ISSN: 0976-7126
Sen *et al.*, 11(10):7042-7064, 2020

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Hormones are the Masterkey in Chemical co–ordination system of body

Kushal Nandi[1], Dhrubo Jyoti Sen[1*] and Dhananjoy Saha[2]

1, Department of Pharmaceutical Chemistry, School of Pharmacy, Techno India University, Kolkata (West Bengal) - India

2, Deputy Director of Technical Education, Directorate of Technical Education, Bikash Bhavan, Kolkata (West Bengal) - India

Article info

Received: 27/09/2020

Revised: 05/10/2020

Accepted: 29/10/2020

www.ijplsjournal.com

Abstract

Hormones are secreted in the body by several glands that are essential for the growth, development, reproduction, etc. They are the chemical substances which coordinate the activities of living organisms and also their growth. They are secreted by special tissues in our body through endocrine glands. Different hormones have different effects on the shape of the body. Some of these hormones work quickly to start or stop a process and some will continually work over a long period of time to perform their functions. They help in body growth, development, metabolism, sexual function, reproduction etc. What happens to the body when these hormones will release in more or less quantity. This article deals with the list of important hormones necessary for our body functions.

Key words: Endocrine glands, Hormones, Thyroxine, Steroids, Insulin, Glucagon

Introduction

List of important hormones and their functions

1. **Hormones of Thyroid:** Thyroid gland basically releases two hormones Triiodothyronine (T_3) and Thyroxine (T_4), which helps in controlling the metabolism of our body. Further, these hormones regulate weight, determines energy levels, internal body temperature, skin, hair etc.

Thyroid hormone

are two hormones produced and released by the thyroid gland, namely triiodothyronine (**T_3**) and thyroxine (**T_4**). They are tyrosine–based hormones that are primarily responsible for regulation of metabolism. T_3 and T_4 are partially composed of iodine. A deficiency of iodine leads to decreased production of T_3 and T_4, enlarges the thyroid tissue and will cause the disease known as simple goitre. The major form of thyroid hormone in the blood is thyroxine (T_4), which has a longer half–life than T_3. In humans, the ratio of T_4 to T_3 released into the blood is approximately 14:1. T_4 is converted to the active T_3 (three to four times more potent than T_4) within cells by deiodinases (5′–iodinase).

These are further processed by decarboxylation and deiodination to produce iodothyronamine (**T_1a**) and thyronamine (**T_0a**). All three isoforms of the deiodinases are selenium–containing enzymes, thus dietary selenium is essential for T_3 production.[1]

***Corresponding Author**
E. Mail: dhrubosen69@yahoo.com

ISSN: 0976-7126
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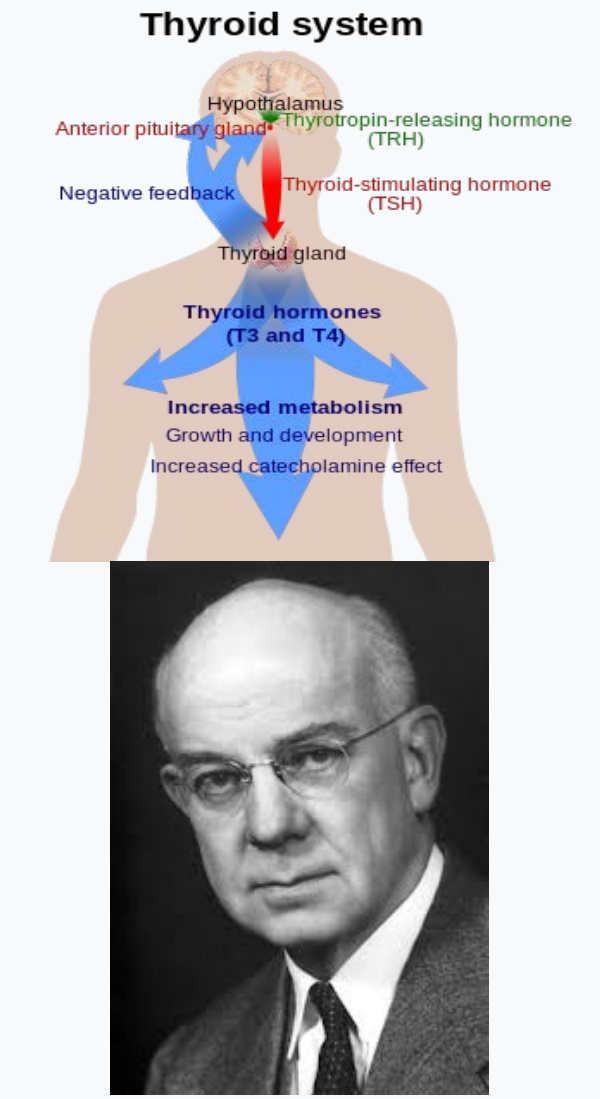
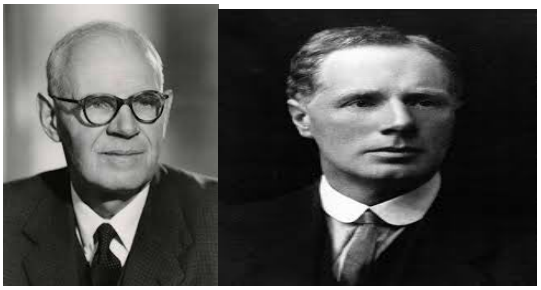


Fig. 1: Thyroid System & Edward Calvin Kendall [Nobel Prize for Thyroid Hormones]

Edward Calvin Kendall was responsible for the isolation of thyroxine in 1915. In 2016 levothyroxine, a manufactured form of thyroxine, was the most prescribed medication in the United States with more than 114 million prescriptions.

Function: The thyroid hormones act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone) and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyroid hormone leads to heat generation in humans. However, the thyronamines function via some unknown mechanism to inhibit neuronal activity; this plays an important role in the hibernation cycles of mammals and the moulting behaviour of birds. One effect of administering the thyronamines is a severe drop in body temperature.

Medical use: Both T_3 and T_4 are used to treat thyroid hormone deficiency (hypothyroidism). They are both absorbed well by the stomach, so can be given orally. Levothyroxine is the pharmaceutical name of the manufactured version of T_4, which is metabolised more slowly than T_3 and hence usually only needs once–daily administration. Natural desiccated thyroid hormones are derived from pig thyroid glands, and are a "natural" hypothyroid treatment containing 20% T_3 and traces of T_2, T_1 and calcitonin. Also available are synthetic combinations of T_3/T_4 in different ratios (such as liotrix) and pure–T_3 medications (INN: liothyronine). Levothyroxine Sodium is usually the first course of treatment tried. Some patients feel they do better on desiccated thyroid hormones; however, this is based on anecdotal evidence and clinical trials have not shown any benefit over the biosynthetic forms. Thyroid tablets are reported to have different effects, which can be attributed to the difference in torsional angles surrounding the reactive site of the molecule.



Charles Robert Harington and George Barger: Inventors of synthetic thyroxine

Thyronamines have no medical usages yet, though their use has been proposed for controlled induction of hypothermia, which causes

ISSN: 0976-7126
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the brain to enter a protective cycle, useful in preventing damage during ischemic shock. Synthetic thyroxine was first successfully produced by Charles Robert Harington and George Barger in 1926.

Formulations

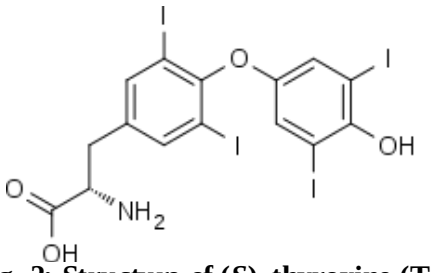

Fig. 2: Structure of (*S*)–thyroxine (T_4)

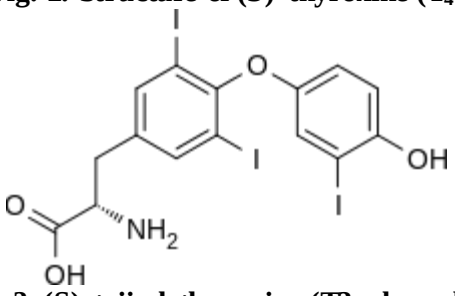

Fig. 3: (S)–triiodothyronine (T3, also called liothyronine)

Most people are treated with levothyroxine, or a similar synthetic thyroid hormone. Different polymorphs of the compound have different solubilities and potencies. Additionally, natural thyroid hormone supplements from the dried thyroids of animals are still available. Levothyroxine contains T_4 only and is therefore largely ineffective for patients unable to convert T_4 to T_3. These patients may choose to take natural thyroid hormone, as it contains a mixture of T_4 and T_3, or alternatively supplement with a synthetic T_3 treatment. In these cases, synthetic liothyronine is preferred due to the potential differences between the natural thyroid products. Some studies show that the mixed therapy is beneficial to all patients, but the addition of liothyronine contains additional side effects and the medication should be evaluated on an individual basis. Some natural thyroid hormone brands are FDA approved, but some are not. Thyroid hormones are generally well tolerated. Thyroid hormones are usually not dangerous for pregnant women or nursing mothers, but should be given under a doctor's supervision. In fact, if a woman who is hypothyroid is left untreated, her baby is at a higher risk for birth defects. When pregnant, a woman with a low–functioning thyroid will also need to increase her dosage of thyroid hormone. One exception is that thyroid hormones may aggravate heart conditions, especially in older patients; therefore, doctors may start these patients on a lower dose and work up to a larger one to avoid risk of heart attack.

Production

Central

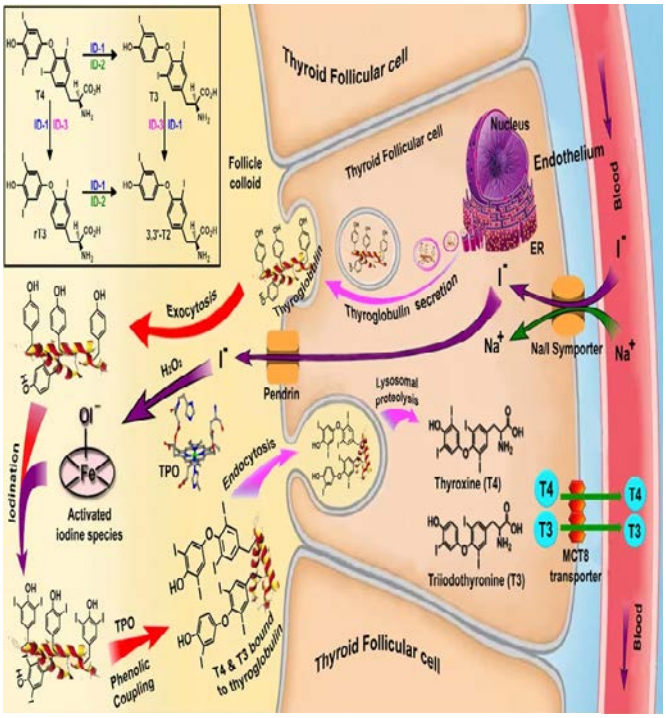

Fig. 4: Biosynthesis of thyroid hormones

Synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell

– Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis.[2]

– Meanwhile, a sodium–iodide (Na/I) symporter pumps iodide (I^-) actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms.

– This iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in a purportedly passive manner.

– In the colloid, iodide (I^-) is oxidized to iodine (I^0) by an enzyme called thyroid peroxidase.

– Iodine (I^0) is very reactive and iodinates the thyroglobulin at tyrosyl residues in its protein

chain (in total containing approximately 120 tyrosyl residues).
– In *conjugation*, adjacent tyrosyl residues are paired together.
– Thyroglobulin re–enters the follicular cell by endocytosis.
– Proteolysis by various proteases liberates thyroxine and triiodothyronine molecules
– Efflux of thyroxine and triiodothyronine from follicular cells, which appears to be largely through monocarboxylate transporter (MCT) 8 and 10, and entry into the blood.

Thyroid hormones (T_4 and T_3) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotropes of the anterior pituitary gland. The effects of T_4 *in–vivo* are mediated via T_3 (T_4 is converted to T_3 in target tissues). T_3 is three to five times as active than T_4.

Thyroxine (3,5,3′,5′–tetraiodothyronine) is produced by follicular cells of the thyroid gland. It is produced as the precursor thyroglobulin (this is *not* the same as thyroxine–binding globulin (TBG)), which is cleaved by enzymes to produce active T_4.

The steps in this process are as follows:

1. The Na^+/I^- symporter transports two sodium ions across the basement membrane of the follicular cells along with an iodide ion. This is a secondary active transporter that utilises the concentration gradient of Na^+ to move I^- against its concentration gradient.
2. I^- is moved across the apical membrane into the colloid of the follicle by pendrin.
3. Thyroperoxidase oxidizes two I^- to form I_2. Iodide is non–reactive, and only the more reactive iodine is required for the next step.
4. The thyroperoxidase iodinates the tyrosyl residues of the thyroglobulin within the colloid. The thyroglobulin was synthesised in the ER of the follicular cell and secreted into the colloid.
5. Iodinated Thyroglobulin binds megalin for endocytosis back into cell.
6. Thyroid–stimulating hormone (TSH) released from the anterior pituitary (also known as the adenohypophysis) binds the TSH receptor (a G_s protein–coupled receptor) on the basolateral membrane of the cell and stimulates the endocytosis of the colloid.
7. The endocytosed vesicles fuse with the lysosomes of the follicular cell. The lysosomal enzymes cleave the T_4 from the iodinated thyroglobulin.
8. The thyroid hormones cross the follicular cell membrane towards the blood vessels by an unknown mechanism. Text books have stated that diffusion is the main means of transport, but recent studies indicate that monocarboxylate transporter (MCT) 8 and 10 play major roles in the efflux of the thyroid hormones from the thyroid cells.

Thyroglobulin (Tg) is a 660 kDa, dimeric protein produced by the follicular cells of the thyroid and used entirely within the thyroid gland. Thyroxine is produced by attaching iodine atoms to the ring structures of this protein's tyrosine residues; thyroxine (T_4) contains four iodine atoms, while triiodothyronine (T_3), otherwise identical to T_4, has one less iodine atom per molecule. The thyroglobulin protein accounts for approximately half of the protein content of the thyroid gland. Each thyroglobulin molecule contains approximately 100–120 tyrosine residues, a small number of which (<20) are subject to iodination catalysed by thyroperoxidase. The same enzyme then catalyses "coupling" of one modified tyrosine with another, via a free–radical–mediated reaction, and when these iodinated bicyclic molecules are released by hydrolysis of the protein, T_3 and T_4 are the result.[Therefore, each thyroglobulin protein molecule ultimately yields very small amounts of thyroid hormone (experimentally observed to be on the order of 5–6 molecules of either T_4 or T_3 per original molecule of thyroglobulin).[3]

More specifically, the monoatomic anionic form of iodine, iodide (I^-), is actively absorbed from the bloodstream by a process called iodide trapping. In this process, sodium is co–transported with iodide from the basolateral side of the membrane into the cell, and then concentrated in the thyroid follicles to about thirty times its concentration in the blood. Then, in the first reaction catalysed by the enzyme thyroperoxidase, tyrosine residues in the protein thyroglobulin are iodinated on their phenol rings, at one or both of the positions *ortho* to the phenolic hydroxyl group,

yielding monoiodotyrosine (MIT)
and diiodotyrosine (DIT), respectively. This introduces 1–2 atoms of the element iodine, covalently bound, per tyrosine residue. The further coupling together of two fully iodinated tyrosine residues, also catalysed by thyroperoxidase, yields the peptidic (still peptide–bound) precursor of thyroxine, and coupling one molecule of MIT and one molecule of DIT yields the comparable precursor of triiodothyronine.

1. peptidic MIT + peptidic DIT → peptidic triiodothyronine (eventually released as triiodothyronine, T_3)
2. peptidic DITs → peptidic thyroxine (eventually released as thyroxine, T_4)

(Coupling of DIT to MIT in the opposite order yields a substance, r–T_3, which is biologically inactive.) Hydrolysis (cleavage to individual amino acids) of the modified protein by proteases then liberates T_3 and T_4, as well as the non–coupled tyrosine derivatives MIT and DIT. The hormones T_4 and T_3 are the biologically active agents central to metabolic regulation.

Peripheral: Thyroxine is believed to be a prohormone and a reservoir for the most active and main thyroid hormone T_3. T_4 is converted as required in the tissues by iodothyronine deiodinase. Deficiency of deiodinase can mimic hypothyroidism due to iodine deficiency. T_3 is more active than T_4, though it is present in less quantity than T_4.

Initiation of production in foetuses: Thyrotropin–releasing hormone (TRH) is released from hypothalamus by 6 – 8 weeks, and thyroid–stimulating hormone (TSH) secretion from foetal pituitary is evident by 12 weeks of gestation, and foetal production of thyroxine (T_4) reaches a clinically significant level at 18–20 weeks. Foetal triiodothyronine (T_3) remains low (less than 15 ng/dL) until 30 weeks of gestation, and increases to 50 ng/dL at term. Foetal self–sufficiency of thyroid hormones protects the foetus against e.g. brain development abnormalities caused by maternal hypothyroidism.

Iodine deficiency: If there is a deficiency of dietary iodine, the thyroid will not be able to make thyroid hormones. The lack of thyroid hormones will lead to decreased negative feedback on the pituitary, leading to increased production of thyroid–stimulating hormone, which causes the thyroid to enlarge (the resulting medical condition is called *endemic colloid goitre*; see goitre). This has the effect of increasing the thyroid's ability to trap more iodide, compensating for the iodine deficiency and allowing it to produce adequate amounts of thyroid hormone.

Circulation and transport

Plasma transport: Most of the thyroid hormone circulating in the blood is bound to transport proteins, and only a very small fraction is unbound and biologically active. Therefore, measuring concentrations of free thyroid hormones is important for diagnosis, while measuring total levels can be misleading.[4]

Thyroid hormone in the blood is usually distributed as follows:

Type	Percent
bound to thyroxine–binding globulin (TBG)	70%
bound to transthyretin or "thyroxine–binding prealbumin" (TTR or TBPA)	10–15%
Albumin	15–20%
unbound T_4 (fT_4)	0.03%
unbound T_3 (fT_3)	0.3%

Despite being lipophilic, T_3 and T_4 cross the cell membrane via carrier–mediated transport, which is ATP–dependent.

T_1a and T_0a are positively charged and do not cross the membrane; they are believed to function via the trace amine–associated receptor *TAAR1* (TAR1, TA1), a G–protein–coupled receptor located in the cytoplasm.

Another critical diagnostic tool is measurement of the amount of thyroid–stimulating hormone (TSH) that is present.

Membrane transport: Contrary to common belief, thyroid hormones cannot traverse cell membranes in a passive manner like other lipophilic substances. The iodine in *o*–position makes the phenolic OH–group more acidic, resulting in a negative charge at physiological pH. However, at least 10 different active, energy–dependent and genetically regulated iodothyronine transporters have been identified in humans. They guarantee that intracellular levels of thyroid hormones are higher than in blood plasma or interstitial fluids.

Intracellular transport: Little is known about intracellular kinetics of thyroid hormones. However, recently it could be demonstrated that the crystallin CRYM binds 3,5,3′–triiodothyronine *in–vivo*.

Mechanism of action: The thyroid hormones function via a well–studied set of nuclear receptors, termed the thyroid hormone receptors. These receptors, together with corepressor molecules, bind DNA regions called thyroid hormone response elements (TREs) near genes. This receptor–corepressor–DNA complex can block gene transcription. Triiodothyronine (T_3), which is the active form of thyroxine (T_4), goes on to bind to receptors. The deiodinase catalyzed reaction removes an iodine atom from the 5′ position of the outer aromatic ring of thyroxine's (T_4) structure. When triiodothyronine (T_3) binds a receptor, it induces a conformational change in the receptor, displacing the corepressor from the complex. This leads to recruitment of coactivator proteins and RNA polymerase, activating transcription of the gene. Although this general functional model has considerable experimental support, there remain many open questions. More recently genetic evidence has been obtained for a second mechanism of thyroid hormone action involving one of the same nuclear receptors, TRβ, acting rapidly in the cytoplasm through the PI3K. This mechanism is conserved in all mammals but not fish or amphibians, and regulates brain development and adult metabolism. The mechanism itself parallels the actions of the nuclear receptor in the nucleus: in the absence of hormone, TRβ binds to PI3K and inhibits its activity, but when hormone binds the complex dissociates, PI3K activity increases, and the hormone bound receptor diffuses into the nucleus.

Thyroxine, iodine and apoptosis: Thyroxine and iodine stimulate the spectacular apoptosis of the cells of the larval gills, tail and fins in amphibian metamorphosis, and stimulate the evolution of their nervous system transforming the aquatic, vegetarian tadpole into the terrestrial, carnivorous frog. In fact, amphibian frog *Xenopus laevis* serves as an ideal model system for the study of the mechanisms of apoptosis.

Effects of triiodothyronine:

Effects of triiodothyronine (T_3) which is the metabolically active form:

1. Increases cardiac output
2. Increases heart rate
3. Increases ventilation rate
4. Increases basal metabolic rate
5. Potentiates the effects of catecholamines (i.e. increases sympathetic activity)
6. Potentiates brain development
7. Thickens endometrium in females
8. Increases catabolism of proteins and carbohydrates

Measurement: Triiodothyronine (T_3) and thyroxine (T_4) can be measured as *free* T_3 and *free* T_4, which are indicators of their activities in the body. They can also be measured as *total* T_3 and *total* T_4, which depend on the amount that is bound to thyroxine–binding globulin (TBG). A related parameter is the free thyroxine index, which is *total* T_4 multiplied by thyroid hormone uptake, which, in turn, is a measure of the unbound TBG. Additionally, thyroid disorders can be detected prenatally using advanced imaging techniques and testing foetal hormone levels.[5]

Related diseases: Both excess and deficiency of thyroxine can cause disorders.

1. Hyperthyroidism (an example is Graves' disease) is the clinical syndrome caused by an excess of circulating free thyroxine, free triiodothyronine, or both. It is a common disorder that affects approximately 2% of women and 0.2% of men. Thyrotoxicosis is often used interchangeably with hyperthyroidism, but there are subtle differences. Although thyrotoxicosis also refers to an increase in circulating thyroid hormones, it can be caused by the intake of thyroxine tablets or by an over–active thyroid, whereas hyperthyroidism refers solely to an over–active thyroid.
2. Hypothyroidism (an example is Hashimoto's thyroiditis) is the case where there is a deficiency of thyroxine, triiodothyronine, or both. Clinical depression can sometimes be caused by hypothyroidism. Some research has shown that T_3 is found in the junctions of synapses, and regulates the amounts and activity of serotonin, norepinephrine, and γ–aminobutyric acid (GABA) in the brain. Hair loss can sometimes be attributed to a malfunction of

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T_3 and T_4. Normal hair growth cycle may be affected disrupting the hair growth. Preterm births can suffer neurodevelopmental disorders due to lack of maternal thyroid hormones, at a time when their own thyroid is unable to meet their postnatal needs. Also in normal pregnancies, adequate levels of maternal thyroid hormone are vital in order to ensure thyroid hormone availability for the foetus and its developing brain. Congenital hypothyroidism occurs in every 1 in 1600–3400 new–borns with most being born asymptomatic and developing related symptoms weeks after birth.

Anti–thyroid drugs: Iodine uptake against a concentration gradient is mediated by a sodium–iodine symporter and is linked to a sodium–potassium ATPase. Perchlorate and thiocyanate are drugs that can compete with iodine at this point. Compounds such as goitrin, carbimazole, methimazole, propylthiou racil can reduce thyroid hormone production by interfering with iodine oxidation.

2. Insulin:

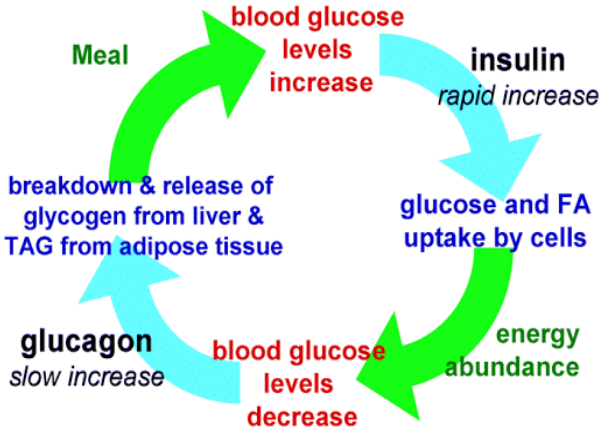

Fig. 5: Insulin Cycle

This hormone is released by the pancreas, a leaf–like gland located in the abdominal cavity behind the stomach. It allows the body to use glucose or sugar from carbohydrates in the food for energy or to store glucose for future use. It helps in keeping blood sugar level from getting too high i.e. hyperglycemia or too low i.e. hypoglycemia.

Evolution and species distribution: Insulin may have originated more than a billion years ago. The molecular origins of insulin go at least as far back as the simplest unicellular eukaryotes. Apart from animals, insulin–like proteins are also known to exist in the Fungi and Protista kingdoms. Insulin is produced by beta cells of the pancreatic islets in most vertebrates and by the Brockmann body in some teleost fish. Cone snails *Conus geographus* and *Conus tulipa*, venomous sea snails that hunt small fish, use modified forms of insulin in their venom cocktails. The insulin toxin, closer in structure to fishes' than to snails' native insulin, slows down the prey fishes by lowering their blood glucose levels.[6]

Gene: The preproinsulin precursor of insulin is encoded by the *INS* gene, which is located on Chromosome 11p15.5.. In some mammals, such as rats and mice, there are two insulin genes, one of which is the homolog of most mammalian genes (*Ins2*), and the other of which is a retroposed copy that includes promoter sequence but that is missing an intron (*Ins1*). Both rodent insulin genes are functional.

Alleles: A variety of mutant alleles with changes in the coding region have been identified. A read–through gene, INS–IGF2, overlaps with this gene at the 5' region and with the IGF2 gene at the 3' region.

Regulation: In the pancreatic β cells, glucose is the primary physiological stimulus for the regulation of insulin synthesis. Insulin is mainly regulated through the transcription factors PDX1, NeuroD1, and MafA.

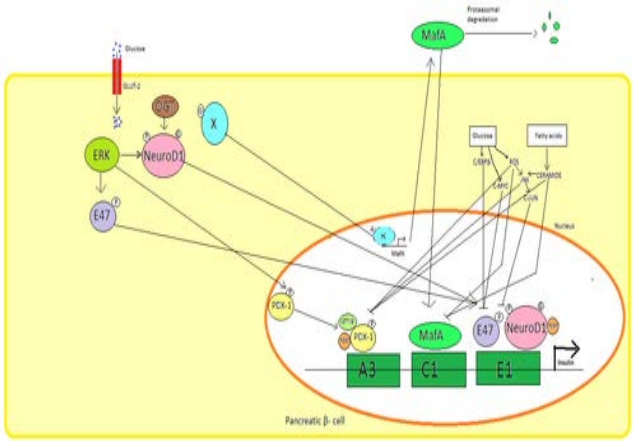

Fig. 6: Diagram of insulin regulation upon high blood glucose

During a low–glucose state, PDX1 (pancreatic and duodenal homeobox protein 1) is located in the nuclear periphery as a result of interaction with HDAC1 and 2, which results in down regulation of insulin secretion. An increase in blood glucose levels
causes phosphorylation of PDX1, which leads it to

undergo nuclear translocation and bind the A3 element within the insulin promoter. Upon translocation it interacts with coactivators HAT p300 and SETD7. PDX1 affects the histone modifications through acetylation and deacetylation as well as methylation. It is also said to suppress glucagon.

NeuroD1, also known as β2, regulates insulin exocytosis in pancreatic β cells by directly inducing the expression of genes involved in exocytosis. It is localized in the cytosol, but in response to high glucose it becomes glycosylated by OGT and/or phosphoryl ated by ERK, which causes translocation to the nucleus. In the nucleus β2 heterodimerizes with E47, binds to the E1 element of the insulin promoter and recruits co–activator p300 which acetylates β2. It is able to interact with other transcription factors as well in activation of the insulin gene. MafA is degraded by proteasomes upon low blood glucose levels. Increased levels of glucose make an unknown protein glycosylated. This protein works as a transcription factor for MafA in an unknown manner and MafA is transported out of the cell. MafA is then translocated back into the nucleus where it binds the C1 element of the insulin promoter.

These transcription factors work synergistically and in a complex arrangement. Increased blood glucose can after a while destroy the binding capacities of these proteins, and therefore reduce the amount of insulin secreted, causing diabetes. The decreased binding activities can be mediated by glucose induced oxidative stress and antioxidants are said to prevent the decreased insulin secretion in glucotoxic pancreatic β cells. Stress signalling molecules and reactive oxygen species inhibits the insulin gene by interfering with the cofactors binding the transcription factors and the transcription factors itself.

Several regulatory sequences in the promoter region of the human insulin gene bind to transcription factors. In general, the A–boxes bind to Pdx1 factors, E–boxes bind to NeuroD, C–boxes bind to MafA, and cAMP response elements to CREB. There are also silencers that inhibit transcription.[7]

Regulatory sequences and their transcription factors for the insulin gene.

Regulatory sequence	binding transcription factors
ILPR	Par1
A5	Pdx1
negative regulatory element (NRE)	glucocorticoid receptor, Oct1
Z (overlapping NRE and C2)	ISF
C2	Pax4, MafA(?)
E2	USF1/USF2
A3	Pdx1
CREB RE	CREB, CREM
A2	–
CAAT enhancer binding (CEB) (partly overlapping A2 and C1)	–
C1	–
E1	E2A, NeuroD1, HEB
A1	Pdx1
G1	–

Table 1: Regulatory sequences and their transcription factors for the insulin gene.

Structure: The structure of insulin. The left side is a space–filling model of the insulin monomer, believed to be biologically active. Carbon is green, hydrogen white, oxygen red, and nitrogen blue. On the right side is a ribbon diagram of the insulin hexamer, believed to be the stored form. A monomer unit is highlighted with the A chain in blue and the B chain in cyan. Yellow denotes disulfide bonds, and magenta spheres are zinc ions.

Contrary to an initial belief that hormones would be generally small chemical molecules, as the first peptide hormone known of its structure, insulin was found to be quite large. A single protein (monomer) of human insulin is composed of 51 amino acids, and has a molecular mass of

5808 Da. The molecular formula of human insulin is $C_{257}H_{383}N_{65}O_{77}S_6$.

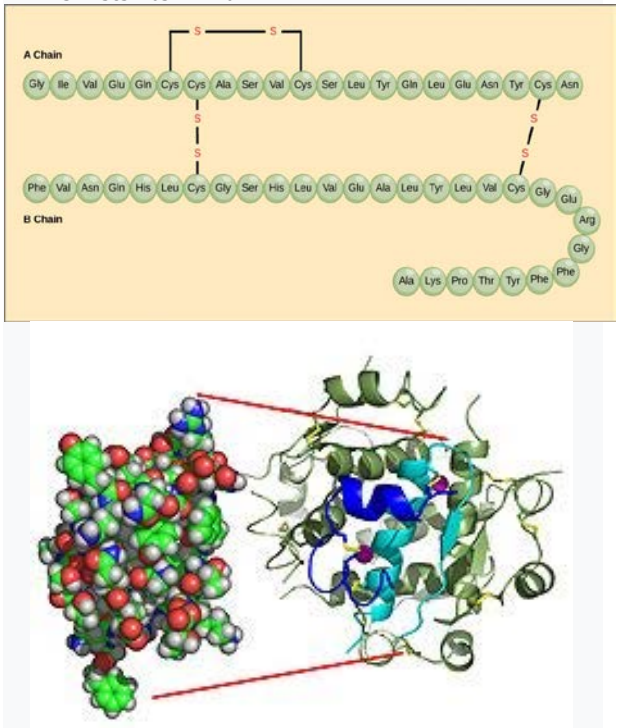

Fig. 7: Peptide bonds in Insulin

It is a combination of two peptide chains (dimer) named an A–chain and a B–chain, which are linked together by two disulfide bonds. The A–chain is composed of 21 amino acids, while the B–chain consists of 30 residues. The linking (interchain) disulfide bonds are formed at cysteine residues between the positions A7–B7 and A20–B19. There is an additional (intrachain) disulfide bond within the A–chain between cysteine residues at positions A4 and A11. The A–chain exhibits two α–helical regions at A1–A8 and A12–A19 which are antiparallel; while the B chain has a central α –helix (covering residues B9–B19) flanked by the disulfide bond on either sides and two β–sheets (covering B7–B10 and B20–B23).[8]

The amino acid sequence of insulin is strongly conserved and varies only slightly between species. Bovine insulin differs from human in only three amino acid residues, and porcine insulin in one. Even insulin from some species of fish is similar enough to human to be clinically effective in humans. Insulin in some invertebrates is quite similar in sequence to human insulin, and has similar physiological effects. The strong homology seen in the insulin sequence of diverse species suggests that it has been conserved across much of animal evolutionary history. The C–peptide of proinsulin, however, differs much more among species; it is also a hormone, but a secondary one.

Insulin is produced and stored in the body as a hexamer (a unit of six insulin molecules), while the active form is the monomer. The hexamer is about 36000 Da in size. The six molecules are linked together as three dimeric units to form symmetrical molecule. An important feature is the presence of zinc atoms (Zn^{2+}) on the axis of symmetry, which are surrounded by three water molecules and three histamine residues at position B10.

The hexamer is an inactive form with long–term stability, which serves as a way to keep the highly reactive insulin protected, yet readily available. The hexamer–monomer conversion is one of the central aspects of insulin formulations for injection. The hexamer is far more stable than the monomer, which is desirable for practical reasons; however, the monomer is a much faster–reacting drug because diffusion rate is inversely related to particle size. A fast–reacting drug means insulin injections do not have to precede mealtimes by hours, which in turn gives people with diabetes more flexibility in their daily schedules. Insulin can aggregate and form fibrillar interdigitated beta–sheets. This can cause injection amyloidosis, and prevents the storage of insulin for long periods.

Synthesis, physiological effects, and degradation:

Synthesis: Insulin is produced in the pancreas and the Brockmann body (in some fish), and released when any of several stimuli are detected. These stimuli include the rise in plasma concentrations of amino acids and glucose resulting from the digestion of food. Carbohydrates can be polymers of simple sugars or the simple sugars themselves. If the carbohydrates include glucose, then that glucose will be absorbed into the bloodstream and blood glucose level will begin to rise. In target cells, insulin initiates a signal transduction, which has the effect of increasing glucose uptake and storage. Finally, insulin is degraded, terminating the response.

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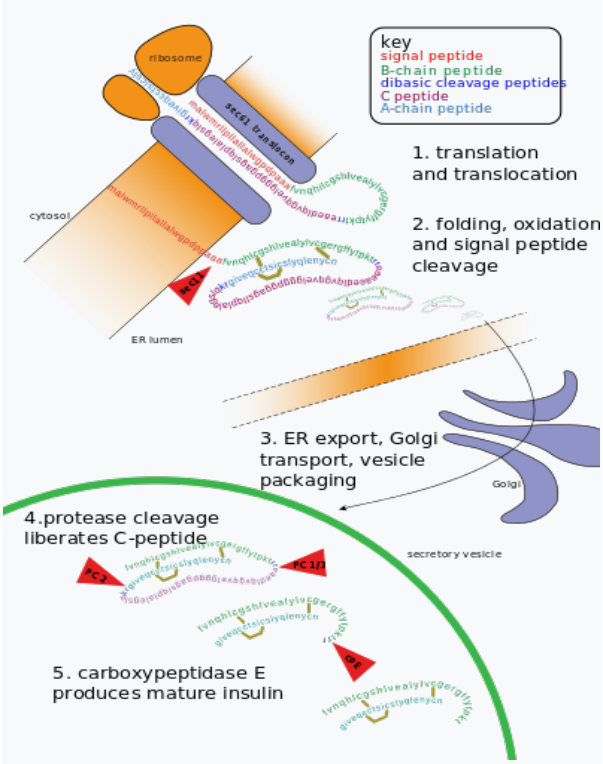

Fig.8: Insulin undergoes extensive posttranslational modification along the production pathway. Production and secretion are largely independent; prepared insulin is stored awaiting secretion. Both C–peptide and mature insulin are biologically active. Cell components and proteins in this image are not to scale

In mammals, insulin is synthesized in the pancreas within the beta cells. One million to three million pancreatic islets form the endocrine part of the pancreas, which is primarily an exocrine gland. The endocrine portion accounts for only 2% of the total mass of the pancreas. Within the pancreatic islets, beta cells constitute 65–80% of all the cells.[9]

Insulin consists of two polypeptide chains, the A– and B– chains, linked together by disulfide bonds. It is however first synthesized as a single polypeptide called preproinsulin in beta cells. Preproinsulin contains a 24–residue signal peptide which directs the nascent polypeptide chain to the rough endoplasmic reticulum (RER). The signal peptide is cleaved as the polypeptide is translocated into lumen of the RER, forming proinsulin. In the RER the proinsulin folds into the correct conformation and 3 disulfide bonds are formed. About 5–10 min after its assembly in the endoplasmic reticulum, proinsulin is transported to the trans–Golgi network (TGN) where immature granules are formed. Transport to the TGN may take about 30 minutes.

Proinsulin undergoes maturation into active insulin through the action of cellular endopeptidases known as prohormone convertases (PC1 and PC2), as well as the exoprotease carboxypeptidase E. The endopeptidases cleave at 2 positions, releasing a fragment called the C–peptide, and leaving 2 peptide chains, the B– and A– chains, linked by 2 disulfide bonds. The cleavage sites are each located after a pair of basic residues (lysine–64 and arginine–65, and arginine–31 and −32). After cleavage of the C–peptide, these 2 pairs of basic residues are removed by the carboxypeptidase. The C–peptide is the central portion of proinsulin, and the primary sequence of proinsulin goes in the order "B–C–A" (the B and A chains were identified on the basis of mass and the C–peptide was discovered later).

The resulting mature insulin is packaged inside mature granules waiting for metabolic signals (such as leucine, arginine, glucose and mannose) and vagal nerve stimulation to be exocytosed from the cell into the circulation.

The endogenous production of insulin is regulated in several steps along the synthesis pathway:

- At transcription from the insulin gene
- In mRNA stability
- At the mRNA translation
- In the posttranslational modifications

Insulin and its related proteins have been shown to be produced inside the brain, and reduced levels of these proteins are linked to Alzheimer's disease. Insulin release is stimulated also by beta–2 receptor stimulation and inhibited by alpha–1 receptor stimulation. In addition, cortisol, glucagon and growth hormone antagonize the actions of insulin during times of stress. Insulin also inhibits fatty acid release by hormone sensitive lipase in adipose tissue.

Release: Beta cells in the islets of Langerhans release insulin in two phases. The first–phase release is rapidly triggered in response to increased blood glucose levels, and lasts about 10 minutes. The second phase is a sustained, slow release of newly formed vesicles triggered independently of sugar, peaking in 2 to 3 hours.

Reduced first–phase insulin release may be the earliest detectable beta cell defect predicting onset of type 2 diabetes. First–phase release and insulin sensitivity are independent predictors of diabetes.
The description of first phase release is as follows:

- Glucose enters the β–cells through the glucose transporters, GLUT2. These glucose transporters have a relatively low affinity for glucose, ensuring that the rate of glucose entry into the β–cells is proportional to the extracellular glucose concentration (within the physiological range). At low blood sugar levels very little glucose enters the β–cells; at high blood glucose concentrations large quantities of glucose enter these cells.
- The glucose that enters the β–cell is phosphorylated to glucose–6–phosphate (G–6–P) by glucokinase (hexokinase IV) which is not inhibited by G–6–P in the way that the hexokinases in other tissues (hexokinase I – III) are affected by this product. This means that the intracellular G–6–P concentration remains proportional to the blood sugar concentration.
- Glucose–6–phosphate enters glycolytic pathway and then, via the pyruvate dehydrogenase reaction, into the Krebs cycle, where multiple, high–energy ATP molecules are produced by the oxidation of acetyl CoA (the Krebs cycle substrate), leading to a rise in the ATP:ADP ratio within the cell.
- An increased intracellular ATP:ADP ratio closes the ATP–sensitive SUR1/Kir6.2 potassium channel (see sulfonylurea receptor). This prevents potassium ions (K^+) from leaving the cell by facilitated diffusion, leading to a buildup of intracellular potassium ions. As a result, the inside of the cell becomes less negative with respect to the outside, leading to the depolarization of the cell surface membrane.
- Upon depolarization, voltage–gated calcium ion (Ca^{2+}) channels open, allowing calcium ions to move into the cell by facilitated diffusion.
- The cytosolic calcium ion concentration can also be increased by calcium release from intracellular stores via activation of ryanodine receptors.
- The calcium ion concentration in the cytosol of the beta cells can also, or additionally, be increased through the activation of phospholipase C resulting from the binding of an extracellular ligand (hormone or neurotransmitter) to a G protein–coupled membrane receptor. Phospholipase C cleaves the membrane phospholipid, phosphatidyl inositol 4,5–bisphosphate, into inositol 1,4,5–trisphosphate and diacylglycerol. Inositol 1,4,5–trisphosphate (IP3) then binds to receptor proteins in the plasma membrane of the endoplasmic reticulum (ER). This allows the release of Ca^{2+} ions from the ER via IP3–gated channels, which raises the cytosolic concentration of calcium ions independently of the effects of a high blood glucose concentration. Parasympathetic stimulation of the pancreatic islets operates via this pathway to increase insulin secretion into the blood.[10]
- The significantly increased amount of calcium ions in the cells' cytoplasm causes the release into the blood of previously synthesized insulin, which has been stored in intracellular secretory vesicles.

This is the primary mechanism for release of insulin. Other substances known to stimulate insulin release include the amino acids arginine and leucine, parasympathetic release of acetylcholine (acting via the phospholipase C pathway), sulfonylurea, cholecystokinin (CCK, also via phospholipase C), and the gastrointestinally derived incretins, such as glucagon–like peptide–1 (GLP–1) and glucose–dependent insulinotropic peptide (GIP).
Release of insulin is strongly inhibited by norepinephrine (noradrenaline), which leads to increased blood glucose levels during stress. It appears that release of catecholamines by the sympathetic nervous system has conflicting influences on insulin release by beta cells, because

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insulin release is inhibited by α_2–adrenergic receptor sand stimulated by β_2–adrenergic receptors. The net effect of norepinephrine from sympathetic nerves and epinephrine from adrenal glands on insulin release is inhibition due to dominance of the α–adrenergic receptors.

When the glucose level comes down to the usual physiologic value, insulin release from the β–cells slows or stops. If the blood glucose level drops lower than this, especially to dangerously low levels, release of hyperglycemic hormones (most prominently glucagon from islet of Langerhans alpha cells) forces release of glucose into the blood from the liver glycogen stores, supplemented by gluconeogenesis if the glycogen stores become depleted. By increasing blood glucose, the hyperglycemic hormones prevent or correct life–threatening hypoglycemia.

Evidence of impaired first–phase insulin release can be seen in the glucose tolerance test, demonstrated by a substantially elevated blood glucose level at 30 minutes after the ingestion of a glucose load (75 or 100 g of glucose), followed by a slow drop over the next 100 minutes, to remain above 120 mg/100 ml after two hours after the start of the test. In a normal person the blood glucose level is corrected (and may even be slightly over–corrected) by the end of the test. An insulin spike is a 'first response' to blood glucose increase, this response is individual and dose specific although it was always previously assumed to be food type specific only.[11]

Oscillations:

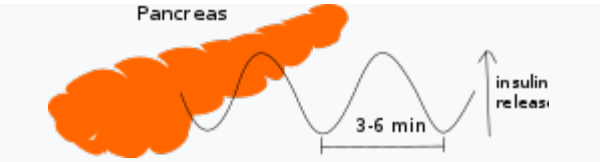

Fig. 9: Insulin release from pancreas oscillates with a period of 3–6 minutes

Even during digestion, in general, one or two hours following a meal, insulin release from the pancreas is not continuous, but oscillates with a period of 3–6 minutes, changing from generating a blood insulin concentration more than about 800 p mol/l to less than 100 pmol/l (in rats). This is thought to avoid downregulation of insulin receptors in target cells, and to assist the liver in extracting insulin from the blood. This oscillation is important to consider when administering insulin–stimulating medication, since it is the oscillating blood concentration of insulin release, which should, ideally, be achieved, not a constant high concentration. This may be achieved by delivering insulin rhythmically to the portal vein, by light activated delivery, or by islet cell transplantation to the liver.

Blood insulin level:

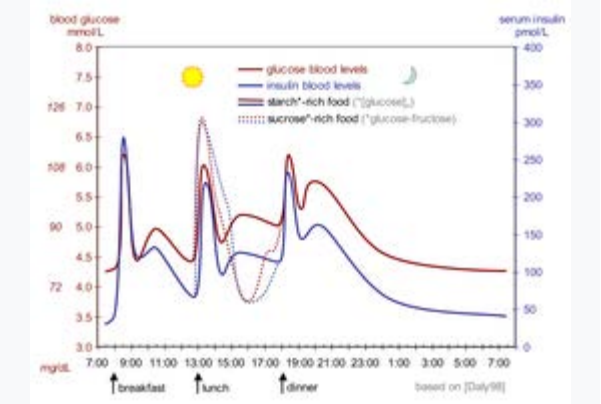

Fig. 10: The idealized diagram shows the fluctuation of blood sugar (red) and the sugar–lowering hormone insulin (blue) in humans during the course of a day containing three meals. In addition, the effect of a sugar–rich versus a starch–rich meal is highlighted

The blood insulin level can be measured in international units, such as µIU/mL or in molar concentration, such as pmol/L, where 1 µIU/mL equals 6.945 pmol/L. A typical blood level between meals is 8–11 µIU/mL (57–79 pmol/L).

Signal transduction: The effects of insulin are initiated by its binding to a receptor present in the cell membrane. The receptor molecule contains an α– and β subunits. Two molecules are joined to form what is known as a homodimer. Insulin binds to the α–subunits of the homodimer, which faces the extracellular side of the cells. The β subunits have tyrosine kinase enzyme activity which is triggered by the insulin binding. This activity provokes the autophosphorylation of the β subunits and subsequently the phosphorylation of proteins inside the cell known as insulin receptor substrates (IRS). The phosphorylation of the IRS activates a signal transduction cascade that leads to the activation of other kinases as well as transcription factors that mediate the intracellular effects of insulin.

The cascade that leads to the insertion of GLUT4 glucose transporters into the cell membranes of muscle and fat cells, and to the synthesis of

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glycogen in liver and muscle tissue, as well as the conversion of glucose into triglycerides in liver, adipose, and lactating mammary gland tissue, operates via the activation, by IRS–1, of phosphoinositol 3 kinase (PI3K). This enzyme converts a phospholipid in the cell membrane by the name of phosphatidylinositol 4,5–bisphosphate (PIP2), into phosphatidylinositol 3,4,5–triphosphate (PIP3), which, in turn, activates protein kinase B (PKB). Activated PKB facilitates the fusion of GLUT4 containing endosomes with the cell membrane, resulting in an increase in GLUT4 transporters in the plasma membrane. PKB also phosphorylates glycogen synthase kinase (GSK), thereby inactivating this enzyme. This means that its substrate, glycogen synthase (GS), cannot be phosphorylated, and remains dephosphorylated, and therefore active. The active enzyme, glycogen synthase (GS), catalyzes the rate limiting step in the synthesis of glycogen from glucose. Similar dephosphorylations affect the enzymes controlling the rate of glycolysis leading to the synthesis of fats via malonyl–CoA in the tissues that can generate triglycerides, and also the enzymes that control the rate of gluconeogenesis in the liver. The overall effect of these final enzyme dephosphorylations is that, in the tissues that can carry out these reactions, glycogen and fat synthesis from glucose are stimulated, and glucose production by the liver through glycogenolysis and gluconeogenesis are inhibited. The breakdown of triglycerides by adipose tissue into free fatty acids and glycerol is also inhibited.[12]

After the intracellular signal that resulted from the binding of insulin to its receptor has been produced, termination of signalling is then needed. As mentioned below in the section on degradation, endocytosis and degradation of the receptor bound to insulin is a main mechanism to end signalling. In addition, the signalling pathway is also terminated by dephosphorylation of the tyrosine residues in the various signalling pathways by tyrosine phosphatases. Serine/Threonine kinases are also known to reduce the activity of insulin.

The structure of the insulin–insulin receptor complex has been determined using the techniques of X–ray crystallography.

Physiological effects:

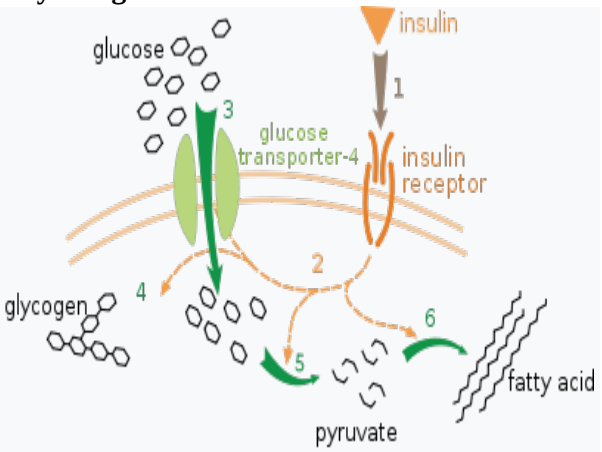

Fig. 11: Effect of insulin on glucose uptake and metabolism

Insulin binds to its receptor (1), which starts many protein activation cascades (2). These include translocation of Glut–4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5) and triglyceride synthesis (6).

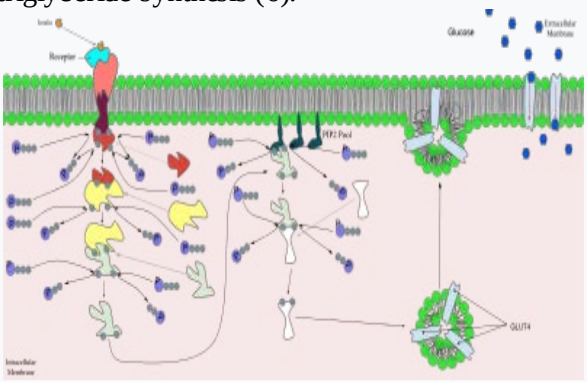

Fig. 12: The insulin signal transduction pathway begins when insulin binds to the insulin receptor proteins. Once the transduction pathway is completed, the GLUT–4 storage vesicles becomes one with the cellular membrane. As a result, the GLUT–4 protein channels become embedded into the membrane, allowing glucose to be transported into the cell

The actions of insulin on the global human metabolism level include:

- Increase of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue (about two–thirds of body cells)
- Increase of DNA replication and protein synthesis via control of amino acid uptake

- Modification of the activity of numerous enzymes.

The actions of insulin (indirect and direct) on cells include:

- Stimulates the uptake of glucose – Insulin decreases blood glucose concentration by inducing intake of glucose by the cells. This is possible because Insulin causes the insertion of the GLUT4 transporter in the cell membranes of muscle and fat tissues which allows glucose to enter the cell.
- Increased fat synthesis – insulin forces fat cells to take in blood glucose, which is converted into triglycerides; decrease of insulin causes the reverse.
- Increased esterification of fatty acids – forces adipose tissue to make neutral fats (i.e., triglycerides) from fatty acids; decrease of insulin causes the reverse.
- Decreased lipolysis – forces reduction in conversion of fat cell lipid stores into blood fatty acids and glycerol; decrease of insulin causes the reverse.
- Induce glycogen synthesis – When glucose levels are high, insulin induces the formation of glycogen by the activation of the hexokinase enzyme, which adds a phosphate group in glucose, thus resulting in a molecule that cannot exit the cell. At the same time, insulin inhibits the enzyme glucose–6–phosphatase, which removes the phosphate group. These two enzymes are key for the formation of glycogen. Also, insulin activates the enzymes phosphofructokinase and glycogen synthase which are responsible for glycogen synthesis.[13]
- Decreased gluconeogenesis and glycogen olysis – decreases production of glucose from noncarbohydrate substrates, primarily in the liver (the vast majority of endogenous insulin arriving at the liver never leaves the liver); decrease of insulin causes glucose production by the liver from assorted substrates.
- Decreased proteolysis – decreasing the breakdown of protein.
- Decreased autophagy – decreased level of degradation of damaged organelles. Postprandial levels inhibit autophagy completely.
- Increased amino acid uptake – forces cells to absorb circulating amino acids; decrease of insulin inhibits absorption.
- Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in microarteries; decrease of insulin reduces flow by allowing these muscles to contract.
- Increase in the secretion of hydrochloric acid by parietal cells in the stomach.
- Increased potassium uptake – forces cells synthesizing glycogen (a very spongy, "wet" substance, that increases the content of intracellular water, and its accompanying K^+ ions) to absorb potassium from the extracellular fluids; lack of insulin inhibits absorption. Insulin's increase in cellular potassium uptake lowers potassium levels in blood plasma. This possibly occurs via insulin–induced translocation of the Na^+/K^+–ATPase to the surface of skeletal muscle cells.
- Decreased renal sodium excretion.

Insulin also influences other body functions, such as vascular compliance and cognition. Once insulin enters the human brain, it enhances learning and memory and benefits verbal memory in particular. Enhancing brain insulin signaling by means of intranasal insulin administration also enhances the acute thermoregulatory and glucoregulatory response to food intake, suggesting that central nervous insulin contributes to the co–ordination of a wide variety of homeostatic or regulatory processes in the human body. Insulin also has stimulatory effects on gonadotropin–releasing hormone from the hypothalamus, thus favouring fertility.

Degradation: Once an insulin molecule has docked onto the receptor and effected its action, it may be released back into the extracellular environment, or it may be degraded by the cell. The two primary sites for insulin clearance are the liver and the kidney. The liver clears most insulin during first–pass transit, whereas the kidney clears most of the insulin in systemic circulation. Degradation normally involves endocytosis of the insulin–receptor complex, followed by the action

of insulin–degrading enzyme. An insulin molecule produced endogenously by the beta cells is estimated to be degraded within about one hour after its initial release into circulation (insulin half–life ~ 4–6 minutes).

Regulator of endocannabinoid metabolism: Insulin is a major regulator of endocannabinoid (EC) metabolism and insulin treatment has been shown to reduce intracellular ECs, the 2–arachidonylglycerol (2–AG) and anandamide (AEA), which correspond with insulin–sensitive expression changes in enzymes of EC metabolism. In insulin–resistant adipocytes, patterns of insulin–induced enzyme expression is disturbed in a manner consistent with elevated EC synthesis and reduced EC degradation. Findings suggest that insulin–resistant adipocytes fail to regulate EC metabolism and decrease intracellular EC levels in response to insulin stimulation, whereby obese insulin–resistant individuals exhibit increased concentrations of ECs. This dysregulation contributes to excessive visceral fat accumulation and reduced adiponectin release from abdominal adipose tissue, and further to the onset of several cardiometabolic risk factors that are associated with obesity and type 2 diabetes.[14]

Hypoglycemia: Hypoglycemia, also known as "low blood sugar", is when blood sugar decreases to below normal levels. This may result in a variety of symptoms including clumsiness, trouble talking, confusion, loss of consciousness, seizures or death. A feeling of hunger, sweating, shakiness and weakness may also be present. Symptoms typically come on quickly. The most common cause of hypoglycemia is medications used to treat diabetes mellitus such as insulin and sulfonylureas. Risk is greater in diabetics who have eaten less than usual, exercised more than usual or have drunk alcohol. Other causes of hypoglycemia include kidney failure, certain tumors, such as insulinoma, liver disease, hypothyroidism, starvation, inborn error of metabolism, severe infections, reactive hypoglycemia and a number of drugs including alcohol. Low blood sugar may occur in otherwise healthy babies who have not eaten for a few hours.

Diseases and syndromes: There are several conditions in which insulin disturbance is pathologic:

1. Diabetes mellitus – general term referring to all states characterized by hyperglycemia. It can be of the following types:

Type 1 – autoimmune–mediated destruction of insulin–producing β–cells in the pancreas, resulting in absolute insulin deficiency

Type 2 – either inadequate insulin production by the β–cells or insulin resistance or both because of reasons not completely understood.

There is correlation with diet, with sedentary lifestyle, with obesity, with age and with metabolic syndrome. Causality has been demonstrated in multiple model organisms including mice and monkeys; importantly, non–obese people do get Type 2 diabetes due to diet, sedentary lifestyle and unknown risk factors.

It is likely that there is genetic susceptibility to develop Type 2 diabetes under certain environmental conditions

Other types of impaired glucose tolerance:

1. Insulinoma – a tumor of beta cells producing excess insulin or reactive hypoglycemia.

2. Metabolic syndrome – a poorly understood condition first called syndrome X by Gerald Reaven. It is not clear whether the syndrome has a single, treatable cause, or is the result of body changes leading to type 2 diabetes. It is characterized by elevated blood pressure, dyslipidemia (disturbances in blood cholesterol forms and other blood lipids), and increased waist circumference (at least in populations in much of the developed world). The basic underlying cause may be the insulin resistance that precedes type 2 diabetes, which is a diminished capacity for insulin response in some tissues (e.g., muscle, fat). It is common for morbidities such as essential hypertension, obesity, type 2 diabetes, and cardiovascular disease (CVD) to develop. Polycystic ovary syndrome – a complex syndrome in women in the reproductive years where anovulation and androgen excess are commonly displayed as hirsutism. In many cases of PCOS, insulin resistance is present.

Medical uses: Biosynthetic human insulin (insulin human rDNA, INN) for clinical use is manufactured by recombinant DNA technology. Biosynthetic human insulin has

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increased purity when compared with extractive animal insulin, enhanced purity reducing antibody formation. Researchers have succeeded in introducing the gene for human insulin into plants as another method of producing insulin ("biopharming") in safflower. This technique is anticipated to reduce production costs.

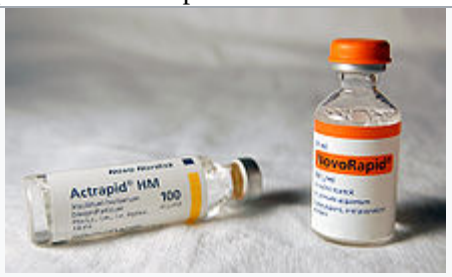

Fig. 13: A vial of insulin. It has been given a trade name, Actrapid, by the manufacturer.

Several analogues of human insulin are available. These insulin analogues are closely related to the human insulin structure, and were developed for specific aspects of glycaemic control in terms of fast action (prandial insulins) and long action (basal insulins). The first biosynthetic insulin analogue was developed for clinical use at mealtime (prandial insulin), Humalog (insulin lispro), it is more rapidly absorbed after subcutaneous injection than regular insulin, with an effect 15 minutes after injection. Other rapid–acting analogues are NovoRapid and Apidra, with similar profiles. All are rapidly absorbed due to amino acid sequences that will reduce formation of dimers and hexamers (monomeric insulins are more rapidly absorbed). Fast acting insulins do not require the injection–to–meal interval previously recommended for human insulin and animal insulins. The other type is long-acting insulin; the first of these was Lantus (insulin glargine). These have a steady effect for an extended period from 18 to 24 hours. Likewise, another protracted insulin analogue (Levemir) is based on a fatty acid acylation approach. A myristic acid molecule is attached to this analogue, which associates the insulin molecule to the abundant serum albumin, which in turn extends the effect and reduces the risk of hypoglycemia. Both protracted analogues need to be taken only once daily, and are used for type 1 diabetics as the basal insulin. A combination of a rapid acting and a protracted insulin is also available, making it more likely for patients to achieve an insulin profile that mimics that of the body's own insulin release.

Insulin is usually taken as subcutaneous injections by single–use syringes with needles, via an insulin pump, or by repeated–use insulin pens with disposable needles. Inhaled insulin is also available in the U.S. market now.

Synthetic insulin can trigger adverse effects, so some people with diabetes rely on animal–source insulin.

Unlike many medicines, insulin cannot be taken by mouth because, like nearly all other proteins introduced into the gastrointestinal tract, it is reduced to fragments, whereupon all activity is lost. There has been some research into ways to protect insulin from the digestive tract, so that it can be administered orally or sublingually.

Oestrogen:

It is a female sex hormone released by the ovaries. It is responsible for the reproduction, menstruation and menopause. Excess of oestrogen in the female body increases the risk of breast cancer, uterine cancer, depression, moodiness etc. If the oestrogen level is less in female body then it leads to acne, skin lesions, thinning skin, hair loss etc.

Types and examples:

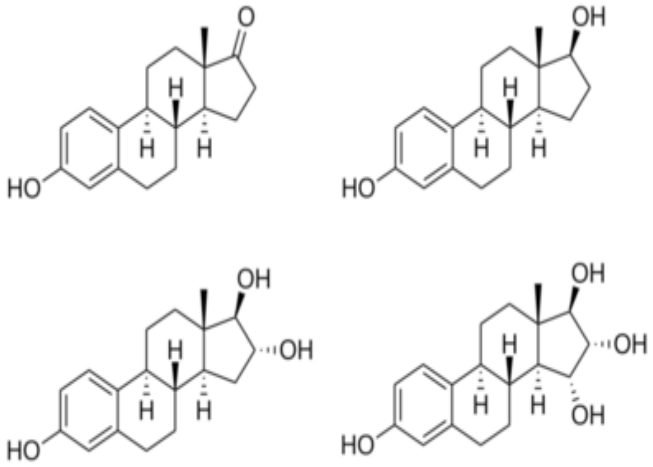

Fig. 14: Estrone (E1), Estradiol (E2), Estriol (E3), Estetrol (E4)

The four major naturally occurring oestrogens in women are estrone (E1), estradiol (E2), estriol (E3), and estetrol (E4). Estradiol is the predominant oestrogen during reproductive years both in terms of absolute serum levels as well as in terms of

oestrogenic activity. During menopause, estrone is the predominant circulating oestrogen and during pregnancy estriol is the predominant circulating oestrogen in terms of serum levels. Given by subcutaneous injection in mice, estradiol is about 10–fold more potent than estrone and about 100–fold more potent than estriol. Thus, estradiol is the most important oestrogen in non–pregnant females who are between the menarche and menopause stages of life. However, during pregnancy this role shifts to estriol, and in postmenopausal women estrone becomes the primary form of oestrogen in the body. Another type of oestrogen called estetrol (E4) is produced only during pregnancy. All of the different forms of oestrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzyme aromatase.

Minor endogenous oestrogens, the biosyntheses of which do not involve aromatase, include 27–hydroxycholesterol, dehydroepiandrosterone (DHEA), 7–oxo–DHEA, 7α–hydroxy–DHEA, 16α–hydroxy–DHEA, 7β–hydroxyepiandrosterone, androstenedione (A4), androstenediol (A5), 3α–androstanediol, and 3β–androstanediol. Some oestrogen metabolites, such as the catechol oestrogens 2–hydroxyestradiol, 2–hydroxyestrone, 4–hydroxyestradiol, and 4–hydroxyestrone, as well as 16α–hydroxyestrone, are also oestrogens with varying degrees of activity. The biological importance of these minor oestrogens is not entirely clear.

Biological function

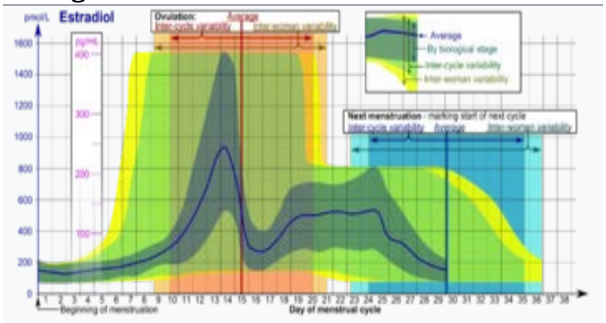

Fig. 15: Reference ranges for the blood content of estradiol, the primary type of oestrogen, during the menstrual cycle

The actions of oestrogen are mediated by the oestrogen receptor (ER), a dimeric nuclear protein that binds to DNA and controls gene expression. Like other steroid hormones, oestrogen enters passively into the cell where it binds to and activates the oestrogen receptor. The oestrogen: ER complex binds to specific DNA sequences called a hormone response element to activate the transcription of target genes (in a study using an oestrogen–dependent breast cancer cell line as model, 89 such genes were identified). Since oestrogen enters all cells, its actions are dependent on the presence of the ER in the cell. The ER is expressed in specific tissues including the ovary, uterus and breast. The metabolic effects of oestrogen in postmenopausal women have been linked to the genetic polymorphism of the ER.

While oestrogens are present in both men and women, they are usually present at significantly higher levels in women of reproductive age. They promote the development of female secondary sexual characteristics, such as breasts, and are also involved in the thickening of the endometrium and other aspects of regulating the menstrual cycle. In males, oestrogen regulates certain functions of the reproductive system important to the maturation of sperm and may be necessary for a healthy libido.

- Structural
    - Anabolic: Increases muscle mass and strength, speed of muscle regeneration, and bone density, increased sensitivity to exercise, protection against muscle damage, stronger collagen synthesis, increases the collagen content of connective tissues, tendons, and ligaments, but also decreases stiffness of tendons and ligaments (especially during menstruation). Decreased stiffness of tendons gives women much lower predisposition to muscle strains but soft ligaments are much more prone to injuries (ACL tears are 2–8x more common among women than men).
    - Anti–inflammatory properties
    - Mediate formation of female secondary sex characteristics

- Accelerate metabolism
- Increased fat storage in some body parts such as breasts, buttocks, and legs but decreased abdominal and visceral fat (androgenic obesity). Estradiol also regulates energy expenditure, body weight homeostasis, and seems to have much stronger anti–obesity effects than testosterone in general.

- Women tend to have lower base strength but on average have about the same increases of muscle mass in responses to resistance training as men and far faster relative increases in strength.
  - Stimulate endometrial growth
  - Increase uterine growth
  - Increase vaginal lubrication
  - Thicken the vaginal wall
  - Maintenance of vessel and skin
  - Reduce bone resorption, increase bone formation
- Protein synthesis
  - Increase hepatic production of binding proteins
- Coagulation
  - Increase circulating level of factors 2, 7, 9, 10, plasminogen
  - Decrease antithrombin III
  - Increase platelet adhesiveness
  - Increase vWF (oestrogen –> Angiotensin II –> Vasopressin)
  - Increase PAI–1 and PAI–2 also through Angiotensin II
- Lipid
  - Increase HDL, triglyceride
  - Decrease LDL, fat deposition
- Fluid balance
  - Salt (sodium) and water retention
  - Increase cortisol, SHBG
- Gastrointestinal tract
  - Reduce bowel motility
  - Increase cholesterol in bile
- Melanin
  - Increase pheomelanin, reduce eumelanin
- Cancer
  - Support hormone–sensitive breast cancers (see section below)
- Lung function
  - Promotes lung function by supporting alveoli (in rodents but probably in humans).
- Uterus lining
  - Oestrogen together with progesterone promotes and maintains the uterus lining in preparation for implantation of fertilized egg and maintenance of uterus function during gestation period, also upregulates oxytocin receptor in myometrium
- Ovulation
  - Surge in oestrogen level induces the release of luteinizing hormone, which then triggers ovulation by releasing the egg from the Graafian follicle in the ovary.
- Sexual behavior
  - Promotes sexual receptivity in estrus, and induces lordosis behavior. In non–human mammals, it also induces estrus (in heat) prior to ovulation, which also induces lordosis behavior. Female non–human mammals are not sexually receptive without the oestrogen surge, i.e., they have no mating desire when not in estrus.
  - Regulates the stereotypical sexual receptivity behavior; this lordosis behavior is oestrogen–dependent, which is regulated by the ventromedial nucleus of the hypothalamus.
  - Sex drive is dependent on androgen levels only in the presence of oestrogen, but without oestrogen, free testosterone level actually decreases sexual desire (instead of increases sex drive), as demonstrated for those women who have hypoactive sexual

desire disorder, and the sexual desire in these women can be restored by administration of oestrogen (using oral contraceptive). In non–human mammals, mating desire is triggered by oestrogen surge in estrus.

Female pubertal development: Oestrogens are responsible for the development of female secondary sexual characteristics during puberty, including breast development, widening of the hips, and female fat distribution. Conversely, androgens are responsible for pubic and body hair growth, as well as acne and axillary odor.

Breast development: Oestrogen, in conjunction with growth hormone (GH) and its secretory product insulin–like growth factor 1 (IGF–1), is critical in mediating breast development during puberty, as well as breast maturation during pregnancy in preparation of lactation and breastfeeding. Oestrogen is primarily and directly responsible for inducing the ductal component of breast development, as well as for causing fat deposition and connective tissue growth. It is also indirectly involved in the lobuloalveolar component, by increasing progesterone receptor expression in the breasts and by inducing the secretion of prolactin. Allowed for by oestrogen, progesterone and prolactin work together to complete lobuloalveolar development during pregnancy.

Androgens such as testosterone powerfully oppose oestrogen action in the breasts, such as by reducing oestrogen receptor expression in them.

Female reproductive system: Oestrogens are responsible for maturation and maintenance of the vagina and uterus, and are also involved in ovarian function, such as maturation of ovarian follicles. In addition, oestrogens play an important role in regulation of gonadotropin secretion. For these reasons, oestrogens are required for female fertility.

Neuroprotection and DNA repair:

Oestrogen regulated DNA repair mechanisms in the brain have neuroprotective effects. Oestrogen regulates the transcription of DNA base excision repair genes as well as the translocation of the base excision repair enzymes between different subcellular compartments.

Brain and behavior

Sex drive: Oestrogens are involved in libido (sex drive) in both women and men.

Cognition: Verbal memory scores are frequently used as one measure of higher-level cognition. These scores vary in direct proportion to oestrogen levels throughout the menstrual cycle, pregnancy, and menopause. Furthermore, oestrogens when administered shortly after natural or surgical menopause prevents decreases in verbal memory. In contrast, oestrogens have little effect on verbal memory if first administered years after menopause. Oestrogens also have positive influences on other measures of cognitive function. However, the effect of oestrogens on cognition is not uniformly favorable and is dependent on the timing of the dose and the type of cognitive skill being measured. The protective effects of oestrogens on cognition may be mediated by oestrogen's anti–inflammatory effects in the brain. Studies have also shown that the Met allele gene and level of oestrogen mediates the efficiency of prefrontal cortex dependent working memory tasks.

Mental health: Oestrogen is considered to play a significant role in women's mental health. Sudden oestrogen withdrawal, fluctuating oestrogen, and periods of sustained low oestrogen levels correlate with significant mood lowering. Clinical recovery from postpartum, perimenopause, and postmenopause depression has been shown to be effective after levels of oestrogen were stabilized and/or restored. Menstrual exacerbation (including menstrual psychosis) is typically triggered by low oestrogen levels, and is often mistaken for premenstrual dysphoric disorder.

Compulsions in male lab mice, such as those in obsessive–compulsive disorder (OCD), may be caused by low oestrogen levels. When oestrogen levels were raised through the increased activity of the enzyme aromatase in male lab mice, OCD rituals were dramatically decreased. Hypothalamic protein levels in the gene COMT are enhanced by increasing oestrogen levels which are believed to return mice that displayed OCD rituals to normal activity. Aromatase deficiency is ultimately suspected which is involved in the synthesis of oestrogen in

humans and has therapeutic implications in humans having obsessive–compulsive disorder.

Local application of oestrogen in the rat hippocampus has been shown to inhibit the re–uptake of serotonin. Contrarily, local application of oestrogen has been shown to block the ability of fluvoxamine to slow serotonin clearance, suggesting that the same pathways which are involved in SSRI efficacy may also be affected by components of local oestrogen signalling pathways.

Parenthood: Studies have also found that fathers had lower levels of cortisol and testosterone but higher levels of oestrogen (estradiol) compared to non–fathers.

Binge eating: Oestrogen may play a role in suppressing binge eating. Hormone replacement therapy using oestrogen may be a possible treatment for binge eating behaviors in females. Oestrogen replacement has been shown to suppress binge eating behaviors in female mice. The mechanism by which oestrogen replacement inhibits binge–like eating involves the replacement of serotonin (5–HT) neurons. Women exhibiting binge eating behaviors are found to have increased brain uptake of neuron 5–HT, and therefore less of the neurotransmitter serotonin in the cerebrospinal fluid. Oestrogen works to activate 5–HT neurons, leading to suppression of binge like eating behaviors.

It is also suggested that there is an interaction between hormone levels and eating at different points in the female menstrual cycle. Research has predicted increased emotional eating during hormonal flux, which is characterized by high progesterone and estradiol levels that occur during the mid–luteal phase. It is hypothesized that these changes occur due to brain changes across the menstrual cycle that are likely a genomic effect of hormones. These effects produce menstrual cycle changes, which result in hormone release leading to behavioral changes, notably binge and emotional eating. These occur especially prominently among women who are genetically vulnerable to binge eating phenotypes.

Binge eating is associated with decreased estradiol and increased progesterone. Klump et al. Progesterone may moderate the effects of low estradiol (such as during dysregulated eating behavior), but that this may only be true in women who have had clinically diagnosed binge episodes (BEs). Dysregulated eating is more strongly associated with such ovarian hormones in women with BEs than in women without BEs. The implantation of 17β–estradiol pellets in ovariectomized mice significantly reduced binge eating behaviors and injections of GLP–1 in ovariectomized mice decreased binge–eating behaviors. The associations between binge eating, menstrual–cycle phase and ovarian hormones correlated.

Masculinization in rodents: In rodents, oestrogens (which are locally aromatized from androgens in the brain) play an important role in psychosexual differentiation, for example, by masculinizing territorial behavior; the same is not true in humans. In humans, the masculinizing effects of prenatal androgens on behavior (and other tissues, with the possible exception of effects on bone) appear to act exclusively through the androgen receptor. Consequently, the utility of rodent models for studying human psychosexual differentiation has been questioned.

Skeletal system: Oestrogens are responsible for both the pubertal growth spurt, which causes an acceleration in linear growth, and epiphyseal closure, which limits height and limb length, in both females and males. In addition, oestrogens are responsible for bone maturation and maintenance of bone mineral density throughout life. Due to hypooestrogenism, the risk of osteoporosis increases during menopause.

Cardiovascular system: Women suffer less from heart disease due to vasculo–protective action of oestrogen which helps in preventing atherosclerosis. It also helps in maintaining the delicate balance between fighting infections and protecting arteries from damage thus lowering the risk of cardiovascular disease. During pregnancy, high levels of oestrogens increase coagulation and the risk of venous thromboembolism.

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Absolute and relative incidence of venous thromboembolism (VTE) during pregnancy and the postpartum period show

ISSN: 0976-7126
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Immune system: Oestrogen has anti–inflammatory properties and helps in mobilization of polymorphonuclear white blood cells or neutrophils.

Associated conditions: Oestrogens are implicated in various oestrogen–dependent conditions, such as ER–positive breast cancer, as well as a number of genetic conditions involving oestrogen signaling or metabolism, such as oestrogen insensitivity syndrome, aromatase deficiency, and aromatase excess syndrome.

Biochemistry:

Biosynthesis

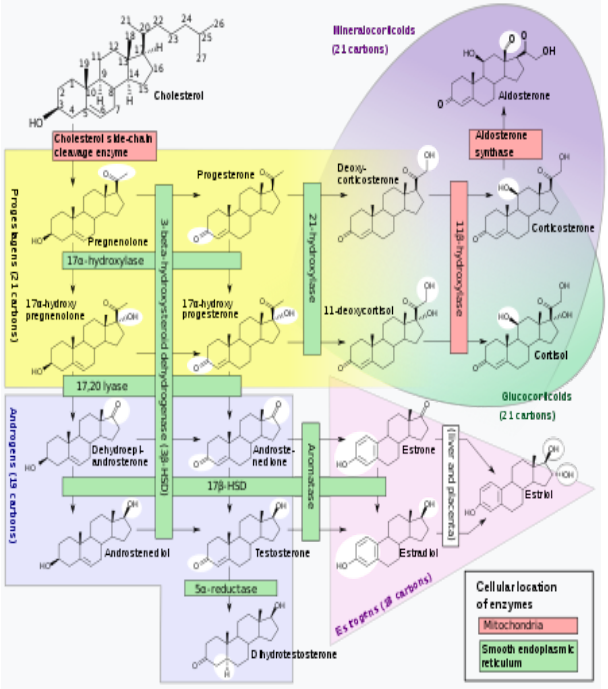

Fig. 16: Steroidogenesis, showing oestrogens at bottom right as in pink triangle

Oestrogens, in females, are produced primarily by the ovaries, and during pregnancy, the placenta. Follicle–stimulating hormone (FSH) stimulates the ovarian production of oestrogens by the granulosa cells of the ovarian follicles and corpora lutea. Some oestrogens are also produced in smaller amounts by other tissues such as the liver, pancreas, bone, adrenal glands, skin, brain, adipose tissue, and the breasts. These secondary sources of oestrogens are especially important in postmenopausal women. The pathway of oestrogen biosynthesis in extragonadal tissues is different. These tissues are not able to synthesize C19 steroids, and therefore depend on C19 supplies from other tissues and the level of aromatase.

In females, synthesis of oestrogens starts in theca interna cells in the ovary, by the synthesis of androstenedione from cholesterol.

Androstenedione is a substance of weak androgenic activity which serves predominantly as a precursor for more potent androgens such as testosterone as well as oestrogen. This compound crosses the basal membrane into the surrounding granulosa cells, where it is converted either immediately into estrone, or into testosterone and then estradiol in an additional step. The conversion of androstenedione to testosterone is catalyzed by 17β–hydroxysteroid dehydrogenase (17β–HSD), whereas the conversion of androstenedione and testosterone into estrone and estradiol, respectively is catalyzed by aromatase, enzymes which are both expressed in granulosa cells. In contrast, granulosa cells lack 17α–hydroxylase and 17,20–lyase, whereas theca cells express these enzymes and 17β–HSD but lack aromatase. Hence, both granulosa and theca cells are essential for the production of oestrogen in the ovaries.

Oestrogen levels vary through the menstrual cycle, with levels highest near the end of the follicular phase just before ovulation. Note that in males, oestrogen is also produced by the Sertoli cells when FSH binds to their FSH receptors.

Distribution: Oestrogens are plasma protein bound to albumin and/or sex hormone–binding globulin in the circulation.

Metabolism: Oestrogens are metabolized via hydroxylation by cytochrome P450 enzymes such as CYP1A1 and CYP3A4 and via conjugation by oestrogen sulfotransferases (sulfation) and UDP–glucuronyltransferases (glucuronidation). In addition, estradiol is dehydrogenated by 17β–hydroxysteroid dehydrogenase into the much less potent oestrogen estrone. These reactions occur primarily in the liver, but also in other tissues.

Excretion: Oestrogens are excreted primarily by the kidneys as conjugates via the urine.

Medical use: Oestrogens are used as medications, mainly in hormonal contraception, hormone replacement therapy, and to treat gender dysphoria in transgender women and

ISSN: 0976-7126
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other transfeminine individuals as part of feminizing hormone therapy.

Progesterone: Progesterone hormone is produced in the ovaries, the placenta when a woman gets pregnant and the adrenal glands. It stimulates and regulates various functions. It plays an important role in maintaining pregnancy. It helps body to prepare for conception, pregnancy and regulates the monthly cycle. When pregnancy doesn't occur, progesterone levels drop and menstrual cycle occurs. It also plays a role in sexual desire.

Composition and Functions of Blood

Prolactin: This hormone is released by the pituitary gland after childbirth for lactation, which enables female to breastfeed. Levels of prolactin hormone rise during pregnancy i.e. it also plays an important role in fertility by inhibiting follicle–stimulating hormone (FSH) and gonadotropin–releasing hormone (GnRH).

Testosterone:

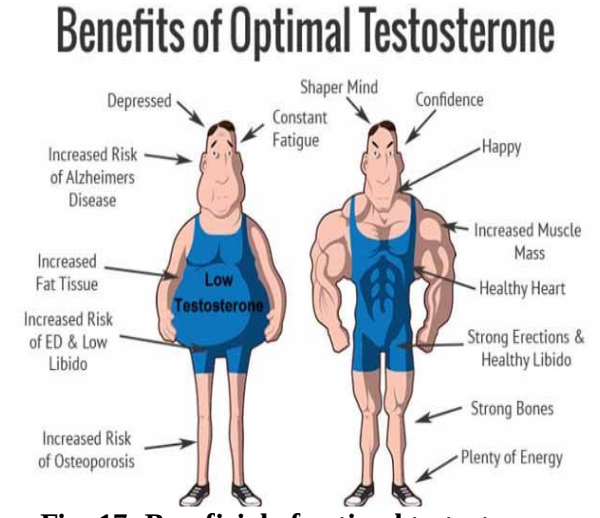

Fig. 17: Beneficial of optimal testosterone

It is a male sex hormone. It is an anabolic steroid by nature which helps in building body muscles. In males, it plays an important role in the development of male reproductive tissues; testes and prostate. It also promotes secondary sexual characteristics like increasing the mass of muscles and bones, growth of body hair etc. If testosterone is secreted insufficient in men then it may lead to abnormalities including frailty and bone loss.

Serotonin: It is a mood–boosting effect hormone or also known as nature's feel–good chemical. It is associated with learning and memory, regulating sleep, digestion, regulates mood, some muscular functions etc. Due to the imbalance of serotonin in the body, brain does not produce enough of the hormone to regulate mood or stress level. Low level of serotonin causes depression, migraine, weight gain, insomnia, craving of carbohydrate etc. Excess level of serotonin in the body causes agitation, stage of confusion, sedation etc.

Cortisol:

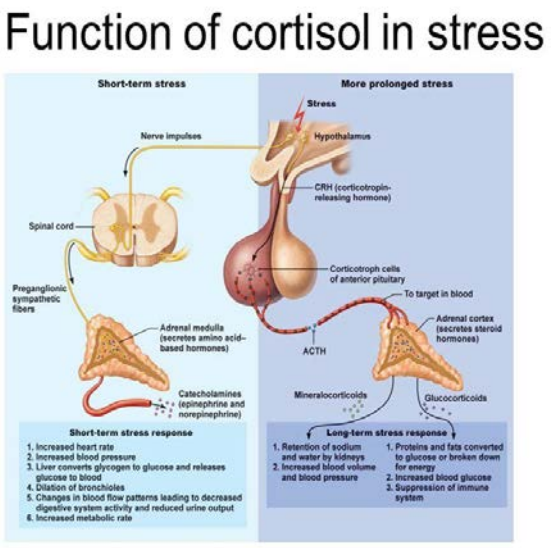

Fig. 18: Function of cortisol in stress

This hormone is produced by the adrenal gland. It helps you stay healthy and energetic. Its main role is to control physical and psychological stress. In danger condition, it increases heart rate, blood pressure, respiration etc. At stressful times body secretes cortisol to cope up with the situation. High level of cortisol consistently causes ulcer, high blood pressure, anxiety, high levels of cholesterol etc. Similarly, a low level of cortisol in the body causes alcoholism, a condition responsible for chronic fatigue syndrome etc.

Adrenaline: Adrenaline hormone is secreted in the medulla in the adrenal gland as well as some of the central nervous system's neurons. It is also known as an emergency hormone because it initiates the quick reaction which makes the individual to think and respond quickly to the stress. It increases the metabolic rate, dilation of blood vessels going to the heart and the brain. During a stressful situation, adrenaline quickly releases into the blood, send impulses to the organs to create a specific response.

Growth Hormone: It is also known as somatotropin hormone. It is basically a protein hormone having 190 amino acids which are synthesised and secreted by the cells called somatotrophs in the anterior pituitary. It stimulates growth, cell reproduction cell regeneration and in boosting metabolism. It is important in human development.

So, now you may have come to know about the various hormones and their functions in the human body.

Conclusion

Hormones are the outcomes of ductless glands which help to make body strong and to combat with physicochemical malfunctions, similarly implementation of basic thinking to culture the knowledge into fruitful outcome is the correlation approach of body engineering with engineering sciences.

References

1. Ryan KJ (1982). "Biochemistry of aromatase: significance to female reproductive physiology". Cancer Research. 42 (8 Suppl): 3342s–3344s.
2. Mechoulam R, Brueggemeier RW, Denlinger DL (2005). "Oestrogens in insects". Cellular and Molecular Life Sciences. 40 (9): 942–944.
3. Burger HG (2002). "Androgen production in women". Fertility and Sterility. 77 Suppl 4: S3–5.
4. Lombardi G, Zarrilli S, Colao A, Paesano L, Di Somma C, Rossi F, De Rosa M (2001). "Oestrogens and health in males". Molecular and Cellular Endocrinology. 178 (1–2): 51–5.
5. Whitehead SA, Nussey S (2001). Endocrinology: an integrated approach. Oxford: BIOS: Taylor & Francis.
6. Soltysik K, Czekaj P (2013). "Membrane oestrogen receptors – is it an alternative way of oestrogen action?". Journal of Physiology and Pharmacology. 64(2): 129–42.
7. Stryer L (1995). Biochemistry (Fourth ed.). New York: W.H. Freeman and Company. pp. 773–74.
8. Sonksen P, Sonksen J (2000). "Insulin: understanding its action in health and disease". British Journal of Anaesthesia. 85 (1): 69–79.
9. Koeslag JH, Saunders PT, Terblanche E (2003). "A reappraisal of the blood glucose homeostat which comprehensively explains the type 2 diabetes mellitus–syndrome X complex". The Journal of Physiology (published 2003). 549(Pt 2): 333–46.
10. Aggarwal SR (2012). "What's fueling the biotech engine–2011 to 2012". Nature Biotechnology. 30 (12): 1191–7.
11. Escobar–Morreale HF, Botella–Carretero JI, Morreale de Escobar G (2015). "Treatment of hypothyroidism with levothyroxine or a combination of levothyroxine plus L–triiodothyronine". Best Practice & Research. Clinical Endocrinology & Metabolism. 29 (1): 57–75.
12. Eliason BC, Doenier JA, Nuhlicek DN (1994). "Desiccated thyroid in a nutritional supplement". The Journal of Family Practice. 38 (3): 287–288.
13. Friesema EC, Jansen J, Jachtenberg JW, Visser WE, Kester MH, Visser TJ (2008). "Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10". Molecular Endocrinology. 22 (6): 1357–1369.
14. Brix K, Führer D, Biebermann H (2011). "Molecules important for thyroid hormone synthesis and action – known facts and future perspectives". Thyroid Research. 4 (Suppl. 1): S9.

Cite this article as:
Nandi K., Sen D.J. and Saha D. (2020). Hormones are the Masterkey in Chemical co–ordination system of body, *Int. J. of Pharm. & Life Sci.*, 11(10): 7042-7064.
Source of Support: Nil
Conflict of Interest: Not declared
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