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11β-Hydroxysteroid Dehydrogenases in the Regulation of Tissue Glucocorticoid Availability

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1. Introduction

The regulation of tissue-specific actions of glucocorticoids (GCs) goes far beyond the effects of the fluctuation of their circulating levels and can be controlled by local intracellular enzymes. In the past few years, evidence is being gathered not only on the relevance of such enzymes to GC physiological actions but also on their involvement in the pathophysiology of certain chronic disease states, in which circulating GC levels are not necessarily altered. These enzymes are hydroxysteroid dehydrogenases (11β-HSDs; EC 1.1.1.146), which interconvert inactive GCs and the active GCs (Gathercole & Stewart, 2010; Seckl & Walker, 2004; Stewart, 2005; Tomlinson et al., 2004).

2. Regulation of glucocorticoid synthesis by the hypothalamus-pituitary-adrenal axis

GCs are part of the hypothalamus-pituitary-adrenal (HPA) axis, a tighly controlled endocrine component with essential roles in the regulation of physiological processes, such as stress responses, energy metabolism, electrolyte levels, blood pressure, immunity, cognitive functions and cell proliferation and differentiation (Atanasov & Odermatt, 2007; Papadimitriou & Priftis, 2009). Cortisol constitutes the main active hormone of the HPA axis and is released by the adrenal gland under the control of the remaining hormones of the axis. Corticotropin-releasing hormone (CRH), produced by hypothalamic neurons, is released onto the anterior pituitary where it stimulates the synthesis and secretion of the adenocorticotropic hormone (ACTH) into the blood. This occurs in a pulsatile man-

ner and with circadian rhythmicity, with higher levels being secreted in early morning and lowering through the afternoon and night (Gathercole & Stewart, 2010; White, 2008b). ACTH acts on the melanocortin 2 receptor (MC2R) in the adrenal cortex and has only a half-life of 10 min. There, it acutely increases cortisol and androgen production as well as the expression of the enzymes involved in their biosynthetic pathways, having a trophic effect on the adrenal cortex. Enhanced production of cortisol negatively regulates the synthesis and release of both CRH and ACTH by the hypothalamus and the pituitary, respectively, despite the ability of the hypothalamus to change the "set point" for the HPA axis to a higher level during severe or chronic stress. Regulators of the HPA axis include neurogenic and systemic stress (White, 2008b).

The zona fasciculata of the adrenal cortex is where the synthesis of most cortisol occurs. Through the stimulating action of ACTH, cholesterol esters, stored in the cytoplasm of these cells, are unsterified by cholesterol ester hydrolase and converted sequentially to pregnenolone [by cytochrome P450 (CYP) 11A1], progesterone [by 3β-hydroxysteroid dehydrogenase (3β-HSD)], 17-hydroxyprogesterone (by CYP17, 17-hydroxylase function), 11-deoxycortisol (by CYP21A2, 21-hydroxylase function) and cortisol (by CYP11B1, 11-hydroxylase function). As a minor pathway in humans, progesterone is converted to 11-deoxycorticosterone (by CYP11B2, 11-hydroxylase function) and then to corticosterone (by CYP11B2, 18-hydroxylase function). Plasma cortisol has a half-life of 70-120 min where it circulates bound to corticosteroid-binding globulin (CBG or transcortin; 90%) and to albumin (5-7%). The remaining constitutes the free, active, fraction (Tomlinson et al., 2004; White, 2008a).

Most cortisol actions take place through binding to GC receptors (GR) and mineralocorticoid receptors (MR) (Dzyakanchuk et al., 2009), nuclear receptors that are members of the steroid hormone receptor family (Gathercole & Stewart, 2010). Cortisol as well as the main GC in rodents, corticosterone, are active steroids whereas cortisone and 11-dehydrocorticosterone, the latter in rodents, are inactive steroids (Tomlinson et al., 2004). Upon GC-binding, the GR moves into the nucleus where it binds specific GC response elements (GRE) and recruits co-activators and co-repressors, which, once bound, enhance or repress gene transcription (Gathercole & Stewart, 2010). Cortisol and corticosterone are secreted in high amounts [15 mg/d (Cope & Black, 1958; Esteban et al., 1991) and 2 mg/d (Peterson & Pierce, 1960), respectively]. Cortisol concentration in the adrenal vein is about 3.7 nmol/mL whereas cortisone level is 0.13 nmol/mL, contrasting with 0.18 nmol/mL and 0.03 nmol/mL, respectively, in the vena cava (Tortorella et al., 1999). However, free cortisone concentrations are similar to those of free cortisol because of the lower binding of the former to CBG (Tomlinson et al., 2004). Cortisol is inactivated in the liver through conjugation with glucuronide and sulfate and subsequently excreted in the urine (Tomlinson et al., 2004; White, 2008b). In the liver, 5α- and 5β-reductases also inactivate cortisol and cortisone, in conjunction with 3α-HSD, to tetrahydrometabolites: 5α-tetrahydrocortisol, 5β-tetrahydrocortisol and tetrahydrocortisone (Campino et al., 2010; Russell & Wilson, 1994).

3. Regulation of tissue glucocorticoid availability

3.1. 11β-Hydroxysteroid dehydrogenase type 2

Cortisol is inactivated to cortisone, in humans, or corticosterone to 11-dehydrocorticosterone, in rodents, in order to avoid deleterious actions of active GCs overstimulation of the MR. This occurs because cortisol and aldosterone have the same *in vitro* affinity for the MR (Arriza et al., 1987; Gathercole & Stewart, 2010). The enzyme responsible for the regulation of active GC availability to the MR is 11β-HSD2, a NAD$^+$ dependent dehydrogenase. Its tissue expression is related to the presence of the MR, the kidney (distal convoluted tubule) being the typical example and the main location of cortisone production (Cooper & Stewart, 2009; Edwards et al., 1988; Gathercole & Stewart, 2010; Walker, B. & Andrew, 2006). However, 11β-HSD2 is also present in other locations such as the colon, salivary and sweat glands, placenta and vascular wall (Anagnostis et al., 2009; Andrews et al., 2003; Edwards et al., 1988; Ferrari, 2010; Funder et al., 1988; Gathercole & Stewart, 2010; Palermo et al., 2004).

Congenital deficiency of 11β-HSD2 in humans (Dave-Sharma et al., 1998; Gathercole & Stewart, 2010; Stewart et al., 1996), transgenic deletion in mice (Kotelevtsev et al., 1999) or pharmacological inhibition of 11β-HSD2 results in a clinical condition termed apparent mineralocorticoid excess (AME) syndrome (Sundbom et al., 2008). Affected subjects, despite having normal circulating levels of cortisol and no disturbances of the HPA axis, present with sodium retention, hypertension and hypokalemia (Anagnostis et al., 2009; Andrews et al., 2003; Edwards et al., 1988; Gathercole & Stewart, 2010; Monder et al., 1986; Mune et al., 1995; Palermo et al., 2004; Quinkler & Stewart, 2003; Stewart et al., 1996; Walker, B. & Andrew, 2006). These alterations arise from the activity of GCs on MR-expressing cells since the lack of GC inactivation allows their mineralocorticoid action. In this sense, AME has been considered a 'Cushing's disease of the kidney' (Stewart, 2005).

3.2. 11β-Hydroxysteroid dehydrogenase type 1

Opposite to 11β-HSD2, 11β-HSD1 reactivates inactive cortisone in humans (11-dehydrocorticosterone in rodents) back into cortisol (corticosterone in rodents) within cells expressing the enzyme (Anagnostis et al., 2009; Chapman et al., 2006; Espindola-Antunes & Kater, 2007; Stewart & Krozowski, 1999). This enzyme is in higher amounts in the liver, adipose tissue (AT), lung and the central nervous system. However, pancreas, kidney cortex, adrenal cortex, cardiac myocytes, bone, placenta, uterus, testis, oocytes and luteinized granulosa cells of the ovary, eye, pituitary, fibroblasts and immune, skeletal and smooth muscle cells are also sites of 11β-HSD1 expression (Anagnostis et al., 2009; Bujalska et al., 1997; Cooper & Stewart, 2009; Espindola-Antunes & Kater, 2007; Stewart & Krozowski, 1999; Tomlinson et al., 2004; Whorwood et al., 2001). In these locations, it is associated with GR rather than with MR (Walker, B. & Andrew, 2006). Acting as a reductase, it assures that GCs have access to GR since GR affinity for cortisol is relatively low, what becomes particularly relevant when cortisol levels are at their lowest due to their circadian variation (while cortisone levels remain constant) (Walker, B. & Andrew, 2006; Walker, B. et al., 1995).

Both 11β-HSD1 and 11β-HSD2 are located in the endoplasmic reticulum (ER). However, 11β-HSD1 is facing the lumen (Gathercole & Stewart, 2010; Ozols, 1995) where hexose-6-phosphate dehydrogenase (H6PDH) coexists and converts glucose-6-phosphate to 6-phosphogluconolactone in a reaction that regenerates NADPH from $NADP^+$ (Atanasov et al., 2008; Bujalska et al., 2005; Draper et al., 2003; Dzyakanchuk et al., 2009). The resulting high concentration of NADPH provides the reducing equivalents necessary for 11β-HSD1 activity. Another advantage of this cellular location is the maintenance of important intra-chain disulfide bonds within the 11β-HSD1 protein (Ozols, 1995; Tomlinson et al., 2004). Human 11β-HSD1 has three putative glycosylation sites: asparagine-X-serine sites at positions 123–125, 162–164 and 207–209 of the protein. However, it seems that, although not required for enzyme activity (Walker, E. et al., 2001) nor correct protein folding, glycosylation of 11β-HSD1 may be necessary for preventing protein aggregation and for stabilizing its structure within the ER (Tomlinson et al., 2004).

11β-HSD1 in intact cells such as hepatocytes (Jamieson et al., 1995) and adipocytes (Bujalska et al., 2002a; Bujalska et al., 2002b) [as well as in myocytes (Whorwood et al., 2001)] works mainly as a reductase, which is revealed by the higher affinity of the enzyme derived from these locations for cortisone than for cortisol (Stewart et al., 1994). However, *in vitro* when deprived of NADPH regeneration (Seckl & Walker, 2001; Walker, B. & Andrew, 2006) or in certain physiological or developmental states, it may work as a dehydrogenase, meaning that the enzyme is bidirectional (Cooper & Stewart, 2009; Tomlinson et al., 2004). This becomes evident when 11β-HSD1 switches from a dehydrogenase to a reductase functioning in human omental adipose stromal cells upon differentiation (Bujalska et al., 2002a; Bujalska et al., 2002b) or when it acts mainly as a dehydrogenase in the liver or AT of the *H6PDH* null mouse (Bujalska et al., 2008; Lavery et al., 2006).

The human *HSD11B1* gene is located in the chromosome 1 (1q32.2–41) and consists of six exons (182, 130, 111, 185, 143 and 617 bp, respectively) and five introns (776, 767, 120, 25,300 and 1,700 bp, respectively) with a total gene size of 30 kb (Draper et al., 2002; Tomlinson et al., 2004). 11β-HSD1 belongs to the short chain dehydrogenase/reductase (SDR) superfamily, a well-established enzyme family of oxido-reductases. Members of this family have a conserved N-terminal cofactor-binding domain, which confers specificity to NADPH, and a centrally located active site (Jornvall et al., 1995), containing invariant tyrosine, lysine and serine residues that consisted of the catalytic triad to which the essential presence of asparagine at 111 position has been added to form a tetrad (Filling et al., 2002; Oppermann et al., 2003; Tomlinson et al., 2004). The rat *HSD11B1* promoter has been cloned from genomic DNA (Moisan et al., 1992) and several transcription factor-binding sites were identified including several GRE consensus half-sites as well as hepatocyte nuclear factor 1, hepatocyte nuclear factor 3 and CCAAT/enhancer-binding proteins (C/EBP) sites (Williams et al., 2000). The importance of C/EBP-α has been highlighted in the regulation of *HSD11B1* transcription (Seckl & Walker, 2001; Wang et al., 1995). In human cell lines, there is evidence that promoter usage in expression of human *HSD11B1* is specific for tissue and differentiation status (Staab et al., 2011).

As with 11β-HSD2, congenital deficiency of 11β-HSD1 has been described in humans and gives rise to the apparent cortisone reductase deficiency syndrome (Phillipov et al., 1996). In this case, the lack of regeneration of cortisol in peripheral tissues results in the compensatory activation of the HPA axis translation into increased secretion of androgens by the adrenals, which, in affected females, originates hirsutism and oligomenorrhea. 11β-HSD1 congenital deficiency does not appear to protect against obesity. Curiously, the co-inheritance of inactivating mutations in both *HSD11B1* and *H6PDH* (Draper et al., 2003), decreasing NADPH supply and switching 11β-HSD1 to the dehydrogenase activity (Lavery et al., 2006), may also be in the origin of the syndrome.

3.3. Glucocorticoid deficiency

GC deficiency, seen in Addison's disease or ACTH deficiency, presents with weight loss and hypoglycemia as clinical features, that seem opposite to those of Cushing's syndrome (Walker, B., 2007). Some of these features of GC deficiency may be recapitulated in animals with type 2 diabetes mellitus (T2DM) and obesity after treatment with the GR antagonist RU38486 (mifepristone), which present with normalized blood glucose and ameliorated insulin resistance (IR) (Bitar, 2001; Gettys et al., 1997; Havel et al., 1996; Jacobson et al., 2005; Kusunoki et al., 1995; Walker, B., 2007; Watts et al., 2005). However, RU38486 may induce compensation from the HPA axis since it blocks GR involved in the HPA axis negative feedback control. Furthermore, progesterone receptor actions of the drug may also influence energy homeostasis (Picard et al., 2002). These effects of GC deficiency are in favor of the usefulness of strategies of reducing GCs action in the management of blood glucose levels and insulin sensitivity and, possibly, body weight.

3.4. Glucocorticoid excess

Although the elevation of GC levels in situations of stress is essential for survival, their chronic augmentation is associated with deleterious health outcomes. Opposite to their deficit, chronically elevated GC levels cause obesity, T2DM, heart disease, mood disorders and memory impairments (Wamil & Seckl, 2007). Elevated GC levels occur in Cushing's syndrome due to increased pathological secretion from the adrenal cortex (endogenous) or from prolonged anti-inflammatory GC treatment (iatrogenic) (Newell-Price et al., 2006). Cushing´s disease, a specific type of ACTH-dependent Cushing's syndrome, is characterized by increased ACTH secretion from a pituitary adenoma that in turn results in higher cortisol secretion from the adrenals (Cushing, 1932). Cushing's syndrome features include hypertension, rapidly accumulating visceral AT, IR (50% develop T2DM or impaired glucose tolerance) and hepatic steatosis (Stewart, 2005); muscle weakness, dyslipidemia, mood disturbances and infertility (Carroll & Findling, 2010; Newell-Price et al., 2006) are also frequently found. Although many of the clinical components (central weight gain, glucose intolerance and hypertension) are seen in other common conditions, identifying features unusual for the patient´s age (e.g. early onset osteoporosis or hypertension), features more specific to Cushing's syndrome (e.g. easy bruising, facial plethora and violaceous striae) and

patients with incidental adrenal mass or polycystic ovary syndrome should be helpful for the diagnosis (Carroll & Findling, 2010).

In effect, Cushing's syndrome represents a secondary cause of metabolic syndrome (MetSyn) (Stewart, 2005). Circulating cortisol concentrations are higher in patients with the MetSyn, hypertension or impaired glucose tolerance compared with healthy subjects, both in basal conditions and during dynamic stimulation (Anagnostis et al., 2009; Duclos et al., 2005; Misra et al., 2008; Phillips et al., 1998; Sen et al., 2008; Weigensberg et al., 2008), despite being within the normal range (Sen et al., 2008; Walker, B., 2006). This suggests increased activity of cortisol in the periphery and dysregulation of the HPA axis (Sen et al., 2008; Walker, B., 2006). However, it has also been proposed that variations in tissue cortisol concentrations could occur without any changes in plasma cortisol levels, provided that the latter are maintained by normal feedback regulation of the HPA axis (Walker, B. & Andrew, 2006). In regard to the visceral AT, this effect has been termed 'Cushing's disease of the omentum' (Bujalska et al., 1997; Stewart, 2005). Increased 11β-HSD1 activity in visceral AT may generate increased cortisol levels within both the AT and the liver and, thereby promotes features of the MetSyn (Walker, B. & Andrew, 2006). The rate of regeneration of cortisol in the visceral AT has been estimated to be sufficient to increase the concentration of cortisol in the portal vein (from about 120 nmol/L in the systemic circulation to about 155 nmol/L in the portal vein) and this has been confirmed in mice overexpressing 11β-HSD1 in the AT (Masuzaki et al., 2001; Walker, B. & Andrew, 2006).

In agreement, transgenic mice overexpressing 11β-HSD1 selectively in the AT or in the liver faithfully recapitulate MetSyn features and *HSD11B1* knockout mice or humans and rodents treated with 11β-HSD1 inhibitors seem to be protected from the cardiometabolic risks of obesity, T2DM and/or MetSyn. Very recently, we have reviewed the involvement of 11β-HSD1 in the pathophysiology of the MetSyn, obesity and T2DM, where a description not only of these animal models [also together with the animal model overexpressing 11β-HSD2 in the AT (phenotypically similar to the *HSD11B1* knockout animals)] but also of the nutritional and pharmacological modulation of 11β-HSD1 is provided (Pereira et al., 2011; 2012).

4. Epigenetics and 11β-hydroxysteroid dehydrogenase type 2

4.1. Epigenetic regulation of gene expression

Epigenetics is not a new area of investigation, as it was first described in the early 1940s (Jablonka & Lamb, 2002), but it is a hot topic of research today, since it became evident that genetic information alone is not sufficient to understand phenotypic manifestations. The way that the DNA code is translated into function depends not only on its sequence but also on the interaction with environmental factors (Ammerpoht & Siebert, 2011; Martin-Subero, 2011).

The word "epigenetic" was first described by Conrad Waddington, in 1942, as "the branch of biology which studies the causal interactions between genes and their products, which

bring the phenotype into being" (Jablonka & Lamb, 2002; Waddington, 1942). Epigenetics may be seen as the link between genotype and phenotype, a phenomenon that changes the final outcome of a locus or chromosome without changing the underlying DNA sequence. In other words, epigenetics studies any potentially stable and, ideally, heritable change in gene expression or cellular phenotype that occurs without changes in Watson-Crick base-pairing of DNA (Goldberg et al., 2007; Jablonka & Lamb, 2002). By controlling gene activity and, therefore, the availability of the final gene product in the cell, epigenetic alterations can have similar effects as classical genetic mutations (Ammerpoht & Siebert, 2011).

Today's epigenetic research is converging in the study of covalent and noncovalent modifications of DNA and histone proteins and the mechanisms by which such modifications influence overall chromatin structure. DNA methylation is perhaps the best characterized chemical modification of chromatin. In mammals, nearly all DNA methylation occurs on cytosine residues of guanidine/cytosine (CpG) dinucleotides. In genome, there are some regions especially rich in CpG in what is called CpG islands, and DNA methylation of these islands correlates with transcriptional repression. DNA methylation plays a role in many cellular processes including X chromosome inactivation in female mammals and mammalian imprinting, which can be both stably maintained (Alikhani-Koopaei et al., 2004; Drake et al., 2012; Goldberg et al., 2007).

Covalent histone modification is another epigenetic mechanism as it changes chromatin conformation, probably because charge-altering modifications, such as acetylation and phosphorylation, which can directly alter the physical properties of the chromatin fiber, lead to changes in higher-order structures. Noncovalent mechanisms such as chromatin remodeling and the incorporation of specialized histone variants provide the cell with additional tools for introducing variation into the chromatin template. Collectively, covalent modifications, nucleosome remodeling and histone variants can work together and introduce meaningful variation into the chromatin fiber. Their collective contribution to epigenetics is being explored (Drake et al., 2012; Goldberg et al., 2007).

4.2. Epigenetic mechanisms of 11β-hydroxysteroid dehydrogenase type 2 regulation

At the present moment, to our knowledge, there is no published research on epigenetic regulation of 11β-HSD1. Nevertheless, besides mutations and environmental factors (like corticosterone hormones, growth factors, shear stress, inflammatory cytokines and hypoxia) (Atanasov et al., 2003; Baserga et al., 2010; Hardy & Yang, 2002) also epigenetic phenomena can regulate 11β-HSD2 abundance and activity (please see below for references).

The *HSD11B2* promoter comprises a highly guanidine + cytosine (G+C)-rich core, containing more than 80% G+C, and two typical CpG islands, bringing the possibility that CpG dinucleotide methylation plays a role in the cell type-specific and, possibly, in the epigenetically determined inter-individual variable expression of *HSD11B2* (Alikhani-Koopaei et al., 2004; Baserga et al., 2010). In this regard, Alikhani-Koopaei *et al* provided *in vitro* and *in vivo* evidence that 11β-HSD2 expression and activity are inversely correlated with the presence of methylation at the *HSD11B2* promoter region. They have found that CpG islands covering the promoter and exon 1 of *HSD11B2* are densely methylated in human tissues and cell lines

with low expression (or activity) [liver, skeletal muscle and renal proximal tubules; MCF-7 and JEG-3 cells (breast adenocarcinoma and placenta choriocarcinoma cell lines, respectively)] but not in those with high expression (or activity) of 11β-HSD2 [placenta and renal distal tubules; SW620 cells (colon carcinoma cell line)]. DNA methyltransferase inhibitors enhance the transcription of *HSD11B2* and the activity of 11β-HSD2 in different human cell types (above mentioned cell lines and primary kidney cells).

Additionally, these inhibitors increase mRNA abundance in various tissues (liver, kidney and lung) and decrease the urinary GC metabolite ratios [corticosterone (THB and 5α-THB)]/[11-dehydrocorticosterone (THA)] in Wistar rats, indicating higher 11β-HSD2 activity (Alikhani-Koopaei et al., 2004).

A decrease in 11β-HSD2 activity, by decreasing renal GC deactivation, is associated with hypertension (Baserga et al., 2010; Pereira et al., 2011; 2012). In order to explore the possible relevance of *HSD11B2* promoter methylation in human blood pressure control, Friso *et al* examined peripheral blood mononuclear cell DNA methylation and urinary tetrahydrocortisol- *versus* tetrahydrocortisone-metabolites (THFs/THE) shuttle as a biochemical indicator of 11β-HSD2 activity. They have found that elevated *HSD11B2* promoter methylation is associated with hypertension developing in GC-treated rheumatoid arthritis patients in parallel with a higher urinary THFs/THE ratio (as a consequence of lower 11β-HSD2 activity). Essential hypertensive patients with elevated urinary THFs/THE ratio also have higher *HSD11B2* promoter methylation (Friso et al., 2008).

So, from animal and human studies it can be hypothesized that changes in the *HSD11B2* gene methylation patterns might explain the inter-individual differences in the expression and activity of 11β-HSD2 in mineralocorticoid target tissues and, consequently, might modulate blood pressure.

4.3. Intrauterine growth restriction and glucocorticoid prenatal overexposure *versus* epigenetic regulation of 11β-hydroxysteroid dehydrogenase type 2

The adverse effects of GC exposure in the prenatal period are related to changes in the expression of the GR and in the intracellular availability and level of GCs, which are modulated by 11β-HSDs as described above. 11β-HSD2, due to its localization to the syncytiotrophoblast layer of the placenta (the site of maternal-fetal exchange), constitutes a functional barrier restricting the free transfer of cortisol (in humans) or corticosterone (in rodents) between the maternal and fetal compartments, by converting maternal active metabolites to the corresponding inactive forms (cortisone and 11-dehydrocorticosterone in humans and rodents, respectively). Thus, the placental 11β-HSD2 protects the fetus from exposure to high levels of maternal GCs, its enzymatic activity being positively correlated with birth weight (in humans and rats) (Albiston et al., 1994; Baserga et al., 2007; Baserga et al., 2010; Benediktsson et al., 1993; Harris & Seckl, 2011; Kajantie et al., 2003; Krozowski et al., 1995; Lesage et al., 2001; Murphy et al., 2002; Pepe et al., 1999; Ronco et al., 2010; Stewart et al., 1995; Wyrwoll et al., 2012).

In line with the above described information, in a well-characterized animal model of intra-uterine growth restriction (IUGR) and adult onset hypertension [after bilateral uterine artery ligation, preformed on day 19 of gestation in Sprague-Dawley rats, uteroplacental insufficiency (UPI) occurs], Baserga *et al* report persistently decreased kidney 11β-HSD2 mRNA and protein levels through day 21 of life (juvenile rat) (Baserga et al., 2007; Baserga et al., 2010). Further developing this study, Baserga *et al* report that IUGR (as a consequence of UPI after surgery on day 19.5 of gestation in Sprague-Dawley rats) a) alters key transcription factors binding to the renal *HSD11B2* promoter [decreases SP1 (specificity protein 1) and NF-kB (nuclear factor-kappaB, p65) binding in males (transcriptional enhancers), while increases Egr-1 (early growth response factor) binding in females and NF-kB (p50) binding in males (transcriptional repressors)]; b) increases CpG methylation status as well as modifies the methylation pattern in several CpG sites of *HSD11B2* promoter at (post-birth) day 0, also in a sex-specific manner; and c) decreases trimethylation of H3K36 in exon 5 of *HSD11B2* at (post-birth) day 0 and day 21 in both genders (which is associated with decreased transcriptional elongation). The authors speculated that alterations in transcription factor binding and chromatin structure may play a role in *in utero* reprogramming (Baserga et al., 2010). In a recent paper, Marsit *et al* demonstrate an inverse association between measures of intrauterine growth, including birth weight and ponderal index, with the extent of DNA methylation of the *HSD11B2* gene promoter region, in the placenta of 185 healthy newborn infants. Growth restricted infants, and particularly those with clinically diagnosed IUGR, show greater methylation than their grown counterparts. An inverse relationship between the extent of *HSD11B2* methylation and infant quality of movement as well as a trend towards a positive correlation between *HSD11B2* methylation and infant attention have been identified. The authors suggested that an adverse intrauterine environment leading to growth restriction may enhance infant cortisol exposure and its downstream effects both by reducing *HSD11B2* expression and by allowing GR expression by maintaining low levels of methylation at that promoter. The enhanced levels of active cortisol and potentially enhanced response may then be responsible for inappropriate programming of the HPA axis as well as altered neuromuscular development in the infant (Marsit et al., 2012).

Very recently, Wyrwoll *et al* reported that, in Wistar rats, prenatal dexamethasone [Dex; subcutaneous injection of 100 μg Dex/kg or vehicle (Veh) from embryonic day (E) 15 to 19] which is not metabolized by 11β-HSD2, has opposite effects on placental choline and folate transport at E20. The placental transport capacity of choline is reduced by Dex, such that the fetus receives less choline/gram of fetal weight. In contrast, Dex increases placental folate transport, such that the Dex-exposed fetuses receive more folate/gram of fetal weight. Placental methionine transport and maternal plasma methionine concentrations are unaffected by Dex exposure, although fetal plasma methionine levels are reduced. As the establishment of epigenetic modifications in the fetus depends on the availability of methyl donors during fetal development, the authors suggested that, altogether, those changes in key components of the methyl donor cycle may explain the impact of prenatal GC overexposure on metabolic programming and disease risk in the offspring (Wyrwoll et al., 2012).

Both syncytialization and Dex stimulate leptin secretion from both the apical and basal surfaces of human choriocarcinoma BeWo cells. Additionally, transport of exogenous leptin is also evident in both the apical to basal and reverse direction, suggesting maternal-fetal exchange of leptin across the human placenta (Wyrwoll et al., 2005). It is recognized that leptin, besides being a proinflammatory cytokine and a regulator of appetite, body fat and bone metabolism, lung development and function, immune and thyroid functions, stress response, metabolic activity by peripheral tissues and energy balance, is also important for the establishment of pregnancy (D'Ippolito et al., 2012; Denver et al., 2011; Malik et al., 2001; Mantzoros et al., 2011; Wyrwoll et al., 2005), being positively associated with fetal (Tsai et al., 2004; Vatten et al., 2002; Wyrwoll et al., 2005) and placental (Jakimiuk et al., 2003; Wyrwoll et al., 2005) growths.

5. Interplay between glucocorticoid availability, diet and fetal programming

Both fetal GC exposure and maternal nutrition contribute to fetal programming. Maternal undernutrition increases cortisol and corticosterone plasma levels (in humans or rats, respectively) in both mothers and growth-retarded fetuses (Lesage et al., 2001).

Exposing rats *in utero* to high levels of Dex also reduces fetal and birth weights, increases blood pressure and causes fasting hyperglycemia, reactive hyperglycemia and hyperinsulinemia on oral glucose loading in the adult offspring (Benediktsson et al., 1993; Lesage et al., 2001; Nyirenda et al., 1998; Wyrwoll et al., 2012; Wyrwoll et al., 2006). Postnatal diet may counteract some of the fetal programming effects (Waddell et al., 2010; Wyrwoll et al., 2006; Wyrwoll et al., 2008).

Accordingly, in pregnant Wistar rats a magnesium-deficient diet (0.003% magnesium *versus* 0.082% magnesium in the control diet) increases methylation of specific CpG dinucleotides in the hepatic *HSD11B2* promoter of neonatal offspring (without gender differences) (Takaya et al., 2011), what might increase hepatic intracellular GC levels [increased GC levels have been associated with IR and T2DM (Pereira et al., 2011; 2012)] and contribute to unravel the mechanisms of the metabolic adverse effects associated to a low magnesium intake (Lecube et al., 2012; Lima et al., 2009; Takaya et al., 2011; Volpe, 2008).

Alterations of *HSD11B2* methylation have been recently reported by Drake *et al* on buffy coat DNA of adult individuals whose mothers ate an unbalanced diet in pregnancy: methylation at specific CpGs in the *HSD11B2* promoter correlates with neonatal anthropometric variables [birth weight (positively) and neonatal ponderal index (negatively)] and CpG methylation within *HSD11B2* and *GR* associates with increased adiposity and blood pressure in adulthood. The authors suggested that these results indicate a persisting epigenetic link between early life maternal diet and/or fetal growth and cardiovascular disease risk in humans (Drake et al., 2012).

Recent studies have identified programming effects of leptin that influence postnatal phenotype (Granado et al., 2012). Wyrwoll *et al* demonstrated that programmed hyperleptinemia
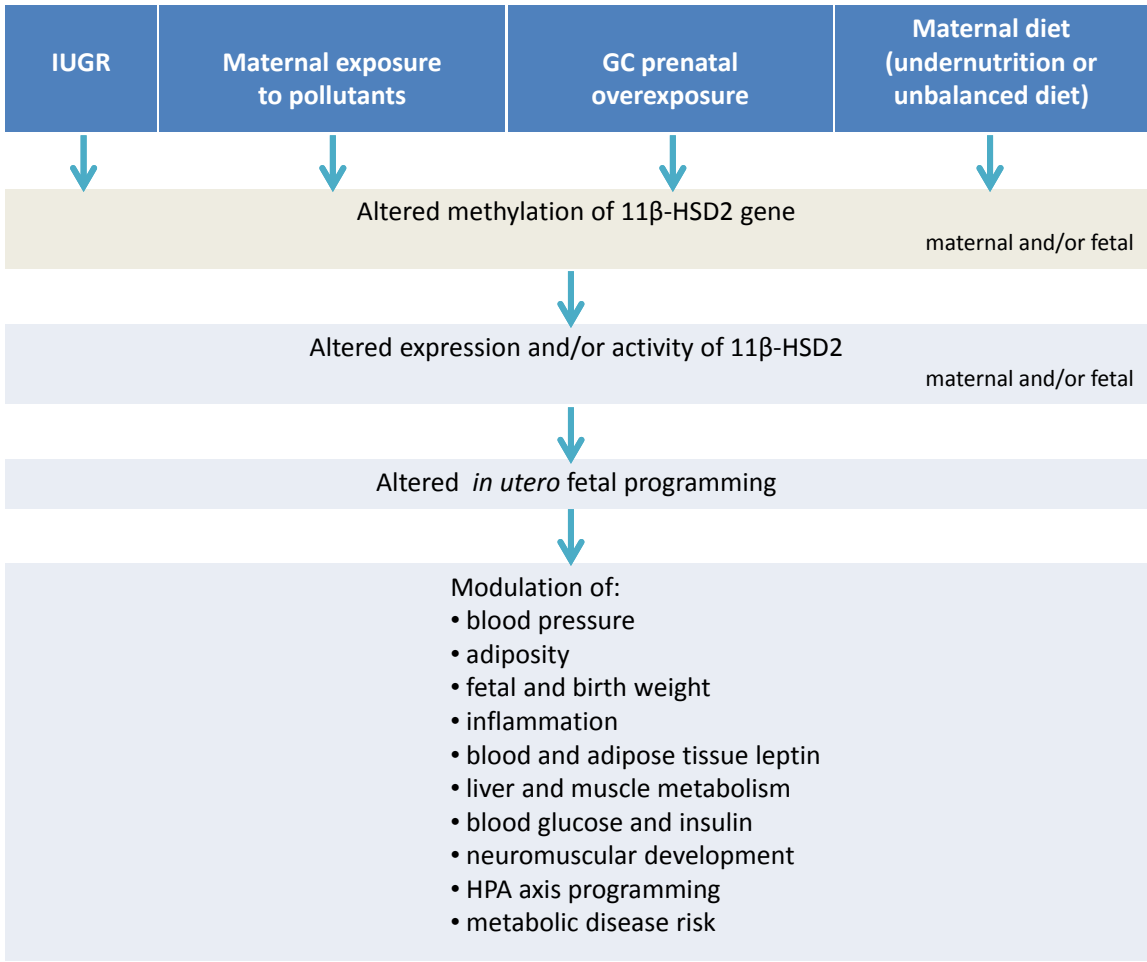
and hypertension, also induced by Dex overexposure *in utero*, in Wistar rats [Dex administered in the drinking water (0.75 μg/mL) from day 13 of pregnancy until birth], are completely blocked in the offspring by a postnatal diet enriched with ω-3 fatty acids (the majority being long chain). In the absence of the ω-3 fatty acid supplementation, programmed hyperleptinemia is totally apparent by 6 months of age (in both sexes), being accompanied by an elevation of leptin mRNA expression in the AT (that is also completely abolished by the dietary supplementation). Programmed hypertension is evident in male offspring by 2 months and in female offspring by 6 months of age. Maternal Dex delays the onset of puberty in the offspring. These results demonstrated, for the first time, that modifications in the postnatal diet can prevent major adverse fetal programming outcomes (by increased GC exposure *in utero*) (Wyrwoll et al., 2006). In the same animal protocol, ω-3 fatty acid supplementation is also effective in preventing the increased plasma fasting insulin and interleukin-1β in the adult offspring exposed to GC excess *in utero*. However, raising animals from birth on a high ω-3 fatty acids diet do not prevent the programmed increase in plasma tumour necrosis factor-α and do not correct disturbances in skeletal muscle expression of SLC2A4 (formerly known as glucose transporter 4, GLUT4), PPAR-δ and uncoupling protein 3 mRNAs (Wyrwoll et al., 2008). However, the protective effects of the high ω-3 fatty acids diet are not mediated by changes in the adrenal function. Waddell *et al* reported that, in the just above mentioned animal protocol, prenatal Dex exposure also increases, in the adult offspring, stimulated urinary corticosterone and aldosterone (after overnight isolation) and plasma corticosterone levels (under anesthesia), what suggests heightened adrenal responsiveness to stress. These effects are not prevented by ω-3 fatty acids supplementation. Key steroidogenic genes expression levels and (adrenal weight)/(body weight) ratio are unaltered by prenatal Dex treatment or postnatal diet. Interestingly, adrenal mRNA expression of both *HSD11B2* and *MC2R* genes increases with Dex treatment. High ω-3 fatty acids diet partially attenuates the Dex-effect on *MC2R* mRNA expression but increases *HSD11B2* mRNA expression (Waddell et al., 2010).

6. Environmental pollutants *versus* epigenetic regulation of 11β-hydroxysteroid dehydrogenase type 2

Cadmium (Cd^{2+}) has been classified as a human carcinogen and has been identified as a new class of endocrine disruptor (Byrne et al., 2009; Henson & Chedrese, 2004; Ronco et al., 2010; Waisberg et al., 2003; Yang et al., 2006). Neonates delivered from mothers who smoked during pregnancy have reduced birth weight, compared to those neonates from non-smoking mothers, what is correlated to placental Cd^{2+} concentration (Ronco et al., 2005; Ronco et al., 2010). Placentas of mothers delivering low birth weight newborns show significantly higher Cd^{2+} concentrations than placentas associated to normal birth weight neonates, what suggests that placental accumulation of heavy metals is related to altered fetal growth mechanisms (Llanos & Ronco, 2009; Ronco et al., 2010). Epigenetic alterations mediate some toxic effects of environmental chemicals like Cd^{2+} (Baccarelli & Bollati, 2009; Ronco et al., 2010),

with paradoxical effects on DNA methylation during Cd^{2+}-induced cellular transformation (Ronco et al., 2010; Takiguchi et al., 2003).

Using primary cultured human trophoblast cells as a model system Yang *et al*, reported that Cd^{2+} exposure results in a time- and concentration-dependent decrease in 11β-HSD2 activity, such that an 80% reduction is observed after 24 h of treatment at 1 μM (with a similar decrease in 11β-HSD2 protein and mRNA levels), suggesting that Cd^{2+} reduces 11β-HSD2 enzyme expression. Furthermore, Cd^{2+} diminishes *HSD11B2* promoter activity, indicative of repression of *HSD11B2* gene transcription. Overall, these results could represent one of the mechanisms involved in the Cd^{2+}-induced reduction in birth weight of smoker's newborns (Ronco et al., 2010; Yang et al., 2006). In line with this, Ronco *et al* observed that a 24 h exposure of JEG-3 cells to 1 μM of Cd^{2+} induces an increase in 11β-HSD2 activity and mRNA expression as well as a reduction in the methylation index of the *HSD11B2* gene. These results suggest that Cd^{2+}-induced endocrine disruptor effects on JEG-3 cells could be mediated by changes in the methylation status of some target genes (Ronco et al., 2010).

IUGR	Maternal exposure to pollutants	GC prenatal overexposure	Maternal diet (undernutrition or unbalanced diet)

Altered methylation of 11β-HSD2 gene
maternal and/or fetal

Altered expression and/or activity of 11β-HSD2
maternal and/or fetal

Altered *in utero* fetal programming

Modulation of:
• blood pressure
• adiposity
• fetal and birth weight
• inflammation
• blood and adipose tissue leptin
• liver and muscle metabolism
• blood glucose and insulin
• neuromuscular development
• HPA axis programming
• metabolic disease risk

Figure 1. Possible factors of epigenetic 11β-HSD2 regulation. 11β-HSD2 - 11β-hydroxysteroid dehydrogenase type 2; GC - glucocorticoids; HPA - hypothalamus-pituitary-adrenal; IUGR - intrauterine growth restriction.

7. Conclusion

The present review highlights the importance of 11β-HSDs for the modulation of tissue CG availability. As depicted above, defects on expression and/or activity of these enzymes can affect physiology and result in clinical conditions related with impaired metabolic or blood pressure control. In this regard, knowing that these enzymes can be differentially modulated within different tissues and that nutritional cues or environmental factors, like pollutants, can modify their activity opens avenues for possible interventions at the level of treatment or prevention of conditions related with dysregulated tissue GC levels.

Furthermore, the contribution of epigenetics to the demonstration that tissue GC levels or their actions can be modified through interference with the expression of *HSD11B1* and *HSD11B2* (data presented here for the 11β-HSD2 enzyme is summarized in Figure 1) or components of the HPA axis, underscores the importance of transgenerational influences of GC level modifiers for the establishment of pathologies of epidemic proportions like obesity, hypertension and MetSyn. This awareness will allow not only to comprehend the pathophysiological processes involved but also paves the way towards the design and implementation of interventions in order to hamper the increase of such pathologies.

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