**ANATOMY – STRUCTURE AND FUNCTION**

- **Hypothalamus/Pituitary/Gonadal Axis**

Reproduction is mediated by a hierarchical system of endocrine glands under CNS control – there are numerous regulatory mechanisms. This system allows amplification, propagation and integration of signals. Signals may be transmitted via neural or humoral pathways.

The hierarchy is not isolated – other inputs (external cues) drive the overall process via the CNS/hypothalamus. These include light, smell, touch, sound and psychosocial inputs (pain and stress). Nutritional status is also an important factor, although it doesn’t input directly.

Important features:

- **Neurosecretion** – neurons (primarily concerned with transmitting electrical signals) also secrete substances that are physiologically active at a site away from the primary cell.
- **Receptor specificity** – information is carried across a range of distances, which influences speed and duration of response. Specificity depends on specific receptor-ligand interactions on, or in target cells.
- **Feedback loops** – information-carrying molecules may feed back to multiple levels of the hierarchy - hormone secretion is typically regulated by feedback action of the target organ hormone or secretory product. Note that positive feedback is involved in the control of the menstrual cycle.
- **Pulsatile release** – hypothalamic secretions are released in discrete bursts, and drive a correspondingly pulsatile release of other hormones i.e. GnRH (hypothalamus) \(\rightarrow\) LH (pituitary). Both frequency and amplitude of pulses are important.

- **Neurosecretion and the Hypothalamus**

**Boundaries:**
- Superior – hypothalamic sulcus (separating it from the thalamus)
- Anterior – lamina terminalis
- Posteriorly – a vertical plane behind the mamillary bodies

The hypothalamus is the primary neurosecretory area in mammals. It is derived from the hindmost part of the forebrain (diencephalons) and consists of:

1. Cell bodies of neurones and axons
2. Axons and terminals of neurones whose soma are outside the hypothalamus
3. Axons passing through the hypothalamus
4. Glial cells (neural connective tissue cells)
5. Blood vessels (especially in the median eminence)

Neurosecretory neurones are aggregated in a number of nuclei – some have specific functions, while other neurosecretory activities may be more diffuse.

1. **Magnocellular nuclei** – paraventricular (PVN) and supraoptic nuclei (SO) – possess long axons that pass as the supraoptico-paraventriculohypophyseal tract to the posterior pituitary. Oxytocin (OT) and antidiuretic hormone (ADH) bound to carrier proteins (neurophysins) pass into the peri-capillary spaces of the neurohypophysis.

2. **Parvicellular nuclei** – infundibular (IN), ventromedial (VMN), dorsomedial (DMN) – consist of small neurons that pass along the tuberoinfundibular tract to intercellular space. Hormones are secreted to capillaries in the region of the median eminence, capillaries lower down the stalk or to capillaries of the median eminence via the lining of the third ventricle (→ tanyocytes).

Hormones secreted by parvicellular nuclei include:

- **Releasing hormones** – TRH, LHRH / GnRH, GHRH, CRH, PRH
- **Inhibitory hormones** – GH-RIH / somatostatin, PIH / dopamine

GnRH producing neurones are stimulated by noradrenaline, and the release of NA from medullary neurons and the sensitivity to NA are controlled by other neurotransmitters. GABA
is inhibitory, dopamine slows the pulse frequency, serotonin sensitises GnRH neurons, and opiates are generally stimulatory (via serotonin).

GABA and noradrenaline producing neurons have oestrogen receptors (feedback).

**Pituitary Gland**

The **pituitary gland** consists of three lobes:
1. **Anterior lobe (adenohypophysis)** – derived from Rathke's pouch (ectoderm)
   a. Pars distalis (anterior)
   b. Pars tuberalis
2. **Intermediate lobe**
   a. Pars intermedia (more a part of the anterior lobe, but generally isn’t present)
3. **Posterior lobe (neurohypophysis)** – extension of the diencephalon and hypothalamus
   a. Median Eminence
   b. Infundibular stalk
   c. Infundibular process (pars nervosa)

The blood supply to the pituitary and hypothalamus is derived from the superior and inferior hypophyseal arteries, which are branches of the internal carotid. Note that:
- The pars distalis lacks direct arterial supply but is well drained
- The median eminence has an arterial supply, but is poorly drained
- There are no significant vascular connections between the ME and hypothalamus
- The neurohypophysis has its own arterial supply and venous drainage.

Colloid tissue may be found between the anterior and posterior lobes. Cell types tend to be separated so that each hormone family has similar cells of origin:
- Anterior pituitary hormones – LH + FSH, GH + PRL, ACTH + TSH
- Posterior pituitary hormones – VP, OT

<table>
<thead>
<tr>
<th>Cells in the pars distalis</th>
<th>Location</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatotroph (GH)</td>
<td>Posterior/lateral parts of pars distalis</td>
<td>50% - round cells</td>
</tr>
<tr>
<td>Lactotroph (PRL)</td>
<td>Diffuse, most in lateral wings. May be associated with LH cells</td>
<td>&lt;20% - variable shape, pleomorphic granules</td>
</tr>
<tr>
<td>Corticotroph (POMC and derivatives)</td>
<td>Anteromedial, some posteromedial</td>
<td>15-20% - variable size</td>
</tr>
<tr>
<td>Thyrotroph (TSH)</td>
<td>Anteromedian basophilic wedge</td>
<td>5% - large and angular with irregular nuclei and peripheral granules</td>
</tr>
<tr>
<td>Gonadotroph (LH, FSH)</td>
<td>Posterolateral, postero-central</td>
<td>5-10% - round/oval, light central granules</td>
</tr>
<tr>
<td>Chromophobes</td>
<td>Dorsal part of the lobe</td>
<td>Degranulated – POMC producers or stellate follicular cells</td>
</tr>
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The **pars tuberalis** is generally chromophobic. It is rich is capillaries, with smaller vessels that may be connected with tanycytes. It produces LH, FSH and TSH (detectable by antibodies).

**The Ovary - Oogenesis**

The **ovary** has two primary roles:
1. Periodic production of gametes (oogenesis)
2. Synthesis and release of oestrogens, androgens, progesterone, inhibin, activin, relaxin, oxytocin and other proteins
Germ cell development:
1. Cells are initially separated 5 weeks in utero, posterosuperior part of the yolk sac
2. They migrate to the midline of the embryo, forming the genital ridges
3. Epithelium surrounds the cells – note that the germ cells are peripheral in the ovary (cortex), and more central in the testis (seminiferous tubules)
4. At 5 months in utero, mitosis stops (~7 million germ cells).
5. Meiosis starts, then is arrested – atresia and necrosis follow

There are a number of morphological and physiological changes during the menstrual cycle (linked closely with the ovarian cycle). The menstrual phase (days 1-5, 1mm endometrium) is followed by the follicular (proliferative) phase. The egg is released on day 14, and the luteal (secretory) phase follows. Ovarian activities in this cycle:
1. Granulosa cell proliferation, growth of a cohort of follicles
2. Selection of the dominant follicle
3. A change from oestrogen to progesterone production with luteinization of the granulosa cells
4. Reinitiation of meiosis at the time of the LH surge
   a. Resumption of meiosis from the dictyate stage (metaphase, anaphase, first polar body)
   b. Second division of meiosis (arrested at metaphase II)
   c. If fertilised → resumption of meiosis (second polar body produced)
5. Breakdown of the follicle wall after a viable oocyte is delivered
6. Development and demise (or maintenance) of the corpus luteum

The primordial follicle is found in the ovarian cortex – the oogonium is an oocyte with squamous follicular cells surrounding it.
1. Follicular cells become cuboidal → primary follicle
2. Oocyte is covered by gelatinous secretion (zona pellucida)
3. Follicular cell proliferation (more layers of granulosa cells) – note basal lamina
4. Fluid-filled antrum develops in stratum granulosa (day 1) → secondary follicle
5. Stomal cells become organised → theca → theca interna, theca externa
6. The mature (Graafian) follicle is 20 mm in diameter – it is fluid filled, with the oocyte surrounded by granulosa (cumulus oophorus). Note the corona radiata – columnar cells near the oocyte send processes through the zona pellucida for nutrition.

The egg is released following follicular rupture – this takes ~36 hours, and mainly involves weakening of the follicle wall (by plasmin and collagenase) in conjunction with follicular expansion. Note that as wall thickness decreases, tension increases without a change in pressure – there is some evidence that the wall decreases most in the region of the stigma.

The remaining part of the follicle is the corpus luteum and consists mainly of granulosa. It releases oestrogen and progesterone to maintain the endometrium. Degeneration → corpus albicans (essentially scar tissue).

- **Hormonal Control of Oogenesis**

Stimulus → decay

The two-cell, two-gonadotrophin concept suggests that LH stimulates androgen production in the theca, while FSH stimulates the conversion of androgens to oestrogens in the granulosa (and synthesis of inhibin). Generally, oestrogens stimulate while androgens inhibit granulosal cell proliferation.

This is not absolute, however, as some oestrogen is produced in the theca, and during folliculogenesis granulosa cells become responsive to LH (which acts via the FSH pathway).

**Early follicular phase:**
1. $E_2$ stimulates granulosal growth → increased aromatase → increased $E_2$
2. Activin inhibits androgen production, as there is insufficient aromatase.
3. Growth of follicle:
   a. $E_2$ stimulates FSH receptors $\rightarrow$ increased $E_2$
   b. FSH induces IGF-1 and receptors
   c. $E_2$ stimulates TGF-β, which acts synergistically with FSH and steroid inducing protein to induce growth
4. IGF-1 $\rightarrow$ growth of the theca $\rightarrow$ increased androgen synthesis

**Late follicular phase:**
1. Inhibin $\rightarrow$ decreased FSH, increased androgens
2. LH receptors are induced by FSH, $E_2$ and prolactin on granulosa cells. LH acts via the FSH pathway (compensating for low FSH) – this is the basis of follicle selection.
3. $E_2$ starts to have a ‘positive’ effect on LH, inhibiting release from pituitary $\rightarrow$ LH/FSH accumulation
4. LH induces increased $P_4$ synthesis by granulosa cells (especially the corpus luteum), and $E_2$ levels fall. Note that the LH surge just precedes the fall in $E_2$.

Note that granulosal cells lack the ability to synthesis cholesterol or other steroids de novo from acetate. Low-density lipoprotein is thus the source of cholesterol for progesterone biosynthesis. LH stimulates activity of cholesterol side-chain cleavage enzymes.

**Selection of the dominant follicle:**
1. Follicular growth is continuous, and gonadotrophin independent to the preantral stage
2. 6-46 follicles are recruited to develop at the beginning of the menstrual cycle – recruitment is due to chance (depends on the stage of the follicle)
3. Decreasing $P_4$ and increasing LH/FSH is the key event
4. Size is the main feature by which the dominant follicle is selected (or mitotic rate)
5. Maintenance of dominance:
   a. High $E_2$ production as FSH falls
   b. Increased inhibin (leading to lowered FSH)
   c. Dominant follicle has enough FSH receptors to overcome the FSH fall – LH receptors are induced on granulosa cells
   d. LH receptors are coupled to the FSH pathway – FSH/LH allows continued growth
   e. FSH induces IGF-1 and receptors by granulosa cells $\rightarrow$ increased androgen production (thecal cells) $\rightarrow$ increased $E_2$ synthesis
   f. Additional gonadotropin (hMG) can induce more than one dominant follicle

**Embryology of the Urogenital Tract**

Germs cells originating from the caudal end of the yolk sac migrate through the mesentery to form the urogenital ridges. The epithelial cells proliferate and surround germ cells (peripheral in females, central/cords in males). Note that the mesonephron includes the Wolffian duct.

The **SRY gene** is a sex determining gene located on the short arm of chromosome Y – it encodes a transcriptional regulation protein.
1. Female tract develops in the absence of SRY
2. SRY is expressed in Sertoli cells in the developing testes
3. SRY can be translocated to the X chromosome $\rightarrow$ sex reversal (very rare)
4. Note that other genes influence the development of the gonads e.g. Turner syndrome $\rightarrow$ death of oocytes

**Development of the urogenital tracts:**
1. **Hormone requirements**
   a. Males – androgen, MIH (Mullerian inhibiting hormone)
   b. Females – no hormones required
2. **Internal tract development** – note that initially tracts are present for both
   a. Males – Mullerian duct degenerates, Wolffian duct persists
      i. Testes begin descending at 12-15 weeks in utero, finish by 30 weeks
1. Crypt orchid testes – only partial descent, surgery required
   ii. Vaginal process closes off, but remains a weakening for herniation
   iii. Appendix of the testis is a remnant of the Mullerian duct
b. Females – Wolffian duct degenerates, Mullerian duct persists
   i. Paramesonephric (Mullerian) ducts are open to the peritoneal cavity; all ducts open into the urogenital sinus
   ii. Mullerian ducts fuse, midline septum degenerates → uterus
   iii. Tissue between Mullerian ducts and the urogenital sinus grows (vaginal plate); urethra separates off anteriorly
   iv. Tissue degeneration → cervix and vagina (hymen is residual tissue)
v. Malformations – bicornuate uterus and vagina

3. External genitalia development
   a. 4 weeks – indifferent stage
   b. 9 weeks
      i. Male – urogenital folds fuse and leave a urethral groove
   c. 11 weeks
      i. Male – urogenital folds fuse to form the scrotum. Urethral groove extends to the glands penis (note possibility of hypospadias)
      ii. Female – no fusion, labia minoris and majoris form – glans clitoris does not increase in size

4. Abnormalities
   a. Intersexuality/hermaphroditism
      i. True hermaphrodites – both ovarian and testicular tissue, ambiguous external genitalia
      ii. Pseudohermaphrodites – either ovaries or testes
         1. Female – 46XY, variable external genitalia
         2. Male – 46XX, male external genitalia (to some extent). Due to suprarenal hyperplasia (increased androgen production)
         3. Testicular feminisation
            a. Normal external female genitalia
            b. Testes internal, no uterus/fallopian tubes
            c. Tissues are androgen insensitive
            d. Presents as primary amenorrhoea

- Uterus and Menstrual Cycle

Concept of cycles:
1. Previously – 30-40 cycles in a lifetime
   a. Puberty later
   b. 1st 1-2 years, cycles are mostly anovulatory
   c. First pregnancy 9/12 with 2-3 years of lactational amenorrhoea → pregnancy
   d. Average 5 children
   e. Shorter lifespan
2. Currently
   a. Average 2 pregnancies
   b. Early abandonment of breastfeeding (~400 cycles)

Note that cycle length varies (with particular relevance to the follicular phase) between and within individuals – generally, cycles become shorter and more regular with age (though less regular as menopause approaches). Basal body temperature also rises during the luteal phase due to progesterone, but this is pretty insignificant (~0.3-0.5°C).

Structure of the uterus:
1. Endometrium – 6-7mm thick consisting of compact, spongy and basal layers
   a. Structure:
      i. Surface epithelium lines glands that extend to the basal layer (allowing regeneration)
      ii. The functional layer consists of spongy and compact layers
iii. Spiral arteries –
   1. Arterial spasm/shutdown at the end of the cycle due to decreasing P₄ and E₂
   2. Æ Ischaemia
   3. Arteries open again, blood pressure bursts the vessel walls
   4. Æ Menstruation as the endometrium (functional layer) is sloughed off

b. E₂ Æ epithelial/stromal cell division/growth, myometrial activity, stromal oedema, serous glandular secretions, synthesis of intracellular P₄ receptors
c. P₄ Æ thick glandular secretins in luteal phase (after E₂ priming), stromal cell proliferation, inhibition of myometrial activity

2. Oviduct – infundibulum, ampulla, isthmus, intramural portion
   a. Structure
      i. Epithelial lining and lamina propria
         1. Ciliated
         2. Secretory (goblet)
         3. Responsive to steroids
      ii. Muscular coat (inner circular, outer longitudinal)
      iii. Serosal coat (peritoneal mesothelium and underlying CT)
   b. E₂ Æ increased epithelial cell size, more cilia, more serous secretory activity, more muscular activity
   c. P₄ Æ less cilia, more cilia beating (after E₂ priming), decreased volume of secretions, decreased muscular activity

3. Cervix
   a. Structure
      i. Endocervix – columnar epithelium, glands/crypts, fibrous stroma (few smooth muscle cells)
      ii. Ectocervix – stratified squamous epithelium
   b. Few changes during menstrual cycle to mucosa and epithelium – most distinct changes involve the cervical mucous
      i. E₂ rise in proliferative phase Æ increased volume, decreased viscosity
         1. Clear, watery, low viscosity, high threadability. Highly receptive to sperm. Micellar structure with oriented channels
         2. Dries in a characteristic pattern called ‘ferning’
      ii. Fall in E₂, increase in P₄ in mid-cycle Æ decreased volume, increased viscosity

4. Vagina
   a. Structure
      i. Mucosa – stratified squamous
      ii. Muscle coat – circular and longitudinal
      iii. Adventitial – dense CT, extensive neural network
   b. Few epithelial changes during cycle, although
      i. A few cornified cells during the late follicular phase
      ii. A few leukocytes during the secretory phase
   c. Secretions – cervical mucous, epithelial transudate, Bartholin’s glands in vestibule

- **Male Tract – Spermatogenesis**

A requirement for normal spermatogenesis (though not endocrine function) is the maintenance of a temperature ~2°C lower than body temperature:

1. Migration of the testes from an intra-abdominal position to the scrotum at approximately 7 months in utero
   a. Cryptorchidism – incompletely descended (unilateral/bilateral) or maldescended (Æ anterior abdominal wall, perineum, thigh) testes. Leads to infertility.
2. Development of a countercurrent heat exchange system (pampiniform plexus)
Cell types in the testis:
1. Spermatogonia – from germ cells
2. Sertoli cells – from epithelial cells of cords (equivalent to granulosa in ovary)
3. Leydig cells – from interstitial tissue between cords (equivalent to theca interna)
   a. Secrete testosterone from 8-10 weeks in utero:
      - 13-15 weeks in utero – 2ng/mL
      - 5-6 months in utero – 0.8ng/mL
      - 2 months post partum – 2-3ng/mL
      - 3-4 months post partum – 0.5ng/mL
      - Pre-puberty – 0.1ng/mL
      - Post-puberty – 2-3ng/mL
      - Adult – 3.9ng/mL
4. Myoid cells – from interstitial tissue between cords

At puberty the spermatogonia proliferate by mitosis and meiosis to form spermatocytes and spermatids. The cords develop a lumen (seminiferous tubules) and sperm production begins.

Components of the testis are the tubules, rete testis, ductuli efferentes, epididymis and vas deferens. It is enclosed in a fibrous capsule (tunica albuginea).

1. Tubular structure
   a. Thick wall containing developing cells
   b. Tails of sperm cells protrude into the lumen
   c. Sertoli cells extend from the basement membrane to the lumen (surrounding the developing sperm)
      i. Junctional complex → tight junctions with neighbour Sertoli cells
      ii. Forms two compartments – spermatogonia are below the junction
      iii. Tight junction opens when the cell is selected to develop – this functions to separate the spermatogonia from the blood stream (preventing immune reactions)

2. Spermiogenesis
   a. Stages – Spermatocytogenesis → meiosis → spermiogenesis
      i. Spermatogonia (type A) → Ad (dark nuclei – stem cells), Ap (pale nuclei)
      ii. Ap cells → 1° spermatocyte
      iii. 1° spermatocyte → 2° spermatocyte (1st division of meiosis)
      iv. 2° spermatocyte → spermatids (2nd division of meiosis)
         1. Golgi phase
         2. Cap phase
         3. Acrosomal phase
         4. Maturation phase
      v. → Spermatozoa
   b. Six cell cohorts (specific cell associations) are recognised in humans
   c. Daughter cells are linked by cytoplasmic bridges throughout division (joint release)
   d. At any one point in a tubule, production is cyclic – but as different segments are at different stages, overall production is continuous.
   e. Transit time from basement membrane to lumen is 74 days
   f. Sperm are released every 16 days from any given point
   g. Production is 150x10^6 (300-600 per second per gram) daily
   h. Normal ejaculate has 200-300x10^6 sperm – <20x10^6 is considered infertile.

- Male Tract – Hormonal Control

The testis is under central hormonal control from the hypothalamus and pituitary with feedback provided by testosterone.

1. Testosterone
   a. Necessary for meiosis (prophase I) and spermatid maturation
   b. Stimulates androgen-binding protein (ABP)
   c. Decreased T → increased LH/FSH
d. Increased T → decreased pulse amplitude

2. FSH
   a. Acts on the Sertoli cells via cAMP to stimulate
      i. Enzymes of spermatogenesis - 5α-reductase
      ii. Spermatid-specific enzymes – inhibin, ABP
   b. Problems:
      i. Obstructed duct – normal levels
      ii. Low sperm count – varies/polymorphic
      iii. Germinal cell arrest – elevated (some normal)
      iv. Inhibin deficiency – increased FSH production

3. Oestrogen
   a. Peripheral conversion of testosterone to oestrogen; negative feedback control
   b. Enhances androgen action:
      i. Fibromuscular growth in male accessory organs
      ii. Induces oestrogen receptors in stromal tissues
      iii. Induces androgen receptors in epithelial cells – note that that this is often the secretory part of glands

• Male Tract – Gamete Transport

Structure of ducts and accessory glands – tests → epididymis → vas deferens (hooks over ureters) → ampulla
   → ejaculatory ducts → urethra
   1. Seminal vesicles – ampulla/ejaculatory duct
   2. Prostatic secretions – prostatic utricle
   3. Bulbourethral (Cowper's) glands – urethra
   4. Glands of Littre – spongy urethra

Sperm maturation:
   1. Takes place in epididymis
   2. Time for passage through epididymis – 2 weeks (1-21 days)
   3. Changes:
      a. Residual portion of cytoplasm absorbed
      b. Increased formation of disulphide bonds in structural proteins increasing stiffness – alters swimming behaviour
      c. Surface charge becomes more negative
      d. Concentration increases 100 fold – 50x10⁶ → 50x10⁸/mL

Seminal fluid
   1. Whole semen – a suspension of sperm in a fluid medium called seminal plasma
      a. Seminal vesicles 13-33%
      b. Prostate 46-80%
      c. Epididymis/Ampullae ~10%
   2. Specific components – note that secretion is apocrine
      a. Seminal vesicles (pH 7.3) – fructose, prostaglandins (13 in total, E₁, E₂)
      b. Prostate (pH 6.5 – acid phosphatase, citric acid, spermine (sperm motility), fibrinolysin
   3. Fractionation
      a. Initially a few drops of fluid from urethral and bulbourethral glands
      b. Prostate rich (pre-sperm)
      c. Middle portion – part seminal vesicles and sperm-rich fraction from epididymis/ampullae
      d. Viscous portion from seminal vesicles (post-sperm)

Reproductive aspects
   1. Erection
530.303 – R+D Lecture Notes

a. Relaxation of smooth muscle in corpora cavernosa → expansion and restriction of venous outflow mediated by nitric oxide and downstream messenger guanosine monophosphate
b. GMP is broken down by phosphodiesterase-5
c. Inhibitor of this phosphodiesterase promotes relaxation → erection (Viagra)

2. Erectile dysfunction – some degree of impotence – 52% (mild – 17%, moderate 25%, complete – 10%). Age 40 complete – 5%, age 70 complete 15%

3. Ejaculation
   a. Emission phase
   b. Expulsion phase
      i. Emission of semen from ducts and glands by contraction of smooth muscle
      ii. Expulsion of semen by contraction of somatic muscles – bulbo- and ischio- cavernosus muscles

4. Neural control – note retrograde ejaculation during expulsion phase (sympathetic)

5. Male fertility
   a. Affected by:
      i. Testosterone levels
      ii. Frequency of emission
      iii. Temperature – 2.2°C lower than body temperature. Heat exchange provided by the pampiniform plexus adjacent to spermatic artery.
   b. If more than three of the following are below optimum, fertilization is not likely (normal values given):
      i. Liquefaction within 15 minutes
      ii. Volume >1mL
      iii. Sperm concentration > 20x10^6/mL
      iv. Motility greater than 40%
      v. Grade of motility – good forward progress, moderate forward progress, poor, ‘twitching’
      vi. Morphology – note that up to 70% abnormal may still allow fertilisation if sperm count is sufficient
      vii. Debris – white blood cells is generally not a good sign

• Fertilisation and the Beginnings of Placenta Formation

Fertilisation
1. Gamete transport
   a. Oocyte – does not require muscle activity
      i. Oestrogen stimulates cilia, increases secretion
      ii. Progesterone (after oestrogen priming) inhibits cilia, decreases secretion – allows 6 day passage along oviduct
   b. Spermatozoa – matures in the epididymis, capacitation in the female
      i. Vagina 2½hr, cervix 48hr, uterus 24hr, oviduct 48hr
      ii. Transport time is 2-7 hours minimum to reach the ampulla
      iii. 50,000 sperm reach the egg (50 million AI, 100,000 GIFT)

2. Prerequisites
   a. Capacitation – stripping of surface glycoproteins (→ receptors)
   b. Acrosome reaction → hyperactivation of sperm and zona degradation
      i. Ca^{2+} increases in response to P_4 from cumulus oophorus
      ii. ZP3 binds sperm, induces acrosome reaction
      iii. ZP2 binds to inner acrosomal membrane, outer membrane fuses with acrosome → channels

3. Pathway
   a. Attachment of the egg (loose, non-specific)
   b. Binding – sperm receptors in the zona pellucida, egg-binding proteins in sperm plasma membrane
   c. Acrosome reaction, zona pellucida penetration
   d. Sperm-egg membrane fusion, release of cortical granules
   e. Blocks to polyspermy
Development prior to implantation
1. Membrane fusion, male pronucleus enters the ovum and decondenses
   a. 16 hrs – 2 unmixed pronuclei
   b. 30 hrs – 2 cells
   c. 72 hrs – 16 cells (morula)
   d. 96 hrs – 60 cells
2. Blastocyst – cavity separates trophoblast (→ placenta) and inner cell mass
3. Zona pellucida splits and blastocyst emerges (~ day 5)
4. Maintenance
   a. Endometrium – HCG
   b. Embryo – varies depending on age/development of enzymes
      1. Pyruvate, oxaloacetate
      2. Lactate, phosphoenol-pyruvate (2 cell)
      3. Glucose (8 cell)

Implantation
1. Posterior part of the fundus is the preferred implantation site – inner cell mass approaches the surface of the endometrium and begins digesting the epithelium
2. Trophoblast → syncytiotrophoblast (tissue without cell walls, invades endometrium for access to glands and blood vessels) and cytotrophoblast
3. Inner cell mass → endoderm and ectoderm (bilaminar embryonic disc – amniotic cavity above, blastocyst cavity below)
4. Amniotic cavity → amnion; blastocyst cavity → primitive yolk sac (both enclosed by mesenchyme)
5. Mesenchyme → extraembryonic coelom

Hormones and Contraception
Factors influencing sperm transport/survival:
1. Endocrine changes
2. Nature of the cervical mucus
3. Sperm numbers and quality
4. Contractile activity of the reproductive tract
5. Immunological factors
6. Infection

Timing – conception can occur 7-8 days into the cycle
1. Sperm survival – 6 days in the female tract, 48 hrs in vitro
2. Egg survival – 24 hours (corpus luteum 14 days)
3. Ovulation occurs ~36 hours after the LH surge

Phases of sperm transport in the female reproductive tract
1. Phase 1 – rapid transport (no time for capacitation)
2. Phase 2 – formation of sperm stores in the cervical crypts
3. Phase 3 – slow release of sperm from cervical crypts

Combined oral contraceptive preparations
1. Actions:
   a. Hypothalamus/pituitary – suppresses LH surge, reduces basal levels of LH/FSH (inhibits selection of dominant follicle)
   b. Local effects on the cervix → hostile mucus (mini-pill P₄ only)
   c. Problems – related to ethynyl estradiol. Note that there is considerable individual variation in serum levels given the same dosage.
      i. → Hepatic effects (clotting factors, angiotensinogen, lipid metabolism, carrier proteins)
      ii. → Sex hormone function effects (CNS/Pituitary, endometrium)
iii. >50mg/pill, smokers – increased risk of venous thromboembolism

2. Generations of pills – more potent progestogens combined with lowering dosage of the oestrogen component. In theory lowered risk of associated side effects.

- **Infertility**

Infertility is defined as the failure to conceive after 12 months of regular unprotected intercourse. Note that it needs to be investigated in both partners.

**Female infertility**
1. Anovulation - clomiphene citrate, gonadotropins, weight gain/loss
2. PCOS – metformin (increases insulin sensitivity)
3. Blocked tubes – surgery, IVF
4. Cervical problems (hostile mucus) – AIH (artificial insemination, husband’s sperm)
5. Endometriosis – danazol, surgery/IVF
6. Premature menopause – donor egg
7. No uterus - surrogacy

**Male infertility**
1. Sperm problems:
   a. Azoospermia – no sperm in ejaculate
   b. Oligospermia – poor sperm count
   c. Asthenospermia – poor sperm motility
   d. Teratospermia – poor quality sperm
2. Congenital absence of vas deferens (cystic fibrosis)

ICSI – intracytoplasmic sperm injection
1. PESA/MESA (percutaneous/microscopic epididymal sperm aspiration)
2. TESA/TESE

- **Placental Structure and Function**

Development of the placenta:
1. **Implantation** (7-11 days)
   a. Bi-layered pre-villous trophoblast → development of lacunae lined with syncytiotrophoblast between cytotrophoblast columns → leakage of maternal blood from the endometrial zona spongiosa
2. **Villous period** (12-20 days)
   a. Cytotrophoblast columns invade the syncytiotrophoblast to form branched/anchored villi (primary/secondary/tertiary).
   b. Decidual reaction (accumulation of glycogen and lipid in stroma) by day 14.
3. **Placental circulation** (21-42 days)
   a. Stem villi → centres of fetal cotyledons
   b. Irregular endometrial invasion leaves placental septa → maternal cotyledons
   c. Decidua basalis, decidua parietalis, chorion frondosum, inter-villous space (from condensation of lacunae), basal plate
4. **Arterial blood in inter-villous space, drainage over basal plates** (45 days to 3 months)
   a. Chorionic and basal plates grow, spiral artery in centre of fetal cotyledons
   b. Chorion laeve develops as decidua capsularis regresses, chorion frondosum associated with decidual basal plate
5. **Uterine lumen obliterated** (4-5 months)
   a. Decidua capsularis (atrophies) and chorion laeve fuse with decidua parietalis of the opposite uterine wall
   b. Decidua basalis reduces in thickness – fetal cotyledons and basal plate separated by reticular space
6. **Mature placenta** (6 months) – 15-20 cm diameter, 2-3 cm thick, 500g
   a. Discoid placenta covers ¼ of inner surface of fetal sack
Functions of the placenta:

1. Self maintenance and renewal
2. Maintenance of the internal environment
3. Regulation of osmotic gradients (active and passive)
4. Synthesis
   a. Production of hCG (blastocyst trophoblast), steroids (feto-placental unit) and protein hormones – important for growth and structural development
5. Maintenance of appropriate immunological environment with the fetus
6. Transport/transfer functions - syncytial placental membrane allows transcellular passage of substances:
   a. Gas transport – O₂ and CO₂
      i. Concentration gradient – uterine arteries Vs umbilical veins
      ii. Fetal Hb has a higher O₂ affinity (shifts dissociation curve left)
      iii. Bohr effect – increased pH due to CO₂ increases fetal O₂ transport
      iv. Haldane effect – capacity for CO₂ binding is increased by O₂ release
   b. Nutrients – water, ions (Ca²⁺, Na⁺, K⁺, Fe, Cl⁻, I⁻, I₂, Pi), carbohydrates, lipids, amino acids, proteins, vitamins, hormones

<table>
<thead>
<tr>
<th>Essential for fetal life</th>
<th>mg/s</th>
<th>Diffusion, active transport</th>
<th>O₂, CO₂, H₂O, urea, electrolytes</th>
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</thead>
<tbody>
<tr>
<td>Nutritional</td>
<td>mg/min</td>
<td>Active transport, facilitated diffusion</td>
<td>Amino acids, iron, vitamins, CHO</td>
</tr>
<tr>
<td>Growth modifying</td>
<td>mg/hr</td>
<td>Slow diffusion</td>
<td>Hormones</td>
</tr>
<tr>
<td>Immune</td>
<td>mg/day</td>
<td>Pinocytosis</td>
<td></td>
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</tbody>
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Fetal membranes – amnion and chorion

Amniotic fluid is a buoyant medium that cushions the embryo, preventing adhesion to the amnion and allowing symmetrical and unimpeded growth (note the musculoskeletal system). It is particularly important in the development of the gastrointestinal tract, respiratory system and the urinary system.

1. Origin
   a. Initially – ultrafiltrate of maternal plasma passing through the trophoblast
   b. 4-20 weeks – contribution from fetus (fetal capillaries) and membranes
   c. 20 weeks – contribution from fetal urine, placenta and cord
2. Volume
   a. 10 weeks – 30mL
   b. 20 weeks – 350mL
   c. 36 weeks – 1000mL
   d. Term – 750mL (less room for fluid)
3. Turnover – last trimester 500mL/day (urine 500mL/day, swallowing 500mL/day)
   a. Hydramnios – excess volume (>2000mL) due to lack of swallowing.
      Oesophageal atresia is a common presentation in premature newborns.
   b. Oligohydramnios – too little volume → adhesion, possible due to poor urination (kidney function)

- Physiology of Pregnancy – Endocrinology of Pregnancy

The feto-placental unit produces marked effects in the homeostasis of the mother important for the maintenance and completion of the pregnancy. Placental hormone production is independent of maternal regulation – important factors are the mass of the trophoblast and blood flows to the feto-placental unit.

1. Effects:
   a. Implantation of the blastocyst
   b. Maternal recognition, maintenance and adaptation to pregnancy
c. Supply of nutrients to the fetus
d. Initiation of parturition
e. Preparation of breasts for lactation

2. Hormones produced – hPL, hCG, inhibin, ACTH, placental specific proteins, oestrogens, progestogens (and GnRH, CRH, TRH and somatostatin)
   a. Functionally obscure hormones (e.g. placental GH variant and HPL) may have evolutionary significance in terms of starvation

**Human chorionic gonadotrophin** is a glycoprotein (M\(_w\) 36,000) consisting of two subunits, which has similar leutetrophic properties as LH (as it binds to the same receptor). The \(\beta\) subunit is produced almost exclusively by the syncytiotrophoblasm of the pre-implantation blastocyst and placenta. hCG is secreted by the trophoblast following implantation.

1. Maintains the corpus luteum, leading to an increase in ovarian secretion of P\(_4\) and E\(_2\) – note that P\(_4\) inhibits myometrial contractility. By 6-8 weeks the placenta takes over.
   a. 15% of pregnancies end in miscarriage – many have low hCG levels
   b. Stage II clinical trials of hCG vaccine for contraception (reversible)
2. Detectable in maternal blood 7-12 days after conception, in urine after 15 days
   a. Immunoassay of serum/urine \(\beta\)hCG is used as a pregnancy test
3. Levels rise exponentially, reaching a peak at 8-10 weeks before dropping off
4. Male fetus – provides a source of testosterone by serving as a LH surrogate and stimulating Leydig cell function at 10-14 weeks
5. Abnormal levels
   a. Increased in females with trophoblastic disease (hydatidiform mole, choriocarcinoma) – can also present with hyperthyroidism due to binding to the TSH receptor
   b. Increased in males with testicular cancers
   c. Increased with multiple pregnancies (more syncytiotrophoblast)
   d. Decreased levels in ectopic pregnancies

**Human placental lactogen** is a polypeptide (M\(_w\) 22,000) with 96% homology with hCG.

1. Detectable in maternal blood from 5 weeks – concentration progressively rises to reach a peak by 38 weeks. It is secreted more than any other hormone near term.
2. Production is directly proportional to placental size
3. Metabolic changes – elevates maternal blood levels of FFAs, glucose and insulin (\(\rightarrow\) gestational diabetes)
4. Normal pregnancies may occur in the absence of plasma/blood hPL – function?

**Progesterone** is synthesised by the syncytiotrophoblast from maternal LDL-cholesterol (cannot use acetate), essentially independent of the fetus. It is secreted to the mother and fetus where it is converted to other steroids (as the placenta does not have 17\(\alpha\)-hydroxylase).

1. Production steadily increases until a few weeks before term where it levels off
2. Levels can be determined by radioimmunoassay, but studies indicate that they are a poor indication of fetal wellbeing (although production is related to size/function)
3. Removal of the ovaries does not compromise pregnancy after 6 weeks – placenta produces adequate levels
4. Functions:
   a. Maintenance of uterine quiescence (and suppression of milk production)
   b. Progesterone receptors expressed by glands and stromal cells in the endometrium and decidua.
   c. Induction of decidua formation – but this is not essential for implantation (ectopic pregnancy). Possibly important to regulate the extent of implantation.

**Oestrogens** are produced by the fetus and are dependent on a live fetus, functioning fetal adrenal glands, and intact feto-placental circulation and a functioning placenta.

1. Oestrogen biosynthesis
   a. Fetal adrenal \(\rightarrow\) produces/secretes dehydroepiandrosterone sulphate (DHEAS) \(\rightarrow\) placenta \(\rightarrow\) conversion to oestrone and oestradiol
      i. Some oestrone/oestradiol \(\rightarrow\) fetus \(\rightarrow\) conversion to oestriol
   b. DHEAS \(\rightarrow\) 16-hydroxylation by the fetal liver \(\rightarrow\) 16OHDHEAS (also produced by the fetal adrenal)
c. Placenta converts 16OHDHEAS to oestriol (first step is removal of sulphate by steroid sulphatase)
   i. DHEAS from the maternal adrenal can also be utilised by the placenta \( \rightarrow \) oestrogens. Last 10 weeks – 50% of oestrone/oestradiol and 10% oestriol come from this source.

2. Production increases almost 100 times during pregnancy – this was used previously as an indication for fetal wellbeing in the third trimester but this is rarely used now.

3. Anencephalic pregnancies usually have low oestrogen levels (atrophic adrenals)

4. Steroid sulphatase deficiency blocks steroid production in the placenta:
   a. Fetal growth and development normal
   b. Fetal DHEASE and 16OHDHEAS normal
   c. Mother has difficulty coming into labour to term, or difficulty with vaginal birth
   d. Dry, scaly skin condition develops at 6 months (X-linked ichthyosis)

Maternal endocrine hormones
1. Anterior pituitary
   a. Prolactin levels increase throughout gestation, then decline after delivery (unless suckling occurs). Rapid withdrawal of P₄ and E₂ allow elevated levels to fall quickly post-partum

2. Posterior pituitary
   a. Oxytocin is a peptide hormone closely related to AVP, and acts on the myometrium to stimulate contractions.
      i. It is produced in high levels, but receptors are not present until mid pregnancy – hence levels needed to induce abortion are higher than those required to augment labour
      ii. It is not necessary for contractions – case study of a woman with diabetes insipidus but with normal labour
      iii. Oxytocin also causes mammary gland contraction during suckling

3. Thyroid
   a. TSH regulates production of T₃ and T₄
   b. Renal clearance of iodide doubles during pregnancy – uptake by the thyroid increases threefold
   c. Increased rate of T₃/T₄ production is thought to be related to hCG
   d. Thyroid hormone binding protein production is enhanced by placental oestrogen – there is a balance between TBG, T₃ and T₄
   e. Thyroxine can cross the placenta, but the fetus is dependent on its own thyroid – inadequate fetal thyroid function \( \rightarrow \) cretinism

4. Adrenal
   a. Cortisol promotes gluconeogenesis, glycogen deposition and has anti-insulin activity
      i. Levels increase steadily in pregnancy due to increased binding protein production. Note that levels also rise in times of stress
   b. Aldosterone regulates sodium/potassium levels and helps regulate blood pressure
      i. Secretion is regulated by the renin \( \rightarrow \) angiotensin I/II system – oestrogen increases the production of the renin substrate, leading to an increase in the production of aldosterone

5. Pancreas
   a. Insulin regulates metabolism by controlling glucose availability
      i. Fetus gets glucose from the mother (fetal levels are 15-20% lower than maternal plasma)
      ii. There is a general insulin resistance in pregnant women, allowing diversion of glucose to the fetus

* Physiology of Pregnancy – Maternal Adaptation to Pregnancy *

The feto-maternal communication system consists of two major arms:
1. Placental arm – e.g. endocrine and nutritional functions
2. Paracrine arm – e.g. acceptance of the semiallogenic fetal graft, maintenance of pregnancy, protection of the fetus and parturition

**Fetal initiatives:**
1. Implantation – apposition of blastocyst, trophoblast invasion, decidualisation
2. Maternal recognition – corpus luteum, immunologic acceptance, decidual quiescence
3. Maintenance – delimit uterotropin/tonin synthesis, myometrial quiescence
4. Endocrine – maternal adaptation, mobilisation of nutrients, mammary development
5. Parturition – retreat from pregnancy maintenance

A number of anatomic, physiological and biochemical changes occur in the mother during pregnancy – notably, these changes reverse relatively rapidly after parturition.

**1. Haematological changes**
   a. Blood volume (particularly plasma) increases ~50%. Haemoglobin, erythrocytes and haematocrit concentrations lower than normal.
      1. Plasma volume increases by 1250mL by 30 weeks
      2. 8-10 weeks there is a 10% increase in plasma volume similar to that seen in the menstrual cycle – so plasma expansion occurs after the changes to the cardiovascular system
   b. Blood loss during delivery (500mL single vaginal, 1000mL twins, 1000mL caesarean) – normally vasoconstriction would be the response, but hypervolaemia of pregnancy modifies this response and this does not occur.
   c. Increased iron requirements due to increased erythrocytes and fetal demand

2. **Cardiovascular changes**
   a. Heart enlarges - cardiac output, stroke volume and pulse rate increase
   b. Peripheral resistance falls (associated with dilation of peripheral vessels and refractoriness to angiotensin II)
   c. Latter part of pregnancy – venous blood pressure increases in lower body due to mechanical and hydrostatic changes → oedema, varicosities
   d. Bioactive substances
      1. Oestrogen – reduces vascular resistance in reproductive tissues (alters the ratio of type I/type III collagen in the brown substance of the vessel wall). Fetal adrenals induce synthesis at 9 weeks.
      3. Nitric oxide – produced by vascular endothelial cells in response to the shear stress of blood flow. Induces arterial relaxation/dilatation; activity of nitric oxide synthetase in some tissues is increased in pregnancy.
   e. Increasing maternal PCO₂ by 1mmHg increases ventilation by 6L/min (1.5L/min in non-pregnant state). Tidal volume increases by 200mL

3. **Immunological changes**
   a. Prior to pregnancy – sperm must survive in the female genital tract, but in most cases a maternal immune response is not stimulated
      1. Seminal plasma – immunosuppressive (cytokines)
      2. Repeated exposure may be protective against preeclampsia
   b. Fetus/placenta is essentially a tissue transplant – there must be a balance allowing the fetus to survive, but allowing normal immune function in the mother. General immunosuppression?
      1. White cells – rise due to expansion of the neutrophil population (starts in the luteal phase, peaks at 30 weeks)
      2. Lymphocyte counts – does not change, may be a bias in the type of T helper (CD4) cells and the cytokines they produce (Th₂ – antibody-mediated response)
      3. Decidua contains almost no B cells. ~10% of leukocytes are T-cells while ~70% are specialised uterine natural killer-like cells (lack CD16 – no antibody-dependent cell mediated cytotoxicity)

4. **Endocrine changes**
   a. Thyroid – moderately enlarged, thyroxine levels increase → basal metabolic rate increases up to 25% (mainly related to fetal O₂ demands)
b. Adrenal cortex – cortisol levels increase (comparable to Cushing’s syndrome)

c. Pituitary – growth hormones 3x, prolactin 10x, FSH/LH suppressed (similar to luteal phase)

5. Metabolic changes – hyperglycaemia, hyperlipidaemia

a. Weight gain – up to 23 kilos, 12.5Kg in primigravida women

b. Water – ~8.5L

c. Insulin – generalised insulin resistance in maternal tissues hence more circulating glucose for fetal uptake. Higher maternal fat utilisation

6. Urinogenital tract changes

a. Ovary – corpus luteum function is maintained six weeks post-ovulation

b. Uterus – increases in size (70g → 1100g), cavity volume (10mL → 5-20L) and contractility. Note that growth is mainly due to stretching of existing cells.

1. Uteroplacental blood flow – minimal during the first trimester, flow into intervillous spaces by 13 weeks

c. Cervix – softening and cyanosis from early pregnancy; collagen-rich connective tissue is rearranged in preparation for dilation; loss of mucus plug – bloody show

d. Vagina – increased vascularity; structural rearrangement of connective tissue and smooth muscle

e. Kidneys – enlarge, creatinine clearance increases (25% at 4 weeks, 45% at 9 weeks) but declines later in pregnancy

7. Abdomen wall and skin

a. Blood flow to the skin is increased – flow to the hands by 6-7 times, raised toe/finger temperature

b. Pigmentation changes in some areas of skin nipples and areola, development of a linea nigra (navel down) and chloasma in neck and face – due to increased secretion of melanocyte stimulating hormone

c. 50% develop striae gravidarum (reddish slightly depressed streaks)

d. Reduced hair loss in pregnancy, excess lost in the puerperium

First pregnancies tend to be more prone to complications of maladaption than subsequent gestations. Preeclampsia is more common in first pregnancies – note that this is now being recognised as a disease of first pregnancy with a given partner (not necessarily maladaption to the pregnancy).

• The Breast and Lactation

Development of the breast

1. Mammary ridges run from the axilla to the groin. In humans there is only one pair – however, supernumerary nipples/breasts may develop along this line. The precise position is variable.

2. Gynaecomastia – breast development in males due to increased oestrogen levels

3. Breast tissue consists of modified sweat glands:

   a. Primary buds (primordium) → mesenchyme

   b. Secondary buds (branching) – note that typical sweat glands coil

   c. Myoepithelial cells surround lactiferous ducts

Structure of the resting gland

1. Oestrogen from the follicle initiates breast development at puberty. Note that the mammary gland is not fully developed until pregnancy.

2. Tubuloalveolar gland – divided into 15-20 lobes (drain to a single duct at the nipple) that can be further divided into lobules consisting of terminal alveolar tissue.

   a. Interseptal tissue - fat and connective tissue present (→ expansion), as well as suspensory ligaments.

   b. Ampulla – swelling in the duct for storage

   c. Lactiferous duct – lined with 2 layers of epithelial cells, drains one lobe

3. The nipple contains the openings for the lactiferous ducts and is richly supplied with nerve endings (Meissner corpuscles). The areola is the pigmented surround area.
Development during pregnancy and the role of hormones:

1. Ducts, then alveoli continue to develop/branch – by the time of parturition there is very little connective tissue left and the breast consists of mainly alveolar tissue.

2. A number of hormones (most importantly prolactin) act synergistically to mediate this:
   a. Growth of duct – oestrogen, growth hormone, adrenal steroids
   b. Lobulo-alveolar growth – oestrogen, progesterone, prolactin, growth hormone, adrenal steroids
   c. Milk secretion – prolactin, adrenal steroids

3. Note:
   a. Hormones may cross the placenta → fetal nipple/breast development
   b. Breast development in males at puberty is inhibited by testosterone
   c. Cyclic breast size/tenderness related to hormones of the menstrual cycle

Colostrum is the very first secretion from mammary tissue, and dominates for the first few days before milk production. It has potent laxative effects.

1. Compared to milk:
   a. More proteins and vitamins (coenzymes required for digestion)
   b. More Na⁺, Cl⁻
   c. Lower in lactose, lipids, potassium
   d. Contains immunoglobulins (mainly IgA – protects against gastroenteritis)

Galactopoiesis occurs a few days after birth and is dependent on prolactin (stimulates mRNA).

1. Prolactin acts on the epithelial cells at a transcriptional level – binds to G-protein coupled receptor → cAMP activation → protein kinases → proteins (e.g. casein)
   a. Prolactin stimulates α-lactalbumin production (blocked by progesterone)
   b. α-lactalbumin + galactosyltransferase → lactose synthetase
   c. Glucose → lactose

Oxytocin is important in the process of milk removal.

1. Reflex arc – suckling induces a neurogenic reflex to the pituitary inducing release from the paraventricular and supraoptic nuclei
2. A conditioned reflex may also occur (e.g. reaction to crying)

Suppression of the menstrual cycle may be achieved through suckling and lactation – serving to space out the interval between births (some evidence as contraceptive method).

1. Mechanism – high levels of prolactin may decrease LH, FSH (directly or indirectly) and ovarian steroid hormones in the mother
2. Role of dopamine – a dopamine antagonist (bromogynocryptine) inhibits prolactin release from the anterior pituitary. Exogenous administration restores the menstrual cycle in women with amenorrhoea.

Parturition and Preterm Birth

Myometrial contractility is a fine balance between local concentrations of uterotonic agents, and factors that regulate myometrial sensitivity.

Preterm birth is defined as delivery prior to 37 weeks completed gestation (clinically relevant cases occur prior to 32 weeks). There is an increased risk of neonatal death and/or morbidity especially before 30 weeks gestation. Worldwide incidence varies (5-6% in Oceania).

1. Risk factors – age/height/weight/parity, socio-economic status, smoking, physical activity, stress, previous preterm birth/abortion, IU/UT infection, faulty placentation, cervical incompetence, antepartum haemorrhage, low rate of weight gain, polyhydramnios/oligohydramnios, fetal anomalies, idiopathic conditions
   a. IU infection can be indicated by analysis of amniotic fluid or the fetal membranes (chorioamnionitis). It is a major cause of early preterm birth.
      i. Stages of ascending intrauterine infection – vagina/cervix → myometrium → amniotic fluid → fetal system
   b. Prediction of preterm birth – risk factor scoring system
530.303 – R+D Lecture Notes

i. Elevated maternal CRH
ii. Cervical assessment – transvaginal ultrasound
iii. Fetal fibronectin (extracellular matrix protein liberated by matrix metalloproteinases) – assay has poor sensitivity
iv. Serum/cervicovaginal IL-6
v. Salivary estriol
vi. Bacterial vaginosis (abnormal flora)

2. Complications
   a. Respiratory distress syndrome
   b. Periventricular haemorrhage
   c. Necrotizing enterocolitis
   d. Fetal/neonatal infection
   e. Bronchopulmonary dysplasia
   f. Patent ductus arteriosus

3. Sequelae include neonatal death or morbidity (blindness, deafness, neurodevelopmental impairment, cerebral palsy). There is a considerable health cost associated, particularly with the ongoing management of neurological conditions.

4. Tocolytic therapies – most have fetal/maternal side-effects
   a. Beta-mimetics (terbutaline/ritodrine) – commonly used to delay birth by 23-48hrs while corticosteroids are administered (lung development)
   b. Magnesium sulphate
   c. Prostaglandin synthesis inhibitors (PGHS-2 inhibitors)
   d. Oxytocin antagonists
   e. Nitric oxide donors (glyceryl trinitrate)
   f. Progesterone
   g. Ca +2 channel blockers

• Fetal Growth and Nutrition

Fetal growth is normally constrained by the maternal environment (maternal constraint) – maternal nutrition does not equal fetal nutrition, and growth is mediated by substrate supply

1. Nutrition → hormones → growth
   a. Insulin is facilitatory/regulatory, though not a primary regulator
      i. Nutrition → fetal glucose → fetal insulin → hormones
   b. GH is high (but there are no receptors on the fetal liver)
   c. IGF (regulated by glucose/insulin) is probably the regulator for fetal growth
   d. Thyroid hormones probably important for fetal O₂ uptake, but doesn’t seem to do much to direct growth (possibly bone maturation)
   e. Glucocorticoids are growth inhibitors – hence requires a placental barrier

2. There is some genetic effect on fetal growth – chromosome abnormalities predispose to low birth weight. Note that genes are more important postnatally.

3. Fetal supply line – causes of poor fetal growth can be placed along this pathway
   a. Nutrition → Maternal circulation
      i. Uterine blood flow
   b. Placental transport
      i. Umbilical blood flow
   c. Fetal circulation → Tissue uptake

4. Smoking – altered nutrition → decreased circulating amino acids, decreased uterine/umbilical blood flow → decreased placental amino acid transport to the fetus

Importance of low birth weight:
1. Affects 6000/year in NZ
2. Increased risk of various diseases – problems can be considered in terms of poor nutrition (CHO, O₂, protein)
   a. Stillbirth rate doubled
   b. Perinatal mortality and asphyxia increased 6-fold
   c. Accounts for ~30% of neonatal unit admissions
   d. Accounts for 21% of short children
   e. On average 8 IQ points lower by adolescence – problems with behaviour
3. Strong relationship between increased blood pressure and low birth weight
   a. Maternal protein intake can affect blood pressure

Barker hypothesis (fetal origins of adult disease):
1. Mechanism ('Thrifty phenotype') – maternal malnutrition → fetal malnutrition
   a. Organ malformation, decreased β-cell mass, insulin resistance, poor vascular development
   b. Hyperlipidaemia, NIIDM, hypertension
   c. Syndrome X
2. Consensus:
   a. Known – consistent, inverse relationship between birth weight and blood pressure, non-insulin dependent diabetes and cardiovascular disease
   b. Probably – not confounded by socio-economic status
   c. Known – selection bias is not a good explanation, effect is modified by attained size
   d. Uncertain – importance of other factors
3. Effects:
   a. Perinatal mortality
   b. Newborn illness (hypoglycaemia, asphyxia)
   c. Poor postnatal growth
   d. Neurological and behavioural problems
   e. Risk of CHD, stroke, obesity, diabetes, syndrome X
   f. Risk of small babies and metabolic disease in further generations

**Puberty**

Puberty is the phase of development in which the gonads secrete hormones in sufficient amounts to mediate accelerated growth of the genital organs and the appearance of secondary sexual characteristics in a characteristic sequence. Important events:
1. Growth and maturation of primary sexual characteristics
2. Appearance of secondary sexual characteristics
3. Growth spurt
4. Psychological changes
5. Ultimately → fertility

Regulation of the onset of puberty:
1. CNS (gonadostat – inhibitory) → hypothalamus (pulsatile GnRH) → anterior pituitary (pulsatile LH/FSH) → gonads (E2, T, inhibin – feedback to all levels)
   a. Fetal/early infancy – few constraints on the HPG axis
   b. Early childhood – inhibition by the CNS (gonadostat), facilitated by inhibitory effects of very small amounts of sex steroids
   c. Puberty – hypothalamus is released from gonadostat inhibition (mechanism unknown), and is less sensitive (as is the pituitary) to sex steroid inhibition
      i. Early puberty – nocturnal pulsatile LH/FSH secretion
      ii. Mid puberty – increase in tonic phase, higher nocturnal LH/FSH
      iii. Late puberty – pulsative LH/FSH secretion day and night
   d. Long-acting GnRH pills may be used to treat precocious puberty
2. Gonadostat
   a. Sex steroid independent
   b. GABA inhibits pulsatile GnRH secretion
   c. Glutamic acid decarboxylase is found in the hypothalamus (→ GABA)
3. Inhibin and activin
   a. Both stimulated by FSH in the Sertoli/granulosa cells
   b. Increase early in puberty
   c. Inhibin inhibits LH/FSH release, activin stimulates release
4. Pubertal growth factors – sex steroids (particularly oestradiol – required by males to fuse the epiphyses), GH, IGF-1, insulin

Important terms:
1. **Andrenarche** – rise in adrenal androgen secretion (DHEAS)
   a. Parallel phenomena (late childhood) – note that the onset of pubic hair development does not necessarily indicate puberty (or vice versa)
   b. Trigger is unknown, but → pubarche
2. **Pubarche** – onset of pubic hair development
3. **Thelarche** – onset of pubertal breast development in the female
4. **Menarche** – onset of menstruation
5. **Gynaecomastia** – palpable or visible breast tissue in the male

### Male puberty
- Note that testosterone levels rise late in puberty
1. **Increased testicular size** is the first physical evidence, occurring at 9.5-13.5 years.
   a. Prepubertal <4mL → 12-15mL adult
   b. Prepubertal has no Leydig cells and solid seminiferous tubules
   c. Spermatozoa can be found in first pass urine in early puberty (little growth)
2. **Maturation of the genitalia** – Tanner stages
   a. Note that stage 1 is prepubertal
   b. Penile enlargement – 9.25-13.75 years
   c. Scrotum enlarges with more rugae and darkening of skin
   d. Full genital development takes 2-5 years – delayed puberty tends to slow progression
3. **Pubic hair** usually appears shortly after genital development. Graded 1-6 clinically.
4. **Axillary** (1-3) and facial hair occurs later in puberty (mean is 14.3 years)
   a. Facial hair begins at 14.9 years at the corner of the mouth and upper lip, spreading to the cheek and chin at 16.2 years. It rarely occurs before the completion of external development
   b. Thickness/distribution of hair is more related to familial traits than hormones
5. **Voice** – begins to break at 14.5 years and is complete a year later
6. **Breast** – areolas increase in diameter, transient gynaecomastia is common (2/3) and may persist for 18-24 months
7. **Sperm production** begins at 13 years
   a. Precedes the peak of the growth spurt
   b. Occur while testicular volume is <10mL

### Female puberty
1. **Breast development** is the first clinical indication (8-13 years) – Tanner stages
   a. May be asymmetrical, Tanner stage 4 is important (but may be fleeting.
   b. Takes 2-6 years to mature
2. **Genitals**
   a. Vagina increases in length before the appearance of secondary sexual characteristics (and continues until menarche)
     i. Mucosa of the vagina and vulva becomes softer and thicker
   b. Mons pubis increases in size (fat deposition), labia majora enlarge
   c. Body of the uterus grows
3. **Pubic hair** usually arises within 6 months of breast development
   a. Increased androgens → pubic and axillary hair, apocrine secretions, acne
4. **Menarche** is usually a late event (10.8-14.6 years)
   a. Most girls begin at Tanner stage 4 (some at stage 3)
   b. Age of menarche is determined by genetic and environmental factors – improving nutrition decreases the age of menarche
   c. Menses may be irregular for the first year

Note that females have their growth spurt ~2 years earlier than males, and in general the length of their growth spurt is slower → ~13cm discrepancy between males and females.

### Postnatal Growth and Development

Growth in the postnatal period is rapid due to hypertrophy and hyperplasia (embryonic – mainly hyperplasia). Genetic, hormonal and environmental factors replace maternal and placental influences of pregnancy.
1. Growth – increase in size
   a. Differential growth – legs get proportionally longer
   b. Organ tissue growth – in general parallels skeletal growth
      i. Brain and eyes well developed, size gain mainly in the first few years
      ii. Reproductive tissues grow at puberty
2. Development – increase in complexity of structure or function

Normal growth patterns
1. Infancy – rapid but decelerating growth
   a. Mean birth weight is ~3.5kg, length 48cm
   b. Linear growth is most rapid immediately after birth
      i. Falls rapidly from 45cm/year to 15cm/year
   c. Growth rate decreases to a nadir just before puberty – if puberty is delayed, this can be very low
2. Childhood – steady growth rate
   a. Fairly consistent growth rate of 5-7.5cm/year
   b. Weight gain of 2-3kg/year
3. Puberty – growth spurt (rapid rise in growth rate)
   a. Peak height velocity of 9.5cm/year in males, 8.2cm/year in females

Factors influencing growth:
1. Genetic and ethnic influences – difficult to assess (nutrition, maternal health, disease)
   a. Fetal genotype makes a minor contribution to size at birth
   b. Postnatal growth and stature is strongly genetically determined
   c. Puberty is also significantly genetically determined
   d. Size is assumed to be a polygenic trait – racial groups vary in size, body proportion and timing of maturation (standards need to be race-relevant)
      i. Social conditions are the main influence of age of menarche
   e. Sex differences:
      i. Male fetuses have a slightly higher birth weight
      ii. Childhood growth – not significantly different (boys slightly taller)
      iii. Pubertal growth spurt is 2 years earlier in females, male growth spurt is slightly longer (13cm height difference)
2. Environment
   a. Nutrition – protein malnutrition is a significant cause of poor growth and failure to reach genetic height potential. Increased height/weight and decreased age of puberty may be attributed to better nutrition
      i. Malnourished children may show catch-up growth (40% incomplete)
      ii. Adaptive response as nutrients are channelled into essential function
      iii. Poor nutrition interacts with the endocrine regulation of growth
   b. Disease may decrease nutrition decreased growth (malabsorption, chronic disease, increased energy demand)
   c. Other factors – disease prevalence, hygiene, SES, physical activity, emotional well-being
3. Hormonal regulation
   a. Growth hormone – pulsatile secretion at night (stages III-IV), low in children, increased in puberty.
      i. Has metabolic and growth effects:
         1. Inhibits glucose uptake, promotes glycogenolysis
         2. Stimulates protein synthesis
         3. Promotes lipolysis insulin resistance
      ii. Secretion increased by sleep, hypoglycaemia, amino acids, malnutrition, exercise, stress, sex steroids
      iii. Secretion decreased by obesity, psychosocial depression
   b. Insulin-like growth factors – note similarity between IGFs and insulin (weak cross-reactivity between receptors).
      i. IGFs are regulated by GH (stimulates) and malnutrition (inhibits)
      ii. Synthesised in the liver (bone, fibroblasts), act on a range of tissues
         1. Autocrine and paracrine actions in bone growth
iii. Lipid, glycogen, protein synthesis → cell proliferation, differentiation
iv. IGF binding proteins (so long half-life) – six characterised
   1. IGFBP-3 – GH dependent, most common
   2. IGFBP-1 – helps regulate glucose, circadian rhythm

c. Sex steroids – promote somatic growth and epiphyseal fusion (E₂, eventually inhibits growth)
d. Thyroid hormones – facilitatory, necessary for GH secretion and growth plate
e. Insulin – facilitatory, provides substrate for growth
f. HPL – facilitatory, homologous to GH (IGF-1)
g. Other growth factors – epidermal growth factor, platelet derived growth factor, fibroblast growth factor. Act at specific cells sites proliferation.
h. Growth-promoting hormones
   i. Fetus – IGF-I and -II, insulin, PDGF, EGF, FGF, (HPL, T₄/₃)
   ii. Child – GH, IGF-I, T₄/₃, (insulin, PDGF, EGF, FGF)
   iii. Puberty – GH, IGF-I, E₂/T, insulin, T₄/₃, (PDGF, EGF, FGF)

Anthropometry (auxology) is the science of the measurement of growth.
1. Height, weight, head circumference measured (others as dictated)
2. Growth charts – constructed from longitudinal and cross-sectional studies
   a. May not be applicable to all racial groups
   b. Height, weight, head circumference charts – 3rd-97th percentile is ‘normal’
      i. Mid-parental height (M + F +/-13 /2) – range +/- 8cm
   c. Height velocity chart – 25th-75th percentile is ‘normal’
      i. Must be assessed over a period of at least 6 months
      ii. Altered in children with delayed puberty
   d. Crossing percentiles alerts to growth disorders
3. Correct measurement techniques are important (supine until 2 years, then standing)

Skeletal maturity (bone age) is important in children with growth disorders, as it indicates biological maturity (as opposed to chronological age). Standardised left hand/wrist x-rays are compared with standards – final height is attained when epiphyseal plates fuse (99% by bone age 15 in females, 17 in males)

Growth disorders – note that many short or tall children are normal variants (normal HV)
1. Evaluation is done in terms of:
   a. Genetic background
   b. Gestational and past medical history
   c. Environment
   d. Physical findings
   e. Growth pattern before birth
2. If height is below the 3rd, or velocity below the 25th percentile → consider pathology
   a. Also for children above the 97th or crossing percentiles upwardly
3. The relation between height and weight percentiles is important
   a. Hypothyroidism – short and overweight
   b. If weight % < height % ‘failure to thrive’ (malabsorption, malnutrition)
4. Minimum normal growth rates:
   a. 2 years 8cm/year
   b. 3 years 7cm/year
   c. 5-9 years 5-6cm/year
   d. Prior to puberty 4cm/year
5. Pathological short stature:
   a. Proportionate – IUGR (intrauterine growth retardation), syndromes, chronic illness, drugs, psychosocial deprivation
   b. Disproportionate – syndromes (particularly Turner S – 50% will not have other clinical presentations), hypoparathyroidism, skeletal dysplasias

Clinical assessment:
1. Medical and social history – antenatal, perinatal, family histories
2. Anthropometry – height, weight, head circumference, parent’s heights
3. Physical examination
4. Bone age
5. Specific evaluation as indicated from preliminary assessment:
   a. Chromosomal
   b. Haematological
   c. Biochemical
   d. Endocrine
   e. Gastrointestinal

- Disorders of Sexual Differentiation

Levels of sexual differentiation – gonads, internal genitalia, external genitalia
1. <6 weeks – bipotential gonads
   a. Intermediate mesoderm → genital ridge + germ cells (from yolk sac endoderm) → bipotential gonad
      i. Transcription factors - steroidogenic factor 1 (SF1), Wilms tumour 1
   b. Gonadal determination:
      i. DAX-1, WnT4 → ovary
      ii. SRY, SOX-9 → testis
2. <7 weeks – bipotential internal genitalia
   a. Male internal genitalia development:
      i. Sertoli cell → AMH → AMH receptor → Mullerian duct regression
      ii. Leydig cell → T → androgen receptor → Wolffian duct stabilisation
         1. Placental HCG and pituitary LH → Leydig cells → T → DHT
   b. Female internal genitalia are retained in the absence of AMH (Sertoli cells)
3. <8 weeks – bipotential external genitalia
   a. Male external genitalia also require androgen effects to maintain/develop
   b. Female external genitalia occur in the absence of androgen effect

Genes important in sexual differentiation:
1. Wilms Tumour 1 (WT-1) – nuclear transcription factor found in urogenital ridge
   a. Initiates transcriptional regulation and differentiation of glomerular epithelial cells and the gonadal primordium
   b. Has repressor and activating components
   c. Acts as a tumour suppressor gene in renal tissue
   d. Loss of function:
      i. Denys-Drash syndrome – gonadal dysgenesis, congenital nephropathy, Wilms tumour
      ii. WAGR syndrome – Wilms tumour, genitourinary abnormalities/gonadoblastoma, aniridia and mental retardation
2. Steroidogenic Factor 1 (SF-1) – orphan (no ligand) nuclear transcription factor, released in the urogenital ridge early in organogenesis, then Sertoli/adrenal
   a. Regulates development at a number of levels:
      i. Gonadal development
      ii. Adrenal development
      iii. Pituitary gonadotroph development (LH and FSH)
      iv. Steroidogenic enzymes – cholesterol side-chain cleavage, 21-hydroxylase, aldosterone synthetase
   b. Expression is sexually dimorphic – low expression in the fetal ovary
   c. Knock-out mouse for SF-1
      i. External genitalia female, internal genitalia mullerian
      ii. Gonads and adrenals absent
      iii. No steroidogenesis, LH/FSH production, AMH production
3. Steel factor and c-kit – growth factor/receptor system involved in haemopoetic cells, melanocytes and germ cells
   a. Receptor (c-kit) on germ cells, ligand (Steel factor) from Sertoli cells
   b. Crucial in the migration of germ cells to the gonadal ridge, important in spermatogenesis
4. SRY Homoeobox-like gene 9 (SOX-9) – SRY-like transcription factor expressed later in testis development (week 18)
a. Loss of function → camptomelic dysplasia, 75% of XY sex reversal
5. Sex-determining Region of the Y chromosome (SRY) – nuclear transcription factor expressed early in the gonadal ridge in pre-Sertoli cells
   a. Target genes unknown but important for testicular development
   b. XX males (1:20,000) – 70% are SRY+
      i. Small testis, functional Leydig/Sertoli cells but azoospermia
   c. XY females (1:100,000) – minority are SRY
      i. Streak gonads, internal and external female genitalia
6. DAX-1 (ridiculously long name) – orphan nuclear transcription factor
   a. Regulates development of adrenals, gonads, pituitary gonadotrophs, hypothalamic GnRH
   b. Loss of function mutation (males) → adrenal underdevelopment, GnRH/LH/FSH deficiency, small phallus
   c. Gene duplication → sex reversal of XY males

In assessing sexual ambiguity at birth, two main tests are required – karyotype and pelvic USS – ultrasound can accurately determine internal female genitalia (uterus) as this develops considerably before birth (maternal oestrogens).
1. Palpable gonad is almost always a testis
2. Virilized girl – implies prenatal androgen exposure
   a. Ovaries, normal female internal genitalia
   b. Fetal – congenital adrenal hyperplasia (most common)
   c. Maternal – ingestion, severe PCOS, androgen secreting tumour
3. Undervirilized male – implies lack of androgen exposure, often hypospadias
   a. Testis, no female internal genitalia
   b. Generally idiopathic
   c. Fetal – LH receptor mutation, steroid defect, androgen receptor mutation
   d. Small phallus – hypothalamic/pituitary defect (e.g. Klinefelter’s)

- Menopause

The total number of follicles peak at around 6-7 weeks in utero, and decline thereafter:
1. Birth – 2 million
2. Puberty – 0.3 million
3. Fertile years (12-50) – 400 menstrual cycles
   a. Younger and older women have more anovulatory cycles (typically >40 days)
4. 50 years – ovary becomes non-functional as there are so few follicles left

The menopause is the last episode of natural menstrual bleeding. It commonly occurs at 50-52 years of age in well-nourished populations, regardless of racial background.
1. Premenopausal – 40 years or older, but still menstruating regularly
2. Menopausal transition – between the 1st break in regularity and menopause
3. Perimenopause – period of erratic hormone fluctuation before the start of menopause, ending with ovarian senescence (following menopause)
4. Postmenopausal – following the menopause

The menopausal transition is marked by 2-3 years of irregular, anovulatory periods. Poor nutrition and smoking contributes towards early onset of menopause. Hormonal features:
1. From 30 years – decreased inhibin → reduced FSH suppression → increased FSH
2. Increased FSH → follicular atresia/depletion in the last 10 years of menstruation
   a. FSH rise is earlier and larger compared to LH – maximum levels are reached 5 years after menopause, then progressively falls
3. Decreased E2 → increased gonadotrophins (sporadic), poor follicular selection
4. Some cycles are paradoxical – increased FSH and E2
5. Hormone levels are generally unpredictable, although ovulation is still possible
   a. 40+ – ¼ chance of conceiving, 4x chance of miscarriage compared to 24-26
Once past the age of 45, a year without periods is 98% indicative that the patient has become postmenopausal. Aromatisation of androstenedione (from the adrenal cortex) in adipose tissue becomes the primary source of oestrogens (primarily oestrone). Symptoms include:
1. Depression
2. Anxiety
3. Insomnia (a cure for this is lectures from Prof France), night sweats, hot flushes
4. Tiredness
5. Loss of libido – treatment with androgens may increase risk of cardiovascular disease
6. Loss of concentration
7. Loss of self-esteem
8. Feelings of social unacceptability

Osteoporosis (loss of bone density) is one of the major consequences of menopause (due to oestrogen deficiency).
1. Divided into two types:
   a. Postmenopausal – trabecular bone loss, fractures in distal radius and vertebrae
   b. Senile – greater proportion of males, trabecular and cortical bone loss, fractures in pelvis
2. Risk factors for primary osteoporosis – note that bone mineral is laid down around ages 15-25, so high calcium intake and exercise at this time is protective
   a. Female, advanced age, white race, thin physique
   b. Early menopause
   c. Family history
   d. Smoking, alcohol, low exercise, low calcium intake
3. HRT for early menopause decreases incidence of hot flushes, vaginal dryness and dyspareunia. It also protects against decreasing bone mineral content (BMC).
   a. May reduce risk of atherosclerosis (controversial), ovarian cancer (40% risk reduction), urinary urgency/cystitis and arthritic pain
   b. Doesn’t increase breast cancer incidence, but may → growth existing cancer
   i. Lower morbidity may just be due to more frequent GP visits
   c. If given with progesterone, doesn’t increase risk of endometrial cancer
   d. Variable effects on psychological and emotional problems

• Ageing

Ageing refers to progressive, universal and irreversible changes that take place in an individual with the passage of time. It may be associated with a reduction in the reserve capacities of the individual, increasing the probability of environmental demands exceeding the individual’s capacity to cope.
1. It is not known how much physiological deterioration is due to age-related lifestyle and disease as opposed to ageing itself
2. Survival curves
   a. Probability of dying doubles every 8 years after age 20
   b. % Survival curves – small initial blip (perinatal mortality) then slow/steady decrease until the 60s where there is a rapid decrease in survival. Mean life span is expressed as 50% survival rate.
   c. Maximum/record age – 120 years 237 days (Japan)
3. Physiological changes:
   a. Nervous system has modest changes
   b. Basal systolic blood pressure rises
   c. Decreased GFR is more likely due to non-clinical disease
4. Tissue component changes:
   a. Collagen → becomes progressively cross-linked (reduced flexibility/diffusion)
   b. Elastin – loss with age, more fragmented (reduced recoil (lung/arterial))
   c. Proteoglycans → increase in sulphated proteoglycans (increased lipid binding), decreased in hyularonic acid (reduced tissue water content)
5. Diseases related to the ageing process that may lead to mortality:
   a. Diseases that are part of the ageing process e.g. atherosclerosis (PUI)
b. Diseases that show increasing incidence e.g. neoplasms (not PUI)
c. Diseases that have more serious consequences for the elderly e.g. respiratory infections, accidents (not PUI)

6. PUI diseases – not fatal, not always related to age
   a. Osteoarthritis
   b. Osteoporosis
   c. Emphysema

Theories of aging:

1. Programmatic theories – due to an inherent genetic programme
   a. Rate of living theory – total metabolic expenditure is species-specific/constant
      i. Flies live 2½ times longer without wings, and live longer in winter
      ii. Hibernating mammals live longer than controls
      iii. Low temperature conditions → shorter life (have to generate heat)
   b. Genetic pleiotropy – ageing in evolutionary and selective terms
   c. Neuro-endocrine-oncogenic – OK for vertebrates
   d. Immunological – age-dependent decline in thymus-dependent system

2. Stochastic theories – results of random environmental influences
   a. Free radical theory – note protective mechanisms & exogenous chemicals
      (superoxide dismutase, catalase, glutathione, S transferases)
      i. Free radicals → protein alterations
      ii. → Increased cross-linking of collagen → impedes nutrient diffusion
      iii. → Inactivates enzymes, breaks DNA, peroxidises lipids, modifies amino acids
      iv. Oxidised proteins and lipids have an increased rate of turnover
   b. Calorie reduction theory – at 30% less caloric intake rate of ageing slows
      i. → Smaller, mature later
      ii. → Decreased blood glucose, insulin
      iii. → Decreased body temperature
      iv. → Increased daytime activity
   c. Amino acid racemisation, non-enzymatic glycosylation → cross-linking of collagen
   d. Somatic mutation and error accumulation – may be associated with the free radical theory

Prospects for an ageing population – optimistic view is that increased longevity will be accompanied by better health, increased number of years free of chronic diseases with reduced health costs. The reality seems to be different e.g. increases in hip fractures and dementia.
Hormones of the Hypothalamus

Peptide hormones secreted by the hypothalamus act by binding to plasma membrane receptors to affect intracellular signalling pathways. They are often synthesised as larger protein precursors, which are then processed via proteolysis within storage vesicles.

pre-protein \rightarrow \text{(signal peptide removal)} \rightarrow \text{peptide hormone precursor} \rightarrow \text{(post-translational modification in Golgi apparatus)} \rightarrow \text{pro-protein} \rightarrow \text{active hormone}

The active hormone is released from the cell via exocytosis, where the secretory granule fuses to the plasma membrane. This is an example of regulated secretion and requires a stimulus for initiation – hence pulsatile release.

These hormones may have several functions:
- **Endocrine** – transported in the blood to allow communication between organs
- **Paracrine** – communication between adjacent cells
- **Autocrine** – cell feeds back on itself
- **Neuroendocrine** – communication between neurons and endocrine cells
- **Neurotransmitter** – communication between neurons

The hypothalamus controls the anterior pituitary by the secretion of hormones into the hypothalamo-hypophyseal portal system. This consists of long portal vessels that carry hormones secreted by the hypothalamus, as well as axons from hypothalamic nuclei that project down the pituitary stalk to release hormones into short portal systems (capillaries).

Many hypothalamic peptides involved with pituitary control have unusual post-translational modifications (e.g. pyroglutamyl residues at the amino-terminus, or amide groups at the carboxy-terminus). These include:
- TRH \{(pyro)-Glu-His-Pro-NH_2\} – stimulates the secretion of TSH and prolactin
- GnRH (LHRH) – stimulates the secretion of LH and FSH
- GRH – stimulates the secretion of growth hormone (somatotropin)
- Somatostatin (SRIH) – inhibits the release of GH
- CRH – stimulates the release of \(?\)-LPH and ACTH (same precursor)

One hypothalamic factor is a peptide – dopamine is a catecholamine that functions as a prolactin-release-inhibiting hormone.

Two groups of hypothalamic nuclei (supraoptic and paraventricular) have long axons that project directly to the neurohypophysis. These are responsible for the synthesis of oxytocin and vasopressin as pro-proteins, and their subsequent processing and release.

Oxytocin – induces smooth muscle contraction in the myoepithelial cells around mammary alveoli. Suckling elicits a neurogenic reflex, releasing oxytocin from the hypothalamus.

Vasopressin – involved in regulating the water content of urine, making more water channels present in collecting tubules (promoting reabsorption). Secretion is triggered by increases in blood osmolarity detected by receptors in the hypothalamus and third ventricle.

Oxytocin is also used clinically to induce labour. While its exact role is unclear, the evidence suggests a paracrine control system – namely, the increase in uterine oxytocin receptors, and the secretion of oxytocin from the fetal chorion at term.

Hormones of the Pituitary: Structure, Action and Control

The pituitary gland secretes a wide variety of hormones – these can be grouped into three families. If the pituitary is removed, the following sequelae are possible:
- Cessation of growth
- Atrophy of thyroid
Atrophy of adrenal cortex
Cessation of lactation
Prevention of skin darkening in lower vertebrates
Disturbance of carbohydrate, fat and protein metabolism
Disturbance of water and salt balance

The growth hormone-prolactin family (GH, PRL and placental lactogen) are structurally related protein hormones consisting of 190-200 amino acids (with two conserved disulfide bonds).

1. **Growth hormone** (somatotropin), synthesised in sommatotropes, is essential for postnatal growth and metabolism.
   a. **Metabolic effects**
      i. Protein synthesis – anabolic (increased translation/transcription – nitrogen balance)
      ii. CHO metabolism – induces insulin resistance (decreased peripheral utilisation, increased hepatic gluconeogenesis)
      iii. Lipid metabolism – promotes release of FFA and glycerol from adipose tissue → greater oxidation in the liver
      iv. Mineral metabolism – promotes positive Ca\(^{2+}\), Mg\(^{2+}\) and PO\(_{4}\)\(^{-2}\) balance by deposition in long bones
      v. Binds to lactogenic receptors and has prolactin-like effects.
   b. **Growth effects** seem to be indirect by GH-dependent secretion of insulin-like growth factor I (IGF-I) by the liver.
      i. In Laron-type dwarves, GH is normal while the ability to produce IGF-I is reduced. hGH is used to promote growth in GH-deficient children (previously from cadaver pituitary glands, but risk of CJD).
      ii. Increased GH production in adults leads to excessive acral bone growth (acromegaly) – jaw, hands, feet and skull.
   c. **Triggers** of GH release are exercise, stress, protein intake (or Arg infusion) and carbohydrate rich meals.
      i. GH is secreted an hour or two after falling asleep
      ii. Dopamine agonists (e.g. levo-dopa) stimulate release; antagonists (e.g. bromocriptine) inhibit release.
   d. **Regulation** of GH release is mediated by GHRH and somatostatin (inhibition)

2. **Prolactin** stimulates lactation in the postpartum period.

3. **Chorionic somatomammotropin** has no defined function, though acts similarly to GH

There are four hormones in the glycoprotein family – these all contain two glycoprotein subunits, a common α chain and a specific β chain.

i. **Thyroid stimulating hormone** binds to plasma membrane receptors on cells producing thyroid hormones, and activates adenylate cyclase. This has short and long term effects, generally stimulating thyroid function (under a highly controlled process).

ii. **Luteinizing hormone** and **follicle stimulating hormone** promote the synthesis of sex steroid hormones and gametogenesis. Effects are mediated by cAMP.

iii. **Human chorionic gonadotrophin** is synthesised by syncytiotrophoblast cells in the placenta – it closely resembles LH and is the basis of many pregnancy tests as it increases in blood after implantation.

The ACTH-related family is derived from proteolytic processing of pre-pro-opiomelanocortin – products include ACTH, endorphins and α-MSH.

i. **ACTH** is a 39 amino acid peptide that regulates the growth and function of the adrenal cortex. All essential amino acids are within the 24 N-terminal amino acids.
   a. ACTH increases steroid hormone production by enhancing the conversion of cholesterol to pregnenolone (cAMP-dependent).
   b. CRH regulates ACTH secretion by stimulating release in a pulsatile manner with a diurnal rhythm (peaks before waking).
   c. Stress stimulates ACTH release – physiological levels of cortisol do no blunt this effect, but very high levels do.
   d. Excess ACTH leads to Cushing’s disease (overproduction of corticosteroids), while low ACTH indicates adrenocortical insufficiency (Addison’s disease).
• **Steroids and Cholesterol**

**Steroids** are compounds that contain the perhydrocyclopentenophenanthrene nucleus, and include a wide variety of biologically significant compounds. They may be named using trivial names for common usage, or by systematic names based on certain fundamental parent structures – which is nice to know, but clinically useless.

**Cholesterol**, produced in the liver, is a key constituent of cell membranes, lipoproteins, bile acids and steroid hormones. While there is no dietary requirement, cholesterol from food (LDL) contributes to the body’s pool.

It is synthesised from acetyl CoA via a number of condensation reactions. Note that the enzyme hydroxymethylglutaryl (HMG) CoA reductase catalyses the rate limiting step.

**Familial hypercholesterolemia** is a condition where there are increased plasma levels of LDL (β) due to absent or abnormal cell membrane receptors on hepatocytes. This may be monogenic or polygenic in origin – most affected individuals are heterozygotes.

Incidence is around 1 in 500, and the disease presents generally between 30 and 50 years of age. There is typically an increased risk of CHD (atherosclerosis). Treatment is by low cholesterol diet, and HMG-CoA reductase inhibitors e.g. simvastatin (ZOCOR).

Note that cholesterol also has an important role as a precursor to many gonadal, adrenal and placental hormones.

Cholesterol utilisation (via LDL) follows a number of steps:
1. LDL binds with coated pit receptor and is internalised
2. Fuses with vesicle of enzymes for degradation, receptosome recycles receptors to cell membrane
3. HMG-CoA reductase in the endoplasmic reticulum → cholesterol
4. Cholesterol → mitochondria, assisted by StAR (steroidogenic acute regulatory protein), which moves cholesterol from the outer to inner membrane by acting on P450 to collapse the membrane.
5. Inversion of cholesterol → pregnenolone is enhanced by various hormones (ACTH, LH, pituitary hormones), increased P450 action, increased LDL uptake and increased StAR.

The conversion of cholesterol to various steroid hormones involves a number of enzymes:
- **Hydroxylases** – substrate specific cytochrome P450 enzymes
- **Oxido-reductases** – hydroxysteroid dehydrogenase (e.g. 17β-oxidoreductase)
- **Isomerases** – function in conjunction with 3β-hydroxysteroid dehydrogenase to transpose C=C bonds (notably Δ5 to Δ4)
- **Reductases** – e.g. Δ5 reductase
- **Desmolase** – cleaves side-chain at C17
- **Aromatase** – aromatises rings → oestrogens (c P450)

• **Male Sex Hormones**

Testosterone and 5αDHT have a number of hormonal actions:
1. **Androgenic actions:**
   a. Development and growth of the male reproductive tract – Wolffian ducts, external genitalia (5αDHT), puberty
   b. Libido and sexual potency in males
   c. Aggressiveness
2. **Anabolic actions:**
   a. Linear body growth and muscular development (nitrogen retention)
   b. Enlargement of larynx, thickening of vocal cords
   c. Beard, axillary, pubic, temporal hair growth
   d. Spermatogenesis
Testosterone (17β-hydroxyandrost-4-en-3-one) is the main secretory product of the testis, though it is also produced in extraglandular tissues from plasma androstenedione (significant pathway in females).

1. Normal levels – 11-40 nmolL⁻¹ males, 0.5-2.5 nmolL⁻¹ females
2. Binding (males) – 2-3% free. 44% sex hormone binding globulin, 54% albumin/other
   a. SHBG (M_W 95,000) is a liver-derived specific transport protein for testosterone. Production is stimulated by oestradiol.

5α-dihydrotestosterone is the main intracellular androgen in a number of tissues (e.g. male reproductive tract, skin). It is twice as potent as testosterone, and mainly arises from local production in target tissues from circulating testosterone and androstenedione:

75% in males: Testosterone (→ 5α-reductase) → 5αDHT
80% in females: Androstenedione (→ 17β-oxidoreductase, 5α-reductase) → 5αDHT

There are two forms of 5α-reductase, and these share only 50% homology in amino acids:
   Type 1. Gene located on the short arm of chromosome 5 – expressed in skin tissue, dominant type in the scalp. Levels are normal in men with congenital 5α-reductase deficiency (Caribbean region – raised as girls, become boys at puberty)
   Type 2. Gene located on the short arm of chromosome 2 – dominant form in male genital tissue (including prostate). Deficient in congenital 5α-reductase deficiency.
   a. Inhibited by finasteride – used in prostatic hyperplasia (Proscar) and hair loss (Propecia – ? effectiveness)

A small amount of oestrogen is secreted by the testes (12% of total circulating levels) – the rest is formed in adipose tissue from the action of aromatase on circulating androstenedione and testosterone.

- Female Sex Hormones

Oestrogens are produced in the ovary where the growing Graafian follicle secretes oestradiol in increasing amounts. After ovulation, the corpus luteum also produces oestradiol but in smaller amounts. Adipose tissue also converts androstenedione and testosterone to oestrogen – this is notable in men and post-menopausal women.

1. E₂ – Oestradiol
   a. Serum levels
      i. Normal females (varies with ovarian cycle) – 200-1100 pmolL⁻¹
      ii. Postmenopausal females - <110 pmolL⁻¹
      iii. Adult males - <160 pmolL⁻¹

2. E₁ – Oestrone (1/10 the activity of E₂)
3. E₃ – Oestriol (1/100 the activity of E₂)

Hormonal actions:
1. Female secondary sexual characteristics (breast development, genitalia maturation)
2. Actions on female reproductive tract are cyclic (more dominant pre-ovulation)
   a. Endometrium – mitotic proliferation of glandular epithelium and stroma
   b. Endocervical canal – secretion of mucus (greater water content – less viscous, more elastic (spinnbarkeit)), softening of the cervical os
   c. Vagina – proliferation of epithelial cells
3. Metabolic effects
   a. Stimulation of osteoblast function (maintain bone formation, density)
   b. Maturation of skeleton/long bones in puberty
   c. Increased plasma triglyceride levels
   d. Increased cortisol-, progesterone-, testosterone-, oestradiol-, thyroxine-binding hormone levels
Progesterone is the main steroid hormone of the corpus luteum and placenta – outside the postovulatory phase, very little progesterone is present in plasma (mainly arising from the adrenal cortex).

1. **Serum levels**
   a. Preovulation to LH surge - <4 nmolL⁻¹
   b. During LH surge – 4-10 nmolL⁻¹
   c. Postovulation – 25-120 nmolL⁻¹

Hormonal actions:
1. Progesterone receptors are induced by oestrogen – hence oestrogen priming
2. Inhibition of mitotic proliferation (anti-oestrogen effect)
3. Endometrium – coiling of the tubular glands, increases water content and vascularity of stroma (maintains endometrial stability)
4. Endocervical canal – inhibits mucus (volume decreases, viscous, less elastic)
5. Increases basal body temperature by 0.3-0.5°C via the CNS

### Clinical Aspects of Adrenal Steroids

**Mechanisms of action:**

1. **Glucocorticoid problem**
   a. Hyponatraemia – unable to excrete a water load (reduced GFR), loss of cortisol inhibition of ADH
   b. Hypoglycaemia – reduced hepatic gluconeogenesis
   c. Hypotension – loss of cortisol effects on vascular tone

2. **Mineralocorticoid problem**
   a. Hyponatraemia – urine Na⁺ loss with intravascular volume contraction and secondary ADH secretion
   b. Hyperkalaemia – reduced renal K⁺ excretion
   c. Metabolic acidosis – reduced renal H⁺ excretion

**Causes of adrenal failure may be**

1. **Primary (adrenal gland)**
   a. POMC → ACTH (contains α-MSH) – regulated by cortisol
   b. Excess pigmentation (due to excess ACTH) apparent in skin flexures, buccal mucosa, old scars, freckles and nails
   c. Aetiology (adults)
      i. Autoimmune (polyendocrine deficiency syndrome) 70%
      ii. TB 20%
      iii. Other
         1. Infections (fungi, meningococcus, AIDS
         2. Metastatic neoplasms
         3. Sarcoidosis

2. **Secondary/tertiary (pituitary or hypothalamus)**
   a. Aetiology
      i. After prolonged administration of exogenous glucocorticoids
      ii. After treatment for Cushing’s syndrome (removal of endogenous glucocorticoids)
      iii. Hypothalamic/pituitary lesions and/or their treatment (rare)

### Inborn Errors of Steroid Hormone Metabolism

**Congenital adrenal hyperplasia** involves a deficiency in one of the enzymes required for normal steroid hormone synthesis in the adrenal cortex. Resultant cortisol deficiency may lead to hyperplasia of the adrenal gland (excess stimulation by ACTH). Adrenogenital syndrome may also be a consequence (virilization of female external genitalia).

1. **21-hydroxylase deficiency** (90% of CAH) is due to the loss of a gene on the short arm of c6, and results in increased androgen production by the adrenal cortex.
   a. Clinical presentation
i. Simple virilization, compensated form – unaffected aldosterone
   ii. Salt-wasting form – virilization, adrenal insufficiency. Decreased aldosterone $\rightarrow$ decreased Na$^+$ and increased K$^+$
   iii. Non-classical form – partial deficiency $\rightarrow$ post-pubertal hirsutism, disturbed menstrual function

b. Diagnosis – high plasma concentrations of 17-hydroxyprogesterone and urinary excretion of pregnanetriol (primary metabolite)
c. Treatment – glucocorticoids (and mineralocorticoids in the salt-wasting form)

2. 11-hydroxylase deficiency results in the hypertensive form of CAH (rare), with decreased cortisol production $\rightarrow$ overproduction of precursors and androgens
   a. Virilization of female external genitalia
   b. Hypertension may be due to excessive 11-desoxycorticosterone (precursor of cortisol with mineralocorticoid properties)

3. 3β-hydroxysteroid deficiency (rare) results in decreased cortisol, aldosterone and androstenedione (and testosterone), but increased dehydroepiandrosterone.
   a. Male pseudohermaphroditism, salt-wasting

4. Other rare enzyme deficiencies
   a. 17-hydroxylase $\rightarrow$ hypertension
   b. Cholesterol desmolase $\rightarrow$ salt-wasting
   c. 18-hydroxysteroid dehydrogenase $\rightarrow$ decreased aldosterone $\rightarrow$ salt-wasting

**Androgen resistance syndromes** stem from the fact that testosterone must be converted to 5α-dihydrotestosterone in target cells.

1. 5α-reductase deficiency (autosomal recessive)
   a. Normal internal male genital tract, feminisation of external genitalia – small hypospadiac phallus, urogenital sinus $\rightarrow$ perineum, blind vaginal pouch
   b. A variable degree of virilization occurs at puberty

2. Disorder of the androgen receptor – at least 4 variants (x-linked)
   a. Symptoms vary – complete testicular feminisation, incomplete testicular feminisation, Reifenstein syndrome, infertile male syndrome

**Steroid sulphatase deficiency** is an x-linked recessive trait that presents in pregnancy as placental sulphatase deficiency ($\rightarrow$ decreased oestrogens, particularly oestradiol). There may be a failure to progress to labour at term. X-linked ichthyosis by 3 months postnatal.

**Aromatase deficiency**

1. Females
   a. Pregnancy – signs of virilization, low maternal oestrogen levels
   b. Birth – ambiguous genitalia
   c. Puberty – hypergonadotrophic hypogonadism with polycystic kidneys, no breast development, enlarged clitoris, short stature with delayed bone age

2. Males
   a. Normal male phenotype and sexual development
   b. Adult – normal sexual orientation/libido, normal testosterone, undetectable oestrogen but impaired spermatogenesis

• **Signal Transduction – Peptide Hormones**

A hormone is a chemical messenger produced by one cell that circulates in the plasma, to cause some effect on a cell in another tissue.

1. **Hydrophilic hormones** (e.g. polypeptides, catecholamines, neurotransmitters) bind to cell-surface receptors without first entering the cell, leading to:
   a. Second messenger activation (e.g. cAMP) $\rightarrow$ further effects
   b. Induction of specific receptors from microsomes to the plasma membrane
   c. Activation of intracellular protein kinases (many by second messengers)
   d. Induction of protein synthesis through transcriptional or post-transcriptional processes
2. **Hydrophobic hormones** (e.g. sex steroids, adrenal steroids) bind to nuclear receptors and must first enter the cell. They modify the transcription of genes by interacting with hormone response elements of the DNA.

### Receptors for hydrophilic hormones

1. **Transmission of chemical information from hormone to cell**
   a. One hormone can interact with different receptors → different effects. The predominant response is determined by the number of each type of receptor.
   b. Several hormones may stimulate the same second messenger (e.g. PTH, adrenalin, calcitonin → adenylate cyclase activation in osteoblasts)

2. **Steps in the isolation of receptors and determination of their properties**
   a. Good source – characterise number/cell, affinity by Scatchard analysis
   b. Identification – radiolabelled hormone + crosslinking → irreversible coupling
   c. Isolation – purification, solubilization (detergent), affinity chromatography, immunoaffinity purification
   d. Determination of primary sequences of receptors and cDNA cloning
   e. Hydrophathy analysis – identification of hormone families

### Types and families of receptors in tissues

1. **Receptors for fast-acting neurotransmitters** (ACh, GABA, glycine)
   a. Act as ion-gated channels – pore in the middle which changes with an appropriate agonist (e.g. 2ACh + nicotinic receptor → cation channel)
   b. Four hydrophobic membrane-spanning parts (peaks on hydrophobicity plot)

2. **G-protein coupled receptors** (peptide hormones, adrenergic, muscarinic, serotonin)
   a. External glycosylated amino-terminal, internal carboxy-terminal
   b. 7-transmembrane domains means that the carboxy-terminal is internal – this often contains serine/threonine residues which can be phosphorylated (temporarily inactivates the receptor)

3. **Single-pass family** (e.g. EGF receptor, insulin receptor)
   a. Hormonal activation → autophosphorylation of an intrinsic tyrosine kinase (on the cytosol side of the receptor)

### G-proteins (guanyl nucleotide binding proteins) act on the 7-pass family of receptors:

1. **Sequence of events**
   a. G-protein consists of three subunits on the internal plasma membrane – note that the α-subunit specifies the identity and function of the G-protein
   b. GDP is bound to the guanyl nucleotide binding site of the α-chain, closely associated with the βγ-subunits
   c. Hormone interaction → exchange of GTP for GDP → α-chain dissociation
   d. α-GTP → increased activity of G-protein coupled enzyme → second messenger
   e. The α-subunit has an intrinsic GTPase activity – this leads to GDP and Pi, and the α-subunit reunits with the βγ-subunits

2. **Examples**
   a. **Cholera toxin**
      i. Diarrhoea by massive water loss from epithelial cells of the colon – huge rise in [cAMP] leading to excessive secretion of Na⁺ and H₂O
      ii. A₁ subunit of the enterotoxin catalyses irreversible ADP-ribosylation of an arginine residue on the α-subunit of G₅
   b. **Pertussis toxin**
      i. ADP-ribosylation of a cysteine residue of the α-subunit of Gₒ, Gᵢ and G₁ but not G₅. This inactivates these subunits, preventing hormone action

### Soluble second messengers are generated by hormone action, and typically phosphorylate enzymes to activate or inhibit (e.g. protein kinases).

1. **cAMP**
   a. Generated from ATP by adenylate cyclase (activated by the Gₛ α-GTP)
   b. Signal is terminated by hydrolysis by cAMP-specific phosphodiesterase
c. There is an amplification cascade involved – 2 molecules with inactive cAMP-dependent Ser/Tyr protein kinase combine to form two catalytic units
d. Signal is rapidly reversed by dephosphorylation (specific for the substrate)
i. Desensitisation/ internalisation of the enzyme-receptor complex
ii. GTPase action of Gs α-subunit
iii. cAMP-specific phosphodiesterase

2. Inositol 1,4,5-triphosphate (IP₃)
a. Generated from a membrane phospholipid by a specific phospholipase C
b. Sensitive to
i. Adrenaline via α₁ adrenergic receptors
ii. ATP via P₂ purinergic receptors
iii. ACh via M₁/M₃ cholinergic receptors
c. Receptors are coupled to phospholipase C through G₁ – note that phospholipase C also produces DAG (another second messenger)
d. IP₃ is inactivated by sequential hydrolysis → IP₂ + P₁ → IP + P₁ → inositol + P₁
e. IP₃ induces the release of ionised Ca²⁺ from stores in the ER

3. Ionised calcium
a. Cytoplasmic Ca²⁺ is maintained by an ATP-driven pump
b. Stimulation → release of Ca²⁺ from ER or ECF
c. Increased [Ca²⁺] leads to changes in calcium-modulated proteins
i. Calmodulin is a highly conserved protein with 4 Ca²⁺ binding sites. When 3 or 4 Ca²⁺ bind, there is a 9% increase in the α-helical content of the secondary structure. It modulates enzyme activity by forming a 1:1 non-covalent molecular complex with it.
d. Changes in [Ca²⁺] can lead to:
   i. Modification of specific enzyme activities
   ii. Interactions with the cAMP-generating/inactivating mechanism → modulation of cAMP-dependent hormone effects
   iii. Change in the phosphorylation (hence activity) of enzymes/cascades

4. Diacylglycerol
a. Generated as a product of hydrolysis of phosphatidylinositol 4,5 diphosphate – lipophilic, so associated with internal membrane
b. Binds to and activates protein kinase C
c. Increases platelet aggregation by phosphorylation of 2 platelet enzymes

Examples of *central metabolic control by phosphorylation*:
1. Glycogen phosphorylase (rate-limiting enzyme for glycogen degradation)
a. cAMP → protein kinase A → phosphorylase B kinase → phosphorylation → activation
2. Glycogen synthetase (rate-limiting enzyme for glycogen synthesis)
a. cAMP → protein kinase A → phosphorylase B kinase → phosphorylation → inactivation
b. Activated by insulin dephosphorylation
3. Hormone-sensitive lipase (rate-limiting enzyme for adipose tissue lipolysis)
a. cAMP → protein kinase A → phosphorylation → activation
4. Pyruvate dehydrogenase (rate-limiting enzyme for glycolysis/fatty acid biosynthesis)
a. Insulin dephosphorylation of the Pyruvate decarboxylase subunit → activation
5. Acetyl CoA carboxylase (rate-limiting enzyme of fatty acid biosynthesis)
a. First site – protein kinase A → phosphorylation → inactivation
b. Second site – insulin → phosphorylation → activation
6. ATP citrate lyase (rate-limiting enzyme of acetyl-CoA transport from mitochondria)
a. Insulin → phosphorylation → activation

**Signal Transduction – Peptide Hormone Action (Gonadotrophins)**

The glycoprotein hormones are all heterodimers consisting of two subunits strongly associated via non-covalent interactions. The β-subunits are responsible for specificity, although there is a certain amount of homology between different gonadotrophins.
Both subunits also contain carbohydrate chains (15-31%) linked to asparagine residues. For example, FSH has 4 N-linked oligosaccharides, two on each subunit. It is speculated that they interact with cell membrane lectins on target tissue, required for linking hormone-receptor complex formation to activation of adenylate cyclase.

Glycoprotein hormones are generally coupled to activation of adenylate cyclase (\( \rightarrow \) enhanced cAMP production). However, note that most effects are long-term (i.e. involve specific gene transcription) – probably involving cAMP-response elements and cAMP-response element binding protein nuclear receptors.

Actions of glycoprotein hormones:

1. **FSH**
   - **Ovary**
     - i. Stimulation of granulosa proliferation/maturation
     - ii. Induction of LH receptors
     - iii. Up-regulation of FSH receptors
     - iv. Induction of p450-dependent aromatase (androgens \( \rightarrow \) oestrogen)
     - v. Production of inhibin (feedback loop \( \rightarrow \) pituitary)
     - vi. Increased activity of plasminogen activator (related to follicle rupture)
     - vii. Actions enhanced by oestradiol, IGF-1
   - **Testis**
     - i. Sertoli cell production of a number of factors
     - ii. Stimulation of testicular steroidogenesis
     - iii. Stimulation of Sertoli cells to enhance 5\( \alpha \)-reductase and aromatase
     - iv. Acts synergistically with androgens on the seminiferous tubules

2. **LH**
   - **Ovary**
     - i. Maintenance of follicular development
       - 1. Production of androgens in the theca
       - 2. Production of oestradiol and progesterone in the mature granulosa
     - ii. Maintenance of the corpus luteum by inducing production of progesterone and 17\( \alpha \)-hydroxyprogesterone – if pregnancy occurs, HCG from the syncytiotrophoblasts take over this role
   - **Testis**
     - i. Maintenance of testosterone production in Leydig cells
       - 1. Increasing cholesterol ester hydrolase activity
       - 2. Increase in lipoprotein B100 receptors (long-term)

3. **HCG** is secreted during the first trimester from syncytiotrophoblast cells – it is detectably by 8 days and peaks around 14 weeks.
   - a. Prevents decline of the corpus luteum by stimulating production of progesterone and 17\( \alpha \)-hydroxyprogesterone
   - b. Production of progesterone (\( \rightarrow \) testosterone) in the testis to signal male sexual differentiation

### Signal Transduction – Steroid Hormones

The hydrophobic steroid hormones act by changing the rate of transcription of specific genes. Most stimulate transcription of enzymes, though corticosteroids also inhibit the transcription of certain enzymes.

Receptors for steroid hormones are found in the nucleus bound to DNA, or in the cytosol bound 1:1 to HSP90 (heat shock protein). Binding of steroid leads to transformation (formation of homodimers in the nucleus) leading to changes in receptor-DNA binding.

**Steroid hormone receptor structure:**

1. Variable domain
a. Amino-terminal region, specific to each receptor

2. DNA binding region
   a. Highly homologous between different steroid receptors
   b. 8 conserved cysteine residues coordinate 2 zinc atoms (zinc fingers) which bind to the major groove of DNA
   c. Each zinc finger binds to a partially palindromic, defined DNA sequence present in genes affected by the hormone (steroid response element)
      i. SRE usually within 200 base pairs of the transcription start site (upstream or downstream)

3. Steroid binding region
   a. Moderate homology between different steroid receptors

Steroid response element occupation leads to altered transcription – note that activation of more than one response element on a single gene can lead to synergistic effects.

• Prostaglandins and Pregnancy

Arachidonic acid metabolites (eicosanoids):
1. Cyclo-oxygenase pathway → prostanoids (prostaglandin, prostacyclin, thromboxane)
   a. Note COX-1 (physiological) and COX-2 (inducible) – COX-1/COX-2 ratio
2. Lipoxygenase → HETEs, leukotrienes
3. Cytochrome P450 → epoxides

Prostaglandins are a group of bioactive lipids derived from endoperoxidase catabolism of arachidonic acid (liberated from glycerophospholipid stores in cell membranes).
1. The variations in the structure of the cyclopentane ring lead to biological specificity and receptor binding affinity
2. The biological half-life is very short – they tend to act close to the site of production
      i. Effects are mediated by smooth muscle contraction/relaxation
   b. PGJ2 acts via nuclear receptors (peroxisome proliferator-activated receptor)

Actions of prostaglandins
1. Non-reproductive
   a. Vascular
      i. Regulation of vascular/brachiotracheal tone
   b. Neurological
      i. Neuromodulator release
   c. Metabolic
      i. Protection of gastric mucosa, liver and brain
      ii. Control of diuresis
      iii. Control of lipolysis
   d. Anti-viral, anti-tumour, anti-inflammatory effects
2. Reproductive
   a. Ovulation – eicosanoid production increases shortly before ovulation
      i. PGs → release of metalloproteins → proteolysis
      ii. PGF2 → decreased progesterone production
      iii. Possible involvement with fallopian transport
   b. Implantation – glandular endometrium, embryo → PGs
      i. Increased PGE2, PGF2α, PGI2 at site of implantation
      ii. Increased vascular permeability, oedema, immunomodulation
         1. PGE → increased PAF production
      iii. Prostaglandin inhibitors prevent implantation
   c. Parturition
      i. IU administration of PGs or AA → abortion/parturition
1. NSAIDS delay onset of labour and birth
   ii. IU infection \( \rightarrow \) increased PG production \( \rightarrow \) pre-term labour
   iii. Increased PGs in blood/AF before labour, progressively increases with cervical expansion and dilatation
   iv. COX-1 and COX-2 levels increase in gestational membranes prior to the onset of labour, COX-2 levels continue to rise during labour
   v. Myometrial PG receptors change with term – more relaxation receptors in the cervix, more contractile receptors in the fundus

d. **Cervical ripening** – PGs produced by the cervix and membranes
   i. Increased PGE\(_2\) \( \rightarrow \) increased leukocytosis and IL-8
   1. PGE\(_2\) administration (PG gel) induces cervical ripening
   2. NSAIDS suppresses cervical ripening

e. **Fetus**
   i. PGE\(_2\) maintains patency of the ductus arteriosus
   ii. Neonates with a patent ductus may have elevated cardiac PGE\(_2\)
   iii. NSAIDs administered during pregnancy can constrict the ductus

### Composition of Human and Cows Milk; Synthesis and Role of Proteins

<table>
<thead>
<tr>
<th>Human</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca/P</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>70g/L</td>
</tr>
<tr>
<td>Fat</td>
<td>38g/L</td>
</tr>
<tr>
<td>Proteins</td>
<td>9.9g/L</td>
</tr>
<tr>
<td>Caseins</td>
<td>4g/L</td>
</tr>
<tr>
<td>( \alpha )-lactalbumin</td>
<td>2.7g/L</td>
</tr>
<tr>
<td>( \beta )-lactoglobulin</td>
<td>-</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.55g/L</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>1.5g/L</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Present</td>
</tr>
<tr>
<td>IgG</td>
<td>-</td>
</tr>
<tr>
<td>IgA</td>
<td>1.1g/L</td>
</tr>
</tbody>
</table>

General properties:
1. Human milk – more lactose, less protein so slower development and larger brains
2. Lysozyme degrades cell coat of gram negative bacteria (limits infection)
3. Hexosamine polysaccharide allows the growth of certain bacteria – e.g. *lactobacillus bifidus* \( \rightarrow \) lactic acid

Proteins (whey = non-casein proteins):
1. Lactoferrin binds Fe\(^{3+}\) (decreased levels - bacteriostatic mechanism)
2. Cow’s milk allergies due to \( \beta \)-lactoglobulin
3. IgA in human milk is absorbed undigested

Infant formulas are usually made from freeze-dried cow’s milk modified to reduce protein and increase sugar (to resemble human milk more closely). Sometimes alternative proteins (e.g. soy) are added.

Protein constituents of milk are stored in secretory granules that fuse with the plasma membrane to release their contents. In contrast, fat globules bud off the surface of the alveolar cell and are released as discrete packages. \( \text{H}_2\text{O} \) is largely an ultrafiltrate of plasma.

Note that there are far fewer types and levels of casein in human milk – this is reflected in phosphate levels. Caseins are degraded initially by rennin, an enzyme generated in the stomachs of young mammals. This is a specific protease that acts on a single phe-met linkage in \( \kappa \)-casein (\( \rightarrow \) \( \text{para} \kappa \)-casein \( \rightarrow \) glycomannopeptide) leading to collapse of the micellar structure of caseins \( \rightarrow \) precipitation \( \rightarrow \) digestion.

### Milk Lactose and Lipids

**Lactose** is the main carbohydrate in milk. It is unique to milk, and not very sweet (1/5 sucrose). Lactase (\( \beta \)-galactosidase) hydrolyses it to glucose and galactose in the jejunum.

1. **Synthesis** – lactose is synthesised by galactosyl transferase
   a. UDP-galactose + glucose \( \rightarrow \) lactose + UDP
   b. UDP-galactose is made from glucose (intermediate \( \rightarrow \) glycogen) in the plasma – this can be a huge drain on maternal glucose
   c. Induction of synthesis:
i. Galactosyl transferase has low affinity for glucose and high affinity for N-acetyl galactosamine (very little produced in the breast)
   1. Galactosyl transferase is found at the same levels in lactating and non-lactating mammary tissue
ii. Lactation – prolactin induces α-lactalbumin production (⇒ milk)
iii. α-lactalbumin (B protein) forms a complex with galactosyl transferase (A protein) ⇒ lactose synthetase ⇒ increased glucose affinity

2. Utilisation in the infant
   a. Lactose is hydrolysed to galactose and glucose in the villous brush-border of the jejunum by lactase
   b. Glucose and galactose ⇒ glycogen in the liver
      i. Galactose ⇒ glucose goes through the UDP-glucose stage (immediate precursor of glycogen)

3. Lactose intolerance
   a. Indigestion ⇒ flatulence and diarrhoea from anaerobic fermentation and osmotic effects
   b. Adults in races with dairying lifestyle are generally lactose tolerant – lactase remains and can be induced by a high-lactose diet
      i. Lactase is de-induced and disappears in all mammalian species on weaning aside from humans
   c. Lactose is present in many convenience foods

Triglycerides make up almost all the lipid in milk – the fatty acid composition of these depends on the source of triglycerides in maternal circulation. Note that short-chain fatty acids not normally present in maternal sources are synthesised within mammary tissue – these are utilised much more rapidly.

1. Synthesis
   a. 40-50% is synthesised by mammary tissue (C_4-C_{16}) exclusively from blood glucose. Synthesis occurs on the same multi-enzyme complex as adipose, but the chain length is shorter – therefore a different deacylase is involved
   b. 50-60% is synthesised from blood lipid constituents (C_{16+})
   c. Lipoprotein lipase activity in mammary tissue is induced in lactation

2. Digestion in the neonate
   a. Neonatal enzymes
      i. Pancreatic lipase (co-lipase dependent) – low activity at birth due to incomplete pancreatic development. Partial hydrolysis.
      ii. Lingual lipase – produced by the serous glands of the tongue and induced by suckling. Relative specificity for short-chain fatty acids, and hydrolyses triglycerides completely
      iii. Gastric lipase – produced by the gastric mucosa and may be identical to lingual lipase (broad specificity, no co-factors, acid pH optimum).
          1. 60-70% of ingested fats are digested in the stomach
   b. Milk-derived enzymes
      i. Lipoprotein lipase (insignificant)
      ii. Bile-salt stimulated enzyme – high concentration in milk of primates only. Activity (requires bile salts acting as true activators) on water-soluble, micellar and emulsified lipids – complete hydrolysis.
SELECTED ASPECTS OF METABOLIC ENDOCRINOLOGY

Steroid Hormones of the Adrenal Cortex

<table>
<thead>
<tr>
<th>Adrenal cortex</th>
<th>Secretory Product</th>
<th>Regulator of Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zona glomerulosa</td>
<td>Aldosterone</td>
<td>Renin-angiotensin system</td>
</tr>
<tr>
<td>Zona fasciculata</td>
<td>Cortisol (major)</td>
<td>ACTH</td>
</tr>
<tr>
<td></td>
<td>DHEA (minor)</td>
<td>ACTH</td>
</tr>
<tr>
<td>Zona reticularis</td>
<td>DHEAS (major)</td>
<td>ACTH</td>
</tr>
<tr>
<td></td>
<td>Cortisol (minor)</td>
<td>ACTH</td>
</tr>
</tbody>
</table>

DHEA(S) – dehydroepiandrosterone (sulphate)

**Cortisol** secretion follows a circadian rhythm with a diurnal pattern (morning maximum, afternoon minimum). Biosynthesis is regulated by ACTH from the pituitary and has feedback on the hypothalamus, anterior pituitary and adrenal cortex.

1. **Cortisol binding globulin** is a glycoprotein (Mw 51,700) that binds ~70% of cortisol in the circulation.
   a. Binds cortisol and progesterone with equal affinity (one binding site)
   b. 20% is bound by albumin, while 8% is free and is hormonally active.

2. **Hormonal actions**
   b. Electrolytes – increases K⁺ excretion by the kidney
   c. Stress – protects against overreaction
   d. Antiinflammatory – inhibits phospholipase A₂
   e. Immunosuppressive
      i. Impairs the production of interleukin I by macrophages, reducing the proliferative effect of antigens (via IL-1) on lymphocytes
      ii. Reduces the number of T and B cells (→ decreased antibodies)
   f. Haematopoiesis
      i. Leukocytosis (increase in number)
      ii. Lymphopenia (decrease in number)
      iii. Eosinopenia (decrease in number)
   g. Calcium metabolism – inhibits bone formation

3. **Figures**
   a. Production rate – 8-25mg/day (22-69μmol/day)
   b. Serum levels – 200-700nmol/L
   c. Urinary free cortisol – 60-300nmol/24hrs
   d. Urinary 17OHCS – 2-10mg/24hrs (5.4-27.6μmol/24hrs)
   e. Urinary 17 ketosteroids
      i. Men – 7-25mg/24hrs (22-88μmol/24hrs)
      ii. Women – 4-16mg/24hrs (14-53μmol/24hrs)

**Aldosterone** regulates salt balance by reducing Na⁺ secretion and increasing K⁺ elimination at the kidney tubule. This influences plasma volume and blood pressure.

1. **Secretion** is controlled by the renin-angiotensin system
   a. Na⁺ depletion, reduction in renal flow rate → stimulation of renin secretion
   b. Renin enzyme action → plasma globulin → angiotensin I
   c. ACE (lung) → angiotensin II → aldosterone secretion, vascular constriction

2. **Regulatory factors** – serum [Na⁺ /K⁺], blood volume, arterial pressure, renal blood flow

3. **Figures**
   a. Production rate – 50-250μg/day (136-694nmol/day)
   b. Serum levels – 140-560pmol/L
   c. Urinary aldosterone – 5-19μg/24hrs (15-43nmol/24hrs)

**DHEA** is the major steroidal product of the adrenal cortex (99% sulphated before secretion). **Androstenedione** also has no defined effects – however, it is converted to testosterone in peripheral/adipose tissue (→ oestradiol).
1. While no specific action has been identified, DHEAS may be a precursor for sex steroid production in the testis and ovary (minor pathway in females, important placental oestrogen pathway in fetus).

2. Figures
   a. DHEA – 7-31nmol/L
   b. DHEAS
      i. Male – 3-9nmol/L
      ii. Female – 1.5-6.5nmol/L
   c. Androstenedione
      i. Male – 2.5-11.0nmol/L
      ii. Female – 3.0-11.5nmol/L

Adrenal hyperfunction (Cushing’s Syndrome)

1. Aetiology
   a. Pituitary tumour (increased ACTH)
   b. Adrenal tumour (increased cortisol)
   c. Adrenal hyperplasia (CAH), ectopic ACTH secretion by a tumour, exogenous glucocorticoids (may be therapy)

2. Clinical features
   a. Fat metabolism – weight gain, facial mooning, truncal obesity (generalised obesity in children), buffalo hump
   b. Protein metabolism – muscle wasting/weakness, thinning of skin (bruising, striae), heightened colour of the face
   c. Calcium metabolism – osteoporosis
   d. Carbohydrate metabolism – decreased glucose tolerance (20% have diabetes mellitus)
   e. Adrenal androgens – acne, hirsutism, infertility
   f. Hypertension, disordered mental function (irritation, depression, psychosis)

3. Biochemical features
   a. Plasma
      i. Cortisol is increased, no diurnal rhythm. Not suppressed by dexamethasone (potent synthetic glucocorticoid)
      ii. ACTH may be elevated or low
      iii. Androgens increased
   b. Urine
      i. Free cortisol increased
      ii. 17OHC increased
      iii. 17-ketosteroids increased

Primary adrenal insufficiency (Addison’s disease)

1. Aetiology – previously thought to be tuberculosis destruction of the adrenal gland, now ‘idiopathic’ atrophy (autoimmune?)
2. Clinical features arise from deficiencies in aldosterone and cortisol
   a. Weight loss, anorexia, weakness, apathy
   b. Elevated ACTH (and α-MSH) → hyperpigmentation
   c. Sodium depletion, liver glycogen depletion, fasting hypoglycaemia, hypotension
3. Biochemical features
   a. Plasma
      i. Cortisol and aldosterone decreased
      ii. ACTH elevated
   b. Urine
      i. Free cortisol low
      ii. 17-hydroxycorticosteroids low
      iii. 17-ketosteroids low

Secondary adrenal insufficiency (consequence of pituitary ACTH deficiency)

1. Clinical features are similar to Addison’s, but there is no hyperpigmentation as α-MSH levels are depressed. Aldosterone production is less affected.
2. Biochemical features are similar to Addison’s, except ACTH is depressed and aldosterone is normal
• Thyroid Hormones

**Thyroid hormones** are derived from tyrosine, and are essential for the normal regulation of basal metabolic rate, growth and metabolism. They contain iodine (note that this represents the body's only requirement) and are made by modifying proteins with many tyrosine residues. Iodine deficiency in utero can lead to problems with brain development.

The thyroid gland is comprised primarily of epithelial cells, with a lumen filled with colloid tissue (thyroglobulin). Thyroid cells are stimulated by TSH, and C-cells produce calcitonin.

1. Underactive gland → small cells, large lumen
2. Overactive gland → large cells, small lumen

**Thyroid hormone production:**

1. Scavenging of iodine and conversion → thyroid hormones
   a. Na⁺ dependent pump drives I⁻ uptake from extracellular space
      i. Mutation of transporter occurs in some disease
   b. I⁻ is oxidised at the inner membrane → I⁺
   c. Thyroglobulin (Mₚ 300,000) acts as a dimer and can be iodinated
      i. Coupling reaction is catalysed by the same enzyme (MIT+/DIT → T₄/T₃)
   d. Increased TSH → pinocytosis
      i. Fuses with lysosomes → 2° lysosomes → degradation of amino acids, T₃ and T₄

2. T₃ Vs T₄
   a. More T₄ is released into blood
      i. 70% bound to thyroxin binding globulin
      ii. Some bond to thyroxin binding prealbumin
      iii. Only free hormone is able to exert actions
   b. T₃ has a short half-life, but is more active
   c. T₄ is deiodinated → T₃ or rT₃ in peripheral tissues
      i. One enzyme in the brain and pituitary, another in the rest of the body

**Thyroid hormone action:**

1. Thyroid hormones act in a similar way to steroids, binding to cytoplasmic receptors → nucleus → affecting transcription
2. GH-IGF axis is affected by thyroid hormones
   a. Increased Na⁺/K⁺ATPase activity – important for increased uptake of nutrients (20% of BMR maintains transmembrane gradient)
   b. Growth-promoting – too much → catabolism/degradation (N excretion)
   c. Breakdown of glycogen stores
   d. Increased plasma cholesterol (less utilisation)

**Disorders** of thyroid function:

1. **Deficiency** – hypothyroidism
   a. Hypothyroid → tiredness, lethargy, sensitivity to cold
      i. Myxoedema (peripheral deposition of mucopolysaccharides)
      ii. Decreased CO₂, decreased heart rate
      iii. Slowed mental/motor function
      1. Slowed relaxation phase of tendon reflexes
   b. Early development – growth and mental retardation (cretinism)
   c. Common in older women
   d. Enlargement of thyroid gland, infiltration by immune cells
   e. May be due to iodine deficiency, circulating antibodies for thyroid hormones, autoimmune disease (e.g. Hashimoto's thyroiditis – lymphocyte infiltration)
      i. Treatment – thyroid replacement therapy
2. **Excess** – hyperthyroidism
   a. Hyperthyroid → agitation, nervousness, irritability, heat sensitivity
i. Greater appetite, but no weight gain – protein catabolism
ii. Tachycardia, arrhythmia
iii. Exophthalmos (mucopolysaccharide deposition in the orbits)
iv. Growth of the thyroid gland due to I₂ deficiency / excess TSH

b. Grave’s disease
i. Autoimmune production of an antibody that mimics TSH
ii. Increased T₃/T₄ with no feedback/regulation

Thyroid hormones cannot be measured directly as they tend to be bound to other molecules (note that changes in the binding proteins may lead to an apparent thyroid hormone abnormality). Instead, an infusion of radiolabelled T₄ is given – this is allowed to equilibrate and the free radioactive T₄ examined (free *T₄ is inversely proportional, to endogenous T₄).

Plasma TSH and thyroxine have an inverse relationship (negative feedback at the level of the hypothalamus and pituitary). An infusion of the hypothalamic hormone TRH may be helpful:
1. Normal – rapid response
2. Primary hypothyroidism – rapid response, elevated output (no feedback)
3. Secondary hypothyroidism:
   a. Hypothalamic hypothyroidism – rapid response, lowered output
   b. Pituitary hypothyroidism – slow/delayed response, low output

Feedback/regulation is complex.

- Adrenal Medulla

The adrenal medulla is the inner part of the adrenal glands and acts as an endocrine tissue as well as an important part of the sympathetic nervous system (along with cholinergic preganglions and adrenergic postganglions).

1. Chromaffin cells comprise ~80% of cells in the adrenal medulla
   a. Phenylalanine → tyrosine (via phenylalanine hydroxylase) to:
      i. Dopa → dopamine (intermediate)
         1. Adrenaline (80%) → fight/flight response
         2. Noradrenaline (20%)
   b. Chromaffin granules have an anti-port
      i. Large dense vesicles 0.05-0.2μ in diameter
      ii. Large ATP content (co-released with catecholamines)
         1. Decreased osmotic pressure via complex with adrenaline → more stable vesicles
         2. ATP-gated ion channels have effects on neighbouring cells
      iii. Store other proteins including encephalins (opioid-like peptides)

2. Release:
   a. ACh from presynaptic neuron binds to receptor, mediating Ca²⁺ release
   b. Increased Ca²⁺ → fusion, then exocytosis of granule contents
   c. Chromaffin granule is recycled to the Golgi apparatus for refilling

3. Regulation:
   a. Tyrosine hydroxylase is rate-limiting
      i. Nerve stimulation → mRNA → increased tyrosine hydroxylase
      ii. Increased cAMP → kinases → phosphorylation of tyrosine hydroxylase → activity
   b. Catecholamines → feedback inhibition
   c. PNMT (methylates noradrenaline to adrenaline) is only found in adrenaline-secreting cells (brainstem, medulla)
      i. Up-regulated by glucocorticoids (e.g. from adrenal cortex)

4. Actions – systemic and local release → α and β receptors that determine effects
   a. General effects – peripheral blood vessel constriction, increased heart rate
   b. α₁-adrenoceptors
      i. Glycogenolysis
      ii. Smooth muscle contraction (blood vessels, urogenital tract)
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c. $\alpha_2$-adrenoceptors
   i. Smooth muscle contraction (some vascular beds)
   ii. Smooth muscle relaxation (GI tract)

d. $\beta_1$-adrenoceptors
   i. Lipolysis
   ii. Myocardial contraction (rate and force)

e. $\beta_2$-adrenoceptors
   i. Hepatic gluconeogenesis
   ii. Hepatic and muscle glycogenolysis
   iii. Increased insulin, glucagon, renin
   iv. Smooth muscle relaxation (bronchi, blood vessels, urogenital tract, gastrointestinal tract)

5. Cessation of actions:
   a. Reuptake via Na$^+$-dependent transporters
      i. Some antidepressants, cocaine – reuptake blockers
      ii. Amphetamines – block granule transporter
   b. Enzymes (work together)
      i. Catechol-O-methyl transferase – cytoplasmic
      ii. Monoamine oxidases
         1. Antidepressants inhibit $\to$ loss of amine moiety

Tyrosine is not an essential amino acid, but phenylalanine is (hydroxlated in the liver to form tyrosine). Phenylalanine hydroxylase is deficient in infants with phenylketonuria.
1. High concentrations of phenylalanine $\to$ metabolised to neurotoxic compounds
2. Tyrosine uptake inhibited $\to$ catecholamine insufficiency
3. Controlled by limiting dietary uptake of phenylalanine (e.g. artificial sweetener)

• Calcium Metabolism I

Total body calcium is approximately 1300g – of this, 99% is found in bone as hydroxyapatite, 0.3% in muscle, 0.7% in extracellular fluid and 0.05% in plasma.
1. Plasma – 2.3-2.6mM
   a. 35-50% bound to albumin
   b. 50-60% ionised (1.4mM)
      i. 4% variation – compare Na$^+$ (9%) and fasting glucose (35%)
   c. 5-10% in organic complexes (e.g. citrate, oxalate, tartrate)
2. Bone (as hydroxyapatite, Ca$\text{_{10}(PO_4)_6(OH)_2}$)
   a. Overall turnover 6-25% a year (0.25-2g a day)
      i. Long bones exchange 5-11% annually
      ii. Ribs exchange 14-44% annually
      iii. Skull has very low turnover
3. Daily Ca$^{2+}$ fluxes
   a. Dietary intake – 0.5-1.0g
   b. Secreted into the gut – 0.3g
   c. Absorbed from the gut (net) – 0.3g
   d. Turnover to bone – 0.25-2.0g
   e. Urinary excretion – 0.1-0.4g

Parathyroid hormone is derived from a pre-pro-protein by removal of a 31+6 amino acid (pre/leader and pro) sequence then secreted as an 84 amino acid peptide into the circulation.
1. Two structured domains, cleaved in the liver and kidney
   a. Amino terminal (1-30) – biologically active, short half life
   b. Carboxy terminal (50-84) – conserved, but not required for biological activity
2. Actions of parathyroid hormone:
   a. Renal effects:
      i. Instant – increase in urinary cAMP (renal origin)
      ii. 8min-2hr – 10x increase in PO$_4$ excretion (lowered tubular resorption)
         1. Psuedo-equilibrium – reciprocal relationship of Ca$^{2+}$/PO$_4$}^{-3}
iii. 3-6hr – activation of $\alpha$-hydroxylase (25-OH vit D $\rightarrow$ 1:25 DHCC)
iv. 12hr-2days – decrease in Ca$^{2+}$ excretion (increase in tubular reabsorption 99$\rightarrow$100%)
b. Effects on bone:
   i. 15-30min – short-lived Ca$^{2+}$ uptake
   ii. 6hrs+ - hypercalcaemia due to increased osteoclast activity
   iii. Days-weeks – low levels, increased bone growth
   iv. Mechanism – osteoblast responds to parathyroid hormone
      1. Low levels $\rightarrow$ enhanced osteoblastic activity (bone growth)
      2. High levels $\rightarrow$ massive bone resorption
         a. $1^\circ$ hyperparathyroidism – osteitis fibrosa cystica
3. Control of ionised plasma Ca$^{2+}$ – note that only ionised calcium is effective
   a. Simple feedback loop – low Ca$^{2+}$ $\rightarrow$ increased parathyroid hormone
   b. Increased PTH $\rightarrow$ increased Ca$^{2+}$:
      i. Increased renal absorption
      ii. Decreased PO$_4$$^{3-}$ $\rightarrow$ upset Ca/P pseudo-equilibrium
      iii. Increased resorption of calcium from bone
      iv. Increased renal 1a-hydroxylase $\rightarrow$ increased 1:25 DHCC $\rightarrow$
         increased absorption from the gut
c. Rise in Ca$^{2+}$ is sensed by the BoPCaR protein which inhibits PTH release

Hyperparathyroidism
1. Primary – tumour (adenoma) of the parathyroid gland. Usually Ca$^{2+}$ is elevated,
   leading to dehydration.
   a. Osteitis fibrosa cystica (advanced disease) $\rightarrow$ pain, demineralisation, cysts
   b. Treatment by removal of one or more glands
2. Secondary – chronic renal disease, pseudohypoparathyroidism
3. Hypercalcaemia of malignancy – generation of PTH-related peptide in some tumours
   (e.g. lung carcinoma, some breast tumours)

Symptoms of hypercalcaemia include (may be asymptomatic):
1. General malaise, fatigue, psychoneurosis, weight loss, pruritus
2. Renal colic, polyuria, polydipsia
3. Constipation, epigastric pain, anorexia, nausea, vomiting
4. Lethargy, muscular weakness, confusion, psychosis, stupor, coma

Hypoparathyroidism
1. Idiopathic – defective PTH, or defect in secretion
   a. Low Ca$^{2+}$, episodes of tetany
   b. Treatment with vitamin D
2. Pseudohypoparathyroidism – defect in the PTH receptor, receptor coupling to the Gs
   protein, or adenylate cyclase in kidney or bone. Usually a mild disorder characterised
   by low intelligence and a short 3$^{\circ}$ metatarsal

Parathyroid related protein (141 amino acids, $M_w$=15,000) is responsible for
hypercalcaemia in some malignancies. It is thought to have arisen from a duplication of the
PTH gene, as it has a striking homology at the amino-terminus (until amino acid 34):
1. Effects:
   a. Activation of adenylate cyclase in the kidney and osteoblasts
   b. Increased bone resorption
   c. Changes the renal handling of Ca/P in the same way as PTH
2. Physiological roles:
   a. Found in skin in spinous keratinocytes and in the inner root of the hair follicle,
      but it’s exact role is not clear
   b. May act as a calcitrophic agent in the fetus (present in the fetal parathyroid)
   c. May act to maintain fetal Ca$^{2+}$ levels by activating a placental Ca pump
   d. High levels of PTHrP in human milk may have a role stimulating Ca$^{2+}$
      transport to milk
Vitamin D (cholecalciferol, D₃) is not significant in the diet, but is synthesised from 8-dehydrocholesterol in the skin following exposure to sunlight. It is subsequently hydroxlated in the liver to 25-hydroxycholecalciferol (25-OH-D₃), which is metabolised in the kidney to active forms. 1α, 25-(OH)₂-D₃ (calcitriol, 1:25 DHCC) is a potent antirachitic.

1. Renal metabolism of 25-OH cholecalciferol
   a. Only a small amount is converted to 1:25 DHCC – most is converted to 24:25 DHCC and 25:26 DHCC, which are both inactive
   b. 1-hydroxylase (as opposed to 24+26) is
      i. Increased by decreased plasma Ca⁺² and increased PTH
      ii. Decreased by increased plasma Ca⁺² and increased 1:25 DHCC

2. Biological actions of 1:25 DHCC
   a. Mediated via the nucleus
      i. Binds to a receptor in all cells (high in intestinal mucosa and bone)
      ii. Calcitriol receptor → transcription of specific genes
      iii. Inhibited by actinomycin D (inhibits DNA-directed mRNA synthesis)
      iv. Calbindins – induced in intestine and bone with Ca⁺²-dependent ATPase and alkaline phosphatase
      v. Osteocalcin – induced in osteoblasts
   b. Not mediated via the nucleus
      i. May induce enhanced generation of ceramide (from membrane sphingomyelin) as a second messenger
      ii. Short-term stimulation of Ca⁺² and PO₄⁻² uptake by enterocytes
      iii. Less specific effects, unknown mechanism
         1. Trophic growth of intestinal villi
         2. Calcification of cartilage
         3. Immunomodulation activities

3. Calbindins
   a. Two calcium binding proteins are induced by calcitriol (function unknown)
      i. Mᵣ 28,000 – widely distributed
      ii. Mᵣ 9,000 – intestine, placenta, kidney
      iii. Only the kidney has both calbindins
   b. Calbindins are part of the calmodulin family of proteins – these have a number of calcium-binding domains linked by short peptide sequences
      i. Calmodulin – 4 binding domains
      ii. Calbindin₂₈K – 2 binding domains
      iii. Calbindin₂₆K – 6 binding domains
   c. Calcitriol also induces alkaline phosphatase, osteocalcin and Ca⁺²-dependent ATPase, which also bind calcium

4. Rickets (juvenile, before epiphyseal fusion) and osteomalacia
   a. Inadequate formation of vitamin D₃ due to insufficient UV exposure – normally cured by adequate exposure to sunlight
   b. Decalcification of bone matrix with thick seams of uncalcified osteoid, leading to bone softening with deformities and fractures
   c. Mild hypocalcaemia → ²° hyperparathyroidism → hypophosphataemia (via enhanced phosphate excretion)
      i. Elevated blood alkaline phosphatase
      ii. Decreased intestinal absorption, and decreased uptake and release of Ca⁺² from bone
   d. Treatment with vitamin D and phosphate → rapid improvement except in vitamin D resistant rickets

5. Vitamin D resistant rickets
   a. Metabolism of vitamin D or some aspect of its action is deficient/absent
   b. Example – nuclear receptor for 1:25 DHCC, 25-hydroxylase, 1-hydroxylase

The structure of calcitonin (small peptide) varies between species, although the disulphide bond and C-terminal prolinamide residue are conserved.

1. Actions of calcitonin
   a. Decreased plasma ionised calcium by
i. Increasing phosphate and calcium excretion from the kidney (Ca opposes PTH, phosphate reinforces PTH)

ii. Inhibiting resorption of bone matrix and mineral (→ decreased urinary excretion of hydroxyproline)

b. Actions on bone over-ride those of PTH in the short-term. Note that this is in conflict with the relative lack of significance of calcitonin in the adult.

2. Secretion of calcitonin

a. Calcitonin is secreted by C-cells in the thyroid

b. Inhibited by low levels of Ca\(^{2+}\), stimulated by high levels and/or pentagastrin

3. Role in the adult

a. Normally undetectable levels in the adult

b. Removal of thyroid gland does not affect calcium balance

c. C-cell carcinoma is not accompanied by any significant changes despite increased calcitonin

d. May have a role in protecting babies from hypercalcaemia (from high levels of calcium in milk)

e. Paget’s disease (benign osteoclast tumour → increased osteoblast activity) have been treated with calcitonin in the past

• Glucose Homeostasis I

Normal regulation of glucose homeostasis:

1. Digestion, absorption, interconversion

a. Complex carbohydrates → component sugars (via amylase/disaccharidases) → liver (via portal blood stream)

b. Major products – glucose (80%), fructose, galactose

c. Fructose/galactose converted to glucose via pathways in the gut wall and liver – glucose is hence the final common transport pathway

2. Transport across the cell membrane

a. Facilitated diffusion is mediated by specific carriers (glucose transporters)

b. Glucose transporters are a family of related proteins

i. GLUT 2 – liver and islet \(\beta\)-cell

ii. GLUT 4 (insulin sensitive) – skeletal muscle and adipose tissue

3. Pattern of carbohydrate utilisation

a. Dietary glucose passes to the systemic circulation where it is taken up by skeletal muscle → phosphorylation → incorporation into glycogen

i. \(\text{Glucose}_{EC} \rightarrow \text{Glucose}_{IC}\)

ii. \(\text{Glucose} + \text{PO}_4^{-3} \rightarrow \text{glucose-6-phosphate}\)

iii. \(\text{Glucose-6-phosphate} \rightarrow \text{glucose-1-phosphate}\)

iv. \(\text{Glucose-1-phosphate} + \text{UTP} \rightarrow \text{UDP-glucose} + \text{PP}_i\)

v. \(\text{UPP-glucose} + \text{glycogen}_{m} \rightarrow \text{glycogen}_{m+1} + \text{UDP}\)

b. Glycogen synthase is regulated by reversible phosphorylation / dephosphorylation induced by insulin, glucagon, and adrenalin

c. Muscle lacks glucose-6-phosphatase and is unable to release glucose back into the circulation – this is important in the development of NIDDM

d. Energy release from skeletal muscle is via 3-carbon containing molecules (e.g. lactate and alanine). These are used as substrates in gluconeogenesis and glycogen synthesis, or fatty acid synthesis and VLDL production

e. Cori cycle: \(\text{glucose}_{muscle} \rightarrow \text{lactate}_{muscle} \rightarrow \text{lactate}_{liver} \rightarrow \text{glucose}_{liver}\)

Insulin is a protein with two subunits – hence it cannot be taken by mouth and must be administered subcutaneously. Note that intramuscular/intravenous insulin (or glucagon) may be used in acute hyperglycaemic coma – ‘brittle diabetes’

1. Secretion of insulin

a. The adult pancreas contains around 150,000-400,000 islets (150-400μm in diameter, ~5000 cells each) consisting of:

i. 80-90% \(\beta\)-cells, producing insulin and amylin

1. Type II diabetes is associated with excessive amyloid
Autoimmune destruction of β-cells is thought to be the mechanism behind type I diabetes – possibly a T-cell mediated event (β-cell ABs not part of the primary process).

10-20% α-cells, producing glucagon

Insulin is released primarily in response to glucose (debated), although some nutrients and drugs (used therapeutically) may also elicit a response.

Mechanism is complex, but involves mobilisation of intracellular calcium and is sensitive to intracellular glucose metabolism

Candidate pathways include the ATP-sensitive β-cell K⁺ channel, and the glucose-sensitive intracellular messenger cyclic ADP-ribose

Cephalic phase – mediated via brain and ANS, important in ensuring normal tissue sensitivity

Insulin stimulates glucose clearance in skeletal muscle and inhibits glucose output from the liver → decreased blood glucose

Insulin binds to the insulin receptor on the cell surface, which activates tyrosine kinase. Structural abnormalities of the receptor are rare.

Glucose uptake in muscle is mediated by GLUT-4 (insulin sensitive)

In skeletal muscle, insulin is thought to stimulate insertion of GLUT-4 molecules into the membrane, as well as stimulating glycogen synthase

Glucose uptake into the liver is mediated by GLUT-2 and is not stimulated directly by insulin – it is driven by the glucose gradient across the membrane

Hepatic glucose production increases in response to hypoglycaemia under the influence of glucagon and decreased insulin. Intrinsic adrenergic agonists (adrenaline, noradrenaline) have a role in severe hypoglycaemia

Mechanism for acute response is hepatic glycogenolysis

Depleted hepatic glycogen stores → easy onset of hypoglycaemia with prolonged/more severe effects

Longer periods of hypoglycaemia → hepatic gluconeogenesis

Glucagon stimulates acute hepatic glucose production by stimulation of glycogenolysis/gluconeogenesis and inhibition of glycogen synthesis, and indirectly by counteracting the effects of insulin.

Cell surface receptor and receptor-mediated stimulation of cAMP → cAMP-dependent protein kinase → reversibly phosphorylation of phosphorylase (rate-limiting enzyme in glycogenolysis)

Intrinsic adrenergic agonists (A, NA) have a role in severe hypoglycaemia

Protect against hypoglycaemia of exercise by decreasing insulin sensitivity of skeletal muscle

Protect against hypoglycaemia of starvation by stimulating lipolysis and supply of FFAs to tissues (preferential use)

Mechanisms via cAMP (β-adrenergic) or activation of intracellular Ca²⁺ and Ca²⁺-dependent protein kinases (α-adrenergic)

Pathology:

IDDM – lack of insulin → lack of stimulation → impaired clearance

NIDDM – insulin resistance. 3 effects – liver, muscle, pancreas – look this up
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a. Primary (idiopathic)
   i. Insulin-dependent (type 1) diabetes mellitus
   ii. Non-insulin dependent (type 2) diabetes mellitus
      1. Impaired glucose tolerance – associated with large vessel disease, but not diabetes-specific microvascular problems. 25% \( \Rightarrow \) NIDDM
      2. Gestational diabetes mellitus – increased risk of fetal (macrosomia) and maternal (preeclampsia) complications
      3. Maturity onset diabetes of the young – may be associated with molecular abnormalities of the islet form of glucokinase

b. Secondary (1%)
   i. Pancreatic disease (e.g. pancreatitis, haemochromatosis)
   ii. Hormonal abnormalities
   iii. Drug or chemical-induced diabetes (especially thiazide/loop diuretics)
   iv. Insulin receptor abnormalities
   v. Genetic syndromes (e.g. Prader-Willi syndrome)
   vi. Other causes

c. Note: Insulin-dependent diabetes mellitus frequently lead to poor peripheral circulation \( \Rightarrow \) ischaemia, necrosis, and gangrene. Diabetes is also associated with:
   a. Macrovascular – other diseases including IGT (arterial obstruction)
   b. Microvascular – specific to diabetes, thought to be caused by exposure of tissues to excess blood glucose concentrations. The primary lesion probably occurs via the mechanism of non-enzymatic glycosylation (covalent attachment of moieties)
      i. Diabetic nephropathy – proteinuria, nephrotic syndrome, renal failure
      ii. Diabetic retinopathy – proliferative or exudative
      iii. Diabetic neuropathy – ‘stocking’ distribution of progressive tingling, pain, numbness, loss of temperature/vibration sensation, ankle jerks
       d. Poor outcome in CV complaints – diabetic cardiomyopathy (diffuse lesion)

d. Non-vascular:
   i. Increased incidence of infections – cellulitis, UTI, balanitis, vaginitis
   ii. Diabetes-associated skin lesions
   iii. Eye disease – cataracts, glaucoma, intermittent blurring of vision

2. Complications of diabetes mellitus frequently lead to poor peripheral circulation \( \Rightarrow \) ischaemia, necrosis, and gangrene. They include:
   a. Macrovascular – other diseases including IGT (arterial obstruction)
   b. Microvascular – specific to diabetes, thought to be caused by exposure of tissues to excess blood glucose concentrations. The primary lesion probably occurs via the mechanism of non-enzymatic glycosylation (covalent attachment of moieties)
      i. Diabetic nephropathy – proteinuria, nephrotic syndrome, renal failure
      ii. Diabetic retinopathy – proliferative or exudative
      iii. Diabetic neuropathy – ‘stocking’ distribution of progressive tingling, pain, numbness, loss of temperature/vibration sensation, ankle jerks

   c. Poor outcome in CV complaints – diabetic cardiomyopathy (diffuse lesion)

3. Diagnosis is based on the demonstration of pathologically elevated blood glucose concentrations as defined by the National Diabetes Data Group and the WHO:
   a. In pregnancy a more stringent definition is applied as a milder elevation of blood glucose can still prove harmful to the fetus
   b. Note distinction between patients with IGT with factors which temporarily worsen glucose levels to diabetic levels (infection, thiazide diuretics)
      i. Elevated fasting or post-prandial blood glucose levels on 2+ times
      ii. Unclear diagnosis (~10% of new cases) – oral glucose tolerance test
         1. Performed in the morning (~9am) as there is a diurnal variation in insulin sensitivity
         2. Administration of an oral glucose/polycose load (75g)
            a. \( \{7/8<x\} \Rightarrow \{11<x\} \) blood glucose – diabetes mellitus
            b. \( \{6<x<8\} \Rightarrow \{8<x<11\} \) – IGT
            c. \( \{x<6\} \Rightarrow \{x<8\} \) – normal
      iii. Note that urine glucose is not useful (different renal thresholds)
   iv. Diagnosis may be obvious, but frequently is obscure (type 2 – silent)
      i. Risk factor approach – includes all health workers
         1. Obesity, older age, positive family history, racial
         2. Random finding of glucosuria
         3. Persons presenting with a known complication
         4. Women with a history of large babies (>4.5Kg)

Insulin-dependent diabetes mellitus
1. Incidence – peaks in Caucasians at age 5 and early adolescence (10% adult-onset)
2. Primary lesion is T-cell mediated destruction of islet β-cells with absolute insulin deficiency – failure to replace insulin leads to diabetic ketoacidosis
3. Mechanism – likely to be mixed autoimmune/genetic
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a. Round cell infiltrates of the islets, anti-islet and primary anti-insulin antibodies
b. Some populations with abnormal residues on the MHC molecule
c. Some interaction between environmental factors and genetic predisposition

4. Insulin therapy – in an adult with no complications, 21 to 24 units per day (2/3 of this given before breakfast, then the remainder later in the day)
   a. Self-blood glucose monitoring to assess control on a daily basis
   b. Measurement of glycosylated protein to monitor control over longer periods
   c. Limiting factor is hypoglycaemia – regular occurrence with insulin therapy

5. Diabetic ketoacidosis is the end result of a complete lack of insulin. This may be the first presentation of IDDM, or in a patient in whom insulin is not working (stopped treatment, concurrent infection inducing progressive resistance)
   a. Inability to utilise glucose → forced oxidation of triglycerides
   c. Mechanism:
      i. Osmotic diuresis – hyperglycaemia → osmotic diuresis → loss of water and electrolytes in the urine
      ii. Ketone body generation - β-hydroxybutyrate and acetoacetate cannot be cleared efficiency, accumulate and add to diuresis
      iii. Metabolic acidsis with initial respiratory compensation
         1. Acidosis with low [bicarbonate] and low pCO₂
         2. Characteristic tachypnoea (Kussmaul breathing)
         3. H⁺ is buffered by extracellular (bicarbonate, protein) and intracellular systems (K⁺, Mg²⁺) → loss of these ions in urine
         4. Progressive dehydration – low blood volume, prerenal failure
            a. Progressive failure of respiratory compensation → secondary respiratory acidosis → unconsciousness
   d. Treatment is based on intravenous fluid and electrolyte replacement, with iv insulin and careful use of bicarbonate in the event of severe acidosis
      i. Elevated intracellular ions on presentation may mask severe whole body deficits. When renal output resumes, there can be rapid shifts of K⁺ back into the intracellular compartment → hypokalaemia

Non-insulin dependent diabetes mellitus – 90% of diabetics in NZ

1. Incidence – later in life, progressive rise in prevalence through the 7th decade. Strong genetic familial tendency (although intrauterine environment may be more important).
   a. Increased prevalence in transplanted third world populations, high prevalence in Maori and Pacific Islanders (NZ), Nauru and Pima Indians
   b. Clustering with obesity, essential hypertension, atherosclerosis
   c. Most NIDDM patient pass through an initial period of IGT (25% → NIDDM)
   d. Rate of development of chronic complications similar to that in IDDM
   e. Initial IGT → increased insulin output with insulin resistance → progressive failure of insulin secretion (requirement for insulin therapy)

2. Mechanisms – not well understood, but characterised by:
   a. Insulin resistance (skeletal muscle, liver)
      i. Not due to abnormal insulin, receptors or glucose transporters in peripheral tissues
   b. Abnormal insulin secretion – progressive loss of first phase of insulin secretion, selective ‘glucose blindness’ of islets
      i. MODY may be related to a defective glucokinase
   c. Islet amyloid – likely role in disruption of islet architecture and late deterioration of insulin secretion

3. Treatment
   a. Diet – reduced total caloric intake, increased intake of complex CHO
   b. Exercise if tolerable
   c. Oral hypoglycaemic agents:
      i. Sulphonylureas
      ii. Biguanides (metformin)
      iii. Glitazones
   d. Failure of hypoglycaemic therapy – switch to insulin therapy
4. **Complications** – chronic vascular complications similar to IDDM
   a. Non-ketotic hyperosmolar coma – similar to IDDM, but no acidosis due to residual insulin production (allows metabolism of ketone bodies)
   b. Lactic acidosis – complication of metformin therapy in association with alcoholism, liver or renal disease

**Hypoglycaemia** is clinically important, as it is a common and major cause of mortality. Blood glucose concentration of <2.0mmol/L leads to impairment of brain function, <1.5mmol/L leads to rapid onset of lethal effects on the brain. It has very rapid effects (similar to hypoxia).

1. With chronic starvation or heavy prolonged exercise glucose can drop below 1.0mmol/L without hypoglycaemic symptoms (due to FFA mobilisation/metabolism)
2. **Symptoms:**
   a. Adrenergic (predominant) – pallor, sweating, tremor, palpitations, dry mouth
   b. Neuroglycopaenic – lack of concentration, slow movements, slurred speech, double vision, personality changes, transient stroke, fits, coma
3. **Sequelaes** – usually no obvious permanent effects. Prognosis for recovery declines with the length of coma – outcome poor if associated with hypoxaemia/hypotension
4. **Classification**
   a. Fasting hypoglycaemia – always indicates an identifiable underlying disease
      i. Excess insulin-like activity – insulin overdose, insulinomas, factitious hypoglycaemia, other substances that stimulate the insulin receptor, sulphonylurea overdose
      ii. Hepatic dysfunction – infection, ethanol toxicity, malaria, parenchymal liver disease, drugs, hormone deficiency
   b. Reactive hypoglycaemia – related to operations which produce excessive gastric emptying, with elevated stimulation to insulin secretion and insulin-induced hypoglycaemia
      i. May occur early in NIDDM development 3-5 hours after a meal
      ii. Occasionally occurs in otherwise normal patients (debatable)
5. **Treatment** – glucose by mouth if tolerated, intravenous if not
   a. 1mg glucagon (intramuscular or intravenous) if glucose not available. This has only a short duration (30min) and is not effective with abnormal liver function or depleted hepatic glycogen stores

Exam format:

6 questions
Essays – some choice
Short notes (7 ½ minutes per answer) – lots of choice, 4 topics
Interpretation – either/or

Q1 – Short notes 4/8 (pairs of choices)
Q2 – Either essay or short notes
Q3 – Interpretation (either/or)
Q4 – Either two part question (small essays) or short notes 4/5
Q5 – Either essay or short notes 4/6
Q6 – Either essay or essay