accelerating wound repair in older patients, whereas elevated 11 β -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). 11 β -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 2000), the involvement of 11 β -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.

Funding

This work was supported by the Department of Defence (grant number W81XWH-11-2-0189) to P M E.

References

- Abbaszade IG, Arensburg J, Park CH, Kasa-Vubu JZ, Orly J & Payne AH 1997 Isolation of a new mouse 3 β -hydroxysteroid dehydrogenase isoform, 3 β -HSD VI, expressed during early pregnancy. *Endocrinology* **138** 1392–1399. (doi:10.1210/endo.138.4.5042)
- Agarwal AK, Monder C, Eckstein B & White PC 1989 Cloning and expression of rat cDNA encoding corticosteroid 11 β -dehydrogenase. *Journal of Biological Chemistry* **264** 18939–18943.
- Ashcroft GS, Horan MA & Ferguson MW 1997 Aging is associated with reduced deposition of specific extracellular matrix components, an upregulation of angiogenesis, and an altered inflammatory response in a murine incisional wound healing model. *Journal of Investigative Dermatology* **108** 430–437. (doi:10.1111/1523-1747.ep12289705)
- Bitar MS 2000 Insulin and glucocorticoid-dependent suppression of the IGF-I system in diabetic wounds. *Surgery* **127** 687–695. (doi:10.1067/msy.2000.105869)
- Brauchle M, Fassler R & Werner S 1995 Suppression of keratinocyte growth factor expression by glucocorticoids *in vitro* and during wound healing. *Journal of Investigative Dermatology* **105** 579–584. (doi:10.1111/1523-1747.ep12323521)
- Brown RW, Chapman KE, Kotelevtsev Y, Yau JL, Lindsay RS, Brett L, Leckie C, Murad P, Lyons V, Mullins JJ *et al.* 1996 Cloning and production of antisera to human placental 11 β -hydroxysteroid dehydrogenase type 2. *Biochemical Journal* **313** 1007–1017.
- Brubaker AL, Rendon JL, Ramirez L, Choudhry MA & Kovacs EJ 2013 Reduced neutrophil chemotaxis and infiltration contributes to delayed resolution of cutaneous wound infection with advanced age. *Journal of Immunology* **190** 1746–1757. (doi:10.4049/jimmunol.1201213)

- Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS & Seckl JR 2013 Changing glucocorticoid action: 11 β -hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. *Journal of Steroid Biochemistry and Molecular Biology* **137** 82–92. (doi:10.1016/j.jsmb.2013.02.002)
- Chedid M, Hoyle JR, Csaky KG & Rubin JS 1996 Glucocorticoids inhibit keratinocyte growth factor production in primary dermal fibroblasts. *Endocrinology* **137** 2232–2237. (doi:10.1210/endo.137.6.8641170)
- Cirillo N & Prime SS 2011 Keratinocytes synthesize and activate cortisol. *Journal of Cellular Biochemistry* **112** 1499–1505. (doi:10.1002/jcb.23081)
- Cooper MS & Stewart PM 2009 11 β -hydroxysteroid dehydrogenase type 1 and its role in the hypothalamus–pituitary–adrenal axis, metabolic syndrome, and inflammation. *Journal of Clinical Endocrinology and Metabolism* **94** 4645–4654. (doi:10.1210/jc.2009-1412)
- Coutinho AE, Brown JK, Yang F, Brownstein DG, Gray M, Seckl JR, Savill JS & Chapman KE 2013 Mast cells express 11 β -hydroxysteroid dehydrogenase type 1: a role in restraining mast cell degranulation. *PLoS ONE* **8** e54640. (doi:10.1371/journal.pone.0054640)
- Dalla Valle L, Belvedere P, Simontacchi C & Colombo L 1992 Extra-glandular hormonal steroidogenesis in aged rats. *Journal of Steroid Biochemistry and Molecular Biology* **43** 1095–1098. (doi:10.1016/0960-0760(92)90337-1)
- Dong DD, Jewell CM, Bienstock RJ & Cidlowski JA 2006 Functional analysis of the LXXLL motifs of the human glucocorticoid receptor: association with altered ligand affinity. *Journal of Steroid Biochemistry and Molecular Biology* **101** 106–117. (doi:10.1016/j.jsmb.2006.06.010)
- Feig PU, Shah S, Hermanowski-Vosatka A, Plotkin D, Springer MS, Donahue S, Thach C, Klein EJ, Lai E & Kaufman KD 2011 Effects of an 11 β -hydroxysteroid dehydrogenase type 1 inhibitor, MK-0916, in patients with type 2 diabetes mellitus and metabolic syndrome. *Diabetes, Obesity and Metabolism* **13** 498–504. (doi:10.1111/j.1463-1326.2011.01375.x)
- Frank S, Madlener M & Werner S 1996 Transforming growth factors β 1, β 2, and β 3 and their receptors are differentially regulated during normal and impaired wound healing. *Journal of Biological Chemistry* **271** 10188–10193. (doi:10.1074/jbc.271.17.10188)
- Gibbs JP, Emery MG, McCaffery J, Smith B, Gibbs MA, Akrami A, Rossi J, Paweletz K, Gastonguay MR, Bautista E *et al.* 2011 Population pharmacokinetic/pharmacodynamic model of subcutaneous adipose 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) activity after oral administration of AMG 221, a selective 11 β -HSD1 inhibitor. *Journal of Clinical Pharmacology* **51** 830–841. (doi:10.1177/0091270010374470)
- Gilmour JS, Coutinho AE, Cailhier JF, Man TY, Clay M, Thomas G, Harris HJ, Mullins JJ, Seckl JR, Savill JS *et al.* 2006 Local amplification of glucocorticoids by 11 β -hydroxysteroid dehydrogenase type 1 promotes macrophage phagocytosis of apoptotic leukocytes. *Journal of Immunology* **176** 7605–7611.
- Hannen RF, Michael AE, Jaulim A, Bhogal R, Burrin JM & Philpott MP 2011 Steroid synthesis by primary human keratinocytes; implications for skin disease. *Biochemical and Biophysical Research Communications* **404** 62–67. (doi:10.1016/j.bbrc.2010.11.059)
- Hardy RS, Seibel MJ & Cooper MS 2013 Targeting 11 β -hydroxysteroid dehydrogenases: a novel approach to manipulating local glucocorticoid levels with implications for rheumatic disease. *Current Opinion in Pharmacology* **13** 440–444. (doi:10.1016/j.coph.2013.03.003)
- Hellmich HL, Rojo DR, Micci MA, Sell SL, Boone DR, Crookshanks JM, DeWitt DS, Masel BE & Prough DS 2013 Pathway analysis reveals common post-survival mechanisms of metyrapone and carbenoxolone after traumatic brain injury. *PLoS ONE* **8** e53230. (doi:10.1371/journal.pone.0053230)
- Hollis G & Huber R 2011 11 β -Hydroxysteroid dehydrogenase type 1 inhibition in type 2 diabetes mellitus. *Diabetes, Obesity and Metabolism* **13** 1–6. (doi:10.1111/j.1463-1326.2010.01305.x)
- Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, Straub RH & Paus R 2005 Human hair follicles display a functional equivalent of the hypothalamic–pituitary–adrenal axis and synthesize cortisol. *FASEB Journal* **19** 1332–1334. (doi:10.1096/fj.04-1968fje)
- Kardon T, Senesi S, Marcolongo P, Legeza B, Bánhegyi G, Mandl J, Fulceri R & Benedetti A 2008 Maintenance of luminal NADPH in the endoplasmic reticulum promotes the survival of human neutrophil granulocytes. *FEBS Letters* **582** 1809–1815. (doi:10.1016/j.febslet.2008.04.045)
- Lakshmi V & Monder C 1988 Purification and characterization of the corticosteroid 11 β -dehydrogenase component of the rat liver 11 β -hydroxysteroid dehydrogenase complex. *Endocrinology* **123** 2390–2398. (doi:10.1210/endo-123-5-2390)
- Lange M, Thulesen J, Feldt-Rasmussen U, Skakkebaek NE, Vahl N, Jørgensen JO, Christiansen JS, Poulsen SS, Sneppen SB & Juul A 2001 Skin morphological changes in growth hormone deficiency and acromegaly. *European Journal of Endocrinology* **145** 147–153. (doi:10.1530/eje.0.1450147)
- Lee B, Vouthounis C, Stojadinovic O, Brem H, Im M & Tomic-Canic M 2005 From an enhancosome to a repressosome: molecular antagonism between glucocorticoids and EGF leads to inhibition of wound healing. *Journal of Molecular Biology* **345** 1083–1097. (doi:10.1016/j.jmb.2004.11.027)
- Liu J, Wang L, Zhang A, Di W, Zhang X, Wu L, Yu J, Zha J, Lv S, Cheng P *et al.* 2011 Adipose tissue-targeted 11 β -hydroxysteroid dehydrogenase type 1 inhibitor protects against diet-induced obesity. *Endocrinology* **150** 199–209. (doi:10.1507/endocrj.110E-318)
- Nabors CJ Jr & Berliner DL 1969 Corticosteroid metabolism during wound healing. *Journal of Investigative Dermatology* **52** 465–473. (doi:10.1038/jid.1969.79)
- Park JS, Rhee SD, Jung WH, Kang NS, Kim HY, Kang SK, Ahn JH & Kim KY 2012 Anti-diabetic and anti-adipogenic effects of a novel selective 11 β -hydroxysteroid dehydrogenase type 1 inhibitor in the diet-induced obese mice. *European Journal of Pharmacology* **691** 19–27. (doi:10.1016/j.ejphar.2012.06.024)
- Pérez P, Page A, Bravo A, Del Río M, Giménez-Conti I, Budunova I, Slaga TJ & Jorcano JL 2001 Altered skin development and impaired proliferative and inflammatory responses in transgenic mice overexpressing the glucocorticoid receptor. *FASEB Journal* **15** 2030–2032. (doi:10.1096/fj.00-0772fje)
- Quinkler M & Stewart PM 2003 Hypertension and the cortisol–cortisone shuttle. *Journal of Clinical Endocrinology and Metabolism* **88** 2384–2392. (doi:10.1210/jc.2003-030138)
- Rohleder N, Kudielka BM, Hellhammer DH, Wolf JM & Kirschbaum C 2002 Age and sex steroid-related changes in glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress. *Journal of Neuroimmunology* **126** 69–77. (doi:10.1016/S0165-5728(02)00062-0)
- Sainte Marie Y, Toulon A, Paus R, Maubec E, Cherfa A, Grossin M, Descamps V, Clemessy M, Gasc JM, Peuchmaur M *et al.* 2007 Targeted skin overexpression of the mineralocorticoid receptor in mice causes epidermal atrophy, premature skin barrier formation, eye abnormalities, and alopecia. *American Journal of Pathology* **171** 846–860. (doi:10.2353/ajpath.2007.060991)
- Shaw AC, Joshi S, Greenwood H, Panda A & Lord JM 2010 Aging of the innate immune system. *Current Opinion in Immunology* **22** 507–513. (doi:10.1016/j.coi.2010.05.003)
- Slominski A, Ermak G & Mihm M 1996 ACTH receptor, CYP11A1, CYP17 and CYP21A2 genes are expressed in skin. *Journal of Clinical Endocrinology and Metabolism* **81** 2746–2749. (doi:10.1210/jcem.81.7.8675607)
- Slominski A, Gomez-Sanchez CE, Foecking MF & Wortsman J 2000a Active steroidogenesis in the normal rat skin. *Biochimica et Biophysica Acta* **1474** 1–4. (doi:10.1016/S0304-4165(99)00215-9)
- Slominski A, Wortsman J, Luger T, Paus R & Solomon S 2000b Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiological Reviews* **80** 979–1020.
- Slominski A, Wortsman J, Foecking MF, Shackleton C, Gomez-Sanchez C & Szczesniwski A 2002 Gas chromatography/mass spectrometry characterization of corticosteroid metabolism in human immortalized

- keratinocytes. *Journal of Investigative Dermatology* **118** 310–315. (doi:10.1046/j.0022-202x.2001.01648.x)
- Slominski A, Zjawiony J, Wortsman J, Semak I, Stewart J, Pisarchik A, Sweatman T, Marcos J, Dunbar C & Tuckey R 2004 A novel pathway for sequential transformation of 7-dehydrocholesterol and expression of the P450scc system in mammalian skin. *European Journal of Biochemistry* **271** 4178–4188. (doi:10.1111/j.1432-1033.2004.04356.x)
- Slominski A, Zbytek B, Szczesniowski A, Semak I, Kaminski J, Sweatman T & Wortsman J 2005a CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH. *American Journal of Physiology. Endocrinology and Metabolism* **288** E701–E706. (doi:10.1152/ajpendo.00519.2004)
- Slominski A, Zbytek B, Semak I, Sweatman T & Wortsman J 2005b CRH stimulates POMC activity and corticosterone production in dermal fibroblasts. *Journal of Neuroimmunology* **162** 97–102. (doi:10.1016/j.jneuroim.2005.01.014)
- Slominski A, Zbytek B, Szczesniowski A & Wortsman J 2006 Cultured human dermal fibroblasts do produce cortisol. *Journal of Investigative Dermatology* **126** 1177–1178. (doi:10.1038/sj.jid.5700204)
- Slominski AT, Zmijewski MA, Skobowiat C, Zbytek B, Slominski RM & Steketee JD 2012 Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system. *Advances in Anatomy, Embryology and Cell Biology* **212** 1–115.
- Stewart PM, Toogood AA & Tomlinson JW 2001 Growth hormone, insulin-like growth factor-I and the cortisol–cortisone shuttle. *Hormone Research* **56**(Suppl 1) 1–6. (doi:10.1159/000048126)
- Terao M, Murota H, Kimura A, Kato A, Ishikawa A, Igawa K, Miyoshi E & Katayama I 2011 11 β -Hydroxysteroid dehydrogenase-1 is a novel regulator of skin homeostasis and a candidate target for promoting tissue repair. *PLoS ONE* **6** e25039. (doi:10.1371/journal.pone.0025039)
- Tiganescu A, Walker EA, Hardy RS, Mayes AE & Stewart PM 2011 Localization, age- and site-dependent expression, and regulation of 11 β -hydroxysteroid dehydrogenase type 1 in skin. *Journal of Investigative Dermatology* **131** 30–36. (doi:10.1038/jid.2010.257)
- Tiganescu A, Tahrani AA, Morgan SA, Otranto M, Desmoulière A, Abrahams L, Hassan-Smith Z, Walker EA, Rabbitt EH, Cooper MS *et al.* 2013 11 β -Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *Journal of Clinical Investigation* **123** 3051–3060. (doi:10.1172/JCI64162)
- Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M & Stewart PM 2004 11 β -hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocrine Reviews* **25** 831–866. (doi:10.1210/er.2003-0031)
- Van Cauter E, Leproult R & Kupfer DJ 1996 Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology and Metabolism* **81** 2468–2473. (doi:10.1210/jcem.81.7.8675562)
- Vukelic S, Stojadinovic O, Pastar I, Rabach M, Krzyzanowska A, Lebrun E, Davis SC, Resnik S, Brem H & Tomic-Canic M 2011 Cortisol synthesis in epidermis is induced by IL-1 and tissue injury. *Journal of Biological Chemistry* **286** 10265–10275. (doi:10.1074/jbc.M110.188268)
- Weaver JU, Thaventhiran L, Noonan K, Burrin JM, Taylor NF, Norman MR & Monson JP 1994 The effect of growth hormone replacement on cortisol metabolism and glucocorticoid sensitivity in hypopituitary adults. *Clinical Endocrinology* **41** 639–648. (doi:10.1111/j.1365-2265.1994.tb01830.x)
- Wilkinson CW, Peskind ER & Raskind MA 1997 Decreased hypothalamic–pituitary–adrenal axis sensitivity to cortisol feedback inhibition in human aging. *Neuroendocrinology* **65** 79–90. (doi:10.1159/000127167)
- Zhang TY, Ding X & Daynes RA 2005 The expression of 11 β -hydroxysteroid dehydrogenase type I by lymphocytes provides a novel means for intracrine regulation of glucocorticoid activities. *Journal of Immunology* **174** 879–889.

Received in final form 9 January 2014

Accepted 23 January 2014

Accepted Preprint published online 24 January 2014