Published in final edited form as:

Clin Pharmacol Ther. 2008 December ; 84(6): 662–673. doi:10.1038/clpt.2008.202.

Hormonal Regulation of Hepatic Drug Metabolizing Enzyme Activity During Adolescence

M.J. Kennedy, Pharm.D.^{1,2}

¹ *Kosair Charities Pediatric Clinical Research Unit, Department of Pediatrics, School of Medicine, University of Louisville, Louisville, KY*

² *Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, Louisville, KY*

Abstract

Activities of drug metabolizing enzymes (DME) are known to change throughout the course of physical and sexual maturation with the greatest variability noted during infancy and adolescence. The mechanisms responsible for developmental regulation of DME are currently unknown. However, the hormonal changes of puberty/adolescence provide a theoretical framework for understanding biochemical regulation of DME activity during growth and maturation. Important information regarding potential influences of growth and sex hormones can also be extrapolated from studies evaluating changes in activities of DMEs occurring as a consequence of physiologic, pathologic and/or pharmacologic hormonal fluctuations. Collectively, current data support the hypothesis that isoform-specific alterations in DME activity during adolescence are mediated via sex and/or growth hormones. Characterization of the underlying biochemical alterations responsible for developmental changes in DME activity will require additional studies in which relationships between DME and important hormonal axes are evaluated during the course of pubertal development.

Keywords

adolescent; hormone; drug metabolism; puberty

Introduction

Normal human development represents a continuum of biologic events enabling somatic growth, sexual maturation and reproduction. In the context of Clinical Pharmacology, the processes of physical and sexual maturation are also responsible for the characteristic changes in drug disposition and action occurring throughout the developmental continuum. As a consequence of recent regulatory initiatives mandating conduct of pediatric clinical trials (1-3), evidence continues to accumulate regarding the need for age-dependent adjustments in drug dosing. Characterization of age-associated pharmacokinetic and pharmacodynamic alterations has also facilitated identification of developmental breakpoints that can be used to stratify children into distinct sub-populations based on definable changes in drug exposure. These changes can be largely attributed to normal developmental alterations in the physiologic factors influencing drug absorption, distribution, metabolism and elimination. (4) There are,

Name and address for correspondence: Mary Jayne Kennedy, Pharm.D. Assistant Professor of Pediatrics and Pharmacology/ Toxicology Department of Pediatrics University of Louisville 231 E. Chestnut Street - N97 Louisville, KY 40202 Phone: (502) 629-5608 Facsimile: (502) 629-5285 Electronic mail: E-mail: mjken07@louisville.edu.

Conflict of Interest / Disclosures
None

however, discrete developmental changes, particularly in drug clearance, that cannot be predicted solely on the basis of physiologic alterations (e.g, increased organ mass, acquisition of functional organ capacity). It is therefore possible that developmental changes in drug disposition may be regulated by specific biochemical signaling events occurring during the processes of physical and sexual maturation.

The use of pharmacologic techniques to map the ontogeny of hepatic drug metabolizing enzyme (DME) activity has generated important information that, in combination with pharmacokinetic data, has facilitated creation of a developmental roadmap which demonstrates distinct, isoform-specific changes in phase I and phase II DME activity. The mechanisms responsible for up- and down-regulation of DME activity during maturation, however, remain unknown. Relationships between the biochemical signaling events of growth and development and age-dependent changes in drug metabolism have also been largely unexplored. Nevertheless, in the absence of mechanistic data, emerging information regarding the ontogenesis of DME activity provides important insight into the biochemical and/or molecular events that may be involved in initiating and regulating developmental changes in hepatic drug metabolism and clearance.

In general, activities of DMEs are low in the fetus and newborn and increase rapidly over the first year of life to levels in toddlers and older children that may exceed adult capacity. (Figure 1) (5) Around the onset of puberty, activities begin a gradual decline that continues throughout adolescence and concludes with attainment of adult capacity at the completion of pubertal development. This ontogenic pattern closely mirrors that of important hormonal axes involved in growth and sexual maturation (6) with an apparent inverse relationship between hormone levels and DME activity. (Figure 2) The periods of greatest change in DME activity also occur during those developmental periods when hormonal fluctuations are most pronounced (i.e., early infancy and adolescence). These extreme discordant developmental phenotypes therefore provide a framework within which hormonal regulation of DME activities can be considered.

Almost 15 years ago (January 20-21, 1994), the Pediatric, Adolescent and Maternal AIDS Branch, Center for Research for Mothers and Children and National Institute of Child Health and Human Development convened a joint workshop entitled "Pharmacokinetics and Pharmacodynamics in Adolescents" which resulted in the development of a research agenda for the study of therapeutic drugs in this patient population. (7) A key component of this agenda included the conduct of studies to characterize the effects of growth and sexual maturation on drug biotransformation. At present, however, information regarding the impact of physical and sexual development DME activity remains limited to a small number of pharmacokinetic studies describing changes in the clearance of multi-enzymatic substrates during adolescence. Evaluation of these pharmacokinetic changes in the context of the significant hormonal changes occurring during puberty, however, provides a theoretical model for considering biochemical regulation of DME activity. Important information regarding potential influences of growth and sex hormones can also be extrapolated from studies evaluating changes in activities of DMEs occurring as a consequence of physiologic (e.g., pregnancy, menopause, sex differences), pathologic (e.g., growth hormone deficiency) and/or pharmacologic (e.g., oral contraceptives, hormone replacement therapy) hormonal fluctuations.

Changes in Hepatic Drug Clearance During Adolescence

Clinical pharmacokinetic studies have consistently demonstrated that the clearances of many hepatically-metabolized drugs, such as theophylline (8), carbamazepine (9), phenytoin (10) and phenobarbital (10), are increased in young children (ages 2-11 years) relative to adults. Consequently, higher mg/kg doses are often required to achieve comparable levels of systemic exposure. By the end of adolescence, however, drug dosing requirements are generally similar

to those in healthy young adults. The relationship between chronologic age and systemic clearance also becomes increasingly non-linear during adolescence (11,12). Adolescence is therefore more than a simple continuum between childhood and adulthood; it is a period of significant change during which maturation of both reproductive and DME functional capacities occur.

There is increasing evidence to suggest that pubertal development *per se* may play an important role in regulating age-dependent changes in hepatic drug biotransformation. Longitudinal data from repeated, within-subject assessments of carbamazepine pharmacokinetics in children have demonstrated that the most significant intra-individual variations in drug clearance occur during adolescence and puberty (ages 9 to 13 years). (11) Significant variability in the steady-plasma clearance of pravastatin (mean $\pm$ SD, 11.0 $\pm$ 11.9 L/min) is similarly observed in adolescents between the ages of 10 and 16 years. (12) Comparison of adolescents during various stages of sexual maturation has also demonstrated differences in the clearances of several drugs eliminated primarily via hepatic biotransformation. A pharmacokinetic study in children with sickle cell crisis (n=24) demonstrated weight normalized morphine total body clearance values that were approximately 30% lower in late or post-pubertal children (Tanner stage 4 or 5) compared to those who were pre-pubertal (Tanner stage 1). (13) Weight-normalized theophylline clearance values in sexually mature adolescents are also reportedly lower (~45%) than those in pre-pubescent individuals. (14) A Tanner stage dependent decrease in CYP1A2-mediated caffeine clearance (measured via the caffeine breath test) has been similarly described in healthy adolescents, with the lowest clearance values observed in the most sexually mature individuals (Tanner stage 4-5). (15)

Previous investigations have demonstrated a temporal relationship between changes in somatic growth and drug clearance suggesting that the process of growth itself may, either directly or indirectly, regulate age-dependent changes in the elimination of agents cleared via hepatic biotransformation. Alterations in antipyrine clearance during sexual maturation have been attributed to changes in body composition coinciding with the pubertal growth spurt. (16) A highly significant positive correlation ($p < 0.001$) between Tanner stage and theophylline elimination half-life ($t_{1/2}$) has also been reported in adolescents with asthma and attributed, at least in part, to alterations in distribution volume resulting from changes in body composition during puberty. (17) Sex-based differences in the ontogeny of CYP1A2 (measured via the caffeine breath test) have been similarly described with lower enzyme activity seen at an earlier stage of sexual maturity in females (Tanner stage 2) than in males (Tanner stage 4-5). (15) The temporal pattern of sex-related divergence in maturation of CYP1A2 activity mirrors that of pubertal growth which peaks occurs early in puberty in females and in mid or late puberty in males. (18)

The physical changes in height, weight and lean body mass occurring during pubertal growth are clearly important factors influencing hepatic drug elimination and clearance during adolescence. However, age-dependent changes in liver size and/or functional capacity may also significantly contribute to observed clearance alterations. When normalized to body weight, liver volume, an estimate of total hepatic parenchymal mass, is greater in young children than in adolescents and adults. (19) The liver to body weight ratio also decreases with increasing age and there are data to suggest that age-dependent changes in this physiologic ratio may be responsible, at least in part, for alterations in drug clearance occurring during adolescence. In a recent pharmacokinetic study of the CYP2C9 substrate, S-warfarin, weight- and body surface area-adjusted oral clearance values in pre-pubertal children were significantly greater than those in pubertal children or adults. (20) When clearance values were adjusted for estimated liver weight, however, differences between groups were no longer apparent. In contrast, systemic clearance of the non-specific CYP450 substrate, antipyrine, was unrelated to liver volume and was significantly greater in young children (< 5 years) than in adolescents

(> 15 years) when normalized for both body weight and liver volume suggesting that changes in the expression and/or activity of DMEs may occur during sexual and physical maturation. (19)

It is likely that changes in hepatic drug clearance during adolescence are mediated by a complex interplay between alterations in body size/composition, liver mass and constitutive DME expression/activity during pubertal development. For some substrates, it appears that changes in body size or composition alone are responsible for changes in systemic clearance. For others, however, there are alterations that occur independently of or in addition to physical or somatic changes. It is therefore possible that the constitutive expression and/or activity of individual DMEs may change during the course of sexual maturation. Given the temporal relationship with the pubertal growth spurt, it is also plausible that these processes (i.e., somatic growth and DME ontogeny) may be regulated by common hormonal or biochemical mechanisms. Consequently, understanding the normal physical and hormonal events of puberty may help elucidate those developmental factors that initiate and control changes in hepatic drug clearance during adolescence.

Endocrinology of Growth and Sexual Maturation

Physical and sexual maturation represent a series of coordinated, hormonally-regulated events that begin during fetal life, continue throughout childhood and conclude during adolescence with the onset and completion of puberty. Alterations in the hormonal milieu underlying pubertal development are therefore not discrete, sudden events but rather represent the culmination of progressive and longitudinal changes occurring during youth. The process of sexual maturation is under endocrine control of the hypothalamic-pituitary-gonadal (HPG) axis, a negative feedback system that mediates its effects via actions of the pituitary gonadotropins [i.e., follicle stimulating hormone (FSH) and leutenizing hormone (LH)] and gonadal sex steroids (i.e., estradiol and testosterone). (21) The hypothalamic hormone, gonadotropin hormone releasing hormone [(GnRH), also known as leutinizing hormone releasing hormone (LHRH)], controls the pituitary release of the gonadotropins which, in turn, regulate sex steroid secretion from the ovary and testes. (Figure 3)

In the fetus, the HPG axis is fully functional and plays an important role in the process of sex differentiation. The HPG axis is also responsible for the postnatal rise in gonadal sex steroid levels occurring around 2 months of age. (22) In both the fetus and the infant, serum gonadotropin and sex steroid levels are equal to or even greater than those in pubertal adolescents. (23) In the months and years after birth, however, there is a progressive decrease in HPG axis activity. By the age of 2 years, the HPG axis enters into a quiescent period commonly referred to as the “juvenile pause” during which circulating plasma levels of gonadotropins and sex steroids are low. (24) This period of relative inactivity continues until around the age of 10 years when there is a reactivation of the HPG axis at the onset of puberty.

The earliest hormonal changes of adolescence occur between the ages of 7 and 8 years when circulating levels of the adrenal androgens, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S), increase. (21) The increase in adrenal androgen secretion signals the onset of adrenarche, the phase of development during which maturation of the hypothalamic pituitary adrenal (HPA) axis occurs. (25) (Figure 3) Adrenal androgen secretion continues to increase throughout adrenarche and into puberty, when plasma DHEA and DHEA-S levels demonstrate a second peak around the ages of 12 to 13 years. Increased secretion of other adrenal androgens (e.g., Δ_4 -androstenedione) also occurs during the immediate pre-pubertal period (ages 8 to 10 years). Although related to HPG maturation and function, adrenarche is a distinct process that appears to be independently regulated by a pituitary stimulating factor that, to date remains unidentified. It is also unknown whether the

changes in adrenal androgen levels occurring during adrenarche have any effect on the subsequent maturation of the HPG axis.

The onset of puberty is characterized by reactivation of the HPG axis which, in turn, leads to an increase in the pulsatile release of GnRH, gonadotropins and sex steroids (i.e., gonadarche). Although the exact mechanisms involved remain unknown, it is postulated that alterations in the balance of inhibitory (e.g., GABA) and excitatory (e.g., glutamate) neurotransmitters may contribute to disinhibition and reactivation of the HPG axis. (23) During the very early stages of puberty, there are distinct hormonal alterations that occur in the absence of any physical signs of sexual maturation. (26) Release of high amplitude gonadotropin pulses are apparent predominantly during sleep and are accompanied by a concomitant increase in circulating early morning sex steroid levels. Continuation of the increased adrenal androgen secretion initiated during adrenarche is also observed. These early hormonal changes precede the appearance of physical alterations by at least 2 years. (27) As pubertal development progresses, however, physical signs of sexual maturation, such as increased testicular volume in males and breast budding in females, become perceptible as pulsatile gonadotropin release continues.

Physical and hormonal changes reflective of sexual maturation continue during mid-puberty and in females, peak height velocity is usually reached during this phase. (26) Sleep augmentation of gonadotropin release persists and higher peak levels are attained. Gonadotropin and sex steroid levels are also increased during the waking period. In late puberty, sleep augmentation of gonadotropin release is maximal and may even exceed that observed in adults. Circulating gonadotropin and sex steroid levels also remain elevated above those observed during the pre-pubertal period. In females, menarche is achieved and in males, peak height velocity occurs. At the completion of puberty, physical changes in both sexes are complete and hormonal levels decrease to those observed in healthy adults. Sleep augmentation of gonadotropin and sex steroid release disappears and reproductive capacity is complete.

The process of somatic growth is under the neuroendocrine control of the growth hormone/insulin like growth factor 1 (IGF-1) axis. (28) The hypothalamic hormone, growth hormone releasing hormone (GHRH) serves as a stimulatory signal for the pituitary release of growth hormone (GH) which, in turn, mediates its growth promoting effects by inducing peptide growth factors such as IGF-1. GH secretion is highly pulsatile and demonstrates significant circadian variation with the majority of secretory bursts occurring at night. Integrated plasma GH levels also vary significantly with age with the highest levels noted at birth and during pubertal growth. During puberty, there is an activation of the GH/IGF-1 axis that is attributed, at least in part, to stimulatory influences of the sex steroids. (29) Specifically, sex steroids cause an increase in the secretion amplitude of the GH pulse resulting in increased circulating GH levels; pulse frequency, in contrast, is not affected. Sex-based differences in GH secretion patterns also become apparent during puberty and mirror that of the differences in growth velocity. In females, circulating GH levels rise significantly during early puberty and peak during mid-puberty. A delayed increase in GH secretion occurs in males with peak levels observed during late puberty. Upon completion of sexual maturation, levels of GH and IGF-1 fall to those observed in pre-pubertal adolescents and adults.

Physical Changes of Adolescence and Puberty and Assessment of Sexual Maturation

The hormonal alterations of puberty are accompanied by profound physical changes in both males and females. The hallmark physical changes of puberty include rapid somatic growth, alterations in body composition and appearance of secondary sex characteristics. In males, one of the earliest physical signs of increased gonadotropin secretion is an increase in testicular volume occurring as a consequence of growth of the seminiferous tubules. (6) Pubic hair begins

to appear at the same time or shortly after testicular growth and is accompanied by penile growth and enlargement of the seminal vesicles, prostate and bulbourethral glands. Appearance of axillary, perineal and facial hair typically begins within 18 to 24 months of pubic hair development. In both sexes, growth of pubic or axillary hair and development of acne and body odor are associated with increasing levels of gonadal and adrenal steroids. Enlargement of the larynx and deepening of the voice in males occur later in puberty following genital and pubic hair development. Peak height velocity or the pubertal “growth spurt” also occurs during late puberty around the age of 14 years and is accompanied by a significant increase in lean body mass and a reduction in body fat.

The first physical sign of puberty in females is either the initiation of breast development (also known as breast budding) or pubic hair development. (30) The relative order of appearance of breast and pubic hair changes exhibits significant inter-individual and inter-ethnic variability with pubic hair development occurring prior to breast budding in most black females and after breast budding in most white females. These early changes are accompanied by less obvious physical changes including enlargement of the ovaries, uterus, labia, and clitoris, and thickening of the endometrium and vaginal mucosa. (6) In general, menses begins approximately 2 years following the onset of breast development and coincides with the attainment of peak height velocity at around 12 years of age. Accumulation of body fat also occurs during the somatic growth spurt in females.

The traditional view of relationships between chronologic age, hormonal alterations and physical/sexual development milestones in males and females are illustrated in Figure 4. It should be recognized, however, that there is significant inter-individual variability with respect to the onset and progression of pubertal changes among individuals of both sexes. Puberty timing is also influenced by a wide range of environmental and genetic factors. Further, a population trend towards an earlier onset of breast development and menarche in females has been observed over the last century, potentially as the result of environmental exposure to endocrine disrupting chemicals, particularly estrogen mimics and antiandrogens. (30) Previous information regarding relationships between chronologic age and physical/sexual development milestones may therefore be inadequate for characterizing pubertal onset and progression in today's adolescent population.

Sexual maturity is most frequently characterized via the direct physical assessment of secondary sexual characteristics. In males, genital and pubic hair development are typically used as surrogates of sexual maturation whereas in females development of breasts and pubic hair are most commonly employed. The status of breast, genital and pubic hair development is typically assessed using progressive sexual maturity rating scales. Although introduced almost 50 years ago, the staging system developed by Tanner and colleagues remains the most commonly used clinical tool for assessing sexual maturity in adolescents. The five Tanner stages range from stage 1 (pre-pubertal) to stage 5 (post-pubertal or adult) and delineate the growth of pubic hair in both boys and girls, breast development in girls and genital development in boys. Standards for pubic hair, breast and genital development are depicted in the classic photographs of Marshall and Tanner (Figure 5). Although somewhat subjective, Tanner staging is well-characterized as a method to assess the level of sexual maturity and to predict timing of growth spurts. However, Tanner staging results are somewhat unreliable given that, in many instances, clinical assessments are usually estimated rather than derived from detailed physical examination. The utility of Tanner staging is also limited since it requires direct physical examination and is rather invasive.

Growth and Sex Hormones as Biochemical Regulators of DMEs

Hormones are important in regulating expression and activity of DMEs and alterations in the clearances of multi-enzymatic drug substrates have been observed in patients with various endocrinopathies (31,32) and conditions associated with hormonal dysregulation. (33-36) Changes in DME activities have also been described during periods of physiologic (37,38) and pharmacologic (39,40) hormonal fluctuation. At present, the impact of developmental alterations in sex and growth hormones on DMEs has not been similarly characterized. Given the striking similarities in the functional ontogenesis of DMEs and the HPG and GH/IGF-1 axes, however, a rational hypothesis regarding the role of hormones in the developmental regulation of DME activity can be formulated.

The ontogenic pattern of the HPG axis is temporally similar to but functionally opposite of that observed for DMEs (Figures 1 and 2). For both systems, the most significant functional variability occurs during early infancy and adolescence. DME functional capacity appears to be greatest, however, during the period of quiescence in HPG axis activity occurring between the ages of 2 to 10 years. (5,21) In contrast, reactivation of the HPG axis coincides with the pharmacokinetic alterations (i.e., reduced clearance) occurring at the onset of puberty and adolescence. It therefore appears that the HPG axis and/or its associated hormonal mediators (GnRH, LH, FSH, estradiol and testosterone) may be important in the developmental regulation of DME activity. Similar parallels are also observed between DME activity and GH levels with the lowest DME functional capacity noted during periods of increased GH secretion (i.e., birth and adolescence). Existence of a temporal relationship between changes in drug clearance and the pubertal growth spurt provides further evidence that alterations in DME activity may be mediated by growth and/or sex hormones.

Data from animal and *in vitro* models have consistently demonstrated an effect of sex steroids on DME expression and activity. Androgenic steroids (e.g., testosterone, DHEA) have been shown to increase activities of hepatic mixed function oxidases in rodents (41) possibly through an increase in constitutive expression of hepatic enzyme protein. (42) Marked variation in CYP3A4-mediated drug biotransformation has also been demonstrated following exposure of human liver microsomes to various endogenous androgens. (43) Estrogens, in contrast, have been shown to decrease hepatic microsomal activity and drug clearance in rodents via a reduction in the amount of microsomal DME protein. (44,45) The reported effects of progesterones on DME activity are variable and inconsistent with some investigations reporting an inhibitory effect (46) and others reporting an inductive effect. (44)

Additional data from animal and *in vitro* models provide convincing evidence that GH and/or its downstream biologic mediators are also important regulators of sex-specific DME expression and activity. (47) In rat liver, expression of CYP2C11 is sexually dimorphic with higher levels observed in males than in females. This dimorphism is thought to be maintained by the differential secretion pattern of GH in animals of different sexes. Sexual dimorphism in GH secretory patterns is also present in humans. In males, GH exhibits a pulsatile GH secretory pattern which results in higher peaks and lower inter-dose concentrations. In females, the GH secretion profile is continuous thereby providing lower peaks with continuous hormone exposure. Sex-specific patterns of GH secretion are maintained via the influences of estrogenic and androgenic sex steroids.

Clinical data from studies evaluating the influence of physiologic, pathologic and/or pharmacologic fluctuations in growth and sex hormone levels on DME activity provide additional insight into those factors which might be important in the developmental regulation of hepatic drug biotransformation. The menstrual cycle represents a normal physiologic process that, much like pubertal development, is regulated by changes in the secretion of

gonadotropins and sex steroids. Although pharmacokinetic (i.e., clearance) differences have been described during the menstrual cycle, the majority of clinical data suggest that activities of individual DME isoforms (phase I and phase II) are unaltered during this physiologic process. (48) However, use of oral contraceptives (OCs) to pharmacologically manipulate gonadotropin secretion during the menstrual cycle results in clinically-apparent and isoform-specific alterations in drug clearance. Pharmacokinetic studies have demonstrated decreased clearances of the CYP1A2 substrates caffeine (55% reduction) and theophylline (29% reduction) following administration of combined OCs. (49,50) Pharmacologic phenotyping studies using specific “probe” substrates have also described decreased activities of CYP2C19 (40) and CYP2B6 (51) during combined OC therapy. In contrast, similar studies revealed no effect of combined OCs on CYP3A4 or CYP2D6. (52) Despite *in vitro* evidence that both the estrogenic and progestogenic components of the combined OCs can reduce DME activity (53), *in vivo* data suggest that it is the estrogenic component that mediates the observed effects. (39) The estrogenic component of OCs has also been shown to increase phase II drug metabolism via conjugation and glucuronidation. (54,55)

Menopause is another physiologic process associated with marked changes in sex hormone levels that occur, at least in part, as a result of normal developmental alterations in HPG axis function. (56) Alterations in ovarian follicle number and function, however, are responsible for the majority of hormonal changes observed during the menopausal period. Post-menopausally, circulating levels of the gonadotropins, FSH and LH, are reduced by up to 10 and 5-fold, respectively. Circulating estradiol levels are also over 90% lower than those observed pre-menopausally. In contrast, circulating androgen levels demonstrate little change during menopause. Pharmacokinetic studies have demonstrated that the clearances of the CYP3A substrates, alfentanil, prednisolone and tirilazad, are lower in post-menopausal women compared to those who are pre-menopausal. (57-60) In women receiving estrogen replacement therapy, reductions in prednisolone clearance were found to be even more pronounced (57) although this finding was not confirmed in a later study. (59) Subsequent pharmacologic phenotyping studies also failed to demonstrate a significant effect of menopausal status (with or without hormone replacement therapy) on activities of intestinal and hepatic CYP3A using the substrates midazolam, erythromycin and endogenous cortisol. (59,61,62) In contrast, a significant reduction in CYP1A2 activity, as measured via the plasma paraxanthine/caffeine metabolic ratio, has been described in post-menopausal women receiving estrogen replacement therapy compared to those who were untreated. (63) Reduced CYP2B6 activity has also been noted following exogenous administration of estrogen and progesterone hormones to healthy, pre-menopausal women. (51) Conversely, increased CYP3A activity (measured via prednisolone clearance and the erythromycin breath test) has been described following intravenous medroxyprogesterone supplementation in post-menopausal women suggesting that both estrogen and progesterone replacement may affect DME. (64) The effect of menopausal status on activities of phase II DMEs have not been characterized to date.

Fluctuations in female sex steroid levels may also be responsible for the observed alterations in DME activity occurring throughout pregnancy. Both estrogen and progesterone are essential for maintaining pregnancy and for normal pregnancy development. Placental human chorionic gonadotropin (hCG), which is measurable in the blood within 48 hours of implantation, is responsible for stimulating increased production and secretion of estradiol and progesterone by the corpus luteum. (65) Levels of both estradiol and progesterone increase until around the 6th to 10th week of gestation until progesterone levels plateau, and in some individuals even decrease, followed by a continuous rise until term. This occurs as a result of the demise of the corpus luteum and the onset of placental progesterone production which occurs around the 8th or 9th week of gestation. Around the 6th to 7th week of gestation there is also an additional increase in estradiol levels occurring as a result of the placental take over of estradiol production

and secretion. Following the onset of placental hormone production, estradiol and progesterone levels continue to rise during the second and third trimesters until term.

Several studies have demonstrated isoform-specific alterations in the activities of phase I and phase II DMEs during pregnancy. (66) Specifically, increases in the activities of CYP3A4, CYP2D6, CYP2C9, CYP2A6, UGT1A1 and UGT2B7 have been described in multiple clinical investigations of pregnant women. In contrast, activities of CYP1A2 and CYP2C19 are generally lower during pregnancy. It is postulated, based on information from animal and *in vitro* models, that the observed increases in the activities of CYP3A4, CYP2C9 and CYP2A6 occur consequent to progesterone-mediated activation of the pregnane X receptor (PXR). (Figure 6) This hypothesis is consistent with data which demonstrated a direct correlation between increased endogenous progesterone levels and CYP3A activity during the mid-luteal phase of the menstrual cycle. (37) Reduced activities of CYP2C19 and CYP1A2 are thought to be mediated by the high levels of estrogen that are present throughout gestation.

Sex-based differences in the disposition and action of pharmacologic agents have been repeatedly described and attributed to underlying differences in sex and growth hormone levels and/or secretion patterns between males and females. Since sex-based differences are most pronounced in rodents, the majority of mechanistic studies regarding sex-dependent differences in DME activity have been performed in this species. Given that the human forms of DMEs differ from those in rats in terms of both isoform composition and catalytic activity, however, sex dependent differences identified in rats often do not translate to humans. (67) Evidence from clinical studies conducted to date suggests that the activities of CYP1A2 and CYP2E1 are higher in males than in females. (52) Conversely, CYP3A activity appears to be greater in females although this result is highly dependent on the substrate used to measure CYP3A activity. It is theorized that sex-specific differences in the expression and activity of P-glycoprotein may account for the variability in results with different CYP3A substrates. (68) Substrate-specific differences in CYP3A may also be related to differential effects of androgenic steroids on the pharmacologic agents investigated. (43) Although several studies have evaluated the effect of sex on activities of CYP2D6, CYP2C19 and CYP2C9, information regarding the influence of sex on these specific isoforms to date are limited and conflicting.

Based on existing clinical data, it appears that sex hormones can significantly influence DME activity in an isoform-specific manner. (Tables 1 and 2) Regulation of constitutive expression and/or activity of certain DMEs (CYP1A2, CYP2B6, CYP2C19 CYP2E1) appears to be estrogen mediated where as others are more significantly influenced by androgens or progesterone (CYP3A). The nature and extent of sex hormone-induced changes in DME activity also seem to differ depending on the underlying mechanisms responsible for the hormonal changes. Normal cyclical variations in gonadotropin, estrogen and progesterone, such as those occurring during the menstrual cycle, appear to have no clinically-relevant effects on DME activity. In contrast, reduced activities of the "estrogen sensitive" DMEs (CYP1A2, CYP2B6, CYP2C19, CYP2E1) have been described during periods when estrogen levels remain persistently elevated for a relatively prolonged period (e.g., pregnancy) or when changes in the normal "baseline" hormone levels occur (e.g., puberty, exogenous hormone administration). This suggests that it is the process of hormonal signaling/secretion itself and not the actual circulating level that is important in DME regulation since changes in activity are most apparent when the normal HPG signaling and feedback processes are altered.

Growth hormone (GH) is known to regulate sex-specific drug metabolizing enzyme (DME) expression (47) and altered clearance of multi-enzymatic substrates following GH replacement has been demonstrated. Previous investigations in children with GH deficiency of various etiologies have reported alterations in amobarbital (39-69% decrease), (35) and antipyrine (4-11% decrease) (36) clearance and in theophylline (30-62% decrease) and amobarbital (34)

(42-200% increase) elimination half-life following GH replacement. Observed alterations appear to be substrate specific in both directions (i.e., increase or decrease) and the extent (50%-200%) of these alterations may cause clinically-relevant changes in systemic drug exposure. Using the caffeine breath test, a single study also noted a significant reduction (~20%) in CYP1A2 activity following 4-weeks of pituitary-derived GH administration. (69) However, using urinary caffeine phenotyping, two subsequent investigations, including one performed by our research group, failed to demonstrate clinically-relevant effects on CYP1A and xanthine oxidase following up to 6 months of rhGH treatment. (70,71) Our study also did not find evidence of a rhGH-mediated effect on activities of N-acetyltransferase 2 or CYP2D6. In a similar study, CYP2A6 activity also remained unaltered following rhGH administration. (72) In contrast, a sex-dependent reduction (~50%) in CYP3A4 activity has been reported in children with GH deficiency (ages 2.9-13.1 years) following 30 days of rhGH treatment. (73) Baseline pharmacokinetic alterations have also been described in adults (74) and children (34-36) with GH deficiency suggesting that the state of relative GH deficiency itself alters DME activity. Recently, our group demonstrated age-adjusted CYP1A2 and XO ratios in GH deficient children that were significantly lower than in healthy controls suggesting activities of these DME isoforms may be reduced by GH dysregulation. (75)

It appears that differential regulation of constitutive DME activity is mediated by the GH pulse pattern created by the influences of endogenous estrogens and androgens. Given that the GH pulse pattern during replacement therapy is determined by the administration schedule it is possible that DME responsiveness to GH-mediated effects differ between states of physiologic variation and pharmacologic replacement. In animal models and children with GH deficiency, exogenous GH administration also does not restore DME activity to the levels observed in normal controls suggesting that GH may serve as an “incomplete” control peptide in the modulation of hepatic DME activity. (75,76) However, GH may very well serve as a final common mediator through which gonadal steroids and other modifiers of pituitary function alter the expression and/or activity of DMEs during pubertal development. Consequently, it is reasonable to speculate that alterations in sex and growth hormones during physical and sexual maturation are responsible for the observed changes in DME occurring during adolescence. Given the known relationships between sex and growth hormones, it is also logical to theorize that observed changes in DME activity during development occur as a result of a complex interaction between the HPG and GH/IGF-1 axes. In order to elucidate the underlying biochemical alterations responsible for the developmental regulation of DME activity it will be necessary to perform a systematic and simultaneous evaluation of the HPG and GH/IGF-1 axes, their associated hormones/biochemical mediators and DME activity throughout the course of pubertal development.

Adolescent Pharmacology and the Current State-of-the-Art

Guidelines for drug dosing in newborns, infants, children and adolescents are currently extrapolated from clinical studies in which subjects are stratified into age groups based on pre-defined “break points” where changes in drug clearance and/or exposure are likely to occur. Implicit in this approach is the assumption that individuals within each age category are similar with respect to those developmental characteristics known to influence drug disposition. Given the substantial variability in the timing and progression of normal developmental events, however, the practice of stratifying children by chronologic age may result in grouping of individuals at different stages of physical and/or sexual maturation. This may be particularly true during periods of rapid growth and development, such as adolescence. The process of pubertal development itself is also highly variable with respect to onset, duration and final outcome and is significantly influenced by genetic, environmental and ethnic factors. It is therefore difficult to predict pubertal status based on chronologic age alone. Additionally, the epidemiology of puberty in the United States, and worldwide, has changed with a trend towards

earlier onset and increased rates of growth noted over the last few decades. Stratification of individuals based on chronologic age alone may therefore result in developmental misclassification.

Despite the significant influence of physical and sexual maturation on drug disposition, many pharmacokinetic and/or pharmacodynamic studies do not account for physical maturation or pubertal status. This can have important clinical implications since changes in drug disposition or clearance occurring during pubertal development may significantly alter drug exposure. Many of the drugs used to treat common chronic illnesses of adolescence (asthma, diabetes, epilepsy, depression) are cleared by DME pathways that can be altered by the hormonal changes of adolescence and puberty. Consequently, doses used prior the onset of puberty may become inappropriately high or low and may either cause toxicity or therapeutic failure. Disease exacerbations occurring during adolescence may also be inappropriately attributed to non-compliance rather than to developmental alterations in drug distribution and/or clearance. Given that puberty-associated hormonal changes are a potentially important source of pharmacokinetic variability in adolescents, they should be accounted for in the development of dosing guidelines for this patient population.

Although developed over 50 years ago, Tanner staging remains the most widely used and clinically accepted measure of sexual maturation. While somewhat subjective, Tanner staging is well-characterized as a method to assess the level of sexual maturity and to predict timing of growth spurts. Since it requires direct physical examination, however, it is somewhat limited for repeated clinical use particularly during the conduct of a clinical pharmacokinetic study. Perhaps more importantly, Tanner stage represents the physical consequences of the underlying hormonal changes occurring during pubertal development and consequently will frequently lag behind endocrine alterations. Significant changes in sex and growth hormones (i.e., gonadarche) also occur approximately 2 years before any physical alterations are apparent; adrenarche occurs even earlier at around 7 to 8 years of age. Tanner staging may therefore not provide an accurate reflection of those biochemical changes thought to be important in the regulation of DME activity.

The concept of developmental age, introduced in 1994 as part of the “Pharmacokinetics and Pharmacodynamics in Adolescents” joint workshop, encompasses the idea that developmental changes in drug pharmacokinetics and pharmacodynamics might be better understood within the context of the underlying mechanisms that are responsible for their occurrence. (77) It also purports that, for those processes that change with development, use of chronologic age as a surrogate or indicator may be very misleading. For example, in children of the same chronologic age, the hormonal milieu may be substantially different depending on the stage of pubertal development. For those processes affected by hormonal fluctuations, such as DME activity, a biomarker that more accurately reflects the underlying hormonal changes should therefore be used.

Future Directions: Reviving the Research Agenda for the Study of Drugs in Adolescents

Although proposed almost 15 years ago, the research agenda for the study of drugs in adolescents (7) has yet to be put into action. While the study of pharmacologic agents in newborns has increased almost exponentially over the last decade there remain few studies assessing the effects of pubertal development on drug disposition and action in the adolescent. Despite an increasing amount of information regarding changes in DME activity during the course of normal growth and development, the mechanisms responsible for up- and down-regulation of DME activity during maturation also remain unknown. Similarly, relationships between the biochemical signaling events of growth and development and age-dependent

changes in drug metabolism are still largely unexplored. The effects of physical and sexual maturation on the expression and activity of important drug transporters and receptors also remain uncharacterized. While a rational hypothesis regarding the regulation of DME activity during development can be developed, systemic evaluations throughout the course of pubertal development will be required in order to fully understand the mechanisms responsible for the observed alterations. The research agenda proposed during the 1994 Pharmacokinetics and Pharmacodynamics in Adolescents joint workshop provides an excellent framework on which these types of studies can be based. (7)

Acknowledgements

Supported in part by the National Institute of Child Health and Human Development (NICHD) Pediatric Pharmacology Research Unit (PPRU) Network (grant numbers 1 U10 HD 0495934-05).

References

1. Best Pharmaceuticals for Children Act. Public Law 107-109.
<http://www.fda.gov/opacom/laws/pharmkids/contents.html>
2. Pediatric Research Equity Act. Public Law 108-155.
http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=108_cong_public_laws&docid=f:publ155.108
3. Food and Drug Administration Modernization Act of 1997 (FDAMA).
<http://www.fda.gov/oc/fdama/default.htm>
4. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *N Engl J Med* 2003;349:1157-67. [PubMed: 13679531]
5. Leeder JS, Kearns GL. Pharmacogenetics in pediatrics. Implications for practice. *Pediatr Clin North Am* 1997;44:55-77. [PubMed: 9057784]
6. Sizonenko PC. Normal sexual maturation. *Pediatrician* 1987;14:191-201. [PubMed: 3331201]
7. Rogers AS. A research agenda for the study of therapeutic agents in adolescents. *J Adolesc Health* 1994;15:672-8. [PubMed: 7696288]
8. Ellis EF, Koysooko R, Levy G. Pharmacokinetics of theophylline in children with asthma. *Pediatrics* 1976;58:542-7. [PubMed: 972795]
9. Battino D, Bossi L, Croci D, Franceschetti S, Gomeni C, Moise A, et al. Carbamazepine plasma levels in children and adults: influence of age, dose, and associated therapy. *Ther Drug Monit* 1980;2:315-22. [PubMed: 7222185]
10. Garrettson LK, Dayton PG. Disappearance of phenobarbital and diphenylhydantoin from serum of children. *Clin Pharmacol Ther* 1970;11:674-9. [PubMed: 5455632]
11. Albani F, Riva R, Contin M, Baruzzi A. A within-subject analysis of carbamazepine disposition related to development in children with epilepsy. *Ther Drug Monit* 1992;14:457-60. [PubMed: 1485365]
12. Wiersma HE, Wiegman A, Koopmans RP, Bakker HD, Kastelein JJ, van Boxtel CJ. Steady-state pharmacokinetics of pravastatin in children with familial hypercholesterolaemia. *Clin Drug Investig* 2004;24:113-20.
13. Robieux IC, Kellner JD, Coppes MJ, Shaw D, Brown E, Good C, et al. Analgesia in children with sickle cell crisis: comparison of intermittent opioids vs. continuous intravenous infusion of morphine and placebo-controlled study of oxygen inhalation. *Pediatr Hematol Oncol* 1992;9:317-26. [PubMed: 1467165]
14. Miles M, Feinstein R, Cutter G, Lawrence C. Developmental pharmacokinetics of theophylline during puberty (abstract). *Clin Pharmacol Ther* 1986;1986:211.
15. Lambert GH, Schoeller DA, Kotake AN, Flores C, Hay D. The effect of age, gender, and sexual maturation on the caffeine breath test. *Dev Pharmacol Ther* 1986;9:375-88. [PubMed: 3102191]

16. Linday LA, Greenblatt DJ, Warren MP, Harmatz JS, DeCresce R, Cicalese C, et al. Changes in salivary antipyrine pharmacokinetics during adolescence, correlated with age, hormonal levels and Tanner stage. *Dev Pharmacol Ther* 1991;16:194–202. [PubMed: 1782837]
17. Cary J, Hein K, Dell R. Theophylline disposition in adolescents with asthma. *Ther Drug Monit* 1991;13:309–13. [PubMed: 1780962]
18. Tanner, J. Growth at adolescence. Vol. 2 edn.. Blackwell; Oxford: 1962.
19. Murry DJ, Crom WR, Reddick WE, Bhargava R, Evans WE. Liver volume as a determinant of drug clearance in children and adolescents. *Drug Metab Dispos* 1995;23:1110–6. [PubMed: 8654200]
20. Takahashi H, Ishikawa S, Nomoto S, Nishigaki Y, Ando F, Kashima T, et al. Developmental changes in pharmacokinetics and pharmacodynamics of warfarin enantiomers in Japanese children. *Clin Pharmacol Ther* 2000;68:541–55. [PubMed: 11103757]
21. Sizonenko PC. Endocrinology in preadolescents and adolescents. I. Hormonal changes during normal puberty. *Am J Dis Child* 1978;132:704–12. [PubMed: 149498]
22. Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *J Clin Invest* 1974;53:819–28. [PubMed: 4812441]
23. Grumbach, M.; Styne, DM. Puberty, ontogeny, neuroendocrinology, physiology and disorderse. In: Larsen, P.; Kroenberg, H.; Melmed, S.; Plonsky, K., editors. *Williams Textbook of Endocrinology*. Vol. 10 edn.. W.B. Saunders Company; Philadelphia: 2002.
24. Styne DM. Physiology of puberty. *Horm Res* 1994;41(Suppl 2):3–6. [PubMed: 8088700]
25. Sizonenko PC, Paunier L. Hormonal changes in puberty III: Correlation of plasma dehydroepiandrosterone, testosterone, FSH, and LH with stages of puberty and bone age in normal boys and girls and in patients with Addison's disease or hypogonadism or with premature or late adrenarche. *J Clin Endocrinol Metab* 1975;41:894–904. [PubMed: 127002]
26. Finkelstein JW. The endocrinology of adolescence. *Pediatr Clin North Am* 1980;27:53–69. [PubMed: 6769088]
27. Mauras N, Rogol AD, Haymond MW, Veldhuis JD. Sex steroids, growth hormone, insulin-like growth factor-1: neuroendocrine and metabolic regulation in puberty. *Horm Res* 1996;45:74–80. [PubMed: 8742123]
28. Rappaport, R.; Souberbielle, J. Growth Disorders. In: Soldin, S.; Rafai, N.; Hicks, J., editors. *Biochemical Basis of Pediatric Disease*. Vol. 3 edn.. AACCC Press; Washington, DC: 1998. p. 141-64.
29. Clark PA, Rogol AD. Growth hormones and sex steroid interactions at puberty. *Endocrinol Metab Clin North Am* 1996;25:665–81. [PubMed: 8879992]
30. Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sorensen TI, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics* 2008;121 (Suppl 3):S172–91. [PubMed: 18245511]
31. Matzke GR, Frye RF, Early JJ, Straka RJ, Carson SW. Evaluation of the influence of diabetes mellitus on antipyrine metabolism and CYP1A2 and CYP2D6 activity. *Pharmacotherapy* 2000;20:182–90. [PubMed: 10678296]
32. Saenger P, Rifkind AB, New MI. Changes in drug metabolism in children with thyroid disorders. *J Clin Endocrinol Metab* 1976;42:155–9. [PubMed: 1249184]
33. Kotlyar M, Carson SW. Effects of obesity on the cytochrome P450 enzyme system. *Int J Clin Pharmacol Ther* 1999;37:8–19. [PubMed: 10027478]
34. Redmond GP, Bell JJ, Nichola PS, Perel JM. Effect of growth hormone on human drug metabolism: time course and substrate specificity. *Pediatr Pharmacol (New York)* 1980;1:63–70. [PubMed: 7346733]
35. Redmond GP, Bell JJ, Perel JM. Effect of human growth hormone on amobarbital metabolism in children. *Clin Pharmacol Ther* 1978;24:213–8. [PubMed: 679599]
36. Rifkind AB, Saenger P, Levine LS, Pareira J, New MI. Effects of growth hormone on antipyrine kinetics in children. *Clin Pharmacol Ther* 1981;30:127–32. [PubMed: 7237892]
37. Zhang Y, Kim MJ, Bertino JS Jr. Nafziger AN, Sellers EM. Use of omeprazole as a CYP3A probe drug: effect of sex and menstrual cycle phase on CYP3A activity in healthy Caucasian adults. *J Clin Pharmacol* 2006;46:345–52. [PubMed: 16490811]

38. Nayak VK, Kshirsagar NA, Desai NK, Satoskar RS. Influence of menstrual cycle on antipyrine pharmacokinetics in healthy Indian female volunteers. *Br J Clin Pharmacol* 1988;26:604–6. [PubMed: 3207565]
39. Palovaara S, Tybring G, Laine K. The effect of ethinylloestradiol and levonorgestrel on the CYP2C19-mediated metabolism of omeprazole in healthy female subjects. *Br J Clin Pharmacol* 2003;56:232–7. [PubMed: 12895199]
40. Laine K, Tybring G, Bertilsson L. No sex-related differences but significant inhibition by oral contraceptives of CYP2C19 activity as measured by the probe drugs mephenytoin and omeprazole in healthy Swedish white subjects. *Clin Pharmacol Ther* 2000;68:151–9. [PubMed: 10976546]
41. Kato R, Chiesara E, Frontino G. Influence of sex difference on the pharmacological action and metabolism of some drugs. *Biochem Pharmacol* 1962;11:221–7. [PubMed: 14454282]
42. Davies DS, Gigon PL, Gillette JR. Sex differences in the kinetic constants for the N-demethylation of ethylmorphine by rat liver microsomes. *Biochem Pharmacol* 1968;17:1865–72. [PubMed: 5688273]
43. Nakamura H, Nakasa H, Ishii I, Ariyoshi N, Igarashi T, Ohmori S, et al. Effects of endogenous steroids on CYP3A4-mediated drug metabolism by human liver microsomes. *Drug Metab Dispos* 2002;30:534–40. [PubMed: 11950784]
44. Blackham A, Spencer PS. The effects of oestrogens and progestins on the response of mice to barbiturates. *Br J Pharmacol* 1969;37:129–39. [PubMed: 5360338]
45. Mackinnon M, Sutherland E, Simon FR. Effects of ethinyl estradiol on hepatic microsomal proteins and the turnover of cytochrome P-450. *J Lab Clin Med* 1977;90:1096–106. [PubMed: 925482]
46. Wilson JT. Developmental pharmacology: a review of its application to clinical and basic science. *Annu Rev Pharmacol* 1972;423–57. [PubMed: 4262145]
47. Waxman DJ, O'Connor C. Growth hormone regulation of sex-dependent liver gene expression. *Mol Endocrinol* 2006;20:2613–29. [PubMed: 16543404]
48. Kashuba AD, Nafziger AN. Physiological changes during the menstrual cycle and their effects on the pharmacokinetics and pharmacodynamics of drugs. *Clin Pharmacokinet* 1998;34:203–18. [PubMed: 9533982]
49. Roberts RK, Grice J, McGuffie C, Heilbronn L. Oral contraceptive steroids impair the elimination of theophylline. *J Lab Clin Med* 1983;101:821–5. [PubMed: 6854122]
50. Balogh A, Klinger G, Henschel L, Borner A, Vollandt R, Kuhn W. Influence of ethinylestradiol-containing combination oral contraceptives with gestodene or levonorgestrel on caffeine elimination. *Eur J Clin Pharmacol* 1995;48:161–6. [PubMed: 7589032]
51. Palovaara S, Pelkonen O, Uusitalo J, Lundgren S, Laine K. Inhibition of cytochrome P450 2B6 activity by hormone replacement therapy and oral contraceptive as measured by bupropion hydroxylation. *Clin Pharmacol Ther* 2003;74:326–33. [PubMed: 14534519]
52. Scandlyn MJ, Stuart EC, Rosengren RJ. Sex-specific differences in CYP450 isoforms in humans. *Expert Opin Drug Metab Toxicol* 2008;4:413–24. [PubMed: 18524030]
53. Guengerich FP. Mechanism-based inactivation of human liver microsomal cytochrome P-450 IIIA4 by gestodene. *Chem Res Toxicol* 1990;3:363–71. [PubMed: 2133086]
54. Waxman DJ. Interactions of hepatic cytochromes P-450 with steroid hormones. Regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of rat P-450 enzyme expression. *Biochem Pharmacol* 1988;37:71–84. [PubMed: 3276320]
55. Back DJ, Orme ML. Pharmacokinetic drug interactions with oral contraceptives. *Clin Pharmacokinet* 1990;18:472–84. [PubMed: 2191822]
56. Burger HG. The endocrinology of the menopause. *J Steroid Biochem Mol Biol* 1999;69:31–5. [PubMed: 10418978]
57. Gustavson LE, Legler UF, Benet LZ. Impairment of prednisolone disposition in women taking oral contraceptives or conjugated estrogens. *J Clin Endocrinol Metab* 1986;62:234–7. [PubMed: 2999180]
58. Rubio A, Cox C. Sex, age and alfentanil pharmacokinetics. *Clin Pharmacokinet* 1991;21:81–2. [PubMed: 1914343]

59. Harris RZ, Tsunoda SM, Mroczkowski P, Wong H, Benet LZ. The effects of menopause and hormone replacement therapies on prednisolone and erythromycin pharmacokinetics. *Clin Pharmacol Ther* 1996;59:429–35. [PubMed: 8612388]
60. Fleishaker JC, Pearson LK, Pearson PG, Wienkers LC, Hopkins NK, Peters GR. Hormonal effects on tirilazad clearance in women: assessment of the role of CYP3A. *J Clin Pharmacol* 1999;39:260–7. [PubMed: 10073325]
61. Burstein AH, Reiss WG, Kantor E, Anderson GD. Cytochrome P450 3A4 activity in premenopausal and postmenopausal women, based on 6-beta-hydroxycortisol:cortisol ratios. *Pharmacotherapy* 1998;18:1271–6. [PubMed: 9855326]
62. Gorski JC, Wang Z, Haehner-Daniels BD, Wrighton SA, Hall SD. The effect of hormone replacement therapy on CYP3A activity. *Clin Pharmacol Ther* 2000;68:412–7. [PubMed: 11061581]
63. Pollock BG, Wylie M, Stack JA, Sorisio DA, Thompson DS, Kirshner MA, et al. Inhibition of caffeine metabolism by estrogen replacement therapy in postmenopausal women. *J Clin Pharmacol* 1999;39:936–40. [PubMed: 10471985]
64. Tsunoda SM, Harris RZ, Mroczkowski PJ, Benet LZ. Preliminary evaluation of progestins as inducers of cytochrome P450 3A4 activity in postmenopausal women. *J Clin Pharmacol* 1998;38:1137–43. [PubMed: 11301566]
65. Schindler AE. Endocrinology of pregnancy: consequences for the diagnosis and treatment of pregnancy disorders. *J Steroid Biochem Mol Biol* 2005;97:386–8. [PubMed: 16216491]
66. Anderson GD. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin Pharmacokinet* 2005;44:989–1008. [PubMed: 16176115]
67. Mugford CA, Kedderis GL. Sex-dependent metabolism of xenobiotics. *Drug Metab Rev* 1998;30:441–98. [PubMed: 9710703]
68. Anthony M, Berg MJ. Biologic and molecular mechanisms for sex differences in pharmacokinetics, pharmacodynamics, and pharmacogenetics: Part I. *J Womens Health Gend Based Med* 2002;11:601–15. [PubMed: 12396893]
69. Levitsky LL, Schoeller DA, Lambert GH, Edidin DV. Effect of growth hormone therapy in growth hormone-deficient children on cytochrome P-450-dependent 3-N-demethylation of caffeine as measured by the caffeine $^{13}\text{CO}_2$ breath test. *Dev Pharmacol Ther* 1989;12:90–5. [PubMed: 2496962]
70. Mayayo-Sinues E, Fanlo A, Sinues B, Mayayo E, Labarta JI, Garcia de Jalon A, et al. Lack of effect of growth hormone replacement therapy on CYP1A2 and xanthine oxidase activities in growth hormone-deficient children. *Eur J Clin Pharmacol* 2006;62:123–7. [PubMed: 16408225]
71. Kennedy MJ, Davis DA, Smith NB, Gaedigk A, Pearce RE, Kearns GL. Absence of Effect of Recombinant Human Growth Hormone (rhGH) on Activities of Cytochrome P4501A2 (CYP1A2), N-acetyltransferase 2 (NAT-2), Xanthine Oxidase (XO) and Cytochrome P4502D6 (CYP2D6) in Pediatric Patients with Growth Hormone Deficiency. *Clinical Therapeutics*. 2008In Press
72. Sinues B, Fanlo A, Vicente J, Mayayo-Sinues E, Mayayo E, Labarta JI, et al. Growth hormone does not alter CYP2A6 activity in growth hormone-deficient children. *Basic Clin Pharmacol Toxicol* 2008;102:45–9. [PubMed: 17927692]
73. Sinues B, Mayayo E, Fanlo A, Mayayo E Jr, Bernal ML, Bocos P, et al. Effects of growth hormone deficiency and rhGH replacement therapy on the 6beta-hydroxycortisol/free cortisol ratio, a marker of CYP3A activity, in growth hormone-deficient children. *Eur J Clin Pharmacol* 2004;60:559–64. [PubMed: 15365655]
74. Cheung NW, Liddle C, Coverdale S, Lou JC, Boyages SC. Growth hormone treatment increases cytochrome P450-mediated antipyrine clearance in man. *J Clin Endocrinol Metab* 1996;81:1999–2001. [PubMed: 8626872]
75. Kennedy MJ, Davis DA, Smith NB, Gaedigk A, Pearce RE, Kearns GL. Reduced Activities of Cytochrome P450 1A2 and Xanthine Oxidase in Children with Growth Hormone Deficiency. *Clin Pharmacol Ther*. 2008In press
76. Zaphiropoulos PG, Mode A, Strom A, Husman B, Andersson G, Gustafsson JA. Sequence and regulation of two growth-hormone-controlled, sex-specific isozymes of cytochrome P-450 in rat liver, P-450(15)beta and P-450(16)alpha. *Acta Med Scand Suppl* 1988;723:161–7. [PubMed: 3164963]

77. Finkelstein JW. The effect of developmental changes in adolescence on drug disposition. *J Adolesc Health* 1994;15:612–8. [PubMed: 7696280]
78. Sizonenko PC. Physiology of puberty. *J Endocrinol Invest* 1989;12:59–63. [PubMed: 2681349]
79. Buck Louis GM, Gray LE Jr, Marcus M, Ojeda SR, Pescovitz OH, Witchel SF, et al. Environmental factors and puberty timing: expert panel research needs. *Pediatrics* 2008;121(Suppl 3):S192–207. [PubMed: 18245512]
80. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291–303. [PubMed: 5785179]
81. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13–23. [PubMed: 5440182]
82. Lazar MA. Gene regulation. One man's food. *Nature* 2000;407:852–3. [PubMed: 11057652]

Figure 1.
Developmental profiles of hypothetical DMEs. Adapted from Leeder (5) with permission.

Figure 2.
Developmental ontogeny of the hypothalamic-pituitary-gonadal (HPG) axis and adrenal androgen secretion. Adapted from Sizonenko (78) with permission.

Figure 3. Regulation of hormonal changes during adolescence and puberty

The earliest hormonal changes of adolescence are mediated via the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis requires signaling from the CNS to the hypothalamus. The hypothalamus releases adrenocorticotropin releasing hormone (CRH) which, in turn, stimulates release of adrenocortical tropic hormone (ACTH) from the pituitary. ACTH activates the adrenal cortex to produce the androgens, DHEA and androstenedione, which initiate armpit and pubic hair growth and skin changes. The onset of puberty is characterized by activation of the HPG axis, a process that is under CNS control. The hypothalamus releases GnRH which stimulates release of LH and FSH from the pituitary. LH and FSH stimulate the gonads to produce sex hormones which in turn initiate pubertal changes in many of the target organs. Positive (+) and negative (-) indicate stimulatory and inhibitory signals, respectively. +/- in the CNS indicates a combination of excitatory and inhibitory neuronal signals. Reprinted from Buck Louis (79) with permission.

Figure 4. Relationships between chronologic age and (i) somatic growth, (ii) sexual maturation and (iii) sex hormone changes in adolescents

Adapted with permission from Finkelstein (77).

Figure 5. Standards for sex maturity ratings in adolescents

(i) Pubic hair changes (stages 2-5) in (A) males and (B) females; (ii) Breast development (stages 1-5) in females (iii) Genital development (stages 1-5) in males. Reprinted with permission from Marshall (80,81).

Figure 6. Potential biochemical mechanism of DME regulation by growth and sex hormones
 Growth and/or sex hormones may serve as biochemical regulators of DME activity via interaction with nuclear hormone receptors such as the pregnane X receptor (PXR) and/or the constitutive androstane receptor (CAR). Up- or down-regulation of DME gene transcription may lead to altered expression of DME protein thereby altering functional DME activity. A schematic of growth and sex hormone regulation of CYP450 is depicted as an example. Adapted from Lazar (82) with permission.

Table 1
Physiologic and Pathologic Alterations in Sex and Growth Hormones and Effects on DME Activity

Condition / Physiologic State	Hormonal Fluctuation(s)	Effects on individual DME Isoforms
Physiologic Alterations		
Puberty / Adolescence	↑ gonadotropins ↑ sex steroids ↑ adrenal androgens ↑ growth hormone	↓ global CYP450 activity (antipyrine) ↓ CYP1A2
Menopause	↓ gonadotropins ↓ estrogen ↔ androgens	↔ hepatic or intestinal CYP3A
Menstrual Cycle	Cyclical changes in gonadotropins, estrogen and progesterone	↔ CYP1A2, CYP2C, CYP2E or CYP3A
Sex	Sex-based differences in estrogen, androgens and growth hormone secretion patterns	↓ CYP1A2 and CYP2E1 in females ↑ CYP3A in females ? CYP2D6, CYP2C19, CYP2C9
Pregnancy	↑ hCG ↑ estrogen ↑ progesterone	↑ CYP3A, CYP2C9, CYP2A6, CYP2D6 ↑ UGT1A1, UGT2B7 ↓ CYP1A2, CYP2C19
Pathologic Alterations		
Growth Hormone Deficiency	↓ growth hormone	↓ CYP1A2, XO

Table 2

Pharmacologic Alterations in Sex and Growth Hormones and Effects on DME Activity

Condition / Physiologic State	Hormonal Fluctuation(s)	Effects on individual DME Isoforms
Hormone Replacement Therapy		
Estrogen Only	↑ estrogen	↓ CYP1A2 ↔ hepatic or intestinal CYP3A
Estrogen + Progesterone	↑ estrogen ↑ progesterone	↓ CYP2B6 ↔ CYP3A
Progesterone Only	↑ progesterone	↑ CYP3A
Combination Oral Contraceptives	↑ estrogen ↑ progesterone ↓ gonadotropins	↓ CYP1A2, CYP2C19, CYP2B6 ↔ CYP3A, CYP2D6
Growth Hormone Replacement	↑ growth hormone	↓ CYP1A2 (caffeine breath test) ↔ CYP1A2 (caffeine urinary phenotyping) ↔ XO, NAT-2, CYP2D6, CYP2A6 ↓ CYP3A (sex-dependent)