Role of sulphate in development

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Abstract

Sulphate contributes to numerous processes in mammalian physiology, particularly during development. Sulphotransferases mediate the sulphate conjugation (sulphonation) of numerous compounds, including steroids, glycosaminoglycans, proteins, neurotransmitters and xenobiotics, transforming their biological activities. Importantly, the ratio of sulphonated to unconjugated molecules plays a significant physiological role in many of the molecular events that regulate mammalian growth and development. In humans, the fetus is unable to generate its own sulphate and therefore relies on sulphate being supplied from maternal circulation via the placenta. To meet the gestational needs of the growing fetus, maternal blood sulphate concentrations double from mid-gestation. Maternal hyposulphataemia has been linked to fetal sulphate deficiency and late gestational fetal loss in mice. Disorders of sulphonation have also been linked to a number of developmental disorders in humans, including skeletal dysplasias and premature adrenarche. While recognised as an important nutrient in mammalian physiology, sulphate is largely unappreciated in clinical settings. In part, this may be due to technical challenges in measuring sulphate with standard pathology equipment and hence the limited findings of perturbed sulphate homeostasis affecting human health. This review article is aimed at highlighting the importance of sulphate in mammalian development, with basic science research being translated through animal models and linkage to human disorders.

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Introduction

In adults and children, approximately one third of sulphate requirements are obtained from the diet (Appel *et al.* 2004), although sulphate intake can vary greatly (1.5–16 mmol/day) and is dependent on the source of drinking water (undetectable to >500 mg/l) and types of food consumed (Allen *et al.* 1989, Florin *et al.* 1991, 1993). Dietary sulphate is absorbed via the intestinal epithelium into the circulation, where it is maintained at ~0.3 mM, making sulphate the fourth most abundant anion in human plasma (Murer *et al.* 1992, Cole & Evroviski 1997). Circulating sulphate levels are maintained by the kidneys, which filter sulphate in the glomerulus and then reabsorb sulphate back into circulation (Ullrich & Murer 1982). The amount of sulphate that is reabsorbed is regulated by sulphate transporter proteins expressed on the plasma membrane of renal epithelial cells.

Sulphate reabsorption occurs in the proximal tubule of the kidney and is mediated by two sulphate transporter proteins, SLC13A1 (aka NaS1, sodium sulphate transporter 1) and SLC26A1 (aka SAT1, sulphate anion transporter 1) (Lee *et al.* 2005). SLC13A1 is expressed on the apical membrane of epithelial cells in the proximal tubule where it mediates the first step of sulphate reabsorption (Lotscher *et al.* 1996), and

SLC26A1 mediates the second step across the basolateral membrane (Karniski *et al.* 1998) (Fig. 1A). Mice lacking the *Slc13a1* or *Slc26a1* genes have sulphate wasting into the urine (hypersulphaturia), which leads to reduced blood sulphate levels (hyposulphataemia) (Dawson *et al.* 2003, 2010). In addition, genetic defects in the *SLC13A1* gene of dogs and sheep also lead to hyposulphataemia (Neff *et al.* 2012, Zhao *et al.* 2012). Humans with loss-of-function mutations (R12X and N174S) in the *SLC13A1* gene exhibit renal sulphate wasting and hyposulphataemia (Bowling *et al.* 2012). This loss of sulphate from circulation reduces sulphate availability to cells throughout the body and leads to a reduced intracellular sulphate conjugation (sulphonation) capacity, as shown in the *Slc13a1*- and *Slc26a1*-null mice (Dawson *et al.* 2003, 2010, Lee *et al.* 2006).

Intracellular sulphonation relies on a sufficient supply of sulphate, which is derived from the uptake of sulphate across the plasma membrane via sulphate transporters. The cell also generates sulphate from the intermediary metabolism of thiol compounds, as well as de-conjugation reactions that are mediated by sulphatases (Fig. 1B). While intracellular oxidation of cysteine provides the majority of sulphate requirements for most cell types, some tissues such as the developing liver and skeleton rely more on extracellular sources of sulphate via sulphate transporters (Ito *et al.* 1982,

Figure 1 Sufficient intracellular sulphate levels need to be maintained for sulphonation reactions to function effectively. (A) In the kidneys, filtered sulphate is reabsorbed through epithelial cells in the proximal tubule via SLC13A1 on the apical membrane and then by SLC26A1 on the basolateral membrane. (B) Intracellular sulphate is obtained from extracellular sources via sulphate transporters and is derived from the metabolism of methionine and cysteine. Sulphate and ATP are converted to the universal sulphonate donor, PAPS. Both (1) sulphonation and (2) de-sulphonation reactions are active within intracellular metabolism. Sulphonated molecules are transported across the plasma membrane of cells via ATP binding cassette (ABC) proteins, sodium-dependent organic anion transporter (SOAT) and organic anion transporter polypeptides (OATPs), where they provide a circulating reservoir for cellular uptake and intracellular de-sulphonation. R-O-SO₃⁻ represents sulphonated substrates, including steroids, neurotransmitters and proteins.

Humphries *et al.* 1988). In addition, the placenta and fetus rely on sulphate supplied from the maternal circulation because these tissues have negligible levels of cystathionase and cysteine oxidase, that are important for generating sulphate from the sulphur-containing amino acids (Gaull *et al.* 1972, Loriette & Chatagner 1978, Dawson 2011). Sulphonation reactions in all organisms require the conversion of sulphate to the universal sulphonate (SO₃⁻) donor, 3'-phosphoadenosine 5'-phosphosulphate (PAPS) (Klassen & Boles 1997). PAPS is generated by the cytosolic enzyme, PAPS synthetase, that sulphurylates ATP to form APS followed by phosphorylation to form PAPS (Venkatachalam

2003; Fig. 1B). The sulphonate group from PAPS is then transferred to the target substrate via sulphotransferase enzymes, which can be grouped into two classes: i) cytosolic sulphotransferases that sulphonate neurotransmitters, bile acids, xenobiotics, and steroids and ii) Golgi-located sulphotransferases that have proteoglycan and lipid substrates (Gamage *et al.* 2006) and rely on PAPS transporters (PAPST1 and PAPST2) to mediate the translocation of PAPS from the cytosol into the Golgi (Sasaki *et al.* 2009). The landmark report of sulphate activation to PAPS (Lipmann 1958) and the subsequent identification of sulphotransferases (Lipmann 1958, Gamage *et al.* 2006), as well as the characterisation of mouse models with perturbed sulphate homeostasis (Bullock *et al.* 1998, Faiyaz ul Haque *et al.* 1998, Ringvall *et al.* 2000, Dawson *et al.* 2003, 2010, Thiele *et al.* 2004, Forlino *et al.* 2005, Tong *et al.* 2005, de Agostini 2006, Habuchi *et al.* 2007, Holst *et al.* 2007, Lum *et al.* 2007, Settembre *et al.* 2007), have led to our current understanding of sulphonation and the physiological importance of this process in modulating the biological activity of steroids (Dawson 2012), thyroid hormone (Richard *et al.* 2001, Wu *et al.* 2005), glycosaminoglycans and xenobiotics (Lee *et al.* 2006, Dawson *et al.* 2010). While the clinical importance of sulphate is currently underappreciated, interest in sulphate and its roles in mammalian reproduction and development has expanded over the past decade.

Sulphate in fertility and maintenance of pregnancy

Several studies have proposed an essential role of sulphate in fertilisation and maintenance of pregnancy. Sulphonation of zona pellucida (ZP) glycoproteins during oocyte maturation contributes to the ZP acquiring the capacity to accept sperm (Lay *et al.* 2011). In addition, sperm from male mice lacking tyrosylprotein sulphotransferase-2 (*Tpst2*) have reduced ability to adhere to the egg plasma membrane, leading to male infertility (Borghei *et al.* 2006). To date, more than 40 tyrosine-sulphonated proteins have been identified in humans and rodents (Stone *et al.* 2009), including the sperm-expressed MFGE8 protein that lacks tyrosine sulphonation in infertile *Tpst2*-deficient male mice (Hoffhines *et al.* 2009). Tyrosine sulphonation of both the luteinizing hormone (LH) receptor and follicle-stimulating hormone (FSH) receptor is required for optimal function of these proteins in reproductive biology (Costagliola *et al.* 2002, Mi *et al.* 2002). Furthermore, sulphonated pregnenolone, but not unconjugated pregnenolone, enhances phospholipase A2 activity, which plays an essential role in eicosanoid production and maintenance of uterine function during gestation (Saitoh *et al.* 1984, Brant *et al.* 2006). Together, these findings show that sulphate plays numerous important physiological roles in mammalian reproductive biology.

Sulphate supply to the fetus in human and animal gestation

During human and rodent pregnancy, maternal circulating sulphate levels increase approximately twofold, with levels peaking in the second and third trimesters (Tallgren 1980, Morris & Levy 1983, Cole *et al.* 1984, Dawson *et al.* 2011). This increase is due to elevated maternal kidney *SLC13A1* and *SLC26A1* gene expression (Lee *et al.* 1999, Dawson *et al.* 2011), which leads to enhanced renal sulphate reabsorption (Cole *et al.* 1985a) in the pregnant mother (Fig. 2). The increased circulating sulphate level in pregnant humans (from ≈ 0.26 to 0.59 mM; Cole *et al.* 1984, 1985b, 1992) and mice (from ≈ 1.0 to 2.3 mM; Dawson *et al.* 2011) provides a reservoir of sulphate for the placenta and fetus and is remarkable as most circulating ions usually decrease slightly due to haemodilution (Lind 1980). As the placenta and fetus have a relatively low capacity to generate sulphate from methionine and cysteine (Gaull *et al.* 1972, Lorient & Chatagner 1978), most of the sulphate in these tissues must come from the maternal circulation (Fig. 2). This is consistent with fetal hyposulphataemia and negligible amniotic fluid sulphate levels in fetuses from pregnant hyposulphataemic *Slc13a1*-null mice (Dawson *et al.* 2011). The reduced fecundity of pregnant female *Slc13a1*-null mice (Dawson *et al.* 2003) is due to fetal death in mid- and late gestation (from embryonic day 12.5; Dawson *et al.* 2011), highlighting the importance for maintaining high maternal blood sulphate levels during pregnancy.

Sulphate is supplied from maternal blood to fetal circulation, via placental sulphate transporters. Recently, the relative abundance and cellular expression of all known sulphate transporters in human and mouse placentae were described (Dawson *et al.* 2012, Simmons *et al.* 2013). Those studies identified *SLC13A4* (aka NaS2) to be the most abundant placental sulphate transporter, which was localised to the syncytiotrophoblasts, where it

is proposed to play an essential role in mediating sulphate supply to the placenta and fetus. The proposed roles of both placental *SLC13A4* and maternal renal *SLC13A1* in meeting the gestational needs of the developing fetus (Fig. 2) warrant future studies of these transporters in pregnant women with perturbed sulphate homeostasis.

Pathophysiology of perturbed sulphate homeostasis in human and animal development

Numerous genes involved in maintaining the required biological ratio of sulphonated to unconjugated molecules have been described in humans and animal models (Table 1). Intracellular sulphate and its sulphate donor PAPS need to be maintained at sufficiently high levels for the sulphonation of xenobiotics, pharmacological drugs, proteoglycans and steroids (Mulder & Jakoby 1990; Fig. 3). Furthermore, the intracellular removal of sulphate from compounds is mediated by a family of 17 sulphatase enzymes (Hanson *et al.* 2004, Sardiello *et al.* 2005), and several of these have been linked to syndromes in humans (Table 1).

Sulphonation of xenobiotics and pharmacological drugs

In human and animal gestation, the fetus has negligible capacity to detoxify xenobiotics and certain drugs via the glucuronidation pathway (Hines & McCarver 2002, McCarver & Hines 2002). However, the fetal liver expresses abundant levels of sulphotransferases, which are important for the sulphonation and clearance of numerous compounds that are potentially detrimental to fetal development (Stanley *et al.* 2005, Alnouti & Klaassen 2006). Both the *Slc13a1* and *Slc26a1* mouse models of hyposulphataemia have a reduced capacity to sulphonate acetaminophen, which leads to enhanced acetaminophen-induced hepatotoxicity (Lee *et al.* 2006, Dawson *et al.* 2010). This may be relevant to earlier

Figure 2 Sulphate is supplied from maternal circulation to placental and fetal tissues, where sulphonation reactions are important for normal growth and development. (A) Increased maternal kidney *SLC13A1* and *SLC26A1* expression from early gestation (in mice from E4.5) enhances renal sulphate reabsorption, leading to (B) a doubling in circulating sulphate concentrations. (C) *SLC13A4* expression in syncytiotrophoblasts mediates sulphate transport (ST) for generation of the universal sulphate donor PAPS. (D) Sulphate is supplied to the fetal blood and (E) taken up by fetal cells. *Intracellular generation of sulphate in fetal and placental cells is negligible.

Table 1 Pathogenetics of perturbed sulphate homeostasis.

Gene	Species and phenotype/syndrome	References
Sulphate transporters		
<i>SLC13A1</i>	Human: renal sulphate wasting, hyposulphataemia	Bowling <i>et al.</i> (2012)
<i>Slc13a1</i>	Mice: renal sulphate wasting, hyposulphataemia, fetal loss, growth retardation, behavioural abnormalities, altered steroid/lipid profiles impaired gastrointestinal function, drug-induced hepatotoxicity	Dawson <i>et al.</i> (2003, 2004, 2005, 2006, 2008, 2009, 2011) and Lee <i>et al.</i> (2006, 2007)
<i>SLC13a1</i>	Dog: hyposulphataemia, osteochondrodysplasia, growth retardation	Neff <i>et al.</i> (2012)
<i>SLC13a1</i>	Sheep: hyposulphataemia, osteochondrodysplasia, growth retardation	Zhao <i>et al.</i> (2012)
<i>Slc26a1</i>	Mice: renal sulphate wasting, hyposulphataemia, urolithiasis	Dawson <i>et al.</i> (2010)
<i>SLC26A2</i>	Human: chondrodysplasias (MED, DTD, AO2, ACG1B)	Dawson <i>et al.</i> (2005)
<i>Slc26a2</i>	Mice: chondrodysplasias	Forlino <i>et al.</i> (2005)
PAPS synthetase and transporter		
<i>PAPSS2</i>	Human: spondyloepimetaphyseal dysplasia, premature pubarche	Faiyaz ul Haque <i>et al.</i> (1998) and Noordam <i>et al.</i> (2009)
<i>Papss2</i>	Mice: brachymorphism	Faiyaz ul Haque <i>et al.</i> (1998)
<i>papst1</i>	Zebrafish: cartilage defects	Clément <i>et al.</i> (2008)
Sulphotransferases		
<i>Sult1e1</i>	Mice: spontaneous fetal loss	Tong <i>et al.</i> (2005)
<i>Tst1</i>	Mice: fetal loss and reduced body weight	Ouyang <i>et al.</i> (2002)
<i>Tpst2</i>	Mice: dwarfism and thyroid hypoplasia	Sasaki <i>et al.</i> (2007)
<i>CHST3</i>	Human: spondyloepimetaphyseal dysplasia	Thiele <i>et al.</i> (2004)
<i>Hs3t1</i>	Mice: reduced fecundity, delayed placental development	de Agostini (2006)
<i>Hs2st (Hs2st1)</i>	Mice: eye and skeletal defects, kidney agenesis, neonatal death	Bullock <i>et al.</i> (1998)
<i>Hs6st (Hs6st1)</i>	Mice: reduced fecundity, perturbed placental development	Habuchi <i>et al.</i> (2007)
<i>Ndst1</i>	Mice: mammary gland development, reduced fecundity, neonatal death	Crawford <i>et al.</i> (2010) and Ringvall <i>et al.</i> (2000)
Sulphatases		
<i>ARSA</i>	Human: metachromatic leucodystrophy	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>ARSB</i>	Human: Maroteaux–Lamy syndrome	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>ARSC</i>	Human: X-linked ichthyosis	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>ARSE</i>	Human: chondrodysplasia punctata 1	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>GALNS</i>	Human: Morquio A syndrome	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>GNS</i>	Human: Sanfilippo D syndrome	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>IDS</i>	Human: Hunter syndrome	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>SGSH</i>	Human: Sanfilippo A syndrome	Diez-Roux & Ballabio (2005) and Sardiello <i>et al.</i> (2005)
<i>SULF1</i>	Human: Mesomelia-synostoses syndrome	Isidor <i>et al.</i> (2010)
<i>Sulf1</i>	Mouse: reduced growth, skeletal defects, perinatal death	Holst <i>et al.</i> (2007)
<i>Sulf2</i>	Mouse: reduced growth, skeletal defects, perinatal death	Holst <i>et al.</i> (2007) and Lum <i>et al.</i> (2007)
<i>SUMF1</i>	Human: Multiple sulphatase deficiencies	Cosma <i>et al.</i> (2003)
<i>Sumf1</i>	Mice: growth retardation, skeletal and neurological abnormalities, mortality	Settembre <i>et al.</i> (2007)

studies that proposed a potential link between human birth defects and a variable acetaminophen sulphonation capacity of the fetal liver (Adjei *et al.* 2008). Nonetheless, sulphonation is an important process for the detoxification of xenobiotics and certain drugs in fetal tissues (reviewed in Gamage *et al.* (2006)).

Sulphonation of proteoglycans

In mammals, sulphonated proteoglycans are an essential component of extracellular matrices throughout the body, particularly in connective tissues (Habuchi *et al.* 2004, Klüppel 2010). Proteoglycan sulphates are also a major component of the mucous barrier in the respiratory, gastrointestinal and reproductive tracts, where they are proposed to play a role in protecting

the underlying epithelium (Nieuw Amerongen *et al.* 1998, Argüeso & Gipson 2006, Dawson *et al.* 2009). The sulphate content of proteoglycans influences cell signalling function and the structural integrity of tissues (Mulder & Jakoby 1990). Highly sulphonated glycoproteins, including heparan sulphate proteoglycans (HSPGs), are critical for maintaining developmental cell signalling pathways in *Drosophila* and *Caenorhabditis elegans* (Sen *et al.* 1998, Lin *et al.* 1999, Dejima *et al.* 2006) and contribute to tissue remodelling of the reproductive tract during gestation (Yanagishita 1994) and fetal kidney and brain development (Bullock *et al.* 1998, Yamaguchi 2001). The importance of HSPG sulphonation in mammalian gestation is highlighted by the phenotypes of heparan sulphotransferase knockout (*Hs3t1*^{−/−}) mice, which exhibit reduced fecundity due

Figure 3 Fetal and placental tissues rely on sulphate supply from maternal stores for numerous physiological roles. (A) Kidney sulphate transporters maintain high maternal circulating sulphate levels during pregnancy, which supply a reservoir of sulphate to the fetus via placental sulphate transport. R, includes steroids, proteoglycans, xenobiotics and pharmacological drugs. (B) Sulphate conjugation in fetal and placental tissues plays an important role in bio-transforming numerous endogenous and exogenous molecules.

to delayed placental development (de Agostini 2006). Sulphate is also important for modulating the physiological roles of chondroitin proteoglycan (CSPG) in numerous developing tissues, with links to modulation of the Indian Hedgehog signalling pathway (Cortes *et al.* 2009). Importantly, sulphonation of CSPGs in chondrocytes is essential for normal skeletal growth and development, and several chondrodysplasias have been attributed to genetic defects that lead to decreased CSPG sulphonation capacity (Table 1).

Chondrocytes rely on an abundant supply of extracellular sulphate to meet the intracellular requirements for CSPG sulphonation. Interestingly, defects in the renal *SLC13a1* gene of dogs and sheep lead to hyposulphaemia and osteochondrodysplasias (Neff *et al.* 2012, Zhao *et al.* 2012), which are most likely to result from a decreased CSPG sulphonation capacity. Sulphate is transported into chondrocytes via the *SLC26A2* sulphate transporter (Rossi *et al.* 1997). Numerous variants in the human *SLC26A2* gene have been linked to chondrodysplasias (Dawson & Markovich 2005), with the underlying metabolic defect being reduced sulphonation of proteoglycans in chondrocytes (Cornaglia *et al.* 2009). Mutant *Slc26a2* mice also exhibit chondrodysplasias, which mimic the biochemical and morphological phenotypes found in humans (Hästbacka *et al.* 1994, Forlino *et al.* 2005, Cornaglia *et al.* 2009). Treatment of the mutant *Slc26a2* mice with *N*-acetyl cysteine showed increased proteoglycan sulphonation and improved skeletal phenotypes, suggesting that thiol-containing compounds can bolster the intracellular sulphate levels needed for sulphonation of CSPGs (Pecora *et al.* 2006).

Loss of PAPS synthetase has also been linked to impaired CSPG sulphonation and skeletal dysplasias (Sugahara & Schwartz 1982). Mammalian genomes contain two PAPS synthetase genes, *PAPSS1* and

PAPSS2 (Kurima *et al.* 1998, Xu *et al.* 2000). However, only *PAPSS2* has been linked to human pathophysiology, with similar skeletal phenotypes found in *Papss2* mutant mice (Faiyaz ul Haque *et al.* 1998, Xu *et al.* 2000). In addition, disruption of the zebrafish PAPS transporter gene (*papst1*, aka *pinscher*) leads to cartilage defects (Clément *et al.* 2008). Skeletal phenotypes are also found in patients with mutations in the chondroitin 6-*O*-sulphotransferase gene (Thiele *et al.* 2004; Table 1), showing that proteoglycan sulphonation is important for maintaining healthy bone development.

Sulphonation of steroids

Early studies proposed that steroid sulphates were end products of metabolism, with the sulphate moiety merely increasing the water solubility of the steroid and enhancing its excretion into the urine (Mulder & Jakoby 1990). However, more recent studies have shown steroid sulphates to be important precursors of biologically active steroids or to have physiological roles that are distinct from non-sulphonated steroids (Strott 1996, 2002). Sulphonation is a molecular mechanism for inactivating steroids, as most steroid sulphonates do not bind their target receptor (Strott 1996). Sulphate also plays an important role in the modulation of cholesterol function. In addition to serving as a substrate for adrenal and ovarian steroidogenesis, cholesterol sulphate has been linked to several biological processes, including regulation of cholesterol synthesis, plasmin and thrombin activities, sperm capacitation and activation of protein kinase C (Reed *et al.* 2005).

When sulphonated, steroids avidly bind to serum proteins, particularly albumin as well as corticosteroid-binding globulin (aka CBG, transcortin) and sex

hormone binding globulin (aka SHBG, androgen-binding protein, testosterone-binding β -globulin) (Puche & Nes 1962, Chader *et al.* 1972, Weiser *et al.* 1979, Dunn *et al.* 1981, Strott 1996). Binding of steroid sulphates to serum proteins decreases their urinary clearance by approximately two orders of magnitude, when compared with unconjugated steroids (Wang *et al.* 1967). This is relevant to circulating steroid sulphate levels being much higher when compared with those of their non-sulphonated forms, as shown for oestrogen sulphate, which has circulating levels several-fold higher than unconjugated oestrogen (Strott 1996, 2002). Circulating albumin-bound steroid sulphates are proposed to provide a pool of inactive steroids, which can be taken up by peripheral target tissues, where de-conjugation via steroid sulphatases generate active steroids.

Synthesised in both maternal and placental tissues, cholesterol sulphate is an essential precursor of steroids for maintaining fetal growth and development. Although fetal steroid biosynthesis is limited, DHEAS is produced in the fetal adrenal gland (zona reticularis) and then circulated to the placenta where it provides the major supply of DHEAS ($\approx 90\%$) for production of oestrone, oestradiol (E_2) and other fetal steroids (Dawson 2011). In addition, DHEAS is also converted to 16α -hydroxy DHEAS in the fetal liver, via 16α -hydroxylase, and subsequently converted to oestriol (> 60 mg/day during the third trimester of human gestation) in the placenta. While decreased levels of oestriol in maternal circulation have been used as a marker for certain developmental disorders, including Down syndrome, trisomy 18, idiopathic pregnancy loss and anencephaly (Aldred *et al.* 2012), the role of perturbed sulphonation of DHEA and oestriol in modifying maternal oestriol levels and potentially human fetal development awaits further investigation.

Disturbances in the balance of sulphonated to unconjugated steroids can have detrimental effects on steroid-responsive events in development (Strott 1996). For example, mid-gestational fetal loss and placental thrombosis have been shown in mice lacking the *Sult1e1* oestrogen sulphotransferase gene (Tong *et al.* 2005). *Sult1e1* is highly expressed in the placenta where it is essential for generating oestrone sulphate, E_2 -3-sulphate and oestriol sulphate.

Perturbed sulphonation capacity, due to inactivating mutations in the *PAPSS2* gene, has also been linked to altered endocrine function in humans (Noordam *et al.* 2009). One XX female patient with premature pubarche had an abnormal endocrine profile with androstenedione and testosterone levels at twofold above the upper limit of normal ranges, a DHEA level at the upper limit of the normal range and DHEAS level at one order of magnitude below the normal range. The clinical presentations of this patient were proposed to be a consequence of reduced DHEA sulphonation, which led

to increased circulating levels of unconjugated DHEA that was converted to androgens. More recent studies have shown a trend ($P=0.06$) for a lower ratio of circulating DHEAS to DHEA, in children with premature adrenarche, and harbouring a polymorphism (rs182420) in the *SULT2A1* gene (Utriainen *et al.* 2012). *SULT2A1* genetic variants have also been associated with reduced DHEAS and inherited adrenal androgen excess in some women with polycystic ovary syndrome (Goodarzi *et al.* 2007). These findings indicate a pathogenetic role of *PAPSS2* and *SULT2A1* in androgen excess disorders. Together, these studies provide evidence that disruption of steroid sulphonation, including decreased sulphate and PAPS supply, as well as steroid sulphotransferase activity, leads to perturbed endocrine profiles and associated clinical manifestations (Table 1).

Summary

Sulphate is an obligate nutrient for healthy fetal growth and development. The field of sulphate research brings together families of genes that encode sulphate transporters, PAPS synthetases and transporters, sulphotransferases and sulphatases. All these contribute to maintaining the required physiological balance of sulphonated and unconjugated compounds. While the importance of sulphate in human growth and development is largely unappreciated, its significance is being realised with the generation of animal models, as well as links to human pathophysiology, particularly chondrodysplasias. These findings warrant future studies to further delineate the roles for sulphate in fetal growth and development, as well as to determine its physiological importance in placental development and maternal adaptation to pregnancy, particularly for the role of steroid sulphonation. Future research will be important to further unravel how perturbed sulphonation affects health outcomes for both mother and child.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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