Biochemistry of Hormones
A case oriented approach

1. **Lecture 1**: Introduction to the Biochemistry of hormones and their mechanism of actions.
   - **Thursday 16/2**

2. **Lecture 2**: Introduction to the Biochemistry of hormones and their mechanism of actions.
   - **Sunday 19/2**

3. **Lecture 3**: Biochemistry and disorders of hormones of the hypothalamus and pituitary gland (hypothalamus-pituitary axis).
   - **Thursday 23/2**

4. **Lecture 4**: Biochemistry and disorders of hormones of the hypothalamus and pituitary gland (hypothalamus-pituitary axis).
   - **Sunday 26/2**

5. **Lecture 5**: Biochemistry and disorders of hormones of the thyroid and parathyroid gland
   - **Sunday 4/3**

6. **Lecture 6**: Biochemistry and disorders of hormones of the pancreas
   - **Thursday 8/3**

7. **Lecture 7**: Biochemistry and disorders of hormones of the adrenal gland
   - **Sunday 11/3**

8. **Lecture 8**: Biochemistry and disorders of hormones of the adipose tissues, heart and kidney
   - **Sunday 18/3**

Aim and objective of the above eight lectures is to understand:

1. The functions of hormones and the mechanisms involved in the regulation of their secretion.
2. The types of receptor-hormone interactions and the specific effect that each type can be produced in the cell.
3. the hypothalamus-pituitary axis.
4. the biochemistry and disorders of hormones secretion from different glands.
5. and to learn the major causes of endocrine disorders by discussing clinical cases.

References:

1. "Biochemistry" by Lubert Stryer *(textbook)*
2. "Textbook of Biochemistry with Clinical Correlations" by T.M.Devlin *(additional reading)*
3. "Lippincott's Illustrated Reviews in Biochemistry" by P.C.Champe, R.A.Harvey and D.R.Ferrier *(additional reading)*
5. "Clinical Laboratory Science Review" By Robert R. Harr *(additional reading)*
INTRODUCTION
TO THE
BIOCHEMISTRY
OF HORMONES
AND THEIR
MECHANISM
OF ACTIONS

2012

Prof. Dr. Hedef Dhafir El-Yassin
The endocrine system is one of the two coordinating and integrating systems of the body. It acts through chemical messengers - hormones – carried in the circulation.

Two systems control all physiologic processes:

- **The nervous system** exerts point-to-point control through nerves, similar to sending messages by conventional telephone. Nervous control is electrical in nature and fast.

- **The endocrine system** broadcasts its hormonal messages to essentially all cells by secretion into blood and extracellular fluid. Like a radio broadcast, it requires a receiver to get the message - in the case of endocrine messages, cells must bear a receptor for the hormone being broadcast in order to respond.

Endocrinology is the study of hormones, their receptors and the intracellular signaling pathways they invoke. Distinct endocrine organs are scattered throughout the body. These are organs that are largely or at least famously devoted to secretion of hormones. In addition to the classical endocrine organs, many other cells in the body secrete hormones. Myocytes in the atria of the heart and scattered epithelial cells in the stomach and small intestine are examples of what is sometimes called the "diffuse" endocrine system. If the term hormone is defined broadly to include all secreted chemical messengers, then virtually all cells can be considered part of the endocrine system.

- *All pathophysiologic events are influenced by the endocrine milieu:* There are no cell types, organs or processes that are not influenced - often profoundly - by hormone signaling.

- *All "large" physiologic effects are mediated by multiple hormones acting in concert:* Normal growth from birth to adulthood, for example, is surely dependent on growth hormone, but thyroid hormones, insulin-like growth
factor-1, glucocorticoids and several other hormones are also critically involved in this process.

- *There are many hormones known and little doubt that others remain to be discovered.*

**Hormones, Receptors and Target Cells**

1. Classes of Hormones

Knowing the basic structure of a hormone gives a considerable knowledge about its receptor and mechanism of action. Like all molecules, hormones are synthesized, exist in a biologically active state for a time, and then degrade or are destroyed. Having an appreciation for the "half-life" and mode of elimination of a hormone aids in understanding its role in physiology and is critical when using hormones as drugs.

**Most commonly, hormones are categorized into four structural groups,** with members of each group having many properties in common:

- Peptides and proteins
- Amino acid derivatives
- Steroids
- Fatty acid derivatives - Eicosanoids

1. **Peptides and Proteins**

Peptide and protein hormones are products of translation. They vary considerably in size and post-translational modifications, ranging from peptides as short as three amino acids to large, multisubunit glycoproteins. Peptide hormones are synthesized in endoplasmic reticulum, transferred to the Golgi and packaged into secretory vesicles for export.
They can be secreted by one of two pathways:

- **Regulated secretion:** The cell stores hormone in secretory granules and releases them in "bursts" when stimulated. This is the most commonly used pathway and allows cells to secrete a large amount of hormone over a short period of time.

- **Constitutive secretion:** The cell does not store hormone, but secretes it from secretory vesicles as it is synthesized.

Most peptide hormones circulate unbound to other proteins, but exceptions exist; for example, insulin-like growth factor-1 binds to one of several binding proteins. In general, the half-life of circulating peptide hormones is only a few minutes.

Several important peptide hormones are secreted from the pituitary gland.

**The anterior pituitary secretes:**

- Luteinizing hormone and follicle stimulating hormone, which act on the gonads.
- prolactin, which acts on the mammary gland,
- adrenocortiotropic hormone (ACTH), which acts on the adrenal cortex to regulate the secretion of glucocorticoids, and
- growth hormone, which acts on bone, muscle and liver.

**The posterior pituitary gland secretes:**

- antidiuretic hormone, also called vasopressin, and
- oxytocin.

Peptide hormones are produced by many different organs and tissues, however, including:

- **the heart** (atrial-natriuretic peptide (ANP) or atrial natriuretic factor (ANF))
- **pancreas** (insulin and somatostatin),
- the gastrointestinal tract cholecystokinin, gastrin, and
- **fat stores** (leptin)
Many neurotransmitters are secreted and released in a similar fashion to peptide hormones, and some ‘neuropeptides’ may be used as neurotransmitters in the nervous system in addition to acting as hormones when released into the blood. When a peptide hormone binds to receptors on the surface of the cell, a second messenger appears in the cytoplasm.

2. **Amino acid derivatives**

There are two groups of hormones derived from the amino acid tyrosine:

- **Thyroid hormones** are basically a "double" tyrosine with the critical incorporation of 3 or 4 iodine atoms.
- **Catecholamines** include epinephrine and norepinephrine, which are used as both hormones and neurotransmitters.

![](image)

The circulating half-life of thyroid hormones is on the order of a few days.

**Two other amino acids are used for synthesis of hormones:**

- **Tryptophan** is the precursor to serotonin and the pineal hormone melatonin
- **Glutamic acid** is converted to histamine

3. **Steroids**

Steroids are lipids and, more specifically, derivatives of cholesterol. Examples include the sex steroids such as testosterone and adrenal steroids such as cortisol. The first and rate-limiting step in the synthesis of all steroid hormones is conversion of cholesterol to pregnenolone, which
demonstrate the system of numbering rings and carbons for identification of different steroid hormones.

Pregnenolone is formed on the inner membrane of mitochondria then shuttled back and forth between mitochondrion and the endoplasmic reticulum for further enzymatic transformations involved in synthesis of derivative steroid hormones. Newly synthesized steroid hormones are rapidly secreted from the cell, with little if any storage. Increases in secretion reflect accelerated rates of synthesis. Following secretion, all steroids bind to some extent to plasma proteins.

Steroid hormones are typically eliminated by inactivating metabolic transformations and excretion in urine or bile.

4. **Fatty Acid Derivatives - Eicosanoids**

Eicosanoids are a large group of molecules derived from polyunsaturated fatty acids. The principal groups of hormones of this class are prostaglandins, prostacyclins, leukotrienes and thromboxanes.

Arachadonic acid is the most abundant precursor for these hormones. Stores of arachadonic acid are present in membrane lipids and released through the action of various lipases.

A great variety of cells produce prostaglandins, including those of the liver, kidneys, heart, lungs, thymus gland, pancreas, brain, and reproductive organs. In contrast to hormones, prostaglandins usually act locally, affecting only adjacent cells or the very cell that secreted it.

Prostaglandins are potent and are presented in very small quantities. They are not stored in cells but rather are synthesized just before release. These hormones are rapidly inactivated by being metabolized, and are typically active for only a few seconds.

Q  How are prostaglandins different from hormones?
A  They do not use the blood for transportation, they act locally and are synthesized in cell membrane just before release
2. Receptors and Target Cells

A given hormone usually affects only a limited number of cells, which are called target cells. A target cell responds to a hormone because it bears receptors for the hormone.

Hormone receptors are found either exposed on the surface of the cell or within the cell, depending on the type of hormone. In very basic terms, binding of hormone to receptor triggers a cascade of reactions within the cell that affects function.

Hormone receptors have two essential qualities:

1. The receptor must be able to recognize a unique binding site within the hormone in order to discriminate between the hormone and all other proteins.
2. The receptor must be able to transmit the information gained from binding to the hormone into a cellular response.

Hormones may be secreted into blood and affect cells at distant sites. Some hormones known to act and affect neighboring cells or even have effects on the same cells that secreted the hormone. Three actions are defined:

- **Endocrine action**: the hormone is distributed in blood and binds to distant target cells.
- **Paracrine action**: the hormone acts locally by diffusing from its source to target cells in the neighborhood.
- **Autocrine action**: the hormone acts on the same cell that produced it.
### Types of hormones

<table>
<thead>
<tr>
<th>Chemical messengers</th>
<th>Description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular</td>
<td>Secreted by cells in a local area and influences the activity of the same</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>chemical signal</td>
<td>cell from which it was secreted</td>
<td></td>
</tr>
<tr>
<td>Autocrine</td>
<td>Produced by a wide variety of tissues and secreted into tissue spaces; has</td>
<td>Histamine, Prostaglandins</td>
</tr>
<tr>
<td></td>
<td>a localized effect on adjacent cells</td>
<td></td>
</tr>
<tr>
<td>Paracrine</td>
<td>Secreted into the blood by specialized cells; travels by the blood to</td>
<td>Thyroxine, Insulin</td>
</tr>
<tr>
<td></td>
<td>target tissues</td>
<td></td>
</tr>
<tr>
<td>Hormone</td>
<td>Produced by neurons and functions like hormones</td>
<td>Oxytocin, Antidiuretic hormone</td>
</tr>
<tr>
<td>Neurohormone</td>
<td>Produced by neurones and secreted into extracellular spaces by nerve</td>
<td>Acetylcholine, norepinephrine</td>
</tr>
<tr>
<td></td>
<td>terminals; travels short distances, influences postsynaptic cells or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>effector cells.</td>
<td></td>
</tr>
</tbody>
</table>

Two important terms are used to refer to molecules that bind to the hormone-binding sites of receptors:

- **Agonists** are molecules that bind the receptor and induce all the post-receptor events that lead to a biologic effect. In other words, they act like the "normal" hormone, although perhaps more or less potently.

- **Antagonists** are molecules that bind the receptor and block binding of the agonist, but fail to trigger intracellular signaling events. Hormone antagonists are widely used as drugs.
Mechanism of Action of Hormone:

Understanding mechanism of action is not only of great interest to basic science, but critical to understanding and treating diseases of the endocrine system, and in using hormones as drugs.

There are two fundamental mechanisms by which a hormone can change its target cell. These mechanisms are:

- **Activation of enzymes and other dynamic molecules**: Most enzymes fluctuate between conformational states that are catalytically active versus inactive. Many hormones affect their target cells by inducing such transitions, usually causing an activation of one or more enzymes. Because enzymes are catalysts and often serve to activate additional enzymes, a seemingly small change induced by hormone-receptor binding can lead to widespread consequences within the cell.

- **Modulation of gene expression**: Stimulating transcription of a group of genes clearly can alter a cell's phenotype by leading to a burst of synthesis of new proteins. Similarly, if transcription of a group of previously active genes is shut off, the corresponding proteins will soon disappear from the cell.

More specifically, **when a receptor becomes bound to a hormone, it undergoes a conformational change which allows it to interact productively with other components of the cells**, leading ultimately to an alteration in the physiologic state of the cell.

Despite the molecular diversity of hormones, all hormone receptors can be categorized into one of two types, based on their location within the cell:
<table>
<thead>
<tr>
<th>Location of Receptor</th>
<th>Classes of Hormones</th>
<th>Principle Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell surface receptors</strong></td>
<td>Proteins and peptides, catecholamines and eicosanoids</td>
<td>Generation of <em>second messengers</em> which alter the activity of other molecules - usually enzymes - within the cell</td>
</tr>
<tr>
<td>(plasma membrane)</td>
<td>(water soluble)</td>
<td></td>
</tr>
<tr>
<td><strong>Intracellular receptors</strong></td>
<td>Steroids and thyroid hormones</td>
<td>Alter transcriptional activity of responsive genes</td>
</tr>
<tr>
<td>(cytoplasm and/or nucleus)</td>
<td>(lipid soluble)</td>
<td></td>
</tr>
</tbody>
</table>

![Diagram showing the location and mechanism of hormone receptors](image-url)
1. Hormones with Cell Surface Receptors

Protein and peptide hormones, catecholamines like epinephrine, and eicosanoids such as prostaglandins find their receptors decorating the plasma membrane of target cells. Binding of hormone to receptor initiates a series of events which leads to generation of so-called second messengers within the cell (the hormone is the first messenger). The second messengers then trigger a series of molecular interactions that alter the physiologic state of the cell. Another term used to describe this entire process is signal transduction.

Structure of Cell Surface Receptors

Cell surface receptors are integral membrane proteins and, as such, have regions that contribute to three basic domains:

- **Extracellular domains**: Some of the residues exposed to the outside of the cell interact with and bind the hormone - another term for these regions is the ligand-binding domain.

- **Transmembrane domains**: Hydrophobic stretches of amino acids are "comfortable" in the lipid bilayer and serve to anchor the receptor in the membrane.

- **Cytoplasmic or intracellular domains**: Tails or loops of the receptor that are within the cytoplasm react to hormone binding by interacting in some way with other molecules, leading to generation of second messengers.

As shown below, some receptors are simple, single-pass proteins; many growth factor receptors take this form. Others, such as the receptor for insulin, have more than one subunit. Another class, which includes the beta-adrenergic receptor, is threaded through the membrane seven times.
Interaction of the hormone-bound receptor with other membrane or cytoplasmic proteins is the key to generation of second messengers and transduction of the hormonal signal.

**Second Messenger Systems**

Nonsteroid hormones (water soluble) do not enter the cell but bind to plasma membrane receptors, generating a chemical signal (second messenger) inside the target cell. Second messengers activate other intracellular chemicals to produce the target cell response.
Currently, four second messenger systems are recognized in cells, as summarized in the table below. Note that not only do multiple hormones utilize the same second messenger system, but a single hormone can utilize more than one system.
### Second Messenger Examples of Hormones Which Utilize This System

<table>
<thead>
<tr>
<th>Second Messenger</th>
<th>Examples of Hormones Which Utilize This System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic AMP</td>
<td>Epinephrine and norepinephrine, glucagon, luteinizing hormone, follicle stimulating hormone, thyroid-stimulating hormone, calcitonin, parathyroid hormone, antidiuretic hormone</td>
</tr>
<tr>
<td>Protein kinase activity</td>
<td>Insulin, growth hormone, prolactin, oxytocin, erythropoietin, several growth factors</td>
</tr>
<tr>
<td>Calcium and/or phosphoinositides</td>
<td>Epinephrine and norepinephrine, antidiuretic hormone, gonadotropin-releasing hormone, thyroid-releasing hormone.</td>
</tr>
<tr>
<td>Cyclic GMP</td>
<td>Atrial naturetic hormone, nitric oxide</td>
</tr>
</tbody>
</table>

In all cases, the seemingly small signal generated by hormone binding its receptor is amplified within the cell into a cascade of actions that changes the cell's physiologic state. Presented below are two examples of second messenger systems commonly used by hormones.

1. **Cyclic AMP Second Messenger Systems**

Cyclic adenosine monophosphate (cAMP) is a nucleotide generated from ATP through the action of the enzyme *adenylate cyclase*. The intracellular concentration of cAMP is increased or decreased by a variety of hormones and such fluctuations affect a variety of cellular processes. One prominent and important effect of elevated concentrations of cAMP is activation of a cAMP-dependent protein kinase called *protein kinase A*.

*Protein kinase A* is nominally in a catalytically-inactive state, but becomes active when it binds cAMP. Upon activation, protein kinase A phosphorylates a number of other proteins, many of which are themselves enzymes that are either activated or suppressed by being phosphorylated. Such changes in enzymatic activity within the cell clearly alter its state.
Simple Example: mechanism of action of glucagon:

- **Glucagon** binds its receptor in the plasma membrane of target cells (e.g. hepatocytes).
- Bound receptor interacts with and, through a set of G proteins, turns on adenylate cyclase, which is also an integral membrane protein.
- Activated adenylate cyclase begins to convert ATP to cyclic AMP, resulting in an elevated intracellular concentration of cAMP.
- High levels of cAMP in the cytosol make it probable that protein kinase A will be bound by cAMP and therefore catalytically active.
- Active protein kinase A "runs around the cell" adding phosphates to other enzymes, thereby changing their conformation and modulating their catalytic activity.
- Levels of cAMP decrease due to destruction by cAMP-phosphodiesterase and the inactivation of adenylate cyclase.

In the above example, the hormone's action was to modify the activity of pre-existing components in the cell. Elevations in cAMP also have important effects on transcription of certain genes.
2. Tyrosine Kinase Second Messenger Systems

The receptors for several protein hormones are themselves protein kinases which are switched on by binding of hormone. The kinase activity associated with such receptors results in phosphorylation of tyrosine residues on other proteins. **Insulin is an example of a hormone whose receptor is a tyrosine kinase.**

The hormone binds to domains exposed on the cell's surface, resulting in a conformational change that activates kinase domains located in the cytoplasmic regions of the receptor. In many cases, the receptor phosphorylates itself as part of the kinase activation process. The activated receptor phosphorylates a variety of intracellular targets, many of which are enzymes that become activated or are inactivated upon phosphorylation. As seen with cAMP second messenger systems, activation of receptor tyrosine kinases leads to rapid modulation in a number of target proteins within the cell. Some of the targets of receptor kinases are protein phosphatases which, upon activation by receptor tyrosine kinase, become competent to remove phosphates from other proteins and alter their activity. Again, a seemingly small change due to hormone binding is amplified into a multitude of effects within the cell.

2. Hormones with Intracellular Receptors

Receptors for steroid and thyroid hormones are located inside target cells, in the cytoplasm or nucleus, and function as **ligand-dependent transcription factors.** The hormone-receptor complex binds to promoter regions of responsive genes and stimulate or sometimes inhibit transcription from those genes. **Thus, the mechanism of action of these hormones is to modulate gene expression in target cells.** By selectively affecting transcription from a battery of genes, the concentration of those respective proteins are altered, which clearly can change the phenotype of the cell.
Structure of Intracellular Receptors

Steroid and thyroid hormone receptors are members of a large group of transcription factors. All of these receptors are composed of a single polypeptide chain that has, three distinct domains:

- **The amino-terminus**: In most cases, this region is involved in activating or stimulating transcription by interacting with other components of the transcriptional machinery. The sequence is highly variable among different receptors.

- **DNA binding domain**: Amino acids in this region are responsible for binding of the receptor to specific sequences of DNA.

- **The carboxy-terminus or ligand-binding domain**: This is the region that binds hormone.
In addition to these three core domains, two other important regions of the receptor protein are a nuclear localization sequence, which targets the protein to nucleus, and a dimerization domain, which is responsible for latching two receptors together in a form capable of binding DNA.
Hormone-Receptor Binding and Interactions with DNA

Being lipids, steroid hormones enter the cell by simple diffusion across the plasma membrane. Thyroid hormones enter the cell by facilitated diffusion. The receptors exist either in the cytoplasm or nucleus, which is where they meet the hormone. When hormone binds to receptor, a characteristic series of events occurs:

- **Receptor activation** is the term used to describe conformational changes in the receptor induced by binding hormone. The major consequence of activation is that the receptor becomes competent to bind DNA.

- **Activated receptors bind to hormone response elements**, which are short specific sequences of DNA which are located in promoters of hormone-responsive genes.

- **Transcription from those genes to which the receptor is bound is affected**. Most commonly, receptor binding stimulates transcription. The hormone-receptor complex thus functions as a transcription factor.
Steroid Hormones

The second mechanism involves steroid hormones, which pass through the plasma membrane and act in a two step process. Steroid hormones bind, once inside the cell, to the nuclear membrane receptors, producing an activated hormone-receptor complex. The activated hormone-receptor complex binds to DNA and activates specific genes, increasing production of proteins.
Control of Endocrine Activity

The physiologic effects of hormones depend largely on their concentration in blood and extracellular fluid.

The concentration of hormone as seen by target cells is determined by three factors:

1. **Rate of production:** Synthesis and secretion of hormones are the most highly regulated aspect of endocrine control. Such control is mediated by positive and negative feedback circuits.

2. **Rate of delivery:** An example of this effect is blood flow to a target organ or group of target cells:

**TRANSPORT OF HORMONES:** hormones must be transported at least some distance to their target organs. The primary transport medium is the plasma, although the lymphatic system and the cerebrospinal fluid are also important. Since delivery of the hormone to its target tissue is required before a hormone can exert its effects, the presence or absence of specific transport mechanisms play a major role in mediating hormonal action.

A) The water-soluble hormones (peptide hormones, catecholamines) are transported in plasma in solution and require no specific transport mechanism. Because of this, the water-soluble hormones are generally short-lived. These properties allow for rapid shifts in circulating hormone concentrations, which is necessary with the pulsatile tropic hormones or the catecholamines. This is consistent with the rapid onset of action of the water-soluble hormones.

B) The lipid-soluble hormones (thyroid hormone, steroids) circulate in the plasma bound to specific carrier proteins. Many of the proteins have a high affinity for specific hormone, such as thyroxine-binding globulin (TBG), sex hormone-binding globulin (SHBG), and cortisol-binding globulin (CBG). Non-specific, low-affinity binding of these hormones to albumin also occurs. Carrier proteins act as reservoirs of hormone. Since it is generally believed that only the free hormone can enter cells, a dynamic equilibrium must exist between the bound and free hormone. Thus, alterations in the amount of binding protein available, or in the affinity of the hormone for the binding protein, can markedly alter the total circulating pool of hormone without affecting the free concentration of hormone.

Carrier proteins act as buffers to both blunt sudden increases in hormone concentration and to diminish degradation of the hormone once it is secreted. Thus, the half-life of hormones that utilize carrier proteins is longer than those that are not protein-
bound. Indeed, carrier proteins have a profound effect on the clearance rate of hormones; the greater the capacity for high affinity binding of the hormone in the plasma, the slower the clearance rate. Also, the carrier proteins allow slow, tonic delivery of the hormone to its target tissue. **This is consistent with the slower onset of action of the lipid-soluble hormones.**

3. **Rate of degradation and elimination**: Hormones, like all biomolecules, have characteristic rates of decay, and are metabolized and excreted from the body through several routes.

**HORMONE METABOLISM**: Clearance of hormones from the circulation plays a critical role in the modulation of hormone levels in response to varied physiologic and pathologic processes. The time required to reach a new steady-state concentration in response to changes in hormone release is dependent upon the half-life of the hormone in the serum. Thus, an increase in hormone release or administration will have a much more marked effect if the hormone is cleared rapidly from the circulation as opposed to one that is cleared more slowly.

Most peptide hormones have a plasma half-life measured in minutes, consistent with the rapid actions and pulsatile nature of the secretion of these hormones. This rapid clearance is achieved by the lack of protein binding in the plasma, degradation or internalization of the hormone at its site of action, and ready clearance of the hormone by the kidney. Binding to serum proteins markedly decreases hormone clearance, as is observed with the steroid hormones and the iodothyronines. Metabolism of the steroid hormones occurs primarily in the liver by reductions, conjugations, oxidations, and hydroxylations, which serve to inactivate the hormone and increase their water-solubility, facilitating their excretion in the urine and the bile. Metabolic transformation also may serve to activate an inactive hormone precursor, such as the deiodination of thyroxine to form T₃. Hormone metabolism is not as tightly regulated as is hormone synthesis and release. However, alterations in the metabolic pathways may be clinically important.

**Feedback Control of Hormone Production**

Feedback circuits are at the root of most control mechanisms in physiology, and are particularly prominent (obvious) in the endocrine system. Instances of positive feedback certainly occur, but negative feedback is much more common. Feedback loops are used extensively to regulate secretion of hormones in the hypothalamic-pituitary axis. An important example of a negative feedback loop is seen in control of thyroid hormone secretion. The **thryoid hormones** thyroxine and triiodothyronine...
("T4 and T3") are synthesized and secreted by thyroid glands and affect metabolism throughout the body. The basic mechanisms for control in this system are:

- Neurons in the hypothalamus secrete thyroid releasing hormone (TRH), which stimulates cells in the anterior pituitary to secrete thyroid-stimulating hormone (TSH).
- TSH binds to receptors on epithelial cells in the thyroid gland, stimulating synthesis and secretion of thyroid hormones, which affect probably all cells in the body.
- When blood concentrations of thyroid hormones increase above a certain threshold, TRH-secreting neurons in the hypothalamus are inhibited and stop secreting TRH. This is an example of "negative feedback".

Inhibition of TRH secretion leads to shut-off of TSH secretion, which leads to shut-off of thyroid hormone secretion. As thyroid hormone levels decay below the threshold, negative feedback is relieved, TRH secretion starts again, leading to TSH secretion...
Biochemistry and Disorders of Hormones of the Hypothalamic and pituitary gland (hypothalamus and pituitary axis)

1. Hormones of the hypothalamus

Prof. Dr. Hedef Dhafir El-Yassin 2012
1. Hormones of the hypothalamus

The hypothalamus is an integral part of the substance of the brain. A small cone-shaped structure, it projects downward, ending in the pituitary stalk, a tubular connection to the pituitary gland, which is a double lobed structure that produces the endocrine secretions when stimulated by the hypothalamus.

Regulation of hormone secretion

The hypothalamus regulates homeostasis. It links the nervous system to the endocrine system. In addition to secreting neurotransmitters and neuromodulators, the hypothalamus synthesizes and secretes a number of neurohormones. The neurons secreting neurohormones are true endocrine cells.
Hormonal cascade of signals from CNS to ultimate hormone.

The target "gland" is the last hormone-producing tissue in the cascade, which is stimulated by an appropriate anterior pituitary hormone. Examples are thyroid gland, adrenal cortex, ovary and testes. Ultimate hormone feedback negatively on sites producing intermediate hormones in the cascade. Amounts (nanogram (ng), microgram (µg), and milligram (mg) represent approximate quantities of hormone released.
The hypothalamus controls each lobe of the pituitary slightly differently.

1. **control of Anterior lobe**
   a. The hypothalamus acts as an endocrine gland.
   b. Hormones are sent from the hypothalamus to the anterior pituitary via a blood vessel called the portal vein.
   c. The target tissue is the anterior lobe of the pituitary e.g. LH, TSH, and FSH.

2. **control of the Posterior lobe**
   d. Neuro-hormones are synthesized in the hypothalamus neurons. They are transported and stored in vesicles in the axon ending located in the posterior pituitary.
   e. Nerve impulses travel down the axon into the posterior pituitary. This causes the release of the vesicles of hormones into the blood stream at the posterior pituitary e.g. oxytocin, and ADH.
Many hormonal systems involve hypothalamus.

Cascade of hormonal responses starting with an external or internal signal. This signal is transmitted first to the CNS and may involve the limbic system, including the hippocampus and amygdala. These components innervate the hypothalamus in a specific region, which responds by secreting (nanogram amounts) a specific releasing hormone. Releasing hormones are transported down a closed portal system to the anterior pituitary, where they cause secretion of microgram amounts of specific anterior pituitary hormones. These access the general circulation through fenestrated local capillaries and trigger release of an ultimate hormone in microgram to milligram daily amounts, which generates its response by binding to receptors in target tissues. Overall, this system is an amplifying cascade. Consequently, the organism is in intimate association with the external environment. Solid arrows indicate a secretory process. Long arrows studded with open or closed circles indicate negative feedback pathways (ultra-short, short, and long feedback loops).
1) **Thyrotropin-releasing hormone (TRH)**
Is the simplest of the hypothalamic neuropeptides. It consists essentially of three amino acids. Its basic sequence is glutamic acid-histidine-proline, although both ends of the peptide are modified. The simplicity of this structure is deceiving for TRH is involved in an extraordinary array of functions. Some of which are:

a. It stimulates the secretion of thyroid-stimulating hormone from the pituitary.

b. It also affects the secretion of prolactin from the pituitary.

The TRH-secreting cells are subject to stimulatory and inhibitory influences from higher centers in the brain and they also are inhibited by circulating thyroid hormone.

2) **Gonadotropin-releasing hormone (GnRH)**
Also known as luteinizing hormone-releasing hormone (LHRH), is a peptide chain of 10 amino acids. It stimulates the synthesis and release of the two pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

3) **Corticotropin-releasing hormone (CRH)**
Is a large peptide consisting of a single chain of 41 amino acids. It stimulates not only secretion of corticotropin in the pituitary gland but also the synthesis of corticotropin in the corticotropin-producing cells (corticotrophs) of the anterior lobe of the pituitary gland. Many factors, both neurogenic and hormonal, regulate the secretion of CRH. Among the hormones that play an important role in modulating the influence of CRH is cortisol, the major hormone secreted by the adrenal cortex, which, as part of the negative feedback mechanism. Vasopressin, the major regulator of the body’s excretion of water, has an additional ancillary role in stimulating the secretion of CRH.

Excessive secretion of CRH leads to an increase in the size and number of corticotrophs in the pituitary gland, often resulting in a pituitary tumor. This, in turn, leads to excessive stimulation of the adrenal cortex, resulting in high circulating levels of adrenocortical hormones, the clinical manifestations of which are known as Cushing’s syndrome. Conversely, a deficiency of CRH-producing cells can, by a lack of stimulation of the pituitary and adrenal cortical cells, result in adrenocortical deficiency.

4) **Growth hormone-releasing hormone (GHRH or GRH)**
Like CRH, growth hormone-releasing hormone (GHRH) is a large peptide. A number of forms have been described that differ from one another only in minor details and in the number of amino acids (varying from 37 to 49). It is stimulated by stresses, including physical exercise, and secretion is blocked by a powerful inhibitor called somatostatin.

Negative feedback control of GHRH secretion is mediated largely through compounds called somatomedins, growth-promoting hormones that are generated when tissues are
exposed to growth hormone itself.
Isolated deficiency of GHRH (in which there is normal functioning of the hypothalamus except for this deficiency) may be the cause of one form of dwarfism, a general term applied to all individuals with abnormally small stature.

5) **Prolactin release factor (PRF):**
Appears to be released from the hypothalamus in a pulsatile fashion and it is the fluctuation in PRF that regulates the circulating level of prolactin.

6) **Somatostatin (Growth hormone release-inhibiting hormone; somatotropin release-inhibiting hormone (GHRIH or SRIH))**
Somatostatin refers to a number of polypeptides consisting of chains of 14 to 28 amino acids. Somatostatin is also a powerful inhibitor of pituitary TSH secretion. Somatostatin, like TRH, is widely distributed in the central nervous system and in other tissues. It serves an important paracrine function in the islets of Langerhans, by blocking the secretion of both insulin and glucagon from adjacent cells. Somatostatin has emerged not only as a powerful blocker of the secretion of GH, insulin, glucagon, and other hormones but also as a potent inhibitor of many functions of the gastrointestinal tract, including the secretion of stomach acid, the secretion of pancreatic enzymes, and the process of intestinal absorption.

7) **Prolactin release-inhibiting hormones (Dopamine and GAP)**
GAP= GnRH-associated peptide
The hypothalamic regulation of prolactin secretion from the pituitary is different from the hypothalamic regulation of other pituitary hormones in two respects:

1. First, the hypothalamus primarily inhibits rather than stimulates the release of prolactin from the pituitary.
2. Second, this major inhibiting factor is not a neuropeptide, but rather the neurotransmitter dopamine. Prolactin deficiency is known to occur, but only rarely. Excessive prolactin production (hyperprolactinemia) is a common endocrine abnormality.
<table>
<thead>
<tr>
<th>Hypothalamic hormones</th>
<th>No. of A.A in structure</th>
<th>Pituitary Hormone Affected$^1$</th>
<th>Target Gland Hormone Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Thyrotropin-releasing hormone (TRH)</td>
<td>3</td>
<td>TSH (PRL)</td>
<td>$T_3$, $T_4$</td>
</tr>
<tr>
<td>2 Gonadotropin-releasing hormone (GnRH)</td>
<td>10</td>
<td>LH, FSH</td>
<td>Androgens, estrogens, progestins</td>
</tr>
<tr>
<td>3 Corticotropin-releasing hormone (CRH)</td>
<td>41</td>
<td>ACTH</td>
<td>Cortisol</td>
</tr>
<tr>
<td>4 Growth hormone-releasing hormone (GHRH or GRH)</td>
<td>49</td>
<td>GH</td>
<td>IGF-1</td>
</tr>
<tr>
<td>5 Prolactin release factor</td>
<td>Not established</td>
<td>PRL</td>
<td>neurohormones</td>
</tr>
<tr>
<td>6 Somatostatin (Growth hormone release-inhibiting hormone; somatotropin release-inhibiting hormone (GHRIH or SRIH)</td>
<td>14</td>
<td>GH (TSH, FSH, ACTH)</td>
<td>IGK-1; $T_3$ and $T_4$</td>
</tr>
<tr>
<td>7 Prolactin- release-inhibiting hormones (Dopamine and GAP) (PRIH or PIH)</td>
<td></td>
<td>PRL</td>
<td>neurohormones</td>
</tr>
</tbody>
</table>

$^1$The hypothalamic hormone has a secondary or lesser effect on the hormones in parentheses.
Biochemistry and Disorders of Hormones of the Hypothalamic and pituitary gland (hypothalamus and pituitary axis)

2. Hormones of the pituitary gland

Prof. Dr. Hedef Dhafir El-Yassin 2012
2. Hormones of the Pituitary gland

The pituitary gland, also known as the hypophysis, is a roundish organ that lies immediately beneath the hypothalamus. Careful examination of the pituitary gland reveals that it composed of two distinctive parts:

- The anterior pituitary (adenohypophysis) is a classical gland composed predominantly of cells that secrete protein hormones.
- The posterior pituitary (neurohypophysis) is not really an organ, but an extension of the hypothalamus. It is composed largely of the axons of hypothalamic neurons which extend downward as a large bundle behind the anterior pituitary.

The target cells for most of the hormones produced in these tissues are themselves endocrine cells.

The pituitary gland is often called the "master gland" of the body. The anterior and posterior pituitary secretes a number of hormones that collectively influence all cells and affect virtually all physiologic processes.
Table: The major hormones synthesized and secreted by the pituitary gland, along with summary statements about their major target organs and physiologic effects.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Major target organ(s)</th>
<th>Major Physiologic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth hormone</strong></td>
<td>Liver, adipose tissue</td>
<td>Promotes growth (indirectly), control of protein, lipid and carbohydrate metabolism</td>
</tr>
<tr>
<td><strong>Thyroid-stimulating h.</strong></td>
<td>Thyroid gland</td>
<td>Stimulates secretion of thyroid hormones</td>
</tr>
<tr>
<td><strong>Adrenocorticotropic h.</strong></td>
<td>Adrenal gland (cortex)</td>
<td>Stimulates secretion of glucocorticoids</td>
</tr>
<tr>
<td><strong>Prolactin</strong></td>
<td>Mammary gland</td>
<td>Milk production</td>
</tr>
<tr>
<td><strong>Luteinizing hormone</strong></td>
<td>Ovary and testis</td>
<td>Control of reproductive function</td>
</tr>
<tr>
<td><strong>Follicle-stimulating h.</strong></td>
<td>Ovary and testis</td>
<td>Control of reproductive function</td>
</tr>
<tr>
<td><strong>Antidiuretic hormone</strong></td>
<td>Kidney</td>
<td>Conservation of body water</td>
</tr>
<tr>
<td><strong>Oxytocin</strong></td>
<td>Ovary and testis</td>
<td>Stimulates milk ejection and uterine contractions</td>
</tr>
</tbody>
</table>

As seen in the table above, the anterior pituitary synthesizes and secreted six major hormones. **Individual cells within the anterior pituitary secrete a single hormone** (or possibly two in some cases). Thus, the anterior pituitary contains at least six distinctive endocrinocytes.

The cells that secrete thyroid-stimulating hormone do not also secrete growth hormone, and they have receptors for thyroid-releasing hormone, not growth hormone-releasing hormone.
Overview of anterior pituitary hormones with hypothalamic releasing hormones and their actions
Anterior Pituitary Hormones

1. Growth Hormone

Growth hormone, also known as somatotropin, is a protein hormone of about 190 amino acids that is synthesized and secreted by cells called somatotrophs in the anterior pituitary. It is a major participant in control of several complex physiologic processes, including growth and metabolism. Growth hormone is also of considerable interest as a drug used in both humans and animals.

Physiologic Effects of Growth Hormone

A critical concept in understanding growth hormone activity is that it has two distinct types of effects:

- **Direct effects** are the result of growth hormone binding its receptor on target cells. Fat cells (adipocytes), for example, have growth hormone receptors, and growth hormone stimulates them to break down triglyceride and suppresses their ability to take up and accumulate circulating lipids.

- **Indirect effects** are mediated primarily by a insulin-like growth factor-1 (IGF-1), a hormone that is secreted from the liver and other tissues in response to growth hormone. A majority of the growth promoting effects of growth hormone is actually due to IGF-1 acting on its target cells. IGF-1 also appears to be the key player in muscle growth. It stimulates amino acid uptake and protein synthesis in muscle and other tissues.

Metabolic Effects

- **Protein metabolism**: In general, growth hormone stimulates protein anabolism in many tissues. This effect reflects increased amino acid uptake, increased protein synthesis and decreased oxidation of proteins.

- **Fat metabolism**: Growth hormone enhances the utilization of fat by stimulating triglyceride breakdown and oxidation in adipocytes.

- **Carbohydrate metabolism**: Growth hormone is one of a battery of hormones that serves to maintain blood glucose within a normal range. Growth hormone is often said to have anti-insulin activity, because it suppresses the abilities of insulin to stimulate uptake of glucose in peripheral tissues and enhance glucose synthesis in the liver.

- **Mineral metabolism**: promotes a positive calcium, magnesium and phosphate balance and causes the retention of Na⁺, K⁺ and Cl⁻.
Control of Growth Hormone Secretion

Production of growth hormone is modulated by many factors, including stress, exercise, nutrition, sleep and growth hormone itself. However, its primary controllers are two hypothalamic hormones and one hormone from the stomach:

- **Growth hormone-releasing hormone** (GHRH) is a hypothalamic peptide that stimulates both the synthesis and secretion of growth hormone.
- **Somatostatin** (SS) is a peptide produced by several tissues in the body, including the hypothalamus. Somatostatin inhibits growth hormone release in response to GHRH and to other stimulatory factors such as low blood glucose concentration.
- **Ghrelin** is a peptide hormone secreted from the stomach. Ghrelin binds to receptors on somatotrophs and potently stimulates secretion of growth hormone.

Growth hormone secretion is also part of a negative feedback loop involving IGF-1. High blood levels of IGF-1 lead to decreased secretion of growth hormone not only by directly suppressing the somatotroph, but by stimulating release of somatostatin from the hypothalamus.

Growth hormone also feeds back to inhibit GHRH secretion and probably has a direct (autocrine) inhibitory effect on secretion from the somatotroph.

Integration of all the factors that affect growth hormone synthesis and secretion lead to a pulsatile pattern of release. In children and young adults, the most intense period of growth hormone release is shortly after the onset of deep sleep.

**Disease States**

A deficiency state can result not only from a deficiency in production of the hormone, but in the target cell's response to the hormone.

Clinically, deficiency in growth hormone or receptor defects are known as growth retardation or dwarfism. The manifestation of growth hormone deficiency depends upon the age of onset of the disorder and can result from either heritable or acquired disease. The effect of excessive secretion of growth hormone is also very dependent on the age of onset and is seen as two distinctive disorders:
• **Gigantism** is the result of excessive growth hormone secretion that begins in young children or adolescents. It is a very rare disorder, usually resulting from a tumor of somatotropes.

• **Acromegaly** results from excessive secretion of growth hormone in adults. The excessive growth hormone and IGF-1 also lead to metabolic derangements, including glucose intolerance.

2. **Thyroid Stimulating Hormone**

Thyroid-stimulating hormone, also known as thyrotropin, is secreted from cells in the anterior pituitary called *thyrotrophs*, finds its receptors on epithelial cells in the thyroid gland, and stimulates that gland to synthesize and release thyroid hormones.

TSH is a glycoprotein hormone composed of two subunits, which are non-covalently bound to one another. The alpha subunit of TSH is also present in two other pituitary glycoprotein hormones, follicle-stimulating hormone and luteinizing hormone. In other words, TSH is composed of alpha subunit bound to the TSH beta subunit, and TSH associates only with its own receptor. Free alpha and beta subunits have essentially no biological activity.

TSH has several acute effects on thyroid function. These occur in minutes and involve increases of all phases of $T_3$ and $T_4$ biosynthesis. TSH also has several chronic effects on the thyroid. These require several days and include increases in the synthesis of proteins, phospholipids, and nucleic acids and in the size of number of thyroid cells.

The most important controller of TSH secretion is thyroid-releasing hormone. Secretion of thyroid-releasing hormone, and hence, TSH, is inhibited by high blood levels of thyroid hormones in a classical *negative feedback loop*. 
3. **Adrenocorticotropic Hormone**

Adrenocorticotropic hormone, stimulates the adrenal cortex by enhancing the conversion of cholesterol to pregnenolone. More specifically, it stimulates secretion of glucocorticoids such as cortisol, and has little control over secretion of aldosterone, the other major steroid hormone from the adrenal cortex. Another name for ACTH is corticotropin.

ACTH is secreted from the anterior pituitary in response to corticotropin-releasing hormone from the hypothalamus. Corticotropin-releasing hormone is secreted in response to many types of stress, which makes sense in view of the "stress management" functions of glucocorticoids. Corticotropin-releasing hormone itself is inhibited by glucocorticoids, making it part of a classical negative feedback loop.

Within the pituitary gland, ACTH is produced in a process that also generates several other hormones. A large precursor protein named proopiomelanocortin (POMC) is synthesized and proteolytically chopped into several fragments as depicted below.

The major attributes of the hormones other than ACTH that are produced in this process are summarized as follows:

- **Lipotropin**: Originally described as having weak lipolytic effects, its major importance is as the precursor to beta-endorphin.
- **Beta-endorphin and Met-enkephalin**: Opioid peptides with pain-alleviation and euphoric effects.
- **Melanocyte-stimulating hormone (MSH)**: Known to control melanin pigmentation in the skin of most vertebrates.
4. **Prolactin**

Prolactin is a single-chain protein hormone closely related to growth hormone. It is secreted by so-called *lactotrophs* in the anterior pituitary. It is also synthesized and secreted by a broad range of other cells in the body. Prolactin is synthesized as a prohormone. Following cleavage of the signal peptide, the length of the mature hormone is between 194 and 199 amino acids, depending on species. Hormone structure is stabilized by three intramolecular disulfide bonds. Overall, several hundred different actions have been reported for prolactin in various species. Some of its major effects are:

1. **Mammary Gland Development, Milk Production and Reproduction**
2. **Effects on Immune Function**

The prolactin receptor is widely expressed by immune cells, and some types of lymphocytes synthesize and secrete prolactin. These observations suggest that prolactin may act as an autocrine or paracrine modulator of immune activity.

**Control of Prolactin Secretion**

In contrast to what is seen with all the other pituitary hormones, the hypothalamus suppresses prolactin secretion from the pituitary. Dopamine serves as the major prolactin-inhibiting factor or brake on prolactin secretion. In addition to inhibition by dopamine, prolactin secretion is positively regulated by several hormones, including thyroid-releasing hormone, gonadotropin-releasing hormone and vasoactive intestinal polypeptide. Estrogens provide a well-studied positive control over prolactin synthesis and secretion.
5. Gonadotropins: Luteinizing and Follicle Stimulating Hormones

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are called gonadotropins because they stimulate the gonads - in males, the testes, and in females, the ovaries. As described for thyroid-simulating hormone, LH and FSH are large glycoproteins composed of alpha and beta subunits. The alpha subunit is identical in all three of these anterior pituitary hormones, while the beta subunit is unique for each hormone with the ability to bind its own receptor.

a. Luteinizing Hormone

In both sexes, LH stimulates secretion of sex steroids from the gonads. In the testes, it stimulates the synthesis and secretion of testosterone. The ovary responds to LH stimulation by secretion of testosterone, which is converted into estrogen by adjacent granulosa cells.

LH is required for continued development and function of corpora lutea. The name luteinizing hormone derives from this effect of inducing luteinization of ovarian follicles.

b. Follicle-Stimulating Hormone

As its name implies, FSH stimulates the maturation of ovarian follicles. FSH is also critical for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation.

Control of Gonadotropin Secretion

The principle regulator of LH and FSH secretion is gonadotropin-releasing hormone or GnRH (also known as LH-releasing hormone). In a classical negative feedback loop, sex steroids inhibit secretion of GnRH and also appear to have direct negative effects on gonadotrophs.

This regulatory loop leads to pulsatile secretion of LH and, to a much lesser extent, FSH. Numerous hormones influence GnRH secretion, and positive and negative control over GnRH and gonadotropin secretion is actually considerably more complex than described in the figure. For example, the gonads secrete at least two additional hormones - inhibin and activin, which selectively inhibit and activate FSH secretion from the pituitary.
Posterior Pituitary Hormones

1. **Antidiuretic Hormone (Vasopressin)**

Roughly, 60% of the mass of the body is water, and despite wide variation in the amount of water taken in each day, body water content remains incredibly stable. Such precise control of body water and solute concentrations is a function of several hormones acting on both the kidneys and vascular system, but there is no doubt that antidiuretic hormone is a key player in this process.

Antidiuretic hormone, also known as vasopressin, is a nine amino acid peptide secreted from the posterior pituitary.

**Physiologic Effects of Antidiuretic Hormone**

The single most important effect of antidiuretic hormone is to conserve body water by reducing the output of urine.

Antidiuretic hormone stimulates water reabsorption by stimulating insertion of "water channels" or aquaporins into the membranes of kidney tubules. These channels transport solute-free water through tubular cells and back into blood, leading to a decrease in plasma osmolarity and an increase osmolarity of urine.

**Control of Antidiuretic Hormone Secretion**

1. The most important variable regulating antidiuretic hormone secretion is **plasma osmolality**, or the concentration of solutes in blood. When plasma osmolality is below a certain threshold, the osmoreceptors are not activated and antidiuretic hormone secretion is suppressed. When osmolality increases above the threshold, the ever-alert osmoreceptors recognize this and stimulate the neurons that secrete antidiuretic hormone. As seen the figure, antidiuretic hormone concentrations rise steeply and linearly with increasing plasma osmolality.

2. Secretion of antidiuretic hormone is also simulated by decreases in blood pressure and volume, conditions sensed by stretch receptors in the heart and large arteries. Changes in blood pressure and volume are not nearly as sensitive a stimulator as increased osmolality, but are nonetheless potent in severe conditions.
For example, Loss of 15 or 20% of blood volume by hemorrhage results in massive secretion of antidiuretic hormone. Another potent stimulus of antidiuretic hormone is nausea and vomiting.

**Disease States**
The most common disease of man and animals related to antidiuretic hormone is diabetes insipidus. This condition can arise from either of two situations:

- **Hypothalamic ("central") diabetes insipidus** results from a deficiency in secretion of antidiuretic hormone from the posterior pituitary. Causes of this disease include head trauma, and infections or tumors involving the hypothalamus.

- **Nephrogenic diabetes insipidus** occurs when the kidney is unable to respond to antidiuretic hormone. Most commonly, this results from some type of renal disease, but mutations in the ADH receptor gene or in the gene encoding aquaporin-2 have also been demonstrated in affected humans.

**The major sign of either type of diabetes insipidus is excessive urine production.**

Some human patients produce as much as 16 liters of urine per day! If adequate water is available for consumption, the disease is rarely life-threatening, but withholding water can be very dangerous.

2. **Oxytocin**

Oxytocin in a nine amino acid peptide that is synthesized in hypothalamic neurons and transported down axons of the posterior pituitary for secretion into blood. Oxytocin differs from antidiuretic hormone in two of the nine amino acids. Both hormones are packaged into granules and secreted along with carrier proteins called neurophysins.

**Control of Oxytocin Secretion**

A number of factors can inhibit oxytocin release, among them acute stress. For example, oxytocin neurons are repressed by catecholamines, which are released from the adrenal gland in response to many types of stress, including fright.
Clinical correlation

Testing Activity of the Anterior Pituitary

Releasing hormones and chemical analogs, particularly of the smaller peptides, are now routinely synthesized. The gonadotropin-releasing hormone, a decapeptide, is available for use in assessing the function of the anterior pituitary. This is of importance when a disease situation may involve either the hypothalamus, the anterior pituitary, or the end organ. Infertility is an example of such a situation. What needs to be assessed in which organ is at fault in the hormonal cascade. Initially, the end organ, in this case the gonads, must be considered. This can be accomplished by injecting the anterior pituitary hormone LH or FSH. If sex hormone secretion is elicited, then the ultimate gland would appear to be functioning properly. Next, the anterior pituitary would need to be analyzed. This can be done by i.v. administration of synthetic GnRH; by this route, GnRH can gain access to the gonadotropic cells of the anterior pituitary and elicit secretion of LH and FSH. Routinely, LH levels are measured in the blood as a function of time after the injection. These levels are measured by radioimmunoassay (RIA) in which radioactive LH or hCG is displaced from binding to an LH-binding protein by LH in the serum sample. The extent of the competition is proportional to the amount of LH in the serum. In this way a progress of response is measured that will be within normal limits or clearly deficient. If the response is deficient, the anterior pituitary cells are not functioning normally and are the cause of the syndrome. On the other hand, normal pituitary response to GnRH would indicate that the hypothalamus was non-functional. Such a finding would prompt examination of the hypothalamus for conditions leading to insufficient availability/production of releasing hormones. Obviously, the knowledge of hormone structure and the ability to synthesize specific hormones permits the diagnosis of these disease states.

Clinical correlation

Hypopituitarism

The hypothalamus is connected to the anterior pituitary by a delicate stalk that contains the portal system through which releasing hormones, secreted from the hypothalamus, gain access to the anterior pituitary cells. In the cell membranes of these cells are specific receptors for releasing hormones. In most cases, different cells express deferent releasing hormone receptors. The connection between the hypothalamus and anterior pituitary can be disrupted by trauma or tumors. Trauma can occur in automobile accidents or other local damaging events that may result in severing of the stalk, thus preventing the releasing hormones from reaching their target anterior pituitary cells. When this happens, the anterior pituitary cells no longer have their signaling mechanism for the release of anterior pituitary hormones. In the case of tumors of the pituitary gland, all of the anterior pituitary hormones may not be shut off to the same degree or the secretion of some may disappear sooner than others.

In any case, if the hypopituitarism occurs, this condition may result in a life-threatening situation in which the clinician must determine the extent of loss of pituitary hormones, especially ACTH. Posterior pituitary hormones (Oxytocin and vasopressin) may also be lost, precipitating a problem of excessive urination (vasopressin deficiency) that must be addressed. The usual therapy involves administration of the end-organ hormones, such as, thyroid hormone, cortisol, sex hormones, and progestin; with female patients it is also necessary to maintain the ovarian cycle. These hormones can be easily administered in the oral form. Growth hormone deficiency is not a problem in the adult but would be an important problem in a growing child.

The patient must learn to anticipate needed increases of cortisol in the face of stressful situations. Fortunately, these patients are usually maintained in reasonably good condition.

**Question:** Hypopituitarism may result from trauma, such as an automobile accident severing the stalk connecting the hypothalamus and anterior pituitary, or from tumors of the pituitary gland. In trauma, usually all of the releasing hormones from hypothalamus fail to reach the anterior pituitary. With a tumor of the gland, some or all of the pituitary hormones may be shut off. Posterior pituitary hormones may also be lost. Hypopituitarism can be life threatening. Usual therapy is administration of end-organ hormones in oral form.

1) If the stalk between the hypothalamus and anterior pituitary is severed, the pituitary would fail to cause the ultimate release of all of the following hormones except:
   a) ACTH.
   b) estradiol.
   c) oxytocin.
   d) testosterone.
   e) thyroxine.

**Answers:**

1) C Oxytocin is released from posterior pituitary. A, B, D, and E all require releasing hormones from hypothalamus for anterior pituitary to release them.
Biochemistry and Disorder of Hormones of the Thyroid and Parathyroid Glands

1. The Thyroid Gland

The thyroid gland (Greek *thyros* “shield”) is shaped like a shield and lies just below the Adam’s apple in the front of the neck.

The thyroid gland secretes thyroxine and smaller amounts of triiodothyronine (T3), which stimulate oxidative respiration in most cells in the body and, in so doing, help set the body’s basal metabolic rate. In children, these thyroid hormones also promote growth and stimulate maturation of the central nervous system. Children with underactive thyroid glands are therefore stunted in their growth and suffer severe mental retardation, a condition called cretinism. This differs from pituitary dwarfism, which results from inadequate GH and is not associated with abnormal intellectual development.

People who are hypothyroid (whose secretion of thyroxine is too low) can take thyroxine orally, as pills. Only thyroxine and the steroid hormones (as in contraceptive pills), can be taken orally because they are nonpolar and can pass through the plasma membranes of intestinal epithelial cells without being digested.

The thyroid gland also secretes calcitonin, a peptide hormone that plays a role in maintaining proper levels of calcium (Ca++) in the blood. When the blood Ca++ concentration rises too high, calcitonin stimulates the uptake of Ca++ into bones, thus lowering its level in the blood.
Thyroid Hormones

1. Thyroxin (T4) and triiodothyronine T3

Biochemistry of Thyroid Hormones

Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The two principal thyroid hormones are:

- **thyroxine** (T₄ or L-3,5,3’,5’-tetraiodothyronine)
- **triiodothyronine** (T₃ or L-3,5,3’-triiodothyronine).

**Thyroid hormones are basically two tyrosines linked together with the critical addition of iodine at three or four positions on the aromatic rings.** The number and position of the iodines is important. Several other iodinated molecules are generated that have little or no biological activity; so called "reverse T₃ "

![Chemical structures of Thyroxine (T4), Triiodothyronine (T3), and Reverse T3](image)

A large majority of the thyroid hormone secreted from the thyroid gland is T₄, but T₃ is the considerably more active hormone. Although some T₃ is also secreted, the bulk of the T₃ is derived by deiodination of T₄ in peripheral tissues, especially liver and kidney. Deiodination of T₄ also yields reverse T₃, a molecule with no known metabolic activity.

Thyroid hormones are poorly soluble in water, and more than 99% of the T₃ and T₄ circulating in blood is bound to carrier proteins. The principle carrier of thyroid hormones is thyroxine-binding globulin, a glycoprotein synthesized in the liver. Another carrier is albumin.
**Synthesis and Secretion of Thyroid Hormones**

The entire synthetic process occurs in three major steps:

- Production and accumulation of the raw materials
- Fabrication or synthesis of the hormones on a backbone or scaffold of precursor
- Release of the free hormones from the scaffold and secretion into blood

**Raw materials:**

- **Tyrosines** are provided from a large glycoprotein scaffold called **thyroglobulin**.
  
  A molecule of thyroglobulin contains 134 tyrosines, although only a handful of these are actually used to synthesize $T_4$ and $T_3$.

- **Iodine**, or more accurately iodide ($I$), is taken up from blood by thyroid epithelial cells, which have on their outer plasma membrane an "iodine trap". Once inside the cell, iodide is transported into the lumen of the follicle along with thyroglobulin.

**Fabrication of thyroid hormones is conducted by the enzyme **thyroid peroxidase**.**

Thyroid peroxidase catalyzes two sequential reactions:

1. Iodination of tyrosines on thyroglobulin (also known as "organification of iodide").
2. Synthesis of thyroxine or triiodothyronine from two iodotyrosines.

**Thyroid hormones are excised from their thyroglobulin scaffold by digestion in lysosomes of thyroid epithelial cells.** Free thyroid hormones apparently diffuse out of lysosomes, through the basal plasma membrane of the cell, and into blood where they quickly bind to carrier proteins for transport to target cells.
Control of Thyroid Hormone Synthesis and Secretion

Each of the processes described above appears to be stimulated by thyroid-stimulating hormone from the anterior pituitary gland. Binding of TSH to its receptors on thyroid epithelial cells stimulates synthesis of the iodine transporter, thyroid peroxidase and thyroglobulin. When TSH levels are low, rates of thyroid hormone synthesis and release diminish. The thyroid gland is part of the hypothalamic-pituitary-thyroid axis, and control of thyroid hormone secretion is exerted by classical negative feedback.

Thyroid Hormone Receptors and Mechanism of Action

Receptors for thyroid hormones are intracellular DNA-binding proteins that function as hormone-responsive transcription factors, very similar conceptually to the receptors for steroid hormones. Thyroid hormones enter cells through membrane transporter proteins. A number of plasma membrane transporters have been identified, some of which require ATP hydrolysis; the relative importance of different carrier systems is not yet clear and may differ among tissues. Once inside the nucleus, the hormone binds its receptor, and the hormone-receptor complex interacts with specific sequences of DNA in the promoters of responsive genes. The effect of receptor binding to DNA is to modulate gene expression, either by stimulating or inhibiting transcription of specific genes.
**Metabolic Effects of Thyroid Hormones**

Thyroid hormones have profound effects on many physiologic processes, such as development, growth and metabolism. They stimulate diverse metabolic activities most tissues, leading to an **increase in basal metabolic rate**. One consequence of this activity is to **increase body heat production**, which seems to result, at least in part, from increased oxygen consumption and rates of ATP hydrolysis. A few examples of specific metabolic effects of thyroid hormones include:

- **Lipid metabolism**: Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels - one diagnostic indication of hypothyroidism is increased blood cholesterol concentration.

- **Carbohydrate metabolism**: Thyroid hormones stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-dependent entry of glucose into cells and increased gluconeogenesis and glycogenolysis to generate free glucose.

**Other Effects**: A few additional, effects of thyroid hormones include:

- **On muscle**: T3 increases glucose uptake by muscle cells it also stimulate protein synthesis and therefore growth of muscle through its stimulatory actions on gene expression. Thyroid hormone sensitizes the muscle cell to the glycogenolytic actions of epinephrine. Glycolysis in muscle is increased by this action of T₃.

- **On the pancreas**: thyroid hormone increases the sensitivity of the β cells of the pancreas to those stimuli that normally promote insulin release and is required for optimal insulin secretion

- **On Cardiovascular system**: Thyroid hormones increases heart rate, cardiac contractility and cardiac output. They also promote vasodilation, which leads to enhanced blood flow to many organs.
• **On Central nervous system:** Both decreased and increased concentrations of thyroid hormones lead to alterations in mental state. Too little thyroid hormone, and the individual tends to feel mentally sluggish, while too much induces anxiety and nervousness.

• **On Reproductive system:** Normal reproductive behavior and physiology is dependent on having essentially normal levels of thyroid hormone. Hypothyroidism in particular is commonly associated with infertility.

**Thyroid Disease States**

1. **Hypothyroidism**
2. **Hyperthyroidism**
   a. Graves's disease.

2. **Calcitonin**

Calcitonin is a hormone secreted from the parafollicular of C cells in the thyroid gland, known to participate in calcium and phosphorus metabolism.

Calcitonin is a 32 amino acid peptide cleaved from a larger prohormone. It contains a single disulfide bond, which causes the amino terminus to assume the shape of a ring.

**Physiologic Effects of Calcitonin**

Calcitonin plays a role in calcium and phosphorus metabolism. In particular, calcitonin has the ability to decrease blood calcium levels at least in part by effects on two well-studied target organs:

- **Bone:** Calcitonin suppresses resorption of bone by inhibiting the activity of osteoclasts, a cell type that "digests" bone matrix, releasing calcium and phosphorus into blood.

- **Kidney:** Calcium and phosphorus are prevented from being lost in urine by reabsorption in the kidney tubules. Calcitonin inhibits tubular reabsorption of these two ions, leading to increased rates of their loss in urine.
Control of Calcitonin Secretion

The most prominent factor controlling calcitonin secretion is the extracellular concentration of ionized calcium. Elevated blood calcium levels strongly stimulate calcitonin secretion, and secretion is suppressed when calcium concentration falls below normal.

Disease States

A large number of diseases are associated with abnormally increased or decreased levels of calcitonin, but pathologic effects of abnormal calcitonin secretion *per se* are not generally recognized.

2. The Parathyroid Glands and Calcium Homeostasis

The parathyroid glands are four small glands attached to the thyroid. Because of their size, researchers ignored them until well into this century.

The hormone produced by the parathyroid glands is a peptide called parathyroid hormone (PTH). It is one of only two hormones in humans that are absolutely essential for survival (the other is aldosterone). PTH is synthesized and released in response to falling levels of Ca++ in the blood.

Hormones that regulate calcium metabolism

Calcium ions regulate a number of important physiological and biochemical process. These include:

1. neuromuscular excitability
2. blood coagulation
3. secretory processes
4. membrane integrity and plasma membrane transport
5. enzyme reactions
6. the release of hormones and neurotransmitters
7. and the intracellular action of a number of hormones.
In addition the proper extracellular fluid and periosteal concentration of $\text{Ca}^{2+}$ and $\text{PO}_4^{-3}$ are required for bone mineralization.
To ensure that these processes operate normally, the plasma Ca\(^{+2}\) concentration is maintained within very narrow limits by the actions of the following hormones:

1. *Parathyroid Hormone (PTH)*

Parathyroid hormone is the most important endocrine regulator of calcium and phosphorus concentration in extracellular fluid. This hormone is secreted from cells of the parathyroid glands and finds its major target cells in bone and kidney. Like most other protein hormones, parathyroid hormone is synthesized as a preprohormone. After intracellular processing, the mature hormone is packaged within the Golgi into secretory vesicles, then secreted into blood by exocytosis. Parathyroid hormone is secreted as a linear protein of 84 amino acids.

**Physiologic Effects of Parathyroid Hormone**

If calcium ion concentrations in extracellular fluid fall below normal, PTH brings them back within the normal range. In conjunction with increasing calcium concentration, the concentration of phosphate ion in blood is reduced. Parathyroid hormone accomplishes its job by stimulating at least three processes:

- **Mobilization of calcium from bone:** Although the mechanisms remain obscure, a well-documented effect of parathyroid hormone is to stimulate osteoclasts to reabsorb bone mineral, liberating calcium into blood.

- **Enhancing absorption of calcium from the small intestine:** Facilitating calcium absorption from the small intestine would clearly serve to elevate blood levels of calcium. Parathyroid hormone stimulates this process, but indirectly by stimulating production of the active form of vitamin D in the kidney. Vitamin D induces synthesis of a calcium-binding protein in intestinal epithelial cells that facilitates efficient absorption of calcium into blood.
• **Suppression of calcium loss in urine:** In addition to stimulating fluxes of calcium into blood from bone and intestine, parathyroid hormone puts a brake on excretion of calcium in urine, thus conserving calcium in blood. This effect is mediated by stimulating tubular reabsorption of calcium. Another effect of parathyroid hormone on the kidney is to stimulate loss of phosphate ions in urine.

**Control of Parathyroid Hormone Secretion**

Parathyroid hormone is released in response to low extracellular concentrations of free calcium. Changes in blood phosphate concentration can be associated with changes in parathyroid hormone secretion, but this appears to be an indirect effect and phosphate *per se* is not a significant regulator of this hormone. When calcium concentrations fall below the normal range, there is a steep increase in secretion of parathyroid hormone. Low levels of the hormone are secreted even when blood calcium levels are high.

**Disease States**

Excessive secretion of parathyroid hormone is seen in two forms:

- **Primary hyperparathyroidism** is the result of parathyroid gland disease.
- **Secondary hyperparathyroidism** is the situation where disease outside of the parathyroid gland leads to excessive secretion of parathyroid hormone. A common cause of this disorder is kidney disease. It can also result from inadequate nutrition - for example, diets that are deficient in calcium or vitamin D, or which contain excessive phosphorus.
Inadequate production of parathyroid hormone - hypoparathyroidism - typically results in decreased concentrations of calcium and increased concentrations of phosphorus in blood. Common causes of this disorder include surgical removal of the parathyroid glands and disease processes that lead to destruction of parathyroid glands.

2. Vitamin D₃

Vitamin D is a fat-soluble steroid hormone precursor that contributes to the maintenance of normal levels of calcium and phosphorus in the bloodstream. It is also known as calciferol.

Vitamin D₃ is produced in the skin by conversion of 7-dehydrocholesterol by UV. Calciferol travels in the blood to the liver where it is converted into 25[OH] Vitamin D₃. This compound travels to the kidney where it is converted into Calcitriol (1,25[OH]₂ Vitamin D₃). This final step is promoted by the PTH.

Although called a vitamin, calciferol and its products fully qualify as hormones because they are:

- Made in certain cells
- Carried in the blood
- Affect gene transcription in target cells

Diseases

Vitamin D deficiency is known to cause several bone diseases, due to insufficient calcium or phosphate in the bones:

- **Rickets:** a childhood disease characterized by failure of growth and deformity of long bones.
- **Osteoporosis:** a condition characterized by fragile bones.
- **Osteomalacia:** a bone-thinning disorder in adults that is characterised by proximal muscle weakness and bone fragility. Osteomalacia can only occur in a mature skeleton.

3. **Calcitonin (discussed earlier)**

The following table summarizes body responses to conditions that would otherwise lead to serious imbalances in calcium and phosphate levels in blood.
<table>
<thead>
<tr>
<th></th>
<th>Calcium Deprivation</th>
<th>Calcium Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parathyroid hormone</strong></td>
<td>Secretion stimulated</td>
<td>Secretion inhibited</td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
<td>Production stimulated by increased parathyroid hormone secretion</td>
<td>Synthesis suppressed due to low parathyroid hormone secretion</td>
</tr>
<tr>
<td><strong>Calcitonin</strong></td>
<td>Very low level secretion</td>
<td>Secretion stimulated high blood calcium</td>
</tr>
<tr>
<td><strong>Intestinal absorption of calcium</strong></td>
<td>Enhanced due to activity of vitamin D on intestinal epithelial cells</td>
<td>Low basal uptake</td>
</tr>
<tr>
<td><strong>Release of calcium and phosphate from bone</strong></td>
<td>Stimulated by increased parathyroid hormone and vitamin D</td>
<td>Decreased due to low parathyroid hormone and vitamin D</td>
</tr>
<tr>
<td><strong>Renal excretion of calcium</strong></td>
<td>Decreased due to enhanced tubular reabsorption stimulated by elevated parathyroid hormone and vitamin D; hypocalcemia also activates calcium sensors in loop of Henle to directly facilitate calcium reabsorption</td>
<td>Elevated due to decreased parathyroid hormone-stimulated reabsorption.</td>
</tr>
<tr>
<td><strong>Renal excretion of phosphate</strong></td>
<td>Strongly stimulated by parathyroid hormone; this phosphaturic activity prevents adverse effects of elevated phosphate from bone resorption</td>
<td>Decreased due to hypoparathyroidism</td>
</tr>
<tr>
<td><strong>General Response</strong></td>
<td>Typically seen near normal serum concentrations of calcium and phosphate due to compensatory mechanisms. Long term deprivation leads to bone thinning (osteopenia).</td>
<td>Low intestinal absorption and enhanced renal excretion guard against development of hypercalcemia.</td>
</tr>
<tr>
<td><strong>Summary</strong></td>
<td><img src="image1.png" alt="Diagram of Calcium Deprivation" /></td>
<td><img src="image2.png" alt="Diagram of Calcium Loading" /></td>
</tr>
</tbody>
</table>
Clinical Correlations
A 48-year-old woman was admitted to the hospital because of weight loss, palpitation, weakness, and exophthalmos. She stated that a goiter, which had been present for years, had recently begun to enlarge. She was extremely irritable, could not tolerate heat and was short of breath. Physical examination revealed bilateral eyelid lag. The thyroid gland was diffusely enlarged, and a bruit was audible over the right lobe. Her heart was enlarged, a prominent apical thrust was noted, and there was a soft systolic heart murmur along the left sternal border. Laboratory examination revealed that the hemoglobin was 1.8 mmol/L and that the hematocrit was 38%. The basal metabolic rate was 145% of normal. plasma T4 and T3 were grossly elevated, and the $^{131}\text{I}$ uptake by the thyroid gland was very high (18% in 4 hr). A diagnosis of hyperthyroidism was made.

Biochemical questions:
1. What are T3 and T4, and how are they related to the thyroid gland?
2. How are TRH and TSH involved in the regulation of thyroid hormone production and secretion?
3. What is the mechanism of increased $^{131}\text{I}$ uptake in hyperthyroidism?

T3 and T4, are the thyroid hormones, triiodothyronine and thyroxine, respectively. Both are tyrosine derivatives. Although lesser amounts of T3 are released by the thyroid gland, it has a more potent effect than T4 in producing the hyper metabolic effects of the thyroid hormones. T4 is converted to T3 in the target cells, and it is likely that T3, actually is the metabolically active form of the thyroid hormone. In this sense, T4 may be considered as a prohormone.

TSH is released from the anterior pituitary and stimulates T3 and T4 production and release. In turn, TSH release is stimulated by TRH, which is made in the hypothalamus. When the plasma T3 and T4 concentrations are elevated, TRH production and release are inhibited. This leads to decreased T3 and T4 release from the thyroid gland.

T3 and T4 contain iodine atoms attached to their phenolic rings. Thyroglobulin, the protein precursor of these hormones that is contained in the thyroid cells, has many iodinated tyrosine residues. The iodine is obtained from iodide ions in the blood plasma, and the thyroid-cells have the capacity to take up and concentrate iodide ions.
In hyperthyroidism the thyroid gland is more active than normal. It synthesizes more thyroglobulin, T3, and T4 and takes up much larger amounts of iodine than in the euthyroid (normal) state. Therefore, when $^{131}$I is administered to a hyperthyroid patient, a larger fraction of the dose is concentrated within the thyroid gland than in a euthyroid subject. This is useful clinically in two ways.

1. Small quantities of $^{131}$I can be administered as a diagnostic test of thyroid function. After administration, the radiation emanating from the thyroid grand can be measured at various times by placing a scanning device over the neck. Greater than normal uptakes occur in hyperthyroidism, and less than normal uptakes occur if the thyroid gland is hypofunctioning (hypothyroidism).

2. The enhanced iodine uptake can be used to treat hyperthyroidism. If larger amounts of $^{131}$I are administered, enough $^{131}$I will concentrate in the thyroid to provide intense but localized radiation to the glandular cells. This will destroy many of the T3- and T4-producing cell, reducing the excessive function of the thyroid and correcting the hyperthyroidism.

As compared with the thyroid, other tissues take up very little iodine. Consequently, most of the $^{131}$I that is not taken up by the thyroid is rapidly excreted in the urine, and there is comparatively little radiation exposure in other tissues. In some respects this is a safer form of treatment than surgical removal of a large portion of the hyperactive gland. It is not without some danger, however, for $^{131}$I treatment can lead in some cases to either hypothyroidism or even thyroid cancer.
Biochemistry and Disorders of Hormones of the Pancreas

The bulk of the pancreas is an exocrine gland secreting pancreatic fluid into the duodenum after a meal.

However, scattered through the pancreas are several hundred thousand clusters of cells called **islets of Langerhans**. The islets are endocrine tissue containing four types of cells. In order of abundance, they are the:

1. **beta** cells, which secrete **insulin** and amylin.
2. **alpha** cells, which secrete **glucagon**;
3. **delta** cells, which secrete **somatostatin**, and
4. **gamma** cells, which secrete pancreatic polypeptide (PP).

**Beta Cells**

**Insulin** is a small protein consisting of

- an alpha chain of 21 amino acids linked by two disulfide (S—S) bridges to a
- beta chain of 30 amino acids.

Beta cells have channels in their plasma membrane that serve as glucose detectors. Beta cells secrete insulin in response to a rising level of circulating glucose ("blood sugar").

![Diagram of insulin production and release](image-url)
Insulin affects many organs.
1. It stimulates skeletal muscle fibers to
   - take up glucose and convert it into glycogen;
   - Take up amino acids from the blood and convert them into protein.
2. acts on liver cells
   - stimulating them to take up glucose from the blood and convert it into glycogen
   - inhibiting production of the enzymes involved in breaking glycogen back down
     ("glycogenolysis") and
   - inhibiting "gluconeogenesis"; that is, the conversion of fats and proteins into glucose.
3. acts on fat (adipose) cells to stimulate the uptake of glucose and the synthesis of fat.
4. acts on cells in the hypothalamus to reduce appetite.

**Actions of Insulin**

<table>
<thead>
<tr>
<th>Metabolic process</th>
<th>Reaction</th>
<th>consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycogenesis</td>
<td>Glucose to Glycogen</td>
<td>(-) Blood glucose</td>
</tr>
<tr>
<td>glycogenolysis</td>
<td>Glycogen to Glucose</td>
<td>(+) Blood glucose</td>
</tr>
<tr>
<td>gluconeogenesis</td>
<td>Amino acids to Glucose</td>
<td>(+) Blood glucose</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Amino acids to protein</td>
<td>(-) Blood amino acids</td>
</tr>
<tr>
<td>Protein degradation</td>
<td>Protein to Amino acids</td>
<td>(+) Blood amino acids</td>
</tr>
<tr>
<td>Fat synthesis (lipogenesis or</td>
<td>Fatty acids and glycerol to</td>
<td>(-) Blood fatty acids</td>
</tr>
<tr>
<td>triglyceride synthesis</td>
<td>triglycerides</td>
<td></td>
</tr>
<tr>
<td>Fat breakdown (lipolysis or</td>
<td>Triglycerides to Fatty acids and glycerol</td>
<td>(+) Blood fatty acids</td>
</tr>
<tr>
<td>triglycerides degradation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(-) decrease in
(+) increase in
Amylin
Amylin is a peptide of 37 amino acids, which is also secreted by the beta cells of the pancreas.
Some of its actions:
• inhibits the secretion of glucagon;
• slows the emptying of the stomach;
• sends a satiety signal to the brain.
Amylin (IAPP) was identified independently by two groups as the major component of diabetes-associated islet amyloid deposits in 1987

Alpha Cells
The alpha cells of the islets secrete glucagon, a polypeptide of 29 amino acids. Glucagon acts principally on the liver where it stimulates the conversion of
• glycogen into glucose ("glycogenolysis") and
• fat and protein into intermediate metabolites that are ultimately converted into glucose ("gluconeogenesis")
In both cases, the glucose is deposited in the blood.
Glucagon secretion is
• stimulated by low levels of glucose in the blood;
• inhibited by high levels of glucose in the blood, and
• inhibited by amylin.
The physiological significance of this is that glucagon functions to maintain a steady level of blood sugar level between meals.

Delta Cells
The delta cells secrete somatostatin. This consists of two polypeptides, one of 14 amino acids and one of 28. Somatostatin has a variety of functions. Taken together, they work to reduce the rate at which food is absorbed from the contents of the intestine. Somatostatin is also secreted by the hypothalamus and by the intestine.

Gamma Cells
The gamma cells of the islets secrete a 36-amino-acid pancreatic polypeptide. Its function is to self regulate the pancreas secretion activities. It also has effects on hepatic glycogen levels and gastrointestinal secretions. Its secretion human is increased after a protein meal, fasting, exercise and acute hypoglycemia and is decreased by somatostatin..
Synthesis and release of insulin and glucagon

Insulin and glucagon are synthesized in different cell types of the endocrine pancreas, which consists of microscopic clusters of small glands (the islets of Langerhans). The α cells secrete glucagon, and the β cells secrete insulin into the hepatic portal vein via the pancreatic veins.

- Synthesis and secretion of Insulin

Insulin is a polypeptide hormone. The active form of insulin is composed of two polypeptide chains (the A-chain and the B-chain) linked by two interchain disulfide bonds. The A-chain has an additional intrachain disulfide bond.

![Diagram of Insulin Synthesis and Secretion](image)

Insulin, like many other polypeptide hormones, is synthesized as a preprohormone that is converted in the rough endoplasmic reticulum (RER) to proinsulin. The "pre" sequence, a short hydrophobic signal sequence at the N-terminal end, is cleaved as it enters the lumen of the RER. Proinsulin folds into the proper conformation and disulfide bonds are formed between the cysteine residues. It is then transported in microvesicles to the Golgi complex. It leaves the Golgi complex in storage vesicles, where a protease removes the C-peptide (a fragment with no hormonal activity) and a few small remnants, resulting in the formation of biologically active insulin. Zinc ions are also transported in these storage vesicles. Cleavage of the C-peptide decreases the solubility of the resulting insulin, which then coprecipitates with zinc. Exocytosis of the insulin storage vesicles from the cytosol of the β cell into the blood is stimulated by rising levels of glucose in the blood bathing the β cells. Glucose enters the β cell via specific glucose transporter proteins known as GLUT2. Glucose is phosphorylated through the action of glucokinase to form glucose 6-phosphate, which is metabolized through glycolysis, the TCA cycle, and oxidative phosphorylation.
These reactions result in an increase in ATP levels within the β cell. As the β cell [ATP]/[ADP] ratio increases, the activity of a membrane-bound, ATP-dependent K⁺ channel is inhibited (i.e., the channel is closed). The closing of this channel leads to a membrane depolarization, which activates a voltage-gated Ca²⁺ channel that allows Ca⁺ to enter the β cell such that intracellular Ca²⁺ levels increase significantly. The increase in intracellular Ca²⁺ stimulates the fusion of insulin containing exocytotic vesicles with the plasma membrane, resulting in insulin secretion. Thus, an increase in glucose levels within the β cells initiates insulin release.

- **Stimulation and inhibition of insulin release**

  The release of insulin occurs within minutes after the pancreas is exposed to a high glucose concentration. The threshold for insulin release is approximately 80 mg/glucose/dL. Above 80 mg/dL, the rate of insulin release is not an all-or-nothing response but is proportional to the glucose concentration up to approximately 300 mg/dL glucose. As insulin is secreted, the synthesis of new insulin molecules is stimulated, so that secretion is maintained until blood glucose levels fall. Insulin is rapidly removed from the circulation and degraded by the liver and to a lesser extent by kidney and skeletal muscle) so that blood insulin levels decrease rapidly.

  A number of factors other than the blood glucose concentration can modulate insulin such as:

  1. neural signals
  2. certain amino acids
  3. gastric inhibitory polypeptide (GIP, a gut hormone released after the ingestion of food)
  4. epinephrine secreted in response to fasting, stress, trauma and vigorous exercise decrease the release of insulin
• Synthesis and secretion of Glucagon

Glucagon, a polypeptide hormone, is synthesized in the α cells of the pancreas by cleavage of the much larger preproglucagon, a 160-amino acid peptide. Like insulin, preproglucagon is produced on the rough endoplasmic reticulum and is converted to proglucagon as it enters the ER lumen. Proteolytic cleavage at various sites produce the mature 29-amino acid glucagon and larger glucagon-containing fragments (named glucagon-like peptides 1 and 2). Glucagon is rapidly metabolized, primarily in the liver and kidneys. Its plasma half-life is only about 3 to 5 minutes.

Glucagon secretion is regulated principally by circulating levels of glucose and insulin. Increasing levels of each inhibit glucagon release. Glucose probably has both a direct suppressive effect on secretion of glucagon from the α cell as well as an indirect effect, the latter being mediated by its ability to stimulate the release of insulin.

Certain hormones stimulate glucagon secretion:
1) catecholamines (epinephrine)
2) cortisol
3) gut hormones
4) Many amino acids also stimulate glucagon release.

**Metabolic effects of glucagon**

1. **Effects on carbohydrate metabolism**: The intravenous administration of glucagon leads to an immediate rise in blood glucose. This results from an increase in the breakdown of liver (not muscle) glycogen and an increase in gluconeogenesis.

2. **Effects on lipid metabolism**: Glucagon favors hepatic oxidation of fatty acids and the subsequent formation of ketone bodies acetyl CoA. The lipolytic effect of glucagon in adipose tissue is minimal in humans.
3. Effects on protein metabolism: Glucagon increases uptake of amino acids by the liver, resulting in increased availability of carbon skeletons for gluconeogenesis. As a consequence plasma levels of amino acids are decreased.

**DIABETES MELLITUS:**
This is a metabolic disorder occurring as a result of an insulin lack or a surplus of insulin antagonists leading to a relative insulin lack. It is characterized by hyperglycemia and glucosuria. Three types of the disease are described-

1. **Juvenile type (type 1)** in children and is due to an absolute deficiency of insulin.

2. **Maturity onset type**: It is usually associated with obesity. A form of diabetes known as MODY (maturity onset diabetes of the young) results from mutations in either pancreatic glucokinase or specific nuclear transcription factors. Thus, although these patients can release insulin, they do so at higher than normal glucose levels, and are therefore almost always in a hyperglycemic state. Interestingly, however, these patients are somewhat resistant to the long-term complications of chronic hyperglycemia.

3. **Secondary diabetes**: There is hyperfunction of one or other of insulin anatgonists leading to a relative insufficiency of insulin eg: acromegaly (excess of growth hormone); Cushing's disease (excess of glucocorticoids); hyperthyroidism etc.

Glucagon is one of the contributory factors in the etiology of diabetes mellitus. Its blood levels are elevated in severe diabetes with ketoacidosis. The α cells seem to be insensitive to the high blood glucose levels in diabetes and continue to secrete large amounts of glucagon.

Somatostatin, a hypothalamic factor inhibiting the release of growth hormone, also inhibits the release of glucagon and is in experimental use as an adjunct to insulin in the control of severe diabetes mellitus.

4. **Type 2 diabetes**: a combination of insulin resistance and dysfunctional β cells.

**Insulin resistance:** Is the decreased ability of target tissues, such as liver adipose and muscle to respond properly to normal circulating concentrations of insulin. For example, insulin resistance is characterized by uncontrolled hepatic glucose production and decreased glucose uptake by muscle and adipose tissue.

In a moderately severe early diabetes mellitus the following features are present-

1. Hyperglycemia.
2. Glycosuria.
3. Loss of weight due to increased breakdown of fat and tissue protein.
4. Increased production of ketone bodies by the liver and their incomplete utilization by tissues leading to their accumulation in blood (ketosis) and elimination in urine (ketonuria).
5. Lowering of the pH of blood due to circulating keto acids (acidosis).
6. Dehydration due to elimination of large amounts of water with glucose in urine.
7. Negative nitrogen balance due to conversion of more amino acids into glucose (increased gluconeogenesis).
8. Increased levels of lipid, fatty, acid and cholesterol in blood (lipemia).
9. Increased tendency to develop cataract in the eye and arteriosclerotic lesions of blood vessels.

Hyperinsulinism; This may occur on account of islet-cell tumors involving the β-cells. There is overproduction of insulin resulting in spontaneous attacks of hypoglycemia associated with sweating, and fainting attacks which are relieved by ingestion of glucose or a lump of sugar.
Insulin and Glucagon receptors

1. Insulin Receptor

**Insulin Binding to its Receptor Followed by Activation of the Receptor:**

1. Insulin binds switching ON the receptor. Once the receptor is ON (catalytically active) insulin may disassociate and be degraded.

2. The Tyr Kinase domain is phosphorylated.

3. A "cascade" of events takes place (see below).

4. Biochemical / Physiological Responses:
Representative pathway for the activation of cAMP-dependent protein kinase, PKA. In this example glucagon binds to its cell-surface receptor, thereby activating the receptor. Activation of the receptor is coupled to the activation of a receptor-coupled G-protein (GTP-binding and hydrolyzing protein) composed of 3 subunits. Upon activation the \( \alpha \)-subunit dissociates and binds to and activates adenylate cyclase. Adenylate cyclase then converts ATP to cyclic-AMP (cAMP). The cAMP thus produced then binds to the regulatory subunits of PKA leading to dissociation of the associated catalytic subunits. The catalytic subunits are inactive until dissociated from the regulatory subunits. Once released the catalytic subunits of PKA phosphorylate numerous substrate using ATP as the phosphate donor.
Clinical cases and correlations

Insulinoma

A 36-year-old woman was referred to a university hospital for evaluation of spells of dizziness and weakness. These spells typically lasted for 10 min and were occurring with increasing frequency. The spells usually came on after a large meal and could be terminated by her eating candy or drinking fruit juice. After each episode the patient was hungry and tired, and her memory was blurred. The patient's physical examination was within normal limits except for mild obesity. She claimed to have gained 20 kg during the preceding 2 yr. After a 13-hr fast her blood glucose concentration was 2.1 mmol/L. After a 5-hr glucose tolerance test, her blood glucose was 2.6 mmol/L.

Celiac angiography revealed an abnormality in the body and tail of the pancreas. The patient developed one of her spells while a medical student was in her room, and he was able to obtain a blood sample during the episode. This sample contained 1.1 mmol/L of glucose. The patient was transferred to the surgical service, and an insulin-secreting pancreatic adenoma (tumor) was removed, requiring resection of 90% of the pancreas.

Biochemical questions
1. An insulinoma is an insulin-secreting tumor. How did the presence of such a tumor explain the patient's symptoms?
2. Proinsulin was found in large quantities in this patient's plasma. What is the relationship of proinsulin to insulin? What is preproinsulin?
3. What effects of increased insulin secretion might have predisposed this woman to obesity?
4. What digestive problems might result from excision of 90% of the pancreas?

The insulinoma was producing insulin. Because of the excessive amount of insulin-secreting tissue, too much insulin was released after dietary carbohydrate intake. This caused hypoglycemia during the 5-hr glucose tolerance test and after meals, producing the spells of weakness and dizziness. In addition, to this normal insulin release when carbohydrate was ingested, the tumor also was secreting some insulin continuously. This inappropriate insulin release caused the low blood glucose concentration during prolonged fasting.

Proinsulin is the prohormone form of insulin that is made in the β-cells of the pancreatic islets. It has no insulin-like action. After synthesis on the ribosomes, the initial precursor, preproinsulin, penetrates through the endoplasmic reticulum into the lumen of this
organelle. The leader sequence is removed in this process, forming proinsulin, which is transported to the Golgi apparatus and stored in granules.

Proinsulin is converted to insulin in these granules by proteolytic cleavage, but the conversion is incomplete. When insulin is discharged from the β-cell, some proinsulin that remains in the granule also is released. Likewise, C-peptide that is split out in the conversion of proinsulin to insulin is released during insulin secretion, but it too, has no insulin-like activity.

Insulin acts on adipocytes, enhancing fatty acid storage as triglyceride. It binds to specific receptors on the cell surface and facilitates glucose entry into the adipocyte, increasing the availability of the triose backbone, glycerol 3-phosphate, needed for triglyceride synthesis. This also provide glucose carbon atoms for fatty acid synthesis.

In addition, it increases the content of lipoprotein lipase in the adipose tissue. This enzyme catalyzes the hydrolysis of chyomicron and VLDL triglycerides, a step that is required to transfer their fatty acids into the adipocytes for resynthesis into triglyceride. Much of the fatty acid stored in the adipose tissue is delivered to the adipocytes in the form of lipoprotein triglycerides, either VLDL from the liver or chylomicrons from the intestine. Therefore the elevated lipoprotein lipase activity also favors triglyceride formation in the adipose tissue. Those adipose tissue effects that were mediated by the excessive insulin production could have contributed to the recent weight gain noted by this patient.

In addition to polypeptide hormones, the pancreas making many digestive enzymes. These include amylase for dietary starches, lipase for triglycerides, chymotrypsin and trypsin for proteins, as well as several others. Since 90%, of the pancrease was excised, the remaining 10% may not produce sufficient amounts of these enzymes to adequately digest large meals. This might lead to malnutrition and weight loss in spite of an adequate diet. Because of this possibility, six or more smaller meals rather than three regular meals each day might be recommended.

Question: Binding of insulin to its receptor:
   a) Occurs on the β-subunit.
   b) **Induces autophosphorylation.**
   c) Reduces binding of cytosolic substrate proteins.
   d) Leads only to phosphorylation of proteins.
   e) Does not lead to release of a second messenger.

Answer: **B** This occurs on tyrosine residues of the β-subunit. A: Binding is to the α-subunit. C: Autophosphorylation facilitates binding. D: Some proteins are dephosphorylated. E: A second messenger may account for short-term metabolic effects.
Hormones of the adrenal gland

1. Hormones of the adrenal cortex
Hormones of the adrenal gland

The structure of the adrenal gland

The two adrenal glands (also called the suprarenal glands) are situated in the abdomen, on either side of the vertebral column, above the kidneys and below the diaphragm. When cut in half each gland consists of

1. An outer cortex, yellow in color and
2. An inner medulla, which is dark red, or grey.
The cortex consists of three distinct zones
1. Zona glomerulosa
2. Zona fasciculata
3. Zona reticularis
Each zone has a characteristic histology and secretes different types of hormones

<table>
<thead>
<tr>
<th>Layer</th>
<th>Name</th>
<th>Primary product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most superficial cortical</td>
<td>Zona glomerulosa</td>
<td>mineralocorticoid (aldosterone) which is responsible for the regulation of salt and water balance in the body</td>
</tr>
<tr>
<td>layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle cortical layer</td>
<td>Zona fasciculata</td>
<td>glucocorticoid (cortisol) which regulates the level of carbohydrate in the body</td>
</tr>
<tr>
<td>Deepest cortical layer</td>
<td>Zona reticularis</td>
<td>sex hormones (progesterone, oestrogen precursors and androgens) which have a role in the development of sexual characteristics</td>
</tr>
</tbody>
</table>
The medulla consists of many large columnar cells called "chromaffin cells". These synthesize and secrete catecholamines when stimulated by the sympathetic nervous system.

**Hormone synthesis**

All adrenocortical hormones are synthesized from cholesterol. Cholesterol is transported into the adrenal gland. Subsequent steps to generate aldosterone and cortisol, primarily occur in the adrenal cortex:

- Progesterone $\rightarrow$ (hydroxylation at C21) $\rightarrow$ 11-Deoxycorticosterone $\rightarrow$ (two further hydroxylations at C11 and C18) $\rightarrow$ Aldosterone

- Progesterone $\rightarrow$ (hydroxylation at C17) $\rightarrow$ 17-alpha-hydroxyprogesterone $\rightarrow$ (hydroxylation at C21) $\rightarrow$ 11-Deoxycortisol $\rightarrow$ (hydroxylation at C11) $\rightarrow$ Cortisol

Cholesterol is converted to pregnenolone (P5) by cytochrome P450 cholesterol side chain cleavage. P5 is the precursor of all the other steroids and stands at the first branch point in the adrenal steroidogenic network.

Steroidogenic defects can cause congenital adrenal hyperplasia (CAH). This condition may cause symptoms ranging from mild acne to salt wasting, depending on the nature of the genetic mutation.

In hypoandrenocorticism (Addison's disease) and CAH the error involves the two enzymes cytochrome P450 17a-hydroxylase/17-20 lyase and cytochrome P450 21-hydroxylase respectively.

Because of a lack of the glucocorticoids and mineralocorticoids the brain signals the adrenal gland with adrenocorticotropic hormone (ACTH) to produce more of the deficient steroids. Consequently, there is an over production of P5 that in turn leads to an over production of DHEA, which is converted to androgens and estrogens outside of the adrenal gland due to the DHEA in circulation diffusing into other steroidogenic tissues with the appropriate activities.

**Hormones secreted by the Adrenal Cortex**

1. **Mineralocorticoids**

The primary mineralocorticoids aldosterone is **aldosterone**. Its secretion is regulated by the oligopeptide angiotensin II (angiotensin II is regulated by angiotensin I, which in turn is regulated by renin). Aldosterone is secreted in response to high extracellular potassium levels, low extracellular sodium levels,
and low fluid levels and blood volume. Aldosterone affects metabolism in different ways:
   a. It increases urinary excretion of potassium ions
   b. It increases interstitial levels of sodium ions
   c. It increases water retention and blood volume

Removal of the adrenal glands leads to death within just a few days. Due to:
   1. the concentration of potassium in extracellular fluid becomes dramatically elevated
   2. urinary excretion of sodium is high and the concentration of sodium in extracellular fluid decreases significantly
   3. volume of extracellular fluid and blood decrease
   4. the heart begins to function poorly, cardiac output declines and shock ensues

Clearly mineralocorticoids are acutely critical for maintenance of life!
Aldosterone and Mineralocorticoid Receptors

Cortisol, have "weak mineralocorticoid activity", which is of some importance because cortisol is secreted very much more abundantly than aldosterone. i.e. a small fraction of the mineralocorticoid response in the body is due to cortisol rather than aldosterone.

The mineralocorticoid receptor binds both aldosterone and cortisol with equal affinity. Moreover, the same DNA sequence serves as a hormone response element for the activated (steroid-bound) forms of both mineralocorticoid and glucocorticoid receptors.

Q: **How can aldosterone stimulate specific biological effects in this kind of system, particularly when blood concentrations of cortisol are something like 2000-fold higher than aldosterone?**

A: In aldosterone-responsive cells, cortisol is effectively destroyed, allowing aldosterone to bind its receptor without competition. Target cells for aldosterone express the enzyme 11-beta-hydroxysteroid dehydrogenase, which has no effect on aldosterone, but converts cortisol to cortisone, which has only a very weak affinity for the mineralocorticoid receptor. In essence, this enzyme "protects" the cell from cortisol and allows aldosterone to act appropriately.

An interesting demonstration of this enzyme protection system is seen in chronic licorice intoxication. The major components of licorice are glycyrrhizic acid and its hydrolytic product, glycyrrhetinic acid (GE). GE is a potent inhibitor of 11ß-hydroxysteroid dehydrogenase. By blocking the activity of this inactivating enzyme, GE facilitates the binding of cortisol to renal mineralocorticoid receptors and hence induces AME syndrome (Apparent Mineralocorticoid Excess Syndrome).

Control of Aldosterone Secretion

The two most significant regulators of aldosterone secretion are:

- **Concentration of potassium ions in extracellular fluid**: Small increases in blood levels of potassium strongly stimulate aldosterone secretion.
- **Angiotensin II**: Activation of the renin-angiotensin system as a result of decreased renal blood flow (usually due to decreased vascular volume) results in release of angiotensin II, which stimulates aldosterone secretion.
Factors which suppress aldosterone secretion include atrial naturetic hormone, high sodium concentration and potassium deficiency.

Disease States: A deficiency in aldosterone can occur by itself or, more commonly, in conjunction with a glucocorticoid deficiency, and is known as hypoadrenocorticism or Addison's disease.

2. Glucocorticoids

Cortisol and Glucocorticoid Receptors
Cortisol binds to the glucocorticoid receptor in the cytoplasm and the hormone-receptor complex is then translocated into the nucleus, where it binds to its DNA response element and modulates transcription from a battery of genes, leading to changes in the cell's phenotype.

Only about 10% of circulating cortisol is free. The remaining majority circulates bound to plasma proteins, particularly corticosteroid-binding globulin (transcortin).

Metabolic Effects of Glucocorticoids
There seem to be no cells that lack glucocorticoid receptors and as a consequence, these steroid hormones have a huge number of effects on physiologic systems.

The name glucocorticoid derives from early observations that these hormones were involved in glucose metabolism.

Cortisol stimulates several processes that collectively serve to increase and maintain normal concentrations of glucose in blood. These effects include:

- **Stimulation of gluconeogenesis, particularly in the liver**: This pathway results in the synthesis of glucose from non-hexose substrates such as amino acids and lipids. Enhancing the expression of enzymes involved in gluconeogenesis is probably the best known metabolic function of glucocorticoids.

- **Mobilization of amino acids from extrahepatic tissues**: These serve as substrates for gluconeogenesis.

- **Inhibition of glucose uptake in muscle and adipose tissue**: A mechanism to conserve glucose.

- **Stimulation of fat breakdown in adipose tissue**: The fatty acids released by lipolysis are used for production of energy in tissues like muscle, and the released glycerol provide another substrate for gluconeogenesis.
Control of Cortisol Secretion

Cortisol and other glucocorticoids are secreted in response to a single stimulator: adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH is itself secreted under control of the hypothalamic peptide corticotropin-releasing hormone (CRH).

Virtually any type of physical or mental stress results in elevation of cortisol concentrations in blood due to enhanced secretion of CRH in the hypothalamus. This fact sometimes makes it very difficult to assess glucocorticoid levels, especially being restrained for blood sampling, is enough stress to artificially elevate cortisol levels several fold!

Cortisol secretion is suppressed by classical negative feedback loops. When blood concentrations rise above a certain threshold, cortisol inhibits CRH secretion from the hypothalamus, which turns off ACTH secretion, which leads to a turning off of cortisol secretion from the adrenal. The combination of positive and negative control on CRH secretion results in pulsatile secretion of cortisol. Typically, pulse amplitude and frequency are highest in the morning and lowest at night.

ACTH, also known as corticotropin, binds to receptors in the plasma membrane of cells in the adrenal. Hormone-receptor engagement activates adenyl cyclase, leading to elevated intracellular levels of cyclic AMP which leads ultimately to activation of the enzyme systems involved in biosynthesis of cortisol from cholesterol.

Disease States

1. Cushings disease or hyperadrenocorticism.
2. Insufficient production of cortisol, often accompanied by an aldosterone deficiency, is called Addison's disease or hypoadrenocorticism.
3. Androgens

The most important androgens include:

1. Testosterone: a hormone with a wide variety of effects, ranging from enhancing muscle mass and stimulation of cell growth to the development of the secondary sex characteristics.

2. Dihydrotestosterone (DHT): a metabolite of testosterone, and a more potent androgen than testosterone in that it binds more strongly to androgen receptors.

3. Androstenedione (Andro): an androgenic steroid produced by the testes, adrenal cortex, and ovaries. While androstenediones are converted metabolically to testosterone and other androgens, they are also the parent structure of estrone.

4. Dehydroepiandrosterone (DHEA): It is the primary precursor of natural estrogens. DHEA is also called dehydroisoandrosterone or dehydroandrostosterone.
2. Hormones of the adrenal Medulla
Hormones secreted by the Adrenal Medulla

Cells in the adrenal medulla synthesize and secrete epinephrine and norepinephrine. Following release into blood, these hormones bind adrenergic receptors on target cells, where they induce essentially the same effects as direct sympathetic nervous stimulation.

Synthesis of catecholamines begins with the amino acid tyrosine, which is taken up by chromaffin cells in the medulla and converted to norepinephrine and epinephrine through the following steps:

Norepinephrine and epinephrine are stored in electron-dense granules which also contain ATP and several neuropeptides. Secretion of these hormones is stimulated by acetylcholine release. Many types of "stresses" stimulate such secretion, including exercise, hypoglycemia and trauma. Following secretion into blood, the catecholamines bind loosely to and are carried in the circulation (50%) by albumin and other serum proteins. Once secreted their half life in the circulation is short (approximately 12 min) but
they have a large effect on heart, vessels, metabolism, brain, muscles etc. all as part of stress responses.

**Adrenergic Receptors and Mechanism of Action**

The physiologic effects of epinephrine and norepinephrine are initiated by their binding to adrenergic receptors on the surface of target cells. These receptors are prototypical examples of seven-pass transmembrane proteins that are coupled to G proteins which stimulate or inhibit intracellular signaling pathways.

There are two major classes of adrenergic receptors these are:

1. $\alpha$ adrenergic receptor (epinephrine and norepinephrine)
   a. $\alpha_1$
   b. $\alpha_2$

2. $\beta$ adrenergic receptor (epinephrine)
   a. $\beta_1$
   b. $\beta_2$

**Control of catecholamine release**

The release of the catecholamines is controlled from nerve cells within the posterior hypothalamus which can ultimately stimulate acetylcholine release from nerve terminals of the sympathetic nerves. This induces depolarization of the chromaffin cells and exocytosis of the catecholamine containing granules following a rise in intracellular calcium concentration.

**Metabolic Effects of catecholamines Hormones**

- In general, circulating epinephrine and norepinephrine released from the adrenal medulla have the same effects on target organs as direct stimulation by sympathetic nerves, although their effect is longer lasting.
- glycogenolysis to provide extra sources of glucose
- Stimulation of lipolysis in fat cells to provided fatty acids for energy production in many tissues and aids in conservation of dwindling reserves of blood glucose.
• Increased metabolic rate due to increased oxygen consumption and heat production increase throughout the body in response to epinephrine binding beta receptors.

• Increased breakdown of glycogen in skeletal muscle to provide glucose for energy production.

**Water and electrolytes metabolism**

• Decreased sodium excretion and glomerular filtration due to direct effects on the kidney

• Effects on renin secretion leads to increased aldosterone production with effects on distal sodium handling

• Serum potassium may be increased

**Catecholamine Degradation**

All catecholamines are rapidly eliminated from target cells and the circulation by three mechanisms:

1. reuptake into secretory vesicles
2. uptake in non-neural cells (mostly liver)
3. degradation.

Degradation relies on two enzymes:

1. catechol O-methyltransferase (COMT) in non-neuronal tissues
2. and monoamine oxidase (MAO) within neurons.

To produce metabolites (metanephrines and vanillylmandelic acid (VMA)) from free catecholamines.

Metabolites and free catecholamine are eliminated by direct filtration into the urine and excreted as:

1) free norepinephrine,
2) conjugated norepinephrine
3) metanephrines and
4) VMA

Urine epinephrine (50%) is converted from norepinephrine by renal (not adrenal) phenylethanolamine N-methyltransferase (PNMT) before excretion.
Clinical cases and Correlations:
Cushing’s syndrome
A 35-year-old man was admitted to the hospital because of irritability and emotional lability together with muscle weakness and easy fatigability. Physical examination revealed that his trunk was obese but his arms and legs were quite lean. He had a rounded facial appearance and a small, nontender hump at the junction of his neck and back. Fullness was noted in the supraclavicular regions, and purple striae were present in the subaxillary areas. Laboratory examination revealed that the plasma cortisol concentration was elevated (0.55 mmol/L), the fasting blood glucose was 8.3 mmol/L, and the urinary excretion of 11-hydroxyandrosterone and 11-hydroxyetiocholanolone were greatly increased. A diagnosis of Cushing's syndrome was made.

Biochemical questions
1. From what substance is cortisol synthesized?
2. How is cortisol synthesis regulated?
3. How is cortisol transported in the blood plasma?
4. What are the metabolic effects of cortisol in humans?
5. How does cortisol function at the molecular level?
6. How are 11-hydroxyandrosterone and 11-hydroxyetiocholanolone related to cortisol and why were they excreted in increased amounts in this patient's urine?
7. Would you expect that excessive quantities of vanillylmandelic acid (VMA) also would be excreted in this patient's urine?

Case discussion:
Cortisol, the main glucocorticoid hormone in humans, is synthesized in the zona fasciculata of the adrenal cortex. Cholesterol is the precursor of cortisol as well as of the approximately 50 other steroids that can be isolated from the adrenal cortex. Most of the cholesterol used for steroid hormone synthesis is taken up from plasma lipoproteins and stored in the adrenal cortical cells as cholesteryl esters. Progesterone is the common intermediate in steroid hormone synthesis.

Regulation of synthesis cortisol synthesis is regulated by ACTH, a polypeptide hormone secreted by the anterior pituitary gland. ACTH release, in turn, is stimulated by CRH, produced in the hypothalamus. Cortisol production is decreased by high concentrations of plasma cortisol or ACTH, both of which inhibit CRH release. ACTH stimulates cortisol production by activating the hydrolysis of cholesteryl esters through a cAMP-dependent mechanism.
**Transport** Like the other steroid hormones, cortisol is transported in the blood by a specific carrier protein, transcortin. This protein is a plasma globulin that is synthesized in the liver. It also transports corticosterone, the other major glucocorticoid produced by the adrenal cortex.

**Metabolic effects** One of the main metabolic effects of the glucocorticoid hormones is to raise the blood glucose concentration by stimulating gluconeogenesis. Body proteins are catabolized to provide the amino acid substrates for gluconeogenesis, leading to decreases in muscle mass and weakness. Glucocorticoids operate through a transcriptional mechanism. This involves binding of the hormone to cytoplasmic protein receptors and transport of the steroid-receptor complex into the cell nucleus. Once inside the nucleus, it activates the transcription of mRNA, which is specific for the synthesis of these amino acid catabolic enzymes. The glucocorticoids have additional actions, such as indirectly facilitating the stimulation of lipolysis, and destruction of lymphocytes.

**Catabolism and excretion** Cortisol is metabolized and the 17-keto metabolites of cortisol are 11-hydroxyandrosterone and 11-hydroxyetiocholanolone. Almost all the cortisol metabolites are excreted in the urine. Since cortisol is overproduced in Cushing’s syndrome, it is understandable that these two cortisol metabolites are excreted in excessive amounts.

VMA is the main urinary excretion product of the catecholamines. It is elevated in diseases associated with excessive catecholamine production, such as pheochromocytoma. If a tumor of the adrenal medulla or other chromaffin tissue is present, VMA excretion would be increased. Cushing's disease results from overproduction of glucocorticoids by the adrenal cortex and, of itself, does not involve the adrenal medulla or other catecholamine-producing tissues. Thus there is no reason to suspect overproduction of either epinephrine or norepinephrine in this case, and one would not expect VMA excretion to be affected if the diagnosis of Cushing's syndrome is correct.
Primary aldosteronism
A 44-year-old man who complained of headaches was discovered to be hypertensive. Hypokalemia was noted; the plasma potassium concentration remained between 2.5 and 3 mmol/L during 3 wk of observation. Peripheral vein renin concentrations were consistently less than 1 µl/L, and they remained low even when the patient was placed on a low-sodium diet. Urinary aldosterone excretion was consistently high, even when a high-sodium diet was given. A venogram showed a questionable lesion in the left adrenal gland. A left adrenalectomy was performed. On follow-up examination 2 months later, the patient was found to be normotensive and normokalemic.

Biochemical questions
1. Explain the hypokalemia in this case. Would you expect any abnormality in total body sodium content?

Apparent Mineralocorticoid Excess Syndrome
Some patients (usually children) exhibit the hypertension, hypokalemia, and suppression of the renin-angiotensin-aldosterone system that would be expected if they were hypersecreting aldosterone. Since assays of plasma and urine may fail to identify, excess mineralocorticoids, these patients are said to have the apparent mineralocorticoid excess (AME) syndrome. This syndrome results from the failure of inactivation of cortisol by 11ß-hydroxysteroid dehydrogenase. Since the plasma levels of cortisol are about 100 times higher than the levels for aldosterone, cortisol saturates the renal mineralocorticoid receptor, causing sodium retention and suppression of the renin-angiotensin-aldosterone axis.

Although this syndrome can result from a congenital defect in renal 11ß-hydroxysteroid dehydrogenase isoform, it can also be caused by ingesting excessive amounts of licorice.
Questions

2) Glucocorticoid receptors are in the cytoplasm. All of the following statements about the process by which the hormone influences transcription are correct except:
   a) The hormone must be in the free state to cross the cell membrane.
   b) Cytoplasmic receptors may be associated with heat shock proteins.
   c) The receptor-hormone complex is not activated/transformed until it is translocated to the nucleus.
   d) In the nucleus, the activated/transformed receptor-hormone complex searches for specific sequences on DNA called HREs (hormone receptor elements).
   e) The activated receptor-hormone complex may either activate or repress transcription of specific genes (only one activity per gene).

   C Dissociation of the heat shock protein from the receptor-hormone complex in the cytosol activates the complex. A: Steroid hormones travel bound to plasma proteins, but some is always free. D: These are consensus sequences in DNA. E: Activation is more common, but glucocorticoids repress transcription of the proopiomelanocortin gene.

3) All of the following are normal events leading to secretion of aldosterone from the adrenal gland except:
   a) Renin is released by the kidney in hypovolemia.
   b) Angiotensinogen binds to membrane receptors.
   c) Ca^{2+} levels in the cell rise.
   d) Aldosterone is secreted into the blood.

   B Angiotensinogen is cleaved by renin to angiotensin I, which must further be cleaved by converting enzyme to active angiotensin II. A: This is a major signal. C: This lead to increased Ca^{2+} and activation of protein kinase C.
Biochemistry and Disorders of Hormones of the Kidney, Heart and Adipose tissue

Lecture 8  Sunday 8/3/2012

1. The Kidney

The human kidney secretes two hormones

- **Erythropoietin**
- **Calcitriol (1,25[OH]_2 Vitamin D_3)**

- **Erythropoietin**
  (EPO) is a glycoprotein hormone that is a growth factor for erythrocyte (red blood cell) precursors in the bone marrow.
  - In adults primarily by peritubular cells in the kidneys, where its production is stimulated by low oxygen levels in the blood.
  - Some EPO is also produced by the liver, which is the primary source in the fetus.

**Actions**

EPO acts by binding to a specific erythropoietin receptor (EpoR) on the surface of red cell precursors in the bone marrow, stimulating them to transform into mature red blood cells. As a result the oxygen level in blood reaching the kidney rises and the amount of EPO produced decreases.

People with failing kidneys can be kept alive by dialysis. But dialysis only cleanses the blood from wastes. Without a source of EPO, these patients suffer from anemia.

2. The Heart

In response to a rise in blood pressure, the heart releases two peptides

- **A-type Natriuretic Peptide (BNP)**
  This hormone of 28 amino acids is released from stretched atria (hence the "A").

- **B-type Natriuretic Peptide (BNP)**
  This hormone (29 amino acids) is released from the ventricles. (It was first discovered in brain tissue; hence the "B")

Both hormones lower blood pressure by:

- Relaxing arterioles
- Inhibiting the secretion of renin and aldosterone
- Inhibiting the reabsorption of sodium ion by the kidneys.
The latter two effects reduce the reabsorption of water by the kidneys. So the volume of urine increases as does the amount of sodium excreted in it. The net effect of these actions is to reduce blood pressure by reducing the volume of blood in the circulating system.

These effects give ANP and BNP their name (natrium = sodium; uresis = urinate)

**Hormones of adipose tissue**

Although the adipocyte's primary role is to store fat, it also functions as an endocrine cell that releases numerous regulatory molecules, such as leptin, adiponectin, and resistin

1. **Leptin**: Studies of the molecular genetics of mouse obesity have led to the isolation of at least six genes associated with obesity. The most well-known mouse gene, named Ob (for obesity), leads to severe hereditary obesity in mice. It has been identified and cloned. In one strain of fat mice, the gene was completely absent, indicating that the gene's protein product is required to keep the animals' weight under control. The product of the Ob gene is a hormone called leptin.

Leptin is produced proportionally to the adipose mass and, thus, informs the brain of the fat store level. It is secreted by fat cells, and acts on the hypothalamus of the brain to regulate the amount of body fat through the control of appetite and energy expenditure. Leptin's secretion is suppressed by depletion of fat stores (starvation) and enhanced by expansion of fat stores (well-fed state). Daily injection of leptin causes overweight mice to lose weight and maintain weight loss. The protein also causes weight loss in mice that are not obese. In humans, leptin increases the metabolic rate and decreases appetite.
However, plasma leptin in obese humans is usually normal for their fat mass, suggesting that resistance to leptin, rather than its deficiency, occurs in human obesity. Other hormones released by adipose tissue, such as adiponectin and resistin, may mediate insulin resistance observed in obesity.

2. Resistin

The hormone resistin is one amongst a novel family of three proteins, known as resistin-like molecules (RELMs). They are cysteine-rich secreted proteins associated with pulmonary inflammation (also known as FIZZ3, found in inflammatory zone). It has 11 cysteine-residues synthesized as a propeptide of 108 amino acids and secreted as a dimer, build by a disulfide bridge of cysteine residues. Beside this intermolecular disulfide bridge, 5 additional intramolecular ones exist.

**Source of resistin**

In humans, resistin expression in adipose tissue can be detected at a low level. It is higher in abdominal fat stores than in thigh adipose tissue, this suggest a potential role in linking central obesity to type 2 diabetes and/or cardiovascular disease. Human resistin is expressed mainly in pancreatic islet, preadiposites, macrophages and bone marrow. So resistin is of relevance for inflammation processes as well as for lipid metabolism.

In mice a correlation between adiposity, insulin resistance and resistin expression was found empirically. In humans respective studies are not clear. Several show an association of resistin serum concentration and adiposity or insulin resistance.
**Resistin putative role(s):**

Relevance of resistin in physiological processes other than energy metabolism was investigated. Experiments with endothelial cells gave interesting results, in which resistin shown to be potentially able to influence endothelial inflammation and thereby atherosclerosis.

Resistin shares some qualities with another protein secreted by fat cells and associated with obesity, the hormone leptin. This hormone, discovered in 1995, seems to regulate food intake.

There is still much to learn about resistin. But with each new piece fitted into the diabetes puzzle, new possibilities arise.

There are two putative roles of resistin:

a. To directly cause insulin resistance
b. To block adipocyte differentiation

The latter might lead to ectopic fat storage (increased amounts of fat in skeletal muscle and liver.

Future work....

Future research in this area aims to establish the role of resistin in human disease. Measurement of resistin in a simple blood test might then be useful in detecting insulin resistance and prediabetic conditions. Looking forward, counteracting resistin's affects on the body might be a new approach to preventing and treating diabetes.