BIOCHEMISTRY OF FASTING – A REVIEW ON METABOLIC SWITCH AND AUTOPHAGY.

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ABSTRACT:
Upavasa, or fasting, is the practice of denying oneself food and water. This is an ancient spiritual practice that has fallen into disuse. As stated by Charaka in his Charaka Samhita, Upavasa is one of the spiritual therapies and a type of langhana (that which produces lightness of the body) therapy. If properly practiced, it keeps our minds calm and gives us control over our senses. The eleventh day of each ascending and descending moon, known as Ekaadashi, is when the majority of people in Bhaarata observe fasts. It is a form of intermittent fasting (IF) that triggers metabolic switching, autophagy, etc. Studies have demonstrated that it has a wide range of positive health effects, including type-2 diabetes reversal, an increase in energy, and weight loss. It is an inexpensive technique to raise ketone bodies, lower lipids, and lower blood sugar levels. Additionally, it slows or stops the progression of illness and ageing. Modern researchers are also studying how fasting affects the body's metabolic processes. This paper intends to elucidate the biochemical aspects of fasting .i.e., the metabolic switch and the autophagy.

Keywords: Fasting, Lipolysis, Ketone body metabolism, Autophagy.

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1. INTRODUCTION:

Preparing our mind can be done in many ways and one of the first steps is to control and rein back our sensory inputs. Amongst the five sensory inputs, food is considered as one of the primary inputs that determine health - mental and physical. Hence, food and fasting have been given significance in ancient customs of India. Eating healthy food in limited quantities – as much as needed by the body, and regular fasting help the body to build its immunity, improve lifespan and importantly, prepares the mind to understand complex subjects, like Self-Knowledge. We can experience this intuitive fact in our daily lives as well. Think of how dull we feel after a heavy meal! (1)

Fasting is partial or total refrain from all foods. It is proven to be a beneficial non-pharmacological strategy in enhancing health.(2) Generally, there are three types of fasting practises; they are a caloric restriction (CR), dietary restriction (DR) and intermittent fasting (IF).(3)

Placing time restrictions on feeding has been shown to have broad systemic effects and trigger similar biological pathways as caloric restriction. (4) It has been demonstrated that IF regimens can improve cardio-metabolic health (5), decrease visceral mass (6) and result in weight reduction that is comparable to CR regimens. Besides weight loss and metabolic improvements, fasting results improvements in lipid profiles, (8) osteoarthritis, (9) thrombophlebitis healing, (9) and many other diseases.

2. METABOLIC SWITCH:

Glucose and fatty acids are the main sources of energy for cells. Generally, glucose is used for energy, and fat is stored in adipose tissue as triglycerides. Energy restriction causes the depletion of liver glycogen reserves as well as the mobilisation of fat from adipose tissue. The hydrolysis of the triglycerides releases free fatty acids and glycerol. These free fatty acids are transported to liver where they are converted to Ketone Bodies (Acetoacetate and β-HB). (10) These ketone bodies are considered to be the major energy source for many of the tissues, especially brain, during fasting. (11) Thus, lipids and ketones replace glucose as the primary energy sources. This is commonly referred to as Metabolic Switch.

2.1. KETONE BODY METABOLISM

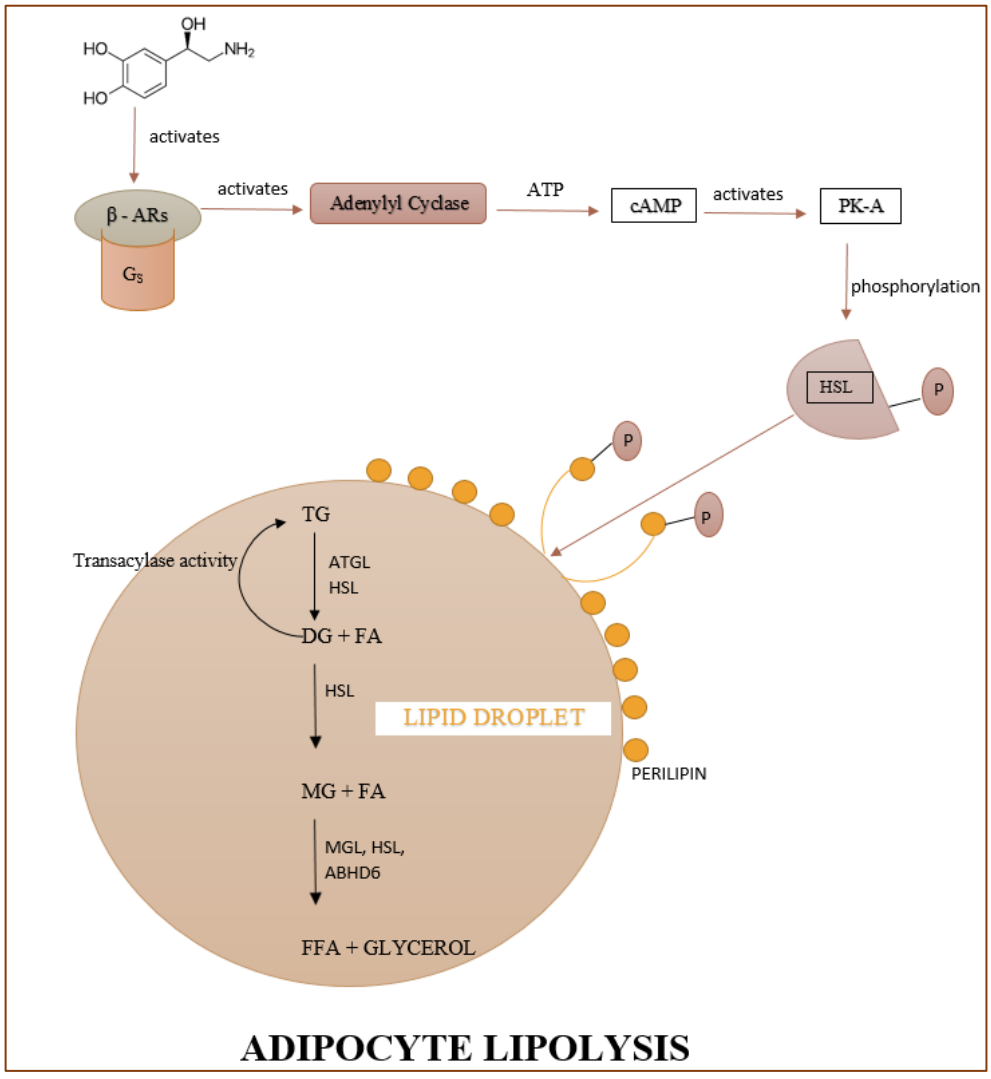
Ketone body metabolism includes its production (ketogenesis) and utilization (ketolysis).

Usually, it happens by undergoing the following important steps:

i. Adipocyte Lipolysis
ii. Mitochondrial entry of Fatty Acids
iii. Ketogenesis
iv. Ketolysis

i. Adipocyte Lipolysis: The FFAs released by adipose tissue serve as the primary substrate for ketogenesis. (12) When there is an energy surplus, adipocytes store the extra energy as TG for use when there is a negative energy balance, such as when fasting, starving, or engaging in prolonged exercise. TG hydrolases, also known as lipases, are required because TG cannot pass through biological membranes and must be broken before entering or leaving cells. (13, 14) Lipolysis is repressed postprandially when plasma insulin concentrations are high and is active during fasting, when plasma insulin concentrations are low (15) Enzyme catalysed TG hydrolysis was discovered in early 20^{th} century, yet it took more than fifty years to characterize the mechanism behind it. (16) In 1964, Steinberg and colleagues discussed the involvement of hormone-sensitive lipase (HSL) as the primary hydrolase in the degradation of TGs and DGs and Monoglyceride lipase (MGL) in the hydrolysis of MG in adipocytes (17) For the next forty years, HSL was believed to be the rate-limiting enzyme for TG hydrolysis, but studies showed that HSL-deficient animals retained hormone-induced FA release in adipose tissue, did not develop obesity, and developed DGs. (18, 19) Finally, a justification was put forward for other enzymes and processes being carried in TG hydrolysis. Following a thorough research for these substitutes, an entirely new enzyme known as adipose triglyceride lipase (ATGL) (37) or Ca^{2+}-independent phospholipase-A2-ζ (iPLA2-ζ) (21), and its coactivator, named α/β hydrolase domain-5 (ABHD-5) were found. (22) Catecholamines are produced by the sympathetic nervous system to start lipolysis during fasting or stressful times. (23) Nor-epinephrine is a key catecholamine that facilitates adipocyte lipolysis (24, 25) and is produced when -adrenergic receptors (β -ARs) are activated. The most potent lipolytic components in humans are the β1- and β2-adrenoceptors, while recent research has shown that β3-adrenergic receptors also play a role. It has been amply demonstrated that human white adipocytes have β3-adrenoceptors, with tissue and subcellular distribution and responses to stimuli being compatible with involvement in lipolysis. (26) Selective stimulation of β3-AR induces lipolysis in human isolated white adipocytes similarly to rats. (27, 28) β1, β2, and β3-ARs, which are coupled to G_s, lead to

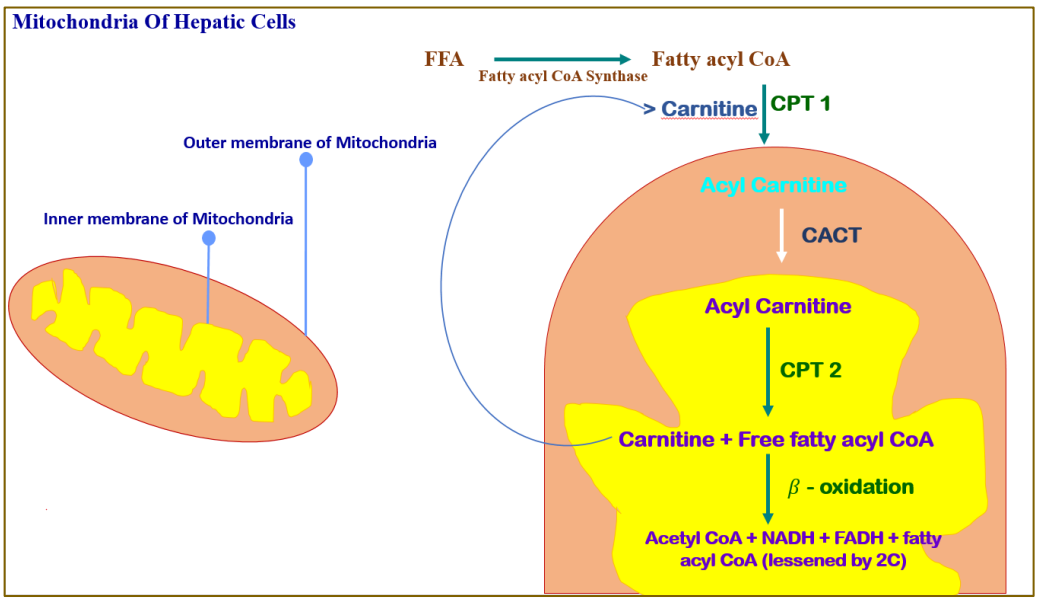
activation of adenylyl cyclase, increasing the levels of the second messenger cyclic AMP (cAMP) to promote Protein kinase A (PKA) activation. (24) PKA phosphorylates HSL, activating it, as well as perilipin, a protein found in adipose tissue that coats lipid droplets to stop them from being hydrolyzed by protecting them from hormone-sensitive lipase. Perilipin is delocalized from the lipid droplet surface as a result of phosphorylation, and active HSL is brought to the droplet surface. (15) It also causes the hydrolysis of TGs by activating the ATGL. A disproportionation reaction between two DG molecules and the enzyme results in the synthesis of TG and MG (21, 29, 30), which is an intriguing transacylase activity. This transacylase activity has remained unsolved. (21) The hydrolysis of TG results in the production of DGs. It promotes DAG hydrolase activity to release an FA and generate MAG. MGL and ABHD-6 hydrolyze MAG to produce a final FA and glycerol. (31)



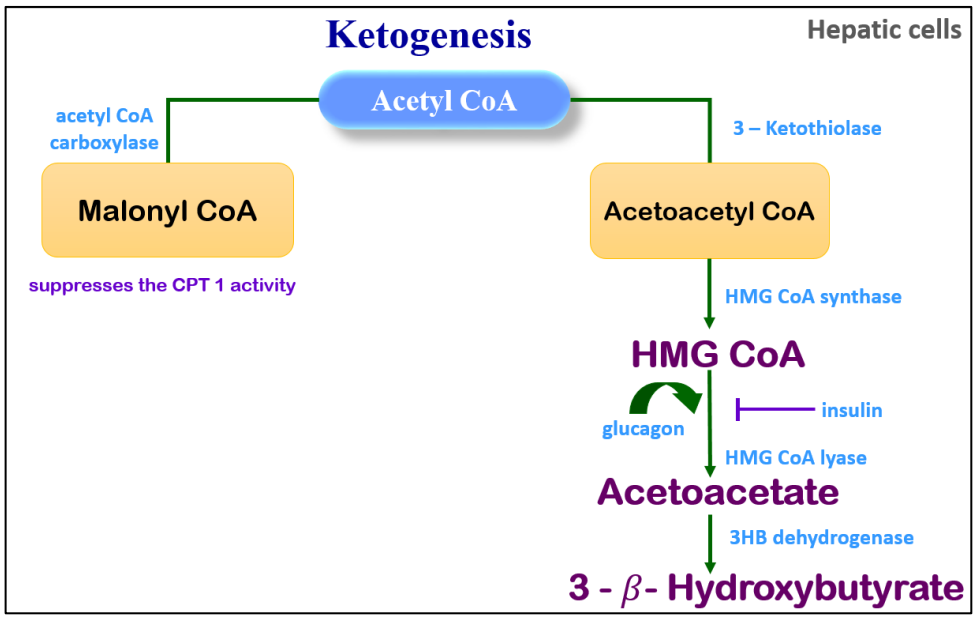
Fatty acids pass through cell membranes and via the bloodstream. Contrary to the notion that the brain cannot use fatty acids for energy and must employ ketone bodies as a route of energy transfer from fat storage, certain tissues, such as skeletal muscle, heart, and liver, may use fatty acids as an energy source.

ii. Mitochondrial entry of fatty acids: When insulin levels are low and fatty acid concentrations are high, fatty acids in the blood get converted to ketone bodies primarily within hepatic cells. Fatty acyl CoA synthase converts fatty acids inside of the cell into long-chain acyl CoA.(15) The carnitine shuttle allows long-chain fatty acyl-CoAs to pass through the inner mitochondrial membrane. Carnitine-palmitoyl transferase I (CPT1) first conjugates acyl-CoA molecules to carnitine. The carnitine-acylcarnitine translocase (CACT) then transports acylcarnitines across the very impermeable inner mitochondrial membrane. Carnitine-palmitoyl transferase 2 (CPT2) then releases free acyl-CoAs into the mitochondrial

matrix while transporting free carnitine back to the cytoplasm. (32, 33, 34) Cycles of b-ox break down fatty acyl-CoAs in the mitochondrial matrix. One molecule of AcCoA, one molecule each of NADH, FADH, and a fatty acyl-CoA with two less carbons are produced throughout each cycle.

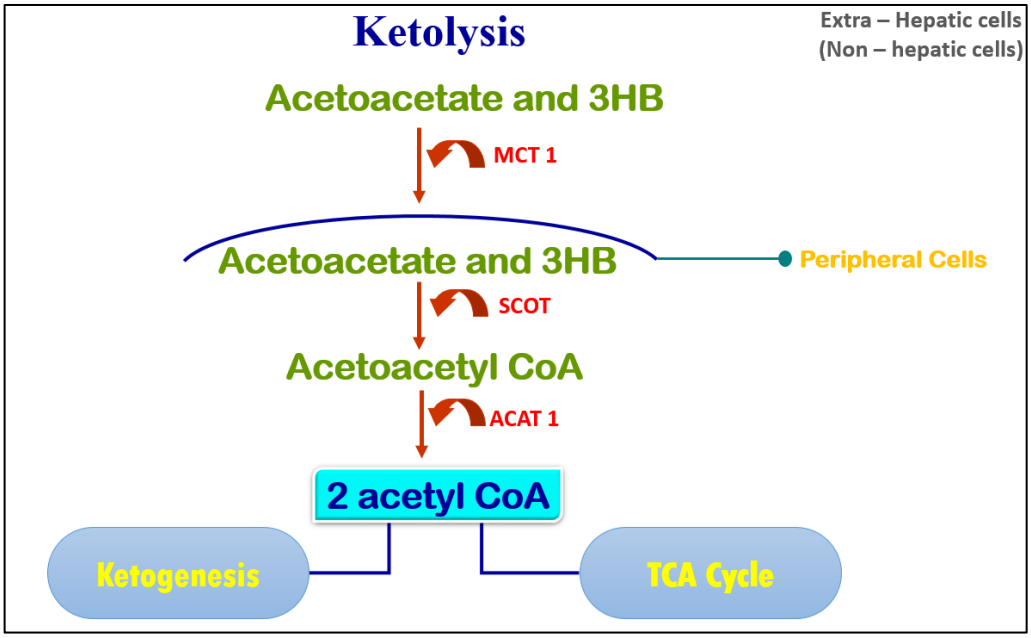


iii. Ketogenesis: Acetyl CoA molecules are either converted to acetoacetyl CoA by 3-ketothiolase or to malonyl CoA by acetyl CoA carboxylase. Malonyl CoA suppresses the activity of liver's CPT-1. HMG CoA synthase then transforms acetoacetyl CoA into 3-hydroxy-3-methylglutaryl CoA (HMG CoA). As the rate-limiting step in the production of ketone bodies, HMG CoA synthase is crucial to this process. Insulin and glucagon have opposing effects on the control of HMG CoA synthase. HMG CoA lyase eventually converts HMG CoA to acetoacetate. Acetoacetate may now be transformed by 3HB dehydrogenase into 3-B-hydroxybutyrate (3HB). Organic acids like acetoacetate and 3HB readily pass through cell membranes and into the blood and other human organs. (15, 35, 36, 37)



iv. Ketolysis: The process of oxidising ketone bodies which occurs in mitochondria is known as ketolysis. Almost all cells (with the exception of hepatocytes and the majority of malignantly altered cells) are capable of ketolysis, in contrast to ketogenesis, which can only be carried out by particularly specialised cells. Monocarboxylate transporter 1 (MCT1), which is expressed in

almost all cells, actively absorbs ketone molecules (bHB and acetoacetate) from blood through the peripheral tissues. BDH (β-hydroxybutyrate dehydrogenase) transforms bHB into acetoacetate. Succinyl-CoA: 3-ketoacid-CoA transferase, or SCOT, converts it next to acetoacetyl-CoA, which is the crucial process that permits ketone bodies to be used as energy substrates. Acetoacetyl-CoA is broken down into two molecules of acetyl-CoA by ACAT1 in the subsequent step. Acetyl-CoA molecules are then oxidised to produce ATP in the TCA cycle and respiratory chain. Alternatively, this acetoacetyl-CoA may, under specific circumstances, be incorporated into lipids (such as cholesterol or fatty acids). Only the liver lacks the expression of SCOT, preventing the ineffective conversion of acetoacetate to HMG-CoA and vice versa. (38)



2.2. SIGNIFICANCE OF METABOLIC SWITCH

2.2.1. METABOLIC SWITCH AND BRAIN METABOLISM

It would be reasonable to assume that KBs may exert their therapeutic impact by directly lowering neuronal firing rates given that ketogenic diets are clinically used to treat epilepsy. In this context, one of the earliest investigations into the metabolism of the brain and the effects of ketogenic diets suggested that switching from glucose to KBs as the source of ATP might lead to higher ATP:ADP ratios. According to the study's findings (39), this increase in the energy levels available may assist preserve neuronal "stability" (i.e., the resting state) and lessen the frequency, length, and/or severity of depolarization events.

BDNF, a member of the nerve growth factor family, is a crucial regulator of glucose metabolism in the body and is essential for maintaining neuronal survival, synaptic function, hippocampal neurogenesis, learning, and memory [40]. One of the most significant neuronal adaptations to IF is the increased synthesis of brain-derived neurotrophic factor (BDNF), which is well supported by the data [41]. In fact, the metabolic switch that occurs during food deprivation stimulates excitatory synaptic activity in neurons, triggering calcium influx through membrane channels and resulting in the activation of multiple kinases and signalling pathways that induce the expression of different genes that ultimately encode proteins involved in cellular stress adaptation, one of which is BDNF [42]. The findings show that the metabolic switch signal for neurons, BHB, which is generally raised during fasting, also acts as a peripheral signal to activate signalling pathways that increase neuronal stress resistance and neuroplasticity. In BDNF-deficient animals fed ad libitum, IF restored BDNF brain levels to the same level as in wild-type mice, and it significantly decreased circulating glucose and insulin levels to restore normal glucose tolerance and insulin tolerance tests [43]. In two further rodent investigations, IF induced the production of BDNF, which improved memory and spatial learning [44, 45] and potentiated hippocampus neurogenesis by increasing the density of dendritic spines in hippocampal dentate granule neurons.

According to a study (46), rats on a ketogenic diet had an accumulation of gamma-amino butyric acid (GABA) in their synaptic terminals. The primary

excitatory neurotransmitter in the human brain, GABA regulates excitability, information processing, synapses between neurons, neuroplasticity, and learning and memory processes [47]. Overfeeding of the Krebs cycle by KBs decreases the amounts of oxaloacetate, which is replaced by aspartate transamination via aspartate transaminase. In order to complete this process, α-ketoglutarate must be converted to glutamate. Glutamate is then catalysed into GABA in GABAergic neurons by the enzyme glutamate decarboxylase. (48) Furthermore, GABA controls how neuronal circuits react to external stressors by activating pathways that govern structural and functional changes, including synaptogenesis, long-term potentiation, and long-term depression, which are crucial for neuroplasticity. (49)

2.2.2. METABOLIC SWITCH AND LOW GRADE INFLAMMATION

Select metabolites, such as lactate, succinate, or fatty acids, among others, can elicit a response from a number of plasma membrane receptors (50, 51). It should be noted that healthy BHB concentrations can activate the G protein-coupled receptor (GPCR) for niacin, also known as GPR109a/HCA2/HM74A/PUMA-G (52). Some hypothalamic neurons may express GPR109a, and its activation may affect endocrine regulation by modifying ERK1/2, namely the growth hormone signalling axis (53). GPR109a is notably concentrated in neutrophils, adipocytes, and macrophages (54). Actually, it has been demonstrated that activating GPR109a has anti-inflammatory effects (55). The question about the physiological significance of the association between KBs and inflammation control emerges in light of these actions of GPR109a and the biological function of KBs as its endogenous ligand. Long-term fasting causes the synthesis of ketone bodies because it lowers glycemia to an extreme degree. It's interesting that prolonged hypoglycemia has been linked to more inflammatory conditions (56). Therefore, it is possible to hypothesise that KBs through GPR109a not only operate as a substitute fuel source during fasting but also help to reduce the immune system's concurrent activity. This is in line with a recent research (57) that found short-term use of a ketogenic diet reduced systemic inflammation in mice.

3. AUTOPHAGY

Over 40 years ago, Christian de Duve first used the term "autophagy," which is derived from the Greek and means "eating of oneself." His theory was largely based on the observation that rat liver lysosomes perfused with the pancreatic hormone glucagon degraded mitochondria and other intracellular structures. (58)

Although the mechanism of autophagy was originally described in the 1960s, important advances in understanding its complicated mechanistic nature only came about with the discovery of autophagy-related genes (ATG genes) in the 1990s(59, 60). Macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA), which are the three main kinds of autophagy, all require delivering substrates to the lysosome for destruction (61, 62).

3.1. CROSS-TALK BETWEEN MACROAUTOPHAGY AND CMA

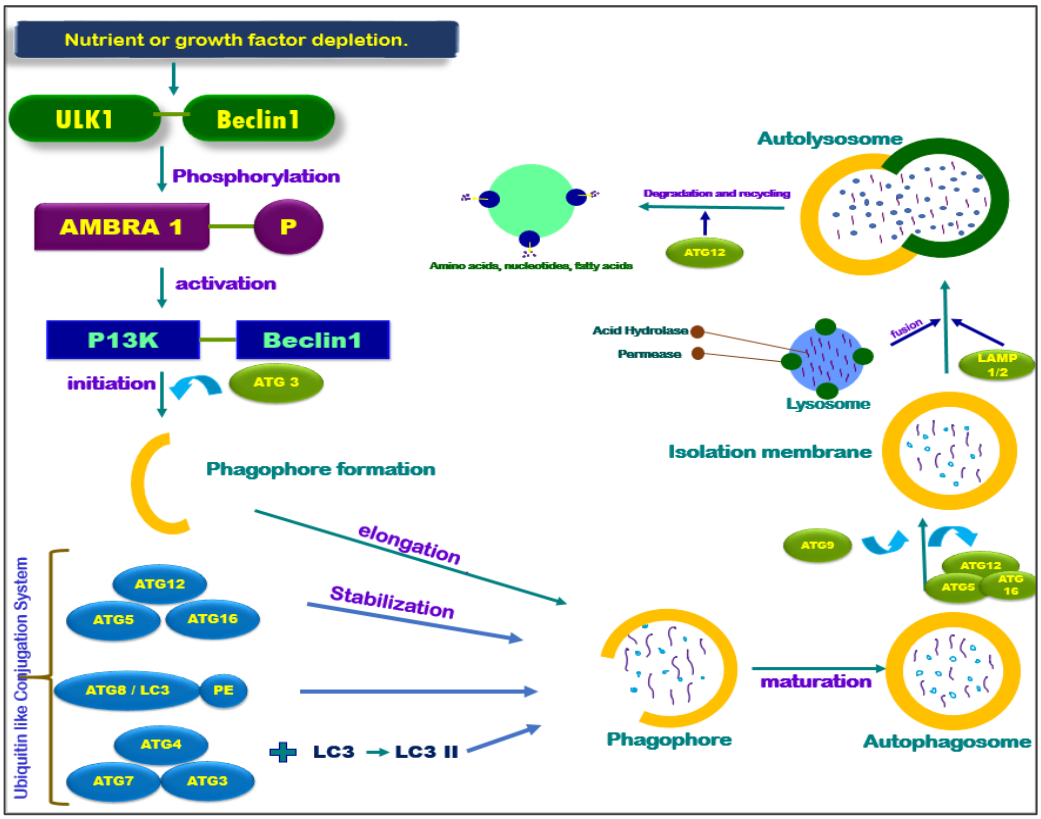
The activation and activity of the three autophagic pathways are coordinated as part of the overall programme for intracellular degradation and the demands of the cell under various situations, rather than functioning in the cell as entirely autonomous entities. For instance, fasting has been shown to trigger CMA and macroautophagy (63). They do not, however, activate at the same time. Instead, macroautophagy begins to function in the early stages of food deprivation, peaks in most cell types at around 4–6 hours, and then progressively falls to baseline levels. Beyond that point, if fast persists, the decline in macroautophagy is accompanied by a gradual rise in CMA activity (64).

Around 12 hours after fast, CMA reaches its peak level of activation in the majority of cells, and it continues to function during this time. In order to access the amino acids needed for cellular fuelling and to maintain protein production under these circumstances, the transition from macroautophagy to CMA may provide better levels of selectivity when determining the cellular components that can be digested. This coordinated process of activation provides compelling evidence that the various forms of autophagy interact with one another on a molecular level. It is conceivable, for instance, that macroautophagy activation aids in the degradation of endogenous CMA inhibitors, which in turn aids in the eventual activation of this route when macroautophagy activity decreases. Alternately, under these circumstances, some of the Atg proteins engaged in macroautophagy might turn into CMA substrates, and hence, the steady rise in CMA activity may result in their depletion and a corresponding decline in macroautophagy. (65)

3.2. MECHANISM OF MACROAUTOPHAGY

Pro-autophagic indications including nutrition or growth factor depletion activate autophagic machinery regulators like the ULK1 and Beclin-1

complexes. (66) The ULK complex is put together, which phosphorylates AMBRA1 and activates the PI3K complex to start the process (67). While PI3K and Beclin 1 mediate membrane nucleation. The Atg5-Atg12-Atg16 complex is attracted to the pre-autophagosomal structure (PAS), where it interacts with the phagophore's outer membrane, thus stopping the early fusion of vesicles and lysosomes (68). The second ubiquitin-like system promotes the interaction of phosphatidylethanolamine (PE) and Atg8/microtubule-associated protein 1 light chain 3 (LC3). When attached to the phagosome (LAPosome), LC3 has a strong affinity for the lysosome, causing any absorbed pathogens to be destroyed and degraded at a faster rate (69). For the growth and completion of the autophagic membrane, Atg4, Atg7, and Atg3 convert LC3 into LC3-II, a molecular marker for autophagosomes (70) that is present on both its inner and outer sides. The Atg5-Atg12-Atg16 complex separates from the autophagosome during autophagosomal closure. Atg9 is necessary for the creation of intraluminal vesicles and is localised inside the autolysosome for acidification (71); Atg9 which is essential for the development of autophagosomes, is also translocated to the site of autophagosome formation where it offers a membrane to extend the limiting membrane, known as the phagophore (72) The autolysosome, which is controlled by lysosomal membrane proteins and cytoskeletal proteins, is created when the autophagosome unites with the lysosome (73). The maturation of autophagosomes is controlled by the LAMP-1/2 protein. The internalised cargo and internal autophagosome membrane are broken down by hydrolytic enzymes within the autolysosome, and the broken-down byproducts, including amino acids, are subsequently released into the cytosol for recycling. Pathways for cell trafficking are also intimately connected to autophagosomes.



3.3. MECHANISM OF CMA

The CMA process begins with the heat-shock protein of 70 kDa (Hsc70), a cytosolic member of the Hsp70 chaperone family that recognises cytosolic proteins with the loose pentapeptide motif KFERQ. This process involves several co-chaperones, including as Hsp40, Hsp90, and Hip. (74) After being bound by hsc70, the substrate proteins are guided to the surface of certain lysosomes that are engaged in the autophagic process. After interacting with the Hsc70 complex, substrates are translocated to the lysosomal membrane surface, where the single-span lysosomal receptor LAMP2A may bind the substrate protein with its 12 amino acid tail exposed in the cytoplasm. (75, 76) LAMP2A is in charge of lysosome internalisation as well as substrate binding. This method relies on LAMP2A levels and conformation state to function. This is a rate-limiting stage in the process that may be altered by

synthesis, degradation, and redistribution. (77) LAMP2A is a monomer that joins with other proteins to create a multimeric complex at the lysosomal membrane. At the lysosomal membrane, CMA substrates bind to monomeric LAMP2A, causing LAMP2A to multimerize and form a 700-kDa complex that is required for substrate translocation into the lysosome. (78) LAMP2A multimerization encourages substrate affinity and probably avoids aggregation as the substrate unfolds. (79) This protein complex permits the substrate protein to enter the lysosome lumen, where it is destroyed by hydrolytic enzymes. The substrate can bind to the receptor while folded, but it must unfold to pass the lysosomal membrane. This process is finished before the LAMP-2A complex is completely put together and is likely mediated by hsc70 and some of its co-chaperones. (76) Complete substrate internalisation also requires the presence of the luminal chaperone lys-Hsc70 within the lysosome. A lysosomal form of hsp90 keeps its stability while LAMP-2A transforms from monomers to multimers. (80) Once the substrate enters the lysosomal matrix, it is broken down into amino acids by lysosomal hydrolytic enzymes, sometimes referred to as cathepsins. Lys-hsc70 (lysosomal HSC70) and EF1α (Elongation Factor 1α) allows LAMP2A to disassemble from its multimeric form into its monomeric form, allowing the next substrate protein to attach to LAMP2A in a new cycle. (81)

Because there is so little known about microautophagy in mammalian cells, nothing is known about the potential connection between macroautophagy and CMA with microautophagy.

3.4. SIGNIFICANCE OF AUTOPHAGY

3.4.1. Anti-Bacterial Role of Autophagy

Autophagy helps to fight infectious illnesses by digesting microorganisms while also stimulating the host immune system (82). This makes it possible to treat diseases both directly by eliminating the infectious agents and subtly by triggering human immunity against pathogens. *Salmonella enterica*, (83) *Listeria monocytogenes*, (84) *Shigella flexneri*, (85) and other bacterial infections are all successfully defended against by the effective intracellular defence system that autophagy offers. Xenophagy is the name given to antibacterial autophagy. (86)

3.4.2. Anti-viral Role of Autophagy

Autophagy has been employed for antiviral immunity because it plays a helpful function in cellular defence against viral invasion. (87) Through a variety of molecular processes, autophagy aids in the removal of viral pathogens during infection, controls immunological responses, and guards against damaging inflammation and over-activation. (88)

3.4.3. Autophagy in Cardiovascular Diseases

Studies demonstrate how CMA protects against processes linked to the aetiology of CVD and how CMA failure contributes to the development of atherosclerosis (89). The two primary cell types implicated in atherogenesis—VSMCs and macrophages—undergo systemic and cell-autonomous alterations in mice with CMA blockage, increasing their susceptibility to proatherosclerotic stimuli. When CMA is lost, VSMCs become more dedifferentiated and more vulnerable to lipid challenges, whereas CMA deficiency in macrophages results in a more proinflammatory phenotype. Overall, proatherosclerotic stressors cause the vasculature to activate CMA, a defence mechanism, and decreased CMA activity makes the vasculature more susceptible to these challenges. (90)

3.4.4. Autophagy in Obesity and Diabetes

Autophagy is involved in obesity (91) and diabetes mellitus (91). Improper lipid and glycogen processing can affect the liver activity and thus, insulin synthesis, resulting in diabetes. Studies have shown that hepatocytes from mouse models of obesity display reduced autophagy, with decreased Atg7 expression causing ER stress and affecting insulin signaling (93). Obesity impairs autophagy in the liver via *S*-nitrosylation, a process induced by nitric oxide (NO). *S*-nitrosylation of the lysosomal enzymes cathepsin B (CTSB) and hexosaminidase subunit β (HexB) impairs normal lysosomal functioning and is carried out by denitrosylation enzymes, particularly *S*-nitrosoglutathione reductase (GSNOR) and thioredoxin (94). Obesity inhibits the denitrosylation ability of the liver, impairing hepatic autophagy and insulin resistance (95). In obese animals, hepatic insulin signaling is impaired by NO-induced hepatic autophagy repression, which ultimately causes the progression of type 2 diabetes (96).

3.4.5. There are several diseases caused by Autophagy Gene Defects such as SENDA (Static Encephalopathy of Childhood with Neurodegeneration in Adulthood) (97), Crohn's disease (98), Danon Disease (99), X-Linked Myopathy with Excessive Autophagy (XMEA) (100) and others.

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