Review

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Influence of intermittent fasting on autophagy in the liver

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SUMMARY Studies have found that intermittent fasting (IF) can prevent diabetes, cancer, heart disease, and neuropathy, while in humans it has helped to alleviate metabolic syndrome, asthma, rheumatoid arthritis, Alzheimer's disease, and many other disorders. IF involves a series of coordinated metabolic and hormonal changes to maintain the organism's metabolic balance and cellular homeostasis. More importantly, IF can activate hepatic autophagy, which is important for maintaining cellular homeostasis and energy balance, quality control, cell and tissue remodeling, and defense against extracellular damage and pathogens. IF affects hepatic autophagy through multiple interacting pathways and molecular mechanisms, including adenosine monophosphate (AMP)-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), silent mating-type information regulatory 2 homolog-1 (SIRT1), peroxisomal proliferator-activated receptor alpha (PPARα) and farnesoid X receptor (FXR), as well as signaling pathways and molecular mechanisms such as glucagon and fibroblast growth factor 21 (FGF21). These pathways can stimulate the pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α), play a cytoprotective role, downregulate the expression of aging-related molecules, and prevent the development of steatosis-associated liver tumors. By influencing the metabolism of energy and oxygen radicals as well as cellular stress response systems, IF protects hepatocytes from genetic and environmental factors. By activating hepatic autophagy, IF has a potential role in treating a variety of liver diseases, including non-alcoholic fatty liver disease, drug-induced liver injury, viral hepatitis, hepatic fibrosis, and hepatocellular carcinoma. A better understanding of the effects of IF on liver autophagy may lead to new approaches for the prevention and treatment of liver disease.

Keywords metabolism, diet, nutrient, NAFLD, HCC, liver disease

1. Introduction

Autophagy is a lysosomal degradation pathway by which cells "self-digest" their own components to provide nutrition under challenging conditions as well as to remove excess and damaged organelles, misfolded proteins, or invasive microorganisms from the cell (*1*). In addition to its role in cellular homeostasis, autophagy also plays an important role in embryonic development, cell differentiation, and regeneration (*2*). The liver is the organ in which autophagy was first studied and has benefited from this deeper understanding of autophagy, a process closely linked to liver pathophysiology (*3-5*). Hepatic autophagy helps to maintain essential liver functions such as lipid, glycogen, and protein regeneration, whereas dysregulated hepatic autophagy is associated with a variety of liver diseases, including alcoholic and non-alcoholic fatty liver disease, pharmacological liver injury, viral hepatitis, hepatic fibrosis, and hepatocellular carcinoma (*5-7*).

The term intermittent fasting (IF) encompasses several different dietary regimens. Current research suggests that IF may reduce the inflammatory response and improve the outcome of diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease (IBD), and allergic contact dermatitis (*8-10*). Studies in animals have demonstrated that IF has anti-aging benefits, and they have revealed its potential applications in neurodegenerative diseases in particular. For example, by attenuating the levels of oxidative stress proteins such as NOX2, NOX4, 8-OHdG, and 4-HNE and inhibiting the levels of the pro-apoptotic factor Bax and cleaved cysteine asparaginase-3, IF had a protective effect against cognitive dysfunction in type 1 diabetic mice (*11*). In addition, IF has the potential to optimize gut microbiota and enhance immune

memory (*12*). A study on the treatment of certain tumors has shown that IF can synergistically enhance the anticancer action of conventional treatments and increase the sensitivity to radiotherapy/chemotherapy (*13*). More importantly, IF is associated with autophagy. Through the regulation of hepatic autophagy, IF has a potentially beneficial role in liver pathophysiology (*14*). The current review summarizes the available evidence on the effects of IF on hepatic autophagy and how this may impact physiopathological changes in the liver.

2. Liver autophagy

2.1. Discovery of autophagy

Autophagy was identified as a mechanism for degrading cytoplasmic components in the late 1950s and early 1960s, following the discovery of lysosomes by Christian de Duve (*3*). Autophagosomes were subsequently identified through electron microscopy of isolated liver tissue under stress conditions, and these structures were found to play an important role in the starvation response (*4*). Autophagy is evolutionarily conserved as a ubiquitous, self-degrading catabolic process. It is necessary to meet the metabolic demands of the cell, maintain genome integrity, regulate innate and adaptive immune processes, modulate pro-inflammatory mediators, and promote cell survival. Constitutive autophagy occurs continuously in nutrient-rich environments to maintain the turnover of cellular components and is a fundamental survival mechanism. However, induced or reactive autophagy occurs in response to stress, including starvation, low amino acid levels, lack of trophic factors or hormones, endoplasmic reticulum (ER) stress, hypoxia, irradiation, drugs, and intracellular pathogens (*15*).

2.2. Diverse forms of autophagy

Autophagy can be classified into three main types based on mechanism of cargo delivery to lysosomes, *i.e.,* macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) of proteins containing lysine-phenylalanine-glutamic acid-arginine-glutamine (KFERQ) -like motifs (Figure 1). Moreover, autophagy can be classified into selective and non-selective autophagy based on the selective and non-selective phagocytosis of substrates. Selective autophagy plays an essential role in maintaining cellular homeostasis by ensuring organelle cycling and degrading bound substrates.

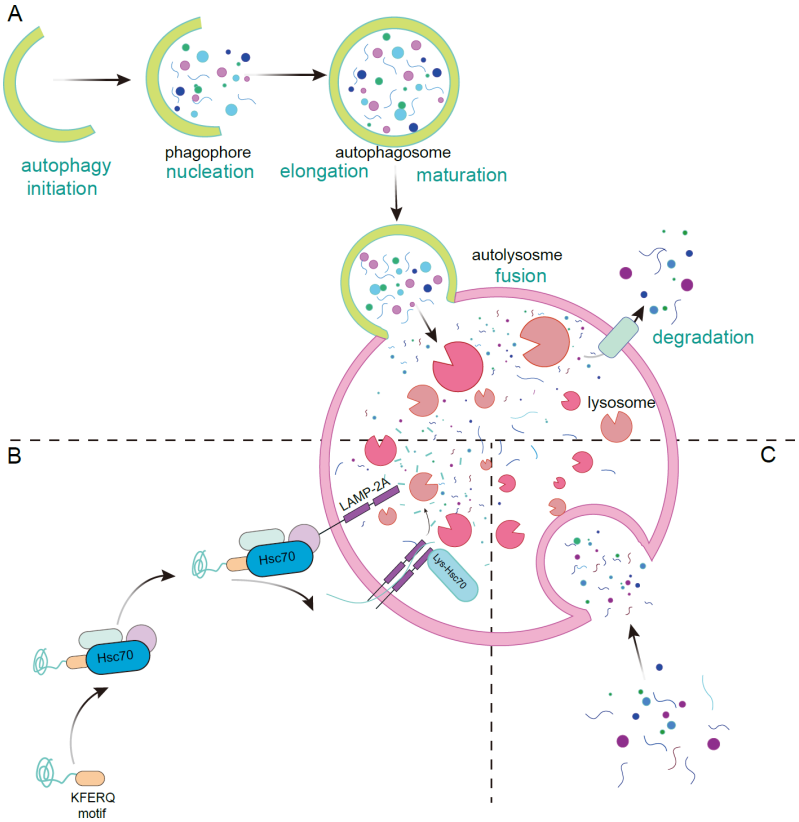

Figure 1. Three main types of autophagy: macroautophagy, CMA, and microautophagy. (A) Macroautophagy is a multistep process involving initiation, nucleation, elongation, maturation, fusion, and finally degradation. **(B)** CMA specializes in chaperone-mediated degradation of cytoplasmic proteins, delivering them to the lysosomal surface where substrate proteins unfold and cross the lysosomal membrane. These substrates, which contain the KFERQ motif, are specifically recognized by Hsc70. Chaperone-targeted proteins bind to LAMP-2A. **(C)** In microautophagy, targeted cargo is directly isolated and subsequently engulfed by lysosomes. Abbreviations: CMA, chaperone-mediated autophagy; Hsc70, heat shock cognate protein of 70 kDa; LAMP-2A, lysosome-associated membrane protein 2A.

2.2.1. Macroautophagy

Macroautophagy is divided into several phases, including autophagy initiation, phagosome nucleation and elongation, autophagosome maturation and fusion with lysosomes, and degradation. The process of autophagy has been extensively described in a number of other reviews and will only be briefly mentioned here. Autophagosomes originate from a membrane called the phagolysosome, a restriction membrane or de novo phagolysosome formed by the assembly of proteins and lipids from various organelles, such as the ER, Golgi apparatus, mitochondria, endocytosis system, or plasma membrane, whose nucleation and elongation are dependent on autophagy-related genes (ATGs) (*16*). More than 40 ATGs have been reported to be involved in autophagy. Once the membrane of the autophagosome is sealed, the cargo-containing vesicle moves along the microtubule to fuse with the lysosome and deliver the loaded cargo to the organelle for further degradation. After degradation, the molecules, amino acids, lipids, and carbohydrates produced are transported to the cytoplasm for recycling, in part by transporter proteins and permeases. Following degradation within autolysosomes, nutrient-supplied reactivation of mammalian target of rapamycin (mTOR) suppresses autophagy initiation and simultaneously initiates autophagic lysosome reformation (ALR), thereby terminating autophagy (*17*).

2.2.2. Chaperone-mediated autophagy (CMA)

Neither CMA nor microautophagy needs autophagosomes. CMA is induced after long-term starvation, and proteins containing the pentapeptide KFERQ motif are recognized by the cytosolic heat shock cognate protein of 70 kDa (Hsc70), which facilitates the translocation of CMA substrates into the lysosomal lumen by binding to lysosome-associated membrane protein 2A (LAMP-2A). Substrate proteins are then rapidly degraded in the lysosomal lumen (*18*). LAMP-2A levels, which limit CMA activity, are controlled both by transcriptional activation and, more commonly, by direct changes in the stability of LAMP-2A at the lysosomal membrane (*19*). CMA was originally described as part of the hepatocyte response to nutritional changes in the liver by replenishing chronically starved cells with amino acids and ATP (*20*). In addition, CMA can act as a defense mechanism against cellular damage, that is by removing damaged proteins and thus maintaining protein homeostasis, maintaining lipid metabolism homeostasis, reprogramming gene transcription, activating the immune response, modulating the cell cycle, and being involved in the regulation of senescence (*18*).

2.2.3. Microautophagy

Microautophagy is the direct phagocytosis of cytoplasmic material by the lysosomal membrane. Based on morphological changes examined using electron microscopy, microautophagy is the major autophagic response in the mouse liver under starvation and refeeding conditions (*21*). Although the first studies of microautophagy were carried out in mammals soon after the identification of the lysosome, many of our previous insights into microautophagy have been gained in yeast, probably because of the relatively large size of the yeast vacuole (analogous to the mammalian lysosome) and the accessibility of the yeast cell (*22*). Most studies of microautophagy have focused on changes associated with the lysosome, vacuole, or endosome. During microautophagy, autophagic cargo is directly engulfed by lysosomes and late endosomes through membrane protrusion and invagination, and the autophagic cargo is then degraded in the lysosomal lumen.

2.2.4. Selective autophagy

Most of the three types of autophagy described above are thought to process, segregate, and degrade some cytoplasmic contents non-selectively. Selective autophagy differs from non-selective autophagy in that it plays a more selective role in response to a variety of physiological stimuli. Depending on the substrate that is being degraded, selective autophagy has been further categorized into mitophagy, ER-phagy, pexophagy, glycophagy, lipophagy, RNautophagy, DNautophagy, ferritinophagy, crinophagy, xenophagy, and aggrephagy. The best described and widely studied types of selective autophagy in the liver are mitochondrial autophagy and lipophagy. Selective autophagy has multiple functions, including protecting mammalian cells from organelle damage by removing dysfunctional organelles (*5*).

2.3. Role of autophagy in liver physiology and metabolism

The liver is the largest solid organ in the human body and plays an important role in various biological activities. The major functions of the liver include bile production, bilirubin metabolism, synthesis of anticoagulant factors and plasma proteins, amino acid and lipid metabolism, vitamin and mineral storage, hormone production, detoxification, and immune response. A point worth noting is that autophagy plays a crucial role in maintaining liver function. Three different types of autophagy coexist in the liver: macroautophagy, microautophagy, and CMA. Autophagy plays an important role in the maintenance of cellular and metabolic homeostasis in the liver.

Hepatic autophagy fluctuates in response to hormonal changes and nutrient availability in the fed and fasted state, as well as circadian rhythmic activity. At the organismal level, liver autophagy varies in response to

pancreatic hormones such as insulin and glucagon and gastric hormones such as growth hormone peptide (GHP) and glucagon-like peptide-1 (GLP-1) analogs, which positively or negatively regulate autophagy in response to feeding and fasting conditions (*23*). Liver autophagy is also regulated by the central nervous system (CNS). Glial cell-derived neurotrophic factor (GDNF) increases hepatic autophagy by inhibiting mTOR (*24*). During nutrient deprivation, adrenaline secretion increases, which ultimately increases hepatic autophagy in response to starvation (*25*). In addition, thyroid hormones (THs) can activate hepatic autophagy to promote fatty acid β-oxidation (*26*).

Nutrient deprivation triggers liver autophagy, which leads to glycogen breakdown, lipolysis, and proteolysis that provide glucose, fatty acids, and amino acids to cells and fuel to other organs. Studies in neonatal and adult mice have shown that autophagy is necessary for the maintenance of amino acid and glucose levels in blood and tissues during fasting (*27*). By converting amino acids to glucose *via* gluconeogenesis, liver-specific autophagy plays an important role in the regulation of blood glucose levels. During starvation, protein homeostasis is maintained by supplementing the intracellular amino acid pool through increased protein degradation in the cytoplasm. Amino acids produced by lysosomal degradation can also participate in the Krebs cycle to promote ATP production or gluconeogenesis (*28*). Moreover, autophagy is involved in lipid metabolism, including lipogenesis, lipolysis, fatty acid oxidation, ketogenesis, and cholesterol efflux. Loss of autophagy in the liver impairs the breakdown of triglycerides into fatty acids and results in steatosis and insulin resistance (*29*). In addition, ketone body production during fasting was significantly impaired by the specific deletion of hepatic *ATG7* or *ATG5* (*20*). Conversely, elevated circulating levels of inulin, glucose, adipokines, regulatory amino acids, and bile acids during feeding inhibit hepatic autophagy (*30*).

Interestingly, autophagy undergoes rhythmic changes that are consistent with circadian patterns in adult mammals. In the liver, cyclic activation of autophagic flux is associated with rhythmic expression of autophagy genes (*31*). The rate of conversion of light chain 3 (LC3)-I to LC3-II in the liver reaches a significant peak during the midday phase and then declines until the dark phase (*32*). A study has shown that the basic helix-loop-helix-PAS transcription factor families BMAL1 and CLOCK, which belong to the clock gene family, activate the transcription of genes involved in the regulation of autophagy (*33*).

In addition, autophagy is essential for the maintenance of protein and organelle homeostasis as well as quality control in hepatocytes. Selective autophagy ensures the removal of specific soluble proteins, protein aggregates, damaged mitochondria, and invasive bacteria from the cell, and defects in autophagy are directly associated with metabolic disorders. For example, mice with liver-specific deletion of *ATG5* or *ATG7* exhibit accumulation of ubiquitinated proteins, abnormal ER, excess peroxisomes, and mitochondrial dysfunction (*34,35*). Autophagy ultimately maintains homeostasis at the subcellular level of the tissue by regulating the renewal of intracellular organelles, including the selective removal of mitochondria (mitophagy), peroxisomes (pexophagy) and the ER (ER-phagy) (*36*). Through these processes, autophagy also has an indirect effect on metabolism and the maintenance of energy homeostasis. In addition, the liver catabolizes organelles and other intracellular components (*e.g.,* specific proteins and invading pathogens) through selective autophagy to maintain cellular homeostasis and protect cells from damage (Figure 2). Mitophagy occurs through many distinct but interrelated mechanisms that can usually be divided into Ub-dependent and Ub-independent pathways. The purpose of mitophagy is to isolate and remove dysfunctional, potentially cytotoxic mitochondria so that they do not cause harm to the host cells. Mitochondrial autophagy plays a key role in maintaining liver homeostasis or in the pathogenesis of liver diseases by selectively targeting damaged/excess mitochondria. Pexophagy removes excess and damaged peroxisomes through P62-dependent or P62-independent pathways to maintain healthy peroxisome homeostasis and it plays a key role in peroxisome biogenesis, reactive oxygen species (ROS) metabolism, fatty acid oxidation, polyamine and D-amino acid oxidation, and synthesis of lipid metabolites, bile acids, and DHA. ER-phagy occurs under normal conditions and is enhanced during starvation. There are two types of ER-phagy: macro-ER-phagy and micro-ER-phagy. In macro-ER-phagy, the autophagic bilayer membrane extends and wraps around the ER fragment, which eventually binds to the lysosome and is degraded. Micro-ER-phagy is a process in which the lysosomal membrane undergoes invagination and extrudes a portion of the ER into the lysosome. ER-phagy plays an important role in the elimination of excess ER, selective removal of misfolded aggregates in the ER, degradation of the nuclear membrane, nutrient supply, quality control, and antioxidant metabolism. The activation of hepatic autophagy protects the liver from oxidative stress, organelle stress, or damage induced by certain xenobiotics (*20*).

2.4. Dysregulation of autophagy in liver pathology

Hepatic autophagy plays a role in the prevention of liver diseases, and disorders or dysfunctions of autophagy play an important role in the pathogenesis of liver diseases such as non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), viral hepatitis, and hepatocellular carcinoma (HCC).

2.4.1. Autophagy in hepatic metabolic diseases

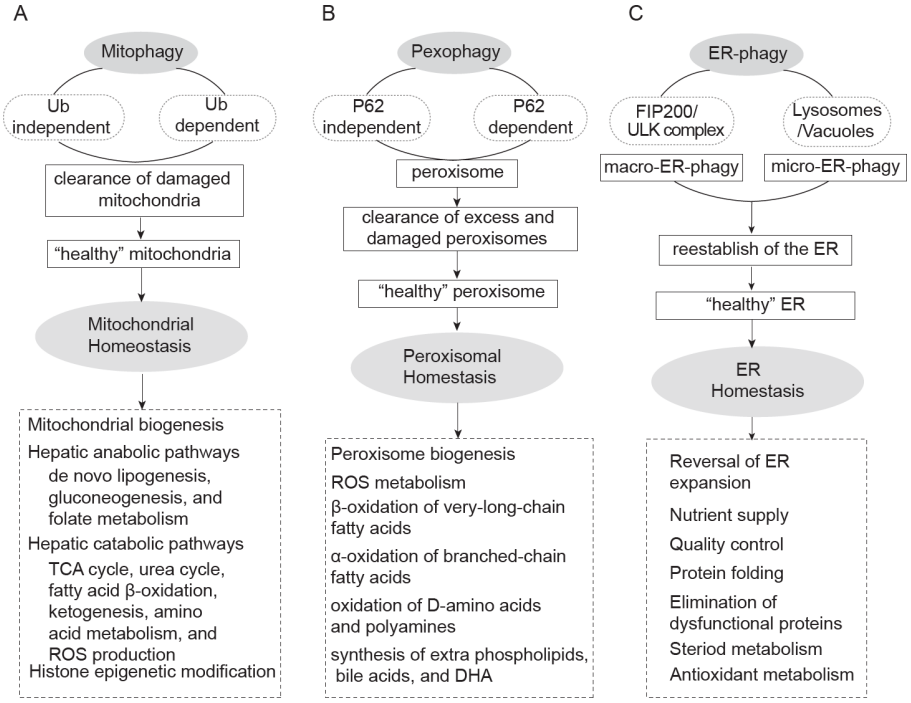

Figure 2. Hepatic autophagy maintains homeostasis through quality control. (A) Mitophagy plays an important role in mitochondrial biogenesis, hepatic anabolism/catabolism, and epigenetic modification of histones by maintaining healthy mitochondrial homeostasis through the clearance of damaged mitochondria, primarily through the Ub-dependent or Ub-independent pathways. **(B)** Pexophagy removes excess and damaged peroxisomes through P62-dependent or P62-independent pathways to maintain healthy peroxisome homeostasis and plays a key role in peroxisome biogenesis, ROS metabolism, fatty acid oxidation, polyamine and D-amino acid oxidation, and synthesis of lipid metabolites, bile acids, and DHA. **(C)** There are two types of ER-phagy: macro-ER-phagy and micro-ER-phagy. By reestablishing the ER through these pathways, ER-phagy plays an important role in the reversal of ER expansion, nutrient supply, quality control, protein folding, elimination of dysfunctional proteins, steroid metabolism, and antioxidant metabolism. Abbreviations: ER, endoplasmic reticulum; ROS, reactive oxygen species; TCA, tricarboxylic acid; ULK complex, unc-51 like autophagy activating kinase complex.

Due to its important role in the pathogenesis of NAFLD, autophagy is gaining attention as a novel therapeutic target. Induction of increased autophagy contributes to lipid degradation in the liver and alleviates disease progression in the early stages of NAFLD. The pathogenesis of NAFLD is due to abnormalities in hepatic lipid metabolism, including increased lipogenesis, increased uptake of free fatty acids (FFAs), and accumulation of hepatocellular lipids. NAFLD comprises a spectrum of liver pathologies, beginning with hepatic steatosis, through inflammatory hepatocellular injury called NASH, to hepatic fibrosis, cirrhosis, and HCC. Autophagy plays a key role in the pathogenesis of NAFLD (*37*). Autophagy facilitates the balance of lipid metabolism through the degradation of metabolic lipid droplets (LDs) and the regulation of LD biogenesis. However, LC3-II and p62 have been observed to accumulate in patients with NASH and correlate with disease severity (*38*). Hepatic metabolic diseases are often associated with metabolic syndrome, and metabolic syndrome has a significant negative impact on autophagy. In the presence of insulin resistance and hyperinsulinemia, there is a loss of regulation of the expression of several ATGs by forkhead box O1 (FoxO1), leading to autophagy dysfunction (*39*). In addition, the expression of tissue proteases has been found to be inhibited in obese mice and NAFLD patients, leading to a reduction in lysosomal activity and ultimately a reduction in lysosomal degradation (*40*). However, the homeostasis of proteins (increased levels of polyubiquitin proteins) and organelles is disturbed when autophagy is blocked. The LD-associated proteins perilipin 2 (PLIN2) and perilipin 3 (PLIN3) are CMA substrates, and CMA can directly degrade LD-associated proteins while increasing LD cytosolic adipose triglyceride lipase (ATGL) and autophagy protein levels, thereby promoting LD lipolysis (*41,42*). However, high-fat diets alter the lysosomal stability of the CMA receptor and reduce the activity of this pathway (*43*). Persistent impairment of hepatic CMA then alters protein homeostasis and metabolic dysregulation, promotes the accumulation of oxidative protein aggregates, and progressively reduces hepatic resistance to stress (*44*). At the same time, changes in intracellular lipid content (*i.e.,* metabolic dysfunction) can have a significant impact on the fusion step of macroautophagy, which in turn affects the overall activity of the protein hydrolysis pathway in that cell, with impaired autophagic flux (*45*). In contrast, impaired hepatic autophagic flux is associated with increased ER stress during the development of NAFLD (*38*). ER stress and ER dysfunction contribute to impaired glucose metabolism in NASH. Healthy mitochondria are critical

for lipid metabolism, and liver lipotoxicity activates a series of mitochondrial dysfunction events, while impaired mitochondrial autophagy has been shown to activate the NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammatory vesicle, facilitating progression from NAFLD to NASH (*46,47*). Lipotoxicity, oxidative stress, and chronic activation of the inflammatory response following autophagy failure usually lead to liver cell death and the features of NASH (*i.e.,* inflammation, oxidative stress, cell death, and fibrosis). Apolipoprotein B (APOB)-directed LDs and bortezomib-induced hepatocyte Mallory-Dunn bodies can be eliminated by lipophagy (*48*). These results further indicate that lipophagy may prevent the development of NAFLD. Moreover, re-establishing mitochondrial autophagy may alleviate NAFLD progression (*49*). Therefore, autophagy has been shown to be a protective factor in the development of fatty liver disease, and boosting autophagy activity has been suggested as a strategy for developing therapeutic approaches to treat fatty liver disease.

2.4.2. Autophagy in HCC

There is a complex relationship between autophagy and the development of HCC (*50*). Autophagy, mitophagy, and lipophagy have ambiguous functions in cancer, acting to inhibit tumor growth in the early phase, but they may contribute to tumor progression by meeting the metabolic needs of tumor cells in the late phase (*51*).

In the liver, changes in autophagic activity are closely linked to tumorigenesis, and one of the ultimate outcomes of metabolic dysfunction is the development of HCC. Liver autophagy can serve as a tumor suppressor, while deletion of *ATG5* or *ATG7* can lead to liver tumor formation (*52*). In addition, reduced expression of autophagy proteins and impaired autophagy have been shown to be associated with tumor malignancy and poor prognosis in HCC (*53*). p62 has been reported to serve as a target of rapamycin complex-1 (mTORC1) on mammalian lysosomes and as a signaling hub for the Keap1 (Kelch-like ECH associated protein 1)-Nrf2 (nuclear factor, erythroid 2 like 2) pathway on autophagic cargo, as well as a receptor for selective autophagy (*54*). Failure of selective autophagy leads to accumulation of SQTSM1/p62, mitochondrial dysfunction, ROS generation, and DNA repair, which ultimately affects tumor susceptibility, as well as sustained activation of downstream Nrf2 and NF-κB pathways (*54*). Activation of Nrf2 can divert glucose and glutamine to anabolic pathways such as the pentose phosphate pathway and purine nucleotide synthesis, thereby facilitating tumor cell proliferation (*55*). In addition, Nrf2-activated cells exhibit a constitutive induction of cytoprotective enzymes and drug efflux pumps, which induce resistance to chemotherapeutic agents in HCC (*56*). There is emerging evidence that HCC cells may also harness autophagy to promote tumor progression; for example, elevated levels of autophagy markers (*e.g.,* LC3) in HCC have been associated with a poor prognosis and higher rates of post-operative recurrence (*57*). Moreover, blocking CMA in cancer cells suppresses the uniquely high rate of aerobic glycolysis in tumors in a p53-dependent manner, leading to a reduction in tumorigenicity and metastatic capacity (*58*).

3. IF

3.1. Overview

IF is an increasingly popular dietary strategy that consists of alternating periods of fasting: periods of complete calorie deprivation alternating with periods of ad libitum consumption. There is growing interest in this type of dietary strategy because several studies have shown that it improves body composition, muscle performance, and clinical parameters (*e.g.,* blood pressure, blood glucose, and cholesterol levels), especially in the absence of weight loss (*59*). IF is a general concept that encompasses a wide range of fasting methods. In general, we can divide IF protocols into three main categories: alternate day fasting (ADF), period fasting (PF) and time-restricted fasting (TRF). There are also reports of certain clinical benefits of religious fasting, such as Ramadan IF (RIF). However, comparing RIF studies to other types of fasting studies is not easy, and we will not discuss them further in this review. All IF protocols include regular fasting, but there are differences in the frequency and duration of fasting in different protocols (Table 1). Complete alternate day fasting is the practice of alternating a whole day of fasting with a whole day of unrestricted feeding, meaning that no energy-containing food or drink is consumed on fasting days and unrestricted food is consumed on feeding days. This is more commonly known as a modified ADF (mADF). On modified fasting days, mADF adherents typically eat a light meal at noon. This meal provides about 20–40% of the daily calorie requirement, or about 400–600 kcal. PF protocols include fasting periods of varying frequency and duration, but each fasting period is usually ≥ 24 hours in length. Other implementations of PF include fasting for 24 hours on consecutive days, and some PF regimens include a complete absence of caloric intake for 1 or 2 consecutive or non-consecutive days per week. Similar to mADF, a popular example of a modified PF protocol is the 5:2 diet, in which participants restrict their calorie intake to 25% of their maintenance requirement on two days of the week, and then maintain their normal eating habits for the remaining five days. Other PF protocols involve not eating for 24 hours once or twice a week. TRF involves fasting for the same period each day, consuming free energy for 6–12 hours, and avoiding energy-dense foods for the remaining 12–18 hours of the 24-hour period.

Table 1. Protocols for intermittent fasting

Category / Intermittent fasting	Description
Complete alternate day fasting	No energy-containing foods or beverages on fasting days, alternating with unrestricted food intake on feeding days.
Modified alternate day fasting	20–40% of energy requirements consumed on fasting days, alternating with unrestricted food intake on feeding days.
Period fasting (5:2)	Intake restricted to 25% or less of caloric needs 2 days per week (consecutive or non-consecutive days), with unrestricted food intake the other 5 days.
Time-restricted fasting	Unlimited energy consumption within 6–12 hours, no energy-containing foods for the remaining 12–18 hours of the day.

3.2. Metabolic effects of IF

3.2.1. Lipid metabolism

IF reduces blood lipids. Results from 2–3 months of ADF trials show that IF deceases low density lipoprotein (LDL) levels (20–25%) and triacylglycerol levels (15–30%) (*60*). Similarly, in normal-weight, overweight, and obese populations, trials of ADF for 3 to 12 weeks appear to be effective in reducing total cholesterol (10–21%) and triglycerides (14–42%). Moreover, results from whole-day fasting trials for 3–6 months show that IF lowers total cholesterol (5–20%) and triglycerides (17–50%) (*59*). The lipid changes that occur during IF are designed to meet metabolic needs and preserve protein reserves (*61*). White adipose tissue serves as an energy reserve and releases long-chain fatty acids into the circulation during fasting. Triglycerides are the main source of fuel for the body during the fasting period (*61*). Previous studies have shown that the rate of lipolysis is almost doubled during 3–4 days of fasting, while gluconeogenesis is reduced by about one-third (*62,63*). In addition, changes in lipid metabolism may occur independently of changes in plasma glucose levels (*64*). The increase in lipid metabolism occurred mainly between 18 and 24 hours after fasting. After 24 hours of fasting, lipid oxidation increased by half, and there was a significant increase in plasma FFAs (*65*). Reduced circulating insulin levels, together with plasma glucose, are thought to play an important role in the regulation of lipolysis (*66*). Insulin is thought to inhibit lipolysis *via* the cyclic AMP (cAMP)/protein kinase A (PKA) pathway, ultimately leading to a reduction in lipids and the activity of hormone-sensitive lipase (HSL) (*67*). Nevertheless, changes in lipid metabolism in response to fasting do not appear to be consistent across populations, such as gender, exercise training status, and body composition.

3.2.2. Glucose metabolism

A study has confirmed that fasting lowers blood glucose. The reduction in glucose production in the early stages of fasting appears to be driven by changes in glycogenolysis and gluconeogenesis and the slow decline in endogenous glucose production (*68*). However, the findings are ambiguous. Most of the reduction in glucose production during the first 48 hours of fasting is due to reduced gluconeogenesis (*65*). In subjects fasted for 60 hours, more than 80% of glucose was produced *via* gluconeogenesis (*69*). Liver biopsy confirmed that only 15% of liver glycogen was preserved after 24 hours of fasting (*70*). Glucose production from gluconeogenesis increased from 67% of total glucose production after 22 hours of fasting to 93% after 42 hours of fasting, with no change in total production, implying a reduced role for glycogenolysis (*71*). During fasting, expression of SIRT1 was found to be upregulated, and SIRT1 inhibited glucose production by inhibiting gluconeogenesis mediated by cAMP response element binding protein (CREB)-regulated transcription coactivator 2 (CRCT2) (*72*). Glucose oxidation was reduced by a factor of 10 between 16 and 22 hours after fasting (*73*). In addition to reduced endogenous glucose production and oxidation, studies have shown that the insulin-stimulated uptake of glucose is reduced by as much as 46% after two days of fasting (*68*). Reduced insulin increases the rate of glycogenolysis and gluconeogenesis mainly by inhibiting the insulin receptor substrate-1 (IRS1)/Akt pathway (*74*).

3.2.3. Ketone metabolism

Fasting is characterized by a markedly increased level of ketones in the blood. The production of ketone bodies in the liver can be stimulated by a number of factors during the fasting period. These include a decrease in the hepatic glycogen level, an increase in the glucagon/insulin ratio, and an increase in plasma FFAs. A study has revealed that levels of blood ketone bodies begin to rise within 8–12 hours after fasting. They can reach to 2–5 mmol/L within 24 hours of fasting and continue to rise until reach a steady state after about 2.5 to 5 days of fasting (*75*). Typically, levels of blood ketone bodies rise between 8 and 24 hours after fasting, and particularly after 20 hours of fasting, when the detectable level of residual liver glycogen is minimal (*65*). Providing available glucose to the CNS may be the main role of increased ketone bodies in the early stages of fasting. In contrast, increased

ketone production may provide an alternative energy source for protein conservation during fasting (*76*).

3.2.4. Protein metabolism

During fasting, proteins are oxidized and broken down for energy. Fasting can alter the levels and types of amino acids. Arginine, alanine, serine, threonine, aspartic acid, and proline decreased significantly during fasting, while levels of other amino acids remained almost unchanged (*77*). Total amino acids and total essential amino acids decreased significantly after 3 hours of fasting (*41*). Moreover, the decrease in the content of essential amino acids was greater than that of the non-essential amino acids (*78*). However, levels of lysine, leucine, isoleucine, and taurine changed in two phases. They were found to be at their lowest level 6 hours after fasting, with a brief recovery occurring 12 hours after fasting.

3.3. The hormonal impact of fasting

3.3.1. Insulin

IF reduces plasma insulin levels and improves insulin sensitivity. Insulin is produced and secreted by the beta cells of the pancreas and is an important metabolic hormone. By inhibiting glucose production in the liver, insulin lowers blood glucose after meals to maintain normal blood glucose levels. During acute fasting, reduced plasma insulin levels and improved insulin sensitivity have been observed in humans. Plasma insulin was reduced by about 35% in the first 24 hours after fasting and by as much as half of the initial level after three days of fasting (*64*). The 22 days of ADF significantly inhibited insulin secretion by 50% (*79*). In a trial of an 8-week TRF, there was also a significant reduction in blood insulin levels and insulin resistance (*80*).

3.3.2. Glucagon

The production of glucagon increases during fasting. As an insulin precursor, glucagon plays a key role in glucose metabolism. Glucagon promotes glucose production in the liver by increasing glycogenolysis and gluconeogenesis. Another important role of glucagon in the liver is to inhibit glucose catabolism by promoting fatty acid oxidation, which is a switch in the coordination of energy demand and energy from glucose production (*81*). Moreover, glucagon is involved in the metabolism of lipids and glucose *via* the cAMP pathway in liver cells (*82*).

3.3.3. Thyroid hormone

Human T3 levels fall rapidly after fasting. Monitoring of thyroid hormones in healthy volunteers who fasted for 80 hours revealed a reduction in T3 and thyroid stimulating hormone (TSH) levels within 48 hours of fasting (*83*). In another study, a decrease in serum T3 of up to 55% was reported after an overnight fasting. Unlike serum T3, TSH remained unchanged after fasting (*84*). Short-term (4 weeks) and long-term (more than 6 months) ADF diets reduced the circulating level of T3 without any change in the level of TSH (*85*). Similar results were also observed in another 8-week TRF study (*86*).

3.3.4. Glucocorticoids

IF increases the level and frequency of cortisol secretion and influences the rhythm of cortisol secretion. Typically, the level of plasma cortisol secretion peaks between 7 and 8 AM and gradually declines until midnight. In humans, cortisol increases immediately after the start of fasting (*87*). Studies have shown that fasting for five days increases cortisol levels and shifts the peak of cortisol secretion from the morning to the afternoon (*88*). Other studies have also noted significant improvements in plasma cortisol after fasting for 2.5 or 6 days (*89*). Consistent with these findings, significantly elevated morning changes in serum cortisol levels were observed after 4 days of early TRF (feeding from 8:00 AM to 2:00 PM) (*90*).

3.3.5. Leptin

Leptin levels significantly decrease after fasting. Leptin is an adipokine secreted by adipose tissue and plays an important role in the regulation of food intake and energy expenditure. After overnight fasting, levels of plasma leptin decreased by more than 54% (*91*). Mechanistic studies suggest that interactions between insulin and catecholamine levels may explain the reduction in leptin during fasting, with insulin secretion stimulating leptin production, whereas increased catecholamines and ketones can inhibit leptin production (*92*).

3.3.6. Growth hormone (GH) and insulin-like growth factor 1 (IGF-1)

Levels of human GH increased during fasting. GH can increase by a multiple of 5-fold after fasting for two days (*93*). A point worth noting is that a shorter period of fasting (one day) increased GH more than a longer period of fasting (two days) (*94*). Moreover, after continuous fasting for three days, the total GH level gradually decreased to the pre-fasting level (*95*). Changes in the response of GH to fasting can be attributed to a number of regulators, such as IGF-1 and IGF-1 binding protein (IGFBP-1). In the rapid response to acute fasting, the regulation of GH and IGF-1 displayed an opposite trend (*96*). In the early stage of fasting, IGF-1 is thought to be inhibited by an increase in the production of IGFBP-1. In fact, the biological activity of IGF-1 decreased after

40 hours of continuous fasting, which was paralleled by an increase in IGFBP-1. In addition, there was little change in total IGF-1 levels within 24 hours of fasting, but free IGF-1 levels were suppressed by up to 50%, demonstrating that circulating free IGF-1 rather than total IGF-1 has a greater effect on the production and action of GH during fasting (*96*).

3.3.7. Fibroblast growth factor 21 (FGF21)

FGF21, originally known as the hunger hormone, is a hormone-like FGF, and circulating FGF21 mainly originates from the liver. FGF21 is directly induced by peroxisomal proliferator-activated receptor alpha (PPARα) in the liver in response to fasting (*97*). Protein restriction for one day can increase blood levels of FGF21 (*98*). In addition, long-term protein restriction can lead to a sustained increase in FGF21 (*99*). FGF21 promotes gluconeogenesis and ketogenesis during prolonged fasting. FGF21 induces the expression of peroxisome proliferator-activated receptor-γ-coactivator-1α (PGC-1α), a transcriptional coactivator protein that interacts with several different DNA-binding proteins to regulate metabolism in response to changes in nutrient status. In the liver, fasting induced PGC-1α activation that was involved in gluconeogenesis, fatty acid oxidation, and ketogenic gene transcription (*97*). An important point worth noting is that FGF21 was unable to induce the expression of genes associated with the gluconeogenic pathway in mice lacking PGC-1α. In addition, FGF21 deficiency failed to fully induce PGC-1α expression in mice in response to prolonged fasting, and gluconeogenesis and ketogenesis were also found to be impaired. FGF21 induces several pancreatic lipases, and the induction of these lipases may contribute to the effect of FGF21 on increasing fatty acid oxidation in the liver. By increasing the expression of glucose transporter 3 (GLUT3) through an insulin-independent pathway, FGF21 can also increase glucose uptake by adipocytes (*100*). FGF21 increased the expression of thermogenic genes in white adipose tissue and decreased the expression of lipogenic genes in the liver (*101*). In addition, FGF21 helps to improve insulin sensitivity by increasing insulin-dependent glucose uptake in adipose tissue and reducing hepatic glucose production (*102*).

3.3.8. Sex hormones

The production and secretion of hormones is partly controlled by circadian rhythms, which are also influenced by the daily pattern of feeding and fasting (*103*). IF may affect the levels of sex hormones in the body by affecting the body's circadian rhythm. TRF can shorten the time window during the day when people eat to better match their circadian biology, which can have a beneficial effect on sex hormone levels. FGF21 increases during TRF and stimulates gonadotropin-releasing hormone (GnRH) neurons to secrete GnRH (*104*). For example, 8-hour restricted feeding has the potential to alleviate hyperandrogenemia by increasing levels of sex hormone-binding globulin (SHBG), which may improve the endocrine and metabolic status of women with anovulatory polycystic ovary syndrome (PCOS) (*105*).

4. Hepatic autophagy mediated by IF: Key events

A study noted the maximum number of autophagic vacuoles before feeding and the minimum number of autophagic vacuoles after feeding (*106,107*). Autophagy, which is influenced by a number of factors including circulating hormones, can be inhibited under energy-rich conditions and can be induced by starvation (*108*). The activation of autophagy in male rats exposed to ADF was studied by Donati *et al.* They assessed the rate of autophagic proteolysis in isolated rat hepatocytes and found that the rate of autophagy was higher in the fasting group than in the control group at all ages (*109*). Krustew *et al.* studied the effects of 1–8 days of fasting on the size and number of lysosomes in rat hepatocytes and noted a significant increase in the number of lysosomes, and particularly secondary lysosomes (*110*). After fasting for 12, 24, 48, and 72 hours, the cytoplasmic volume fraction of autophagic vacuoles in liver cells increased significantly compared to feeding for 3 hours. Moreover, serum insulin levels gradually decreased throughout the fasting period (*107*). Fasting may induce anti-stress mechanisms that influence cellular proliferation and energy metabolism and prevent inflammatory and oxidative stress (*10*). Fasting has also been proven to prevent liver ischemia-reperfusion injury in mice by inducing autophagy (*111*).

4.1. IF mediates autophagy in the liver *via* nutrient-sensing pathways

During fasting, a high AMP/ATP ratio, nutrient deprivation, and/or reduced levels of growth factors combine to activate autophagy in the liver (*112*). The key nutrient-sensing pathways involved in fasting-regulated autophagy include mTOR, AMPK, and SIRT1, where the activation of SIRT1 and AMPK is a positive regulator of autophagy while the activation of mTOR is a negative regulator (Figure 3). Moreover, PPARα and farnesoid X receptor (FXR) play a key integrative role in the induction of liver autophagy by IF.

4.1.1. AMPK

A cellular energy sensor, AMPK is a serine/threonine kinase consisting of a functional complex with a catalytic protein subunit (α) and two regulatory protein subunits (β and γ) (*113*). AMPKβ1 is essential for the maintenance of AMPK activity in tissues such as the liver. AMPK activation is triggered by a variety of metabolic

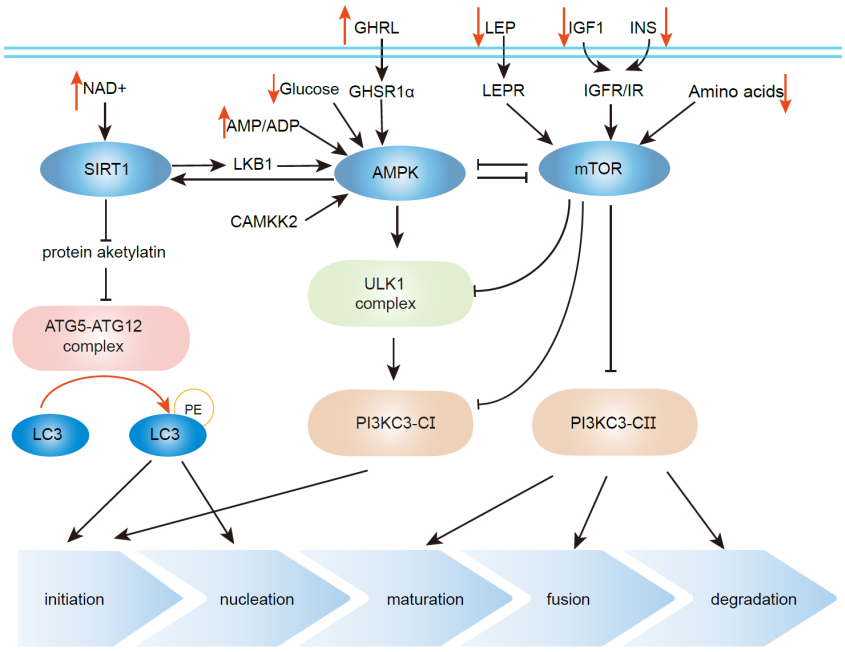

Figure 3. Intermittent fasting regulates autophagy in the liver. The key nutrient-sensing pathways involved include mTOR, AMPK and SIRT1, where the activation of SIRT1 and AMPK is a positive regulator of autophagy while the activation of mTOR is a negative regulator. Intermittent fasting may also affect hepatic autophagy through hormonal changes in the body. Abbreviations: AMPK, AMP-activated protein kinase; CaMKK2, calcium/calmodulin-dependent protein kinase 2; GHRL, growth hormone releasing peptide (ghrelin); GHSR1α, growth hormone secretagogue receptor subtype 1α; IGF1, insulin-like growth factor-1; INS, insulin; IGFR/IR, IGF-I receptor/ insulin receptor; LEP, leptin; LEPR, leptin receptor; LKB1, liver kinase B1; mTOR, mammalian target of rapamycin; PI3KC3-CI, class III phosphatidylinositol-3 kinase complex I; PI3KC3-CII, class III phosphatidylinositol-3 kinase complex II; SIRT1, silent mating-type information regulatory 2 homolog-1; ULK1, unc-51 like autophagy activating kinase 1.

stresses, including amino acid deprivation, hypoxia or hypoglycemia, extracellular matrix (ECM) depletion, physical exertion, and mitochondrial damage. AMP and adenosine diphosphate (ADP) are the primary signals for activation of AMPK. Under low energy conditions, AMP/ADP binds to the cystathionine-β-synthase domains (CBS domains) present in the γ subunit. This induces conformational changes in the catalytic domain of the subunit-α, which is then phosphorylated at threonine 172 by upstream kinases such as liver kinase B1 (LKB1), calcium/calmodulin-dependent protein kinase 2 (CaMKK2), and TGFβ-activated kinase 1 (TAK1) (*113*).

4.1.2. mTOR

Numerous studies have shown that mTORC1 is a key regulator of autophagy, regulating different steps in the autophagy process, such as nucleation, autophagic extension, autophagic maturation, and autophagic termination. Under nutrient-rich conditions, mTORC1 is recruited to the lysosomal surface by amino acid-regulated heterodimers of the Rag family of small GTPases (*114*). mTORC1 subsequently phosphorylates unc-51 like autophagy activating kinase 1 (ULK1) at Ser758 to inhibit its interaction with AMPK, thereby preventing autophagy. During fasting, however, AMPK inhibits mTORC1 and thus ULK1 phosphorylation at Ser758, allowing ULK1 to interact with AMPK. In turn, AMPK promotes the phosphorylation of ULK1 at Ser317 and Ser777 to activate it. Activated ULK1 is then recruited to the vacuolar protein sorting 34 (VPS34) complex. There, it phosphorylates Bcl-2-interacting protein (Beclin-1) at Ser15, which is essential for the initiation of the formation of the autophagosome in autophagy (*115*). Moreover, mTORC1 can indirectly regulate autophagy by repressing the transcription of genes required for lysosome biogenesis, such as transcription factor EB (TFEB) (*116*). As a key transcriptional regulator of lysosomal biogenesis and autophagy genes, TFEB upregulates a number of genes associated with autophagosome formation, those associated with fusion of autophagosomes with lysosomes, and genes required for lysosome biogenesis, such as *UVRAG*, *WIPI*, *MAPLC3B*, *SQSTM1*, *VPS11*, *VPS19*, and *ATG9B*. TFEB is phosphorylated at multiple sites, promoting its nuclear export and switching off the transcription of autophagy genes; this is how mTORC1 negatively regulates autophagy at the transcriptional level (*117*).

4.1.3. SIRT1

Sirtuins are nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases whose activity is regulated by nutrient availability (*118*). Nicotinamide phosphoribosyltransferase (NAMPT) catalyses the conversion of nicotinamide (NAM) to NAD, activating SIRT-1 through the elevation of cellular NAD and the reduction of NAM. Fasting enhances SIRT-1 activity

by increasing the levels of NAD and reducing the levels of NAM and NADH, thereby increasing SIRT-1 activity. The activity of AMPK is linked to SIRT1 since SIRT1 deacetylates and activates LKB1, the upstream factor of AMPK, which ultimately activates AMPK (*119*). A recent study has shown that SIRT1 affects the formation of autophagosomes through its interactions with ATG5 and ATG7 and through the lipidation of the microtubule-associated protein LC3 (*120*). SIRT1 is known to be activated in response to nuclear perilipin5 (PLIN5) under fasting conditions; in male mouse hepatocytes, SIRT1 promotes the transcriptional network (PGC1α/PPARα), induces autophagy, and alleviates the inflammatory response of the liver (*121*). SIRT3 is located in mitochondria, which are particularly critical in the liver, and it regulates fatty acid oxidation during fasting by deactivating mitochondrial enzymes (*122*). SIRT1 is found to deacetylate forkhead box O3a (FoxO3a), thereby increasing transcription of the *BNIP3* gene and initiating autophagy through Beclin-1 (*123*). Glutamine synthase is a target of FoxO3 and when FoxO3 is activated, the levels of cellular glutamine increase and thus inhibit mTORC1 signaling activity and reduce its negative modulation of autophagy (*123*). In the context of fasting, nuclear FoxOs transactivate genes that determine the formation of autophagosomes and their fusion with lysosomes, and cell membrane FoxO proteins can induce autophagy through direct interaction with autophagy proteins. In addition, cytosolic FoxO1 has been reported to induce autophagy through a transcriptionally independent pathway (*124*).

4.1.4. PPARα and FXR

The nuclear receptor PPARα is activated during fasting while the nuclear receptor FXR is activated during feeding; both are involved in regulating hepatic autophagy. Activated by fatty acids during fasting, PPARα is a nuclear receptor that plays a critical role in lipid metabolism and glucose homeostasis and that can activate or repress genes involved in inflammation, adipogenesis, and energy homeostasis (*125*). FXR and PPARα counter-regulate autophagy to maintain energy homeostasis. FXR has four distinct isoforms (FXRα1-4) that originate from differential promoter usage and alternative splicing of the same gene (*126*). These isoforms are species-specific. They are differentially expressed in different organs depending on the bile acid composition. In the human liver, FXRα1/2 predominates, with FXRα2 being the most transcriptionally active form (*127*). Feeding and fasting cycles dynamically regulate FXR splicing in mice. Fasting can increase the FXRα2 isoform in the liver, facilitating fatty acid β-oxidation, decreased hepatic lipogenesis, enhanced glycerate metabolism, and ammonia clearance (*128*). FXR and the fasting transcriptional activator CREB work in a coordinated manner to regulate the hepatic autophagy gene network (*128*). G protein bile acid receptor 1 (GPBAR1) is a receptor for secondary bile acids. It is mainly expressed in non-parenchymal cells, including macrophages and sinusoidal cells, and in biliary cells. During fasting, GPBAR1 acts genomically *via* the cAMP-CREB pathway and it functions as a positive modulator of liver autophagy; its ligands reverse the repressive effects of FXR on liver autophagy flux during feeding (*129*).

4.2. IF mediates liver autophagy *via* an endocrine pathway

Somatically, hepatic autophagy fluctuates in response to hormonal signals, negatively or positively regulating feeding and fasting autophagy. Activation of the hypothalamic-pituitary-adrenal (HPA) axis is essential for the metabolic adaptation that occurs in response to fasting. Chen *et al.* found that fasting in mice activates autophagy in the liver in parallel with activation of hypothalamic agouti-related protein (AgRP) neurons (*130*). Adrenaline secretion promotes hepatic autophagy in response to starvation (*37*). In contrast, during feeding, hepatic autophagy is inhibited in favor of anabolism (*46*).

4.2.1. Glucagon

Increased circulating glucagon during fasting, together with decreased insulin and amino acids, activates hepatic autophagy. Glucagon and insulin are important regulators of autophagy. The role of glucagon in the induction of hepatic autophagy has been well established for over 60 years (*4*). Subsequent *in vivo* studies further confirmed the role of glucagon in the induction of hepatic autophagy. The glucagon receptor (G protein-coupled receptor) on hepatocytes recognizes glucagon and subsequently inhibits salt-inducible kinase (SIK) *via* the cAMP/PKA pathway. PKA then phosphorylates CREB and SIK dephosphorylates CREB-regulated transcription co-activator (CRTC). Phosphorylated CREB and CRTC act synergistically to upregulate the expression of TFEB, which in turn regulates the expression of autophagy protein genes (*131*). Glucagon may also induce autophagy by increasing the size and number of autophagic vesicles (*132*). During fasting, glucagon stimulates hepatic autophagy, providing substrates for gluconeogenesis/ketogenesis to maintain systemic glucose homeostasis. Glycogen particles are selectively encapsulated by autophagosomes for degradation and metabolism in a process known as "glycophagy". Glucagon induces calcium signaling with subsequent phosphorylation of O-linked β-N-acetylglucosamine (O-GlcNAc) transferase (OGT) by CaMKK2 and it further promotes O-GlcNAc modification and activation of ULK proteins by enhancing AMPK-dependent phosphorylation to promote autophagy (*133*).

4.2.2. FGF21

Liver-derived FGF21 is highly inducible by fasting and is linked to many critical metabolic pathways that are altered under nutrient stress. FGF21 can promote autophagy in liver cells through the AMPK/mTOR signaling pathway, accelerating regeneration of the damaged liver (*134*). Mechanistically, this is closely related to increased FGF21 expression, enhanced AMPKα phosphorylation, and stimulation of autophagy. FGF21 can also induce autophagy by promoting SIRT1 expression (*135*). Epigenetic studies have revealed that the FGF21-JMJD3 signaling pathway links nutrient deprivation to hepatic autophagy (*136*). The impairment of autophagy in *FGF21*-KO mice strongly suggests that physiological levels of hepatic FGF21 during fasting may activate autophagy in an autocrine/paracrine manner.

5. Importance of IF-mediated liver autophagy

By activating hepatic autophagy, IF plays a role in both the physiology and pathology of the liver. The activation of hepatic autophagy mediated by IF may help to maintain the energy balance, improve mitochondrial function, control the quality of the liver, maintain cell homeostasis, and protect cells from harmful factors. IF is a promising intervention for improving liver function (Figure 4).

5.1. IF activates liver autophagy to recycle nutrients

Autophagy is an important regulator of metabolism at the cellular and organismal levels. During fasting, autophagy provides an important source of internal nutrients to maintain cell integrity and survival (Figure 5). Autophagy, which has been shown to be critical for survival in adult mice, is required to maintain circulating nutrients during fasting. Autophagosome membranes engulf glycogen, LDs, or proteins, which are then

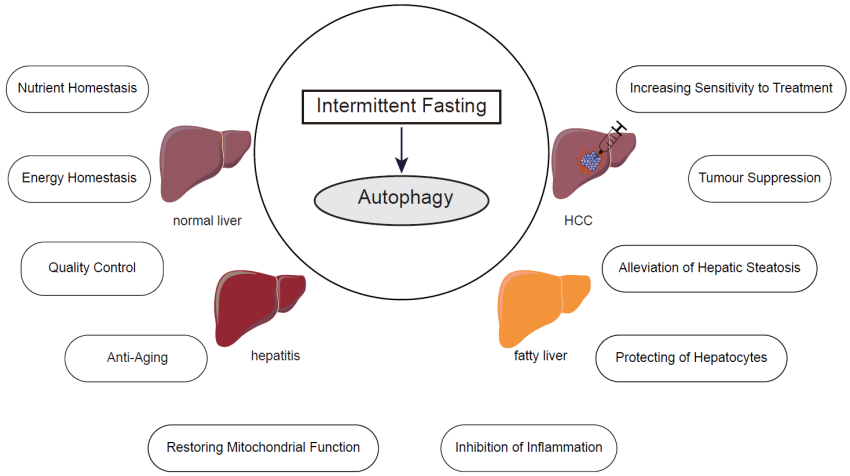

Figure 4. Importance of intermittent fasting in hepatic autophagy. By activating hepatic autophagy, intermittent fasting plays a role in liver physiology and pathology. Abbreviations: HCC, hepatocellular carcinoma.

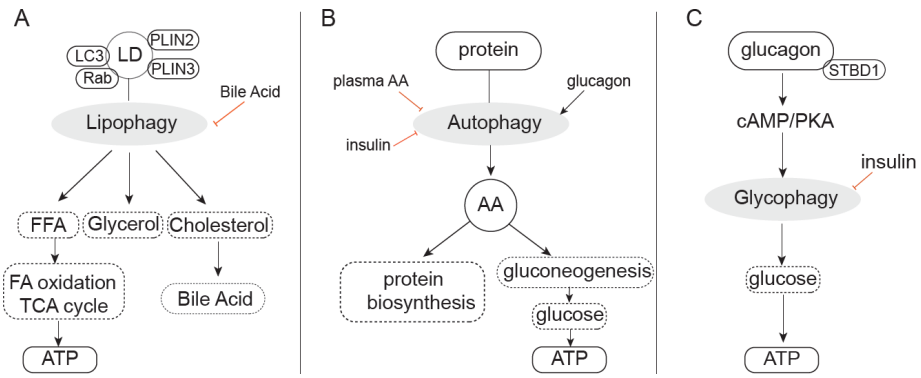

Figure 5. Intermittent fasting activates hepatic autophagy to maintain homeostasis of nutrient and energy metabolism. (A) LDs are selectively removed by lipophagy to produce FFAs, which are continuously released to undergo mitochondrial β-oxidation, supplying ATP. **(B)** Under the influence of hormonal and nutrient signals, hepatic autophagy is activated to provide amino acids to the body through protein catabolism and to maintain glucose homeostasis and energy balance through gluconeogenesis. **(C)** Glycophagy plays an important role in maintaining blood glucose and energy homeostasis. During glycophagy, STBD1 anchors glycogen to the lysosome and ultimately produces glucose through the cAMP/PKA signaling pathway to maintain glucose and energy homeostasis. Abbreviations: AA, amino acid; FA, fatty acid; FFA, free fatty acid; LC3, light chain 3; LD, lipid droplet; PLIN2, perilipin 2; PLIN3, perilipin 3; STBD1, starch-binding domain-containing protein 1; TCA cycle, tricarboxylic acid cycle.

degraded in lysosomes to maintain nutrient cycling and key metabolic pathways. Glycophagy and protein autophagy promote nutrient mobilization during early starvation. After 8 hours of fasting, however, there is a gradual switch from these autophagy substrates to LD degradation. This sequence of selective degradation of autophagy substrates during starvation is somewhat consistent with the progression of conventional metabolic degradation processes in the liver in response to prolonged nutrient deprivation: first glycogenolysis and gluconeogenesis and then mitochondrial β-oxidation, ketogenesis, and gluconeogenesis. Glucose homeostasis during fasting, maintained by autophagy induced by fasting, is an important contribution of liver-specific metabolism to energy homeostasis. In addition, autophagy indirectly affects the metabolic activity of cells by regulating the degradation of intracellular organelles.

5.1.1. Maintenance of balanced glucose metabolism in the liver

Autophagy in the liver plays an important role in blood glucose homeostasis. In addition to the classic process of glycogen degradation, glycophagy plays an important role in maintaining energy homeostasis in the liver. During glycophagy, starch-binding domain-containing protein 1 (STBD1) is thought to anchor glycogen to lysosomes. STBD1 contains a C-terminal CBM20 glycan-binding domain that binds glycogen and an ATG8 interaction motif (AIM) that can bind GABA type A receptor-associated protein-like 1 (GABARAPL1, an Atg8 family member) (*137*). By interacting with GABARAPL1, STBD1 recruits glycogen to the forming phagosome. Following autophagosome-lysosome fusion, acid α-glucosidase mediates lysosomal glycogen degradation. The cAMP/PKA signaling pathway has been found to play a key role in glycophagy. In contrast, insulin is involved in the regulation of glycophagy by indirectly activating mTOR and inhibiting autophagy through the I-PI3-kinase (PI3K-Akt)/protein kinase B (PKB)-mTOR signaling pathway (*138*). By inhibiting protein phosphatase 2a (PP2A), activated mTOR can inhibit glycophagy.

5.1.2. Maintenance of homeostasis in amino acid and protein metabolism

The liver is the main source of serum protein and provides the body with amino acids for energy in response to prolonged fasting by catabolizing protein. Under nutrient-rich conditions, constitutive autophagy degrades about 1.5% of total liver protein per hour, but during fasting, the rate of induced autophagy increases to three times the basic rate (*139*). After one day of fasting, there was an increase in amino acid levels in liver tissue and circulation, and blood glucose levels were within the normal range. Amino acids released by autophagy may be responsible for blood glucose levels (*77*). Further analysis showed that 11 of 18 amino acids (valine, leucine, isoleucine, serine, threonine, methionine, asparagine, phenylalanine, tyrosine, lysine, and arginine) increased in mice fasting for one day (*27*). The glycogenic amino acids released by hepatic autophagy are partially converted to glucose by gluconeogenesis and secreted into the circulation, while the amino acids generated by hepatic autophagy are also used for protein biosynthesis (*77*). After two days of fasting, approximately 40% of total liver protein was catabolized by activated autophagy (*140*). Both hormones and plasma amino acid levels are involved in the regulation of protein autophagy in the liver. Compared to the influence of changes in plasma amino acid levels, insulin and glucagon may be more important in regulating protein autophagy. Insulin has been reported to inhibit liver autophagy by activating the Akt/mTOR pathway (*27*). Studies have found that inhibition of serum insulin in rats increases protein autophagy by approximately 70%. Glucagon remained at high and stable levels during 24-hour fasting. Glucagon not only induces the formation of autophagosomes but also upregulates acid phosphatase and cathepsin D in lysosomes and increases lysosomal susceptibility in the liver (*141*). Plasma amino acid levels could be a regulator of hepatic autophagy independent of hormonal influence.

Hepatic autophagy breaks down not only protein aggregates but also metabolic enzymes involved in lipid, carbohydrate, and amino acid metabolism. During nutrient deprivation, CMA removes glycolytic enzymes to reduce the use of hepatic glucose, allowing it to be recruited to other organs (*29*). For example, the degradation of hexokinase 2 (HK2) may have a direct effect on glycolysis in the liver (*142*). Protein is degraded by autophagy to maintain the intracellular amino acid pool and plasma amino acid levels. In addition to providing metabolic substrates, protein autophagy may also contribute to protein quality control by removing misfolded and aggregated proteins and alleviating protein toxicity caused by abnormal protein levels. Degradation of p62/SQSTM1, a receptor for selective autophagy, is not only part of the regular autophagy process but is also key to preventing inappropriate activation of Nrf2, which would otherwise be the underlying mechanism for severe liver pathology (*143*).

5.1.3. Lipophagy promotes lipid metabolism in the liver

The liver is crucial to the processing and storage of lipids, and hepatic lipid homeostasis is maintained by coordinating lipid uptake, de novo synthesis, storage, and catabolism. The uptake of LDs by autophagy is an alternative pathway to mobilize lipid storage and intracellular LD degradation, a process known as lipophagy that is a subtype of macroautophagy. Portions

of cytosolic LDs are engulfed by lipo-autophagosomes and transported to lysosomes, where triacylglycerols and other lipids are subjected to acid lipolysis by lysosomal acid lipase. Lipophagy is essential for the maintenance of lipid homeostasis (*42*). The lipid content of hepatocyte double-membrane autophagosomes, a hallmark of macroautophagy, was evident in mice fasted for as short as 6 hours (*42*). In addition, inhibition of autophagy increased triglyceride storage in LDs both *in vivo* and *in vitro*. CMA and lipophagy play a synergistic role in the clearance of LDs. CMA-mediated PLIN degradation appears to be a key event in the initiation of lipophagy. CMA targets and degrades the LD-associated proteins PLIN2 and PLIN3, which increase during fasting. The degradation of PLIN proteins appears to be essential for promoting LD degradation by allowing the entry of adipose triglyceride lipase (ATGL) and autophagy proteins to the LD surface. Moreover, inhibition of the CMA process leads to a reduction in both lipase-mediated lipolysis and lipophagy. As the major Rab protein on the surface of LDs, Rab7 helps to regulate lysosome-autophagosome interaction. During fasting, the Rab7 GTP enzyme on the surface of LDs is activated and promotes lipophagy. Rab10, another Rab family protein, co-localizes with autophagy membrane markers (such as LC3 and Atg16) and is involved in lipid degradation during fasting. The results of LD proteomic screening suggest that several other Rab proteins, such as Rab32, Rab18, and Rab25, may also play an important role in lipophagy (*42*).

5.2. Potential impact of IF-mediated liver autophagy on liver pathophysiology

Besides regulating energy and nutrient homeostasis in response to fasting, IF-mediated hepatic autophagy is essential for maintaining organelle homeostasis and quality control in hepatocytes. Autophagy plays a critical role in cellular quality control by regulating the regeneration of intracellular organelles. Misfolded proteins can be toxic to cells, a phenomenon known as proteotoxicity. Autophagy can prevent the degradation of normal long-lived proteins and misfolded proteins, so both correctly folded and misfolded proteins increase significantly in cells lacking autophagy. In addition, many target molecules are overproduced as a result of sustained Nrf2 activation (*56*). Proteomic analysis of autophagy-deficient hepatocytes revealed an increase in the total amount of protein without specific changes in protein composition. Excess proteins, even when properly folded, can be detrimental to cells and this adverse effect can manifest in hepatocyte hypertrophy, hepatomegaly, and liver injury.

5.2.1. Restoring mitochondrial function

Mitophagy is an evolutionarily conserved degradation mechanism responsible for the selective removal of damaged or excess mitochondria to maintain liver function and protect tissues from damage (*144*). Conversely, dysregulation of mitophagy has been shown to contribute to the development of liver diseases such as alcoholic liver disease (ALD), NAFLD, viral hepatitis, drug-induced liver injury (DILI), liver fibrosis, and HCC, suggesting that it plays an important role in maintaining liver homeostasis. After alcohol ingestion, autophagy decreases in hepatocytes. This may be due to reduced intracellular AMPK and altered vesicle transport in hepatocytes (*145*). Alcohol can also damage mitochondria, sometimes by depolarizing the inner mitochondrial membrane and changing mitochondrial permeability (*146*). IF may prevent alcohol-induced acute liver injury by activating AMPK activity, restoring mitochondrial function, and stimulating autophagy, offering a potential therapeutic benefit to patients with ALD.

5.2.2. Alleviation of hepatic steatosis

In mice fed a high-fat diet, TRF reversed the expression of genes involved in fatty acid metabolism, β-oxidation, and antioxidant defense in the liver (*147*). TRF also enhanced the circadian rhythm of expression of a number of different genes in liver cells, including genes encoding PER2, BMAL1, and CRY1, and it prevented fat accumulation in the livers of mice fed a high-fat diet. Tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and interleukin 1β (IL-1β) were found to decrease in the livers of mice treated with TRF. Moreover, metabolomic analysis revealed that changes in liver metabolites (oleates, palmitates and palmitoleates), bioenergy pathway molecules (citrate, glucose-6-phosphate, and phthalates) and an antioxidant (glutathione) induced by a high-fat diet were reversed by TRF (*38*). Mounting evidence has revealed that autophagy is impaired in NAFLD (*148*). Upregulation of autophagy transcription factors such as FoxO1 and TFEB can prevent liver steatosis and reduce apoptosis. By upregulating liver-specific *ATG7* expression in ob/ob mice (genetic models of murine obesity), liver autophagy can help to alleviate metabolic stress and reduce hepatic steatosis (*148*). Due to increased autophagy, ADF can help to ameliorate elevated serum lactate dehydrogenase (LDH) and liver histological changes in the mouse model of NASH induced with a high-fat-fructose (HFF) diet (*149*). A study has reported that IF reduced lipid accumulation, activated AMPK/ULK1 signaling, inhibited mTOR phosphorylation, and prevented NAFLD progression in the liver of mice with diet-induced obesity (DIO) *via* autophagy (*150,151*). In short, findings suggested that IF may play an active role in liver steatosis.

5.2.3. Inhibition of liver inflammation

A recent study has shown that fasting can reduce the number of circulating inflammatory monocytes and inhibit their pro-inflammatory activity by activating liver AMPK and inhibiting chemokine ligand 2 (CCL2) production (*152*). SIRT1 is known to be activated in response to nuclear PLIN5 under fasting conditions. In male mouse liver cells, it can promote transcription of PGC1α/PPARα, induce autophagy, and alleviate liver inflammation (*121*). A study has shown that FGF-21 supplementation can effectively increase the expression of LC3-II and Beclin-1, a key molecule in autophagy, while reducing liver toxicity in wild-type mice exposed to carbon tetrachloride (CCl4) (*135*). FGF21 significantly reduced levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), IL-6, and TNF-α in acute liver injury.

5.2.4. Protecting hepatocytes

In models of liver ischemia-reperfusion injury, IF may have a beneficial protective effect on hepatocytes *via* autophagy mediated by the SIRT1/FoxO3a pathway (*153*). Moreover, in models of hepatic ischemia-reperfusion injury, IF may also protect hepatocytes from damage *via* the AMPK/mTOR autophagy pathway (*154*). In addition, the FoxO-autophagy axis has been identified as playing a cytoprotective role in both alcohol-induced hepatotoxicity and hepatic ischemia-reperfusion injury (*155,156*).

5.2.5. Tumor suppression

Mitochondrial dysfunction, oxidative stress, and DNA damage, all of which are key factors in tumorigenesis, are present in autophagy-deficient hepatocytes. Autophagy has been implicated as a tumor suppressor, and impaired autophagy may promote tumorigenesis in the liver. A study has shown that the AMPK/mTOR pathway is involved in the metabolism and tumorigenesis of HCC (*157*). Activation of AMPK is related to inhibition of HCC cell migration and invasion. By activating the transcription factor CCAAT/enhancer binding protein δ (CEBPD) and increasing LC3B expression, AMPK can induce autophagy and apoptosis in HCC (*158*). Conversely, loss of AMPK in HCC cells can promote cell progression, survival, migration, and invasion through various oncogenic molecules and pathways (*159*). A study has reported that fasting can promote autophagy and p62 degradation, increase AMPKα phosphorylation, upregulate FGF21 expression, downregulate the expression of aging-related molecules, and prevent the development of steatosis-associated liver tumors in hepatitis C virus core gene transgenic (HCVcpTg) mice (*160*).

5.2.6. Improving the sensitivity of tumor therapy

IF has also been proven to improve the sensitivity of radiotherapy for liver cancer. In liver cancer cells, mTORC1 is activated during fasting, thereby increasing their sensitivity to radiation (*161*). Moreover, sorafenib resistance of liver cancer cells is associated with a low level of activity of AMPK and CCAAT/CEBPD and insufficient activation of autophagy (*162*). Fasting is beneficial in improving the efficacy of sorafenib and has a synergistic effect in sensitizing sorafenib-resistant HCC (*163,164*).

6. Conclusion

IF is emerging as a simple but attractive strategy to combat human diseases, including aging, cancer, neurodegenerative diseases, and metabolic disorders. Fasting induces a number of adaptive changes, such as lowering the basal metabolic rate, inducing lipolysis and ketogenesis, regulating hormone levels, and reducing oxidative stress and inflammation. Numerous preclinical and clinical studies have shown that fasting plays a positive role in preventing a wide range of diseases. In particular, preclinical and clinical studies have demonstrated the clinical benefits of this dietary strategy in liver metabolic disorders (*165*).

IF regulates hepatic autophagy primarily *via* the nutritional and hormonal pathways. The regulation of liver autophagy by IF is crucial for liver physiology and pathology. The activation of liver autophagy mediated by IF can maintain the energy balance, improve mitochondrial function, control liver quality, maintain cell homeostasis, and protect cells from harmful factors. In addition, IF-mediated liver autophagy may also help to alleviate liver metabolic diseases, ameliorate liver inflammation, inhibit the development of liver cancer to some extent, and improve efficacy and reduce tumor resistance in combination with chemotherapy/radiotherapy.

IF is a promising intervention to improve liver function. However, research on its effectiveness and safety in people with liver disease is still limited. To determine whether IF can prevent liver diseases or improve their outcomes, randomized controlled trials need to be conducted in people at high risk or in the early stages of liver diseases. Moreover, the mechanisms by which IF works involve multiple pathways and are not well understood. Currently, there are technical barriers to measuring autophagic flux in the human body. As a result, the effects and mechanisms of IF on autophagy in the liver are not well understood at a systemic level. Further research also needs to be conducted to determine whether there is a link between the benefits of IF and refeeding. The safety of IF protocols in clinical practice is a major concern. At present, one of the main problems with the use of IF in clinical practice is the lack of guidelines. Wherever possible, guidance should be provided on how to select an IF regimen that is more appropriate for a particular goal. This should include the

types of foods that should be consumed, how physical activity should be performed, and how long such a regimen should last. The efficacy and safety of different IF protocols in different populations also need to be continually evaluated. More attention should be paid to advances in the research and use of IF in the near future.

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References

1. Lei Y, Klionsky DJ. The emerging roles of autophagy in human diseases. Biomedicines. 2021; 9:1651.
2. Boya P, Codogno P, Rodriguez-Muela N. Autophagy in stem cells: Repair, remodelling and metabolic reprogramming. Development. 2018; 145:dev146506.
3. De Duve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem J. 1955; 60:604-617.
4. Ashford TP, Porter KR. Cytoplasmic components in hepatic cell lysosomes. J Cell Biol. 1962; 12:198-202.
5. Alim Al-Bari A, Ito Y, Thomes PG, Menon MB, Garcia-Macia M, Fadel R, Stadlin A, Peake N, Faris ME, Eid N, Klionsky DJ. Emerging mechanistic insights of selective autophagy in hepatic diseases. Front Pharmacol. 2023; 14:1149809.
6. Udoh US, Rajan PK, Nakafuku Y, Finley R, Sanabria JR. Cell autophagy in NASH and NASH-related hepatocellular carcinoma. Int J Mol Sci. 2022; 23:7734.
7. Zhao H, Liu H, Yang Y, Wang H. The role of autophagy and pyroptosis in liver disorders. Int J Mol Sci. 2022; 23:6208.
8. Han SC, Kang JI, Choi YK, Boo HJ, Yoon WJ, Kang HK, Yoo ES. Intermittent fasting modulates immune response by generating tregs *via* TGF-β dependent mechanisms in obese mice with allergic contact dermatitis. Biomol Ther (Seoul). 2023. doi: 10.4062/biomolther.2023.053. Epub ahead of print.
9. Lavallee CM, Bruno A, Ma C, Raman M. A review of the role of intermittent fasting in the management of inflammatory bowel disease. Therap Adv Gastroenterol. 2023; 16:17562848231171756.
10. Longo VD, Mattson MP. Fasting: Molecular mechanisms and clinical applications. Cell Metab. 2014; 19:181-192.
11. Xiong F, Jiang K, Wu Y, Lou C, Ding C, Zhang W, Zhang X, Li C, Zheng H, Gao H. Intermittent fasting alleviates type 1 diabetes-induced cognitive dysfunction by improving the frontal cortical metabolic disorder. Biochim Biophys Acta Mol Basis Dis. 2023 ;1869:166725.
12. Ma RX, Hu JQ, Fu W, Zhong J, Cao C, Wang CC, Qi SQ, Zhang XL, Liu GH, Gao YD. Intermittent fasting protects against food allergy in a murine model *via* regulating gut microbiota. Front Immunol. 2023; 14:1167562.
13. Schmidt MW, Brenner W, Gebhard S, *et al.* Effects of intermittent fasting on quality of life tolerance of chemotherapy in patients with gynecological cancers: Study protocol of a randomized-controlled multi-center trial. Front Oncol. 2023; 13:1222573.
14. Lavallee CM, Bruno A, Ma C, Raman M. The role of intermittent fasting in the management of nonalcoholic fatty liver disease: A narrative review. Nutrients. 2022; 14:4655.
15. Komatsu M, Ueno T, Waguri S, Uchiyama Y, Kominami E, Tanaka K. Constitutive autophagy: Vital role in clearance of unfavorable proteins in neurons. Cell Death Differ. 2007; 14:887-894.
16. Mizushima N, Komatsu M. Autophagy: Renovation of cells and tissues. Cell. 2011; 147:728-741.
17. Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, Mi N, Zhao Y, Liu Z, Wan F, Hailey DW, Oorschot V, Klumperman J, Baehrecke EH, Lenardo MJ. Termination of autophagy and reformation of lysosomes regulated by mTOR. Nature. 2010; 465:942-946.
18. Kaushik S, Cuervo AM. Chaperone-mediated autophagy: A unique way to enter the lysosome world. Trends Cell Biol. 2012; 22:407-417.
19. Cuervo AM, Dice JF. Regulation of lamp2a levels in the lysosomal membrane. Traffic. 2000; 1:570-583.
20. Schneider JL, Cuervo AM. Liver autophagy: Much more than just taking out the trash. Nat Rev Gastroenterol Hepatol. 2014; 11:187-200.
21. de Waal EJ, Vreeling-Sindelárová H, Schellens JP, Houtkooper JM, James J. Quantitative changes in the lysosomal vacuolar system of rat hepatocytes during short-term starvation. A morphometric analysis with special reference to macro- and microautophagy. Cell Tissue Res. 1986; 243:641-648.
22. Uttenweiler A, Mayer A. Microautophagy in the yeast Saccharomyces cerevisiae. Methods Mol Biol. 2008; 445:245-259.
23. Zhang Y, Fang F, Goldstein JL, Brown MS, Zhao TJ. Reduced autophagy in livers of fasted, fat-depleted, ghrelin-deficient mice: Reversal by growth hormone. Proc Natl Acad Sci U S A. 2015; 112:1226-1231.
24. Mwangi SM, Li G, Ye L, Liu Y, Reichardt F, Yeligar SM, Hart CM, Czaja MJ, Srinivasan S. Glial cell line-derived neurotrophic factor enhances autophagic flux in mouse and rat hepatocytes and protects against palmitate lipotoxicity. Hepatology. 2019; 69:2455-2470.
25. Mortimore GE, Poso AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. Annu Rev Nutr. 1987; 7:539-564.
26. Sinha RA, You SH, Zhou J, Siddique MM, Bay BH, Zhu X, Privalsky ML, Cheng SY, Stevens RD, Summers SA, Newgard CB, Lazar MA, Yen PM. Thyroid hormone stimulates hepatic lipid catabolism *via* activation of autophagy. J Clin Invest. 2012; 122:2428-2438.
27. Ezaki J, Matsumoto N, Takeda-Ezaki M, *et al.* Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. Autophagy. 2011; 7:727-736.
28. Singh R, Cuervo AM. Autophagy in the cellular energetic balance. Cell Metab. 2011; 13:495-504.
29. Madrigal-Matute J, Cuervo AM. Regulation of liver metabolism by autophagy. Gastroenterology. 2016; 150:328-339.
30. Mortimore GE, Poso AR. Lysosomal pathways in hepatic protein degradation: Regulatory role of amino acids. Fed Proc. 1984; 43:1289-1294.
31. Li S, Lin JD. Transcriptional control of circadian metabolic rhythms in the liver. Diabetes Obes Metab. 2015; 17 Suppl 1:33-38.

32. Czaja MJ, Ding WX, Donohue TM Jr, Friedman SL, Kim JS, Komatsu M, Lemasters JJ, Lemoine A, Lin JD, Ou JH, Perlmutter DH, Randall G, Ray RB, Tsung A, Yin XM. Functions of autophagy in normal and diseased liver. Autophagy. 2013; 9:1131-1158.
33. Dong C, Gongora R, Sosulski ML, Luo F, Sanchez CG. Regulation of transforming growth factor-beta1 (TGF-β1)-induced pro-fibrotic activities by circadian clock gene BMAL1. Respir Res. 2016; 17:4.
34. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, Chiba T. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol. 2005; 169:425-434.
35. Ni HM, Boggess N, McGill MR, Lebofsky M, Borude P, Apte U, Jaeschke H, Ding WX. Liver-specific loss of Atg5 causes persistent activation of Nrf2 and protects against acetaminophen-induced liver injury. Toxicol Sci. 2012; 127:438-450.
36. Green DR, Levine B. To be or not to be? How selective autophagy and cell death govern cell fate. Cell. 2014; 157:65-75.
37. Amir M, Czaja MJ. Autophagy in nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol. 2011; 5:159-166.
38. Gonzalez-Rodriguez A, Mayoral R, Agra N, *et al.* Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. Cell Death Dis. 2014; 5:e1179.
39. Liu HY, Han J, Cao SY, Hong T, Zhuo D, Shi J, Liu Z, Cao W. Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia: Inhibition of FoxO1-dependent expression of key autophagy genes by insulin. J Biol Chem. 2009; 284:31484-31492.
40. Inami Y, Yamashina S, Izumi K, Ueno T, Tanida I, Ikejima K, Watanabe S. Hepatic steatosis inhibits autophagic proteolysis *via* impairment of autophagosomal acidification and cathepsin expression. Biochem Biophys Res Commun. 2011; 412:618-625.
41. Kaushik S, Cuervo AM. Degradation of lipid droplet-associated proteins by chaperone-mediated autophagy facilitates lipolysis. Nat Cell Biol. 2015; 17:759-770.
42. Schulze RJ, Drizyte K, Casey CA, McNiven MA. Hepatic lipophagy: New insights into autophagic catabolism of lipid droplets in the liver. Hepatol Commun. 2017; 1:359-369.
43. Rodriguez-Navarro JA, Kaushik S, Koga H, Dall'Armi C, Shui G, Wenk MR, Di Paolo G, Cuervo AM. Inhibitory effect of dietary lipids on chaperone-mediated autophagy. Proc Natl Acad Sci U S A. 2012; 109:E705-E714.
44. Schneider JL, Villarroya J, Diaz-Carretero A, Patel B, Urbanska AM, Thi MM, Villarroya F, Santambrogio L, Cuervo AM. Loss of hepatic chaperone-mediated autophagy accelerates proteostasis failure in aging. Aging Cell. 2015; 14:249-264.
45. Koga H, Kaushik S, Cuervo AM. Altered lipid content inhibits autophagic vesicular fusion. FASEB J. 2010; 24:3052-3065.
46. Mansouri A, Gattolliat CH, Asselah T. Mitochondrial dysfunction and signaling in chronic liver diseases. Gastroenterology. 2018; 155:629-647.
47. Zhang NP, Liu XJ, Xie L, Shen XZ, Wu J. Impaired mitophagy triggers NLRP3 inflammasome activation during the progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis. Lab Invest. 2019; 99:749-763.
48. Harada M. Autophagy is involved in the elimination of intracellular inclusions, Mallory-Denk bodies, in hepatocytes. Med Mol Morphol. 2010; 43:13-18.
49. Li R, Xin T, Li D, Wang C, Zhu H, Zhou H. Therapeutic effect of Sirtuin 3 on ameliorating nonalcoholic fatty liver disease: The role of the ERK-CREB pathway and Bnip3-mediated mitophagy. Redox Biol. 2018; 18:229-243.
50. Liu L, Liao JZ, He XX, Li PY. The role of autophagy in hepatocellular carcinoma: Friend or foe. Oncotarget. 2017; 8:57707-57722.
51. Sun K, Guo XL, Zhao QD, *et al.* Paradoxical role of autophagy in the dysplastic and tumor-forming stages of hepatocarcinoma development in rats. Cell Death Dis. 2013; 4:e501.
52. Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N. Autophagy-deficient mice develop multiple liver tumors. Genes Dev. 2011; 25:795-800.
53. Ding ZB, Shi YH, Zhou J, Qiu SJ, Xu Y, Dai Z, Shi GM, Wang XY, Ke AW, Wu B, Fan J. Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma. Cancer Res. 2008; 68:9167-9175.
54. Katsuragi Y, Ichimura Y, Komatsu M. p62/SQSTM1 functions as a signaling hub and an autophagy adaptor. FEBS J. 2015; 282:4672-4678.
55. Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, Yamamoto M, Motohashi H. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell. 2012; 22:66-79.
56. Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, Asamura H, Yamamoto M, Hirohashi S. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. Proc Natl Acad Sci U S A. 2008; 105:13568-13573.
57. Yang JD, Seol SY, Leem SH, Kim YH, Sun Z, Lee JS, Thorgeirsson SS, Chu IS, Roberts LR, Kang KJ. Genes associated with recurrence of hepatocellular carcinoma: Integrated analysis by gene expression and methylation profiling. J Korean Med Sci. 2011; 26:1428-1438.
58. Kon M, Kiffin R, Koga H, Chapochnick J, Macian F, Varticovski L, Cuervo AM. Chaperone-mediated autophagy is required for tumor growth. Sci Transl Med. 2011; 3:109ra117.
59. Tinsley GM, La Bounty PM. Effects of intermittent fasting on body composition and clinical health markers in humans. Nutr Rev. 2015; 73:661-674.
60. Varady KA, Bhutani S, Klempel MC, Kroeger CM, Trepanowski JF, Haus JM, Hoddy KK, Calvo Y. Alternate day fasting for weight loss in normal weight and overweight subjects: A randomized controlled trial. Nutr J. 2013; 12:146.
61. Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr. 2006; 26:1-22.
62. Horowitz JF, Coppack SW, Paramore D, Cryer PE, Zhao G, Klein S. Effect of short-term fasting on lipid kinetics in lean and obese women. Am J Physiol. 1999; 276:E278-E284.
63. Jahoor F, Klein S, Wolfe R. Mechanism of regulation of glucose production by lipolysis in humans. Am J Physiol. 1992; 262:E353-E358.
64. Klein S, Sakurai Y, Romijn JA, Carroll RM. Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. Am J Physiol. 1993; 265:E801-E806.

65. Browning JD, Baxter J, Satapati S, Burgess SC. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. J Lipid Res. 2012; 53:577-586.
66. Cahill GF, Jr., Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA, Jr., Kipnis DM. Hormone-fuel interrelationships during fasting. J Clin Invest. 1966; 45:1751-1769.
67. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. Prog Lipid Res. 2009; 48:275-297.
68. Clore JN, Glickman PS, Helm ST, Nestler JE, Blackard WG. Accelerated decline in hepatic glucose production during fasting in normal women compared with men. Metabolism. 1989; 38:1103-1107.
69. Landau BR, Chandramouli V, Schumann WC, Ekberg K, Kumaran K, Kalhan SC, Wahren J. Estimates of Krebs cycle activity and contributions of gluconeogenesis to hepatic glucose production in fasting healthy subjects and IDDM patients. Diabetologia. 1995; 38:831-838.
70. Nilsson LH, Hultman E. Liver glycogen in man--the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. Scand J Clin Lab Invest. 1973; 32:325-330.
71. Landau BR. Quantifying the contribution of gluconeogenesis to glucose production in fasted human subjects using stable isotopes. Proc Nutr Soc. 1999; 58:963-972.
72. Walker AK, Yang F, Jiang K, *et al.* Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. Genes Dev. 2010; 24:1403-1417.
73. Romijn JA, Godfried MH, Hommes MJ, Endert E, Sauerwein HP. Decreased glucose oxidation during short-term starvation. Metabolism. 1990; 39:525-530.
74. Samuel VT, Shulman GI. Mechanisms for insulin resistance: Common threads and missing links. Cell. 2012; 148:852-871.
75. Stratton MT, Albracht-Schulte K, Harty PS, Siedler MR, Rodriguez C, Tinsley GM. Physiological responses to acute fasting: Implications for intermittent fasting programs. Nutr Rev. 2022; 80:439-452.
76. Norrelund H, Nair KS, Nielsen S, Frystyk J, Ivarsen P, Jorgensen JO, Christiansen JS, Moller N. The decisive role of free fatty acids for protein conservation during fasting in humans with and without growth hormone. J Clin Endocrinol Metab. 2003; 88:4371-4378.
77. Ueno T, Ezaki J, Kominami E. Metabolic contribution of hepatic autophagic proteolysis: Old wine in new bottles. Biochim Biophys Acta. 2012; 1824:51-58.
78. Wang Y, Wu R. The effect of fasting on human metabolism and psychological health. Dis Markers. 2022; 2022:5653739.
79. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: Effects on body weight, body composition, and energy metabolism. Am J Clin Nutr. 2005; 81:69-73.
80. Cienfuegos S, Gabel K, Kalam F, Ezpeleta M, Wiseman E, Pavlou V, Lin S, Oliveira ML, Varady KA. Effects of 4- and 6-h time-restricted feeding on weight and cardiometabolic health: A randomized controlled trial in adults with obesity. Cell Metab. 2020; 32:366-378.e3.
81. Sandoval DA, D'Alessio DA. Physiology of proglucagon peptides: Role of glucagon and GLP-1 in health and disease. Physiol Rev. 2015; 95:513-548.
82. Capozzi ME, Svendsen B, Encisco SE, Lewandowski SL, Martin MD, Lin H, Jaffe JL, Coch RW, Haldeman JM, MacDonald PE, Merrins MJ, D'Alessio DA, Campbell JE. β cell tone is defined by proglucagon peptides through cAMP signaling. JCI Insight. 2019; 4:e126742.
83. Gardner DF, Kaplan MM, Stanley CA, Utiger RD. Effect of tri-iodothyronine replacement on the metabolic and pituitary responses to starvation. N Engl J Med. 1979; 300:579-584.
84. Merimee TJ, Fineberg ES. Starvation-induced alterations of circulating thyroid hormone concentrations in man. Metabolism. 1976; 25:79-83.
85. Stekovic S, Hofer SJ, Tripolt N, *et al.* Alternate day fasting improves physiological and molecular markers of aging in healthy, non-obese humans. Cell Metab. 2019; 30:462-476.e6.
86. Moro T, Tinsley G, Bianco A, Marcolin G, Pacelli QF, Battaglia G, Palma A, Gentil P, Neri M, Paoli A. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. J Transl Med. 2016; 14:290.
87. Hojlund K, Wildner-Christensen M, Eshoj O, Skjaerbaek C, Holst JJ, Koldkjaer O, Moller Jensen D, Beck-Nielsen H. Reference intervals for glucose, beta-cell polypeptides, and counterregulatory factors during prolonged fasting. Am J Physiol Endocrinol Metab. 2001; 280:E50-E58.
88. Bergendahl M, Vance ML, Iranmanesh A, Thorner MO, Veldhuis JD. Fasting as a metabolic stress paradigm selectively amplifies cortisol secretory burst mass and delays the time of maximal nyctohemeral cortisol concentrations in healthy men. J Clin Endocrinol Metab. 1996; 81:692-699.
89. Johnstone AM, Faber P, Andrew R, Gibney ER, Elia M, Lobley G, Stubbs RJ, Walker BR. Influence of short-term dietary weight loss on cortisol secretion and metabolism in obese men. Eur J Endocrinol. 2004; 150:185-194.
90. Jamshed H, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM. Early time-restricted feeding improves 24-hour glucose levels and affects markers of the circadian clock, aging, and autophagy in humans. Nutrients. 2019; 11:1234.
91. Grinspoon SK, Askari H, Landt ML, Nathan DM, Schoenfeld DA, Hayden DL, Laposata M, Hubbard J, Klibanski A. Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. Am J Clin Nutr. 1997; 66:1352-1356.
92. Fain JN, Cowan GS, Jr., Buffington C, Li J, Pouncey L, Bahouth SW. Synergism between insulin and low concentrations of isoproterenol in the stimulation of leptin release by cultured human adipose tissue. Metabolism. 2000; 49:804-809.
93. Hartman ML, Veldhuis JD, Johnson ML, Lee MM, Alberti KG, Samojlik E, Thorner MO. Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. J Clin Endocrinol Metab. 1992; 74:757-765.
94. Beer SF, Bircham PM, Bloom SR, Clark PM, Hales CN, Hughes CM, Jones CT, Marsh DR, Raggatt PR, Findlay AL. The effect of a 72-h fast on plasma levels of pituitary, adrenal, thyroid, pancreatic and gastrointestinal hormones in healthy men and women. J Endocrinol. 1989; 120:337-350.
95. Palmblad J, Levi L, Burger A, Melander A, Westgren U, von Schenck H, Skude G. Effects of total energy withdrawal (fasting) on the levels of growth hormone,

thyrotropin, cortisol, adrenaline, noradrenaline, T4, T3, and rT3 in healthy males. Acta Med Scand. 1977; 201:15-22.
96. Norrelund H, Frystyk J, Jorgensen JO, Moller N, Christiansen JS, Orskov H, Flyvbjerg A. The effect of growth hormone on the insulin-like growth factor system during fasting. J Clin Endocrinol Metab. 2003; 88:3292-3298.
97. Kliewer SA, Mangelsdorf DJ. A dozen years of discovery: Insights into the physiology and pharmacology of FGF21. Cell Metab. 2019; 29:246-253.
98. Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, Munzberg H, Hutson SM, Gettys TW, Schwartz MW, Morrison CD. FGF21 is an endocrine signal of protein restriction. J Clin Invest. 2014; 124:3913-3922.
99. Kliewer SA, Mangelsdorf DJ. Fibroblast growth factor 21: From pharmacology to physiology. Am J Clin Nutr. 2010; 91:254S-257S.
100. Kharitonenkov A, Shiyanova TL, Koester A, *et al.* FGF-21 as a novel metabolic regulator. J Clin Invest. 2005; 115:1627-1635.
101. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A. Fibroblast growth factor 21 corrects obesity in mice. Endocrinology. 2008; 149:6018-6027.
102. Xu J, Lloyd DJ, Hale C, *et al.* Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes. 2009; 58:250-259.
103. Cienfuegos S, Corapi S, Gabel K, Ezpeleta M, Kalam F, Lin S, Pavlou V, Varady KA. Effect of intermittent fasting on reproductive hormone levels in females and males: A review of human trials. Nutrients. 2022; 14:2343.
104. Hua L, Feng B, Huang L, Li J, Luo T, Jiang X, Han X, Che L, Xu S, Lin Y, Fang Z, Wu D, Zhuo Y. Time-restricted feeding improves the reproductive function of female mice *via* liver fibroblast growth factor 21. Clin Transl Med. 2020; 10:e195.
105. Li C, Xing C, Zhang J, Zhao H, Shi W, He B. Eight-hour time-restricted feeding improves endocrine and metabolic profiles in women with anovulatory polycystic ovary syndrome. J Transl Med. 2021; 19:148.
106. Mizushima N, Murphy LO. Autophagy assays for biological discovery and therapeutic development. Trends Biochem Sci. 2020; 45:1080-1093.
107. Kovacs AL, Laszlo L, Fellinger E, Jakab A, Orosz A, Rez G, Kovacs J. Combined effects of fasting and vinblastine treatment on serum insulin level, the size of autophagic-lysosomal compartment, protein content and lysosomal enzyme activities of liver and exocrine pancreatic cells of the mouse. Comp Biochem Physiol B. 1989; 94:505-510.
108. White E, Mehnert JM, Chan CS. Autophagy, metabolism, and cancer. Clin Cancer Res. 2015; 21:5037-5046.
109. Donati A, Cavallini G, Bergamini E. Effects of aging, antiaging calorie restriction and *in vivo* stimulation of autophagy on the urinary excretion of 8OHdG in male Sprague-Dawley rats. Age (Dordr). 2013; 35:261-270.
110. Krustev LP, Tashev TA, Popov AA, Apostolov JZ, Borov BJ, Stefanova MS. Histochemical, biochemical and ultrastructural studies on the liver of fasted rats. Nahrung. 1976; 20:597-603.
111. Rickenbacher A, Jang JH, Limani P, Ungethum U, Lehmann K, Oberkofler CE, Weber A, Graf R, Humar B, Clavien PA. Fasting protects liver from ischemic injury through Sirt1-mediated downregulation of circulating HMGB1 in mice. J Hepatol. 2014; 61:301-308.
112. Meijer AJ, Codogno P. Autophagy: Regulation by energy sensing. Curr Biol. 2011; 21:R227-R229.
113. Jeon SM. Regulation and function of AMPK in physiology and diseases. Exp Mol Med. 2016; 48:e245.
114. Condon KJ, Sabatini DM. Nutrient regulation of mTORC1 at a glance. J Cell Sci. 2019; 132.
115. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011; 13:132-141.
116. Peña-Llopis S, Vega-Rubin-de-Celis S, Schwartz JC, Wolff NC, Tran TA, Zou L, Xie XJ, Corey DR, Brugarolas J. Regulation of TFEB and V-ATPases by mTORC1. EMBO J. 2011; 30:3242-3258.
117. Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, Huynh T, Ferron M, Karsenty G, Vellard MC, Facchinetti V, Sabatini DM, Ballabio A. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome *via* mTOR and TFEB. EMBO J. 2012; 31:1095-1108.
118. Carafa V, Rotili D, Forgione M, Cuomo F, Serretiello E, Hailu GS, Jarho E, Lahtela-Kakkonen M, Mai A, Altucci L. Sirtuin functions and modulation: From chemistry to the clinic. Clin Epigenetics. 2016; 8:61.
119. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, Ido Y. AMPK and SIRT1: A long-standing partnership? Am J Physiol Endocrinol Metab. 2010; 298:E751-E760.
120. Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, Tsokos M, Alt FW, Finkel T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proc Natl Acad Sci U S A. 2008; 105:3374-3379.
121. Zhang E, Cui W, Lopresti M, Mashek MT, Najt CP, Hu H, Mashek DG. Hepatic PLIN5 signals *via* SIRT1 to promote autophagy and prevent inflammation during fasting. J Lipid Res. 2020; 61:338-350.
122. Ahn BH, Kim HS, Song S, Lee IH, Liu J, Vassilopoulos A, Deng CX, Finkel T. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc Natl Acad Sci U S A. 2008; 105:14447-14452.
123. Kim JY, Mondaca-Ruff D, Singh S, Wang Y. SIRT1 and autophagy: Implications in endocrine disorders. Front Endocrinol (Lausanne). 2022; 13:930919.
124. Zhao Y, Yang J, Liao W, Liu X, Zhang H, Wang S, Wang D, Feng J, Yu L, Zhu WG. Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. Nat Cell Biol. 2010; 12:665-675.
125. Mandal SK, Puri S, Kumar BK, Muzaffar-Ur-Rehman M, Sharma PK, Sankaranarayanan M, Deepa PR. Targeting lipid-sensing nuclear receptors PPAR (α, γ, β/δ): HTVS and molecular docking/dynamics analysis of pharmacological ligands as potential pan-PPAR agonists. Mol Divers. 2023.
126. Panzitt K, Wagner M. FXR in liver physiology: Multiple faces to regulate liver metabolism. Biochim Biophys Acta Mol Basis Dis. 2021; 1867:166133.
127. Ramos Pittol JM, Milona A, Morris I, Willemsen ECL, van der Veen SW, Kalkhoven E, van Mil SWC. FXR isoforms control different metabolic functions in liver cells *via* binding to specific DNA motifs. Gastroenterology. 2020; 159:1853-1865.e10.
128. Correia JC, Massart J, de Boer JF, Porsmyr-Palmertz M, Martínez-Redondo V, Agudelo LZ, Sinha I, Meierhofer D, Ribeiro V, Björnholm M, Sauer S, Dahlman-Wright K, Zierath JR, Groen AK, Ruas JL. Bioenergetic cues shift

FXR splicing towards FXRα2 to modulate hepatic lipolysis and fatty acid metabolism. Mol Metab. 2015; 4:891-902.
129. Carino A, Marchianò S, Biagioli M, Scarpelli P, Bordoni M, Di Giorgio C, Roselli R, Fiorucci C, Monti MC, Distrutti E, Zampella A, Fiorucci S. The bile acid activated receptors GPBAR1 and FXR exert antagonistic effects on autophagy. FASEB J. 2021; 35:e21271.
130. Chen W, Mehlkop O, Scharn A, Nolte H, Klemm P, Henschke S, Steuernagel L, Sotelo-Hitschfeld T, Kaya E, Wunderlich CM, Langer T, Kononenko NL, Giavalisco P, Brüning JC. Nutrient-sensing AgRP neurons relay control of liver autophagy during energy deprivation. Cell Metab. 2023; 35:786-806.e13.
131. Ueno T, Komatsu M. Autophagy in the liver: Functions in health and disease. Nat Rev Gastroenterol Hepatol. 2017; 14:170-184.
132. Kanasaki K, Kawakita E, Koya D. Relevance of autophagy induction by gastrointestinal hormones: Focus on the incretin-based drug target and glucagon. Front Pharmacol. 2019; 10:476.
133. Ruan HB, Ma Y, Torres S, Zhang B, Feriod C, Heck RM, Qian K, Fu M, Li X, Nathanson MH, Bennett AM, Nie Y, Ehrlich BE, Yang X. Calcium-dependent O-GlcNAc signaling drives liver autophagy in adaptation to starvation. Genes Dev. 2017; 31:1655-1665.
134. Qiang W, Shen T, Noman M, Guo J, Jin Z, Lin D, Pan J, Lu H, Li X, Gong F. Fibroblast growth factor 21 augments autophagy and reduces apoptosis in damaged liver to improve tissue regeneration in zebrafish. Front Cell Dev Biol. 2021; 9:756743.
135. Yang X, Jin Z, Lin D, Shen T, Zhang J, Li D, Wang X, Zhang C, Lin Z, Li X, Gong F. FGF21 alleviates acute liver injury by inducing the SIRT1-autophagy signalling pathway. J Cell Mol Med. 2022; 26:868-879.
136. Byun S, Seok S, Kim YC, Zhang Y, Yau P, Iwamori N, Xu HE, Ma J, Kemper B, Kemper JK. Fasting-induced FGF21 signaling activates hepatic autophagy and lipid degradation *via* JMJD3 histone demethylase. Nat Commun. 2020; 11:807.
137. Koutsifeli P, Varma U, Daniels LJ, Annandale M, Li X, Neale JPH, Hayes S, Weeks KL, James S, Delbridge LMD, Mellor KM. Glycogen-autophagy: Molecular machinery and cellular mechanisms of glycophagy. J Biol Chem. 2022; 298:102093.
138. Zhao H, Tang M, Liu M, Chen L. Glycophagy: An emerging target in pathology. Clin Chim Acta. 2018; 484:298-303.
139. Komatsu M. Liver autophagy: Physiology and pathology. J Biochem. 2012; 152:5-15.
140. Mortimore GE, Hutson NJ, Surmacz CA. Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. Proc Natl Acad Sci U S A. 1983; 80:2179-2183.
141. Ke PY. Diverse functions of autophagy in liver physiology and liver diseases. Int J Mol Sci. 2019; 20.
142. Jiao L, Zhang HL, Li DD, *et al.* Regulation of glycolytic metabolism by autophagy in liver cancer involves selective autophagic degradation of HK2 (hexokinase 2). Autophagy. 2018; 14:671-684.
143. Lee YJ, Kim JK, Jung CH, *et al.* Chemical modulation of SQSTM1/p62-mediated xenophagy that targets a broad range of pathogenic bacteria. Autophagy. 2022; 18:2926-2945.
144. Ke PY. Mitophagy in the pathogenesis of liver diseases. Cells. 2020; 9:831.
145. You M, Matsumoto M, Pacold CM, Cho WK, Crabb DW. The role of AMP-activated protein kinase in the action of ethanol in the liver. Gastroenterology. 2004; 127:1798-1808.
146. Kim JS, Nitta T, Mohuczy D, O'Malley KA, Moldawer LL, Dunn WA, Jr., Behrns KE. Impaired autophagy: A mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. Hepatology. 2008; 47:1725-1736.
147. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, Leblanc M, Chaix A, Joens M, Fitzpatrick JA, Ellisman MH, Panda S. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab. 2012; 15:848-860.
148. Wu WKK, Zhang L, Chan MTV. Autophagy, NAFLD and NAFLD-related HCC. Adv Exp Med Biol. 2018; 1061:127-138.
149. Elsayed HRH, El-Nablaway M, Khattab BA, Sherif RN, Elkashef WF, Abdalla AM, El Nashar EM, Abd-Elmonem MM, El-Gamal R. Independent of calorie intake, short-term alternate-day fasting alleviates NASH, with modulation of markers of lipogenesis, autophagy, apoptosis, and inflammation in rats. J Histochem Cytochem. 2021; 69:575-596.
150. Yang W, Cao M, Mao X, Wei X, Li X, Chen G, Zhang J, Wang Z, Shi J, Huang H, Yao X, Liu C. Alternate-day fasting protects the livers of mice against high-fat diet-induced inflammation associated with the suppression of Toll-like receptor 4/nuclear factor kappaB signaling. Nutr Res. 2016; 36:586-593.
151. Zhang W, Wang J, Wang L, Shi R, Chu C, Shi Z, Liu P, Li Y, Liu X, Liu Z. Alternate-day fasting prevents non-alcoholic fatty liver disease and working memory impairment in diet-induced obese mice. J Nutr Biochem. 2022; 110:109146.
152. Jordan S, Tung N, Casanova-Acebes M, *et al.* Dietary intake regulates the circulating inflammatory monocyte pool. Cell. 2019; 178:1102-1114 e1117.
153. Khader A, Yang WL, Godwin A, Prince JM, Nicastro JM, Coppa GF, Wang P. Sirtuin 1 stimulation attenuates ischemic liver injury and enhances mitochondrial recovery and autophagy. Crit Care Med. 2016; 44:e651-e663.
154. Liu H, Dong J, Song S, Zhao Y, Wang J, Fu Z, Yang J. Spermidine ameliorates liver ischaemia-reperfusion injury through the regulation of autophagy by the AMPK-mTOR-ULK1 signalling pathway. Biochem Biophys Res Commun. 2019; 519:227-233.
155. Chen Y, Lv L, Pi H, Qin W, Chen J, Guo D, Lin J, Chi X, Jiang Z, Yang H, Jiang Y. Dihydromyricetin protects against liver ischemia/reperfusion induced apoptosis *via* activation of FoxO3a-mediated autophagy. Oncotarget. 2016; 7:76508-76522.
156. Ni HM, Du K, You M, Ding WX. Critical role of FoxO3a in alcohol-induced autophagy and hepatotoxicity. Am J Pathol. 2013; 183:1815-1825.
157. Jansen M, Ten Klooster JP, Offerhaus GJ, Clevers H. LKB1 and AMPK family signaling: The intimate link between cell polarity and energy metabolism. Physiol Rev. 2009; 89:777-798.
158. Tsai HH, Lai HY, Chen YC, Li CF, Huang HS, Liu HS, Tsai YS, Wang JM. Metformin promotes apoptosis in hepatocellular carcinoma through the CEBPD-induced autophagy pathway. Oncotarget. 2017; 8:13832-13845.
159. Jiang X, Tan HY, Teng S, Chan YT, Wang D, Wang N. The role of AMP-activated protein kinase as a potential target

of treatment of hepatocellular carcinoma. Cancers (Basel). 2019; 11.
160. Jia F, Diao P, Wang X, Hu X, Kimura T, Nakamuta M, Nakamura I, Shirotori S, Sato Y, Moriya K, Koike K, Gonzalez FJ, Nakayama J, Aoyama T, Tanaka N. Dietary restriction suppresses steatosis-associated hepatic tumorigenesis in hepatitis C virus core gene transgenic mice. Liver Cancer. 2020; 9:529-548.
161. Murata Y, Uehara Y, Hosoi Y. Activation of mTORC1 under nutrient starvation conditions increases cellular radiosensitivity in human liver cancer cell lines, HepG2 and HuH6. Biochem Biophys Res Commun. 2015; 468:684-690.
162. Lai HY, Tsai HH, Yen CJ, Hung LY, Yang CC, Ho CH, Liang HY, Chen FW, Li CF, Wang JM. Metformin resensitizes sorafenib-resistant HCC cells through AMPK-dependent autophagy activation. Front Cell Dev Biol. 2020; 8:596655.
163. Lo Re O, Panebianco C, Porto S, Cervi C, Rappa F, Di Biase S, Caraglia M, Pazienza V, Vinciguerra M. Fasting inhibits hepatic stellate cells activation and potentiates anti-cancer activity of sorafenib in hepatocellular cancer cells. J Cell Physiol. 2018; 233:1202-1212.
164. Krstic J, Reinisch I, Schindlmaier K, *et al.* Fasting improves therapeutic response in hepatocellular carcinoma through p53-dependent metabolic synergism. Sci Adv. 2022; 8:eabh2635.
165. Kord-Varkaneh H, Salehi-Sahlabadi A, Tinsley GM, Santos HO, Hekmatdoost A. Effects of time-restricted feeding (16/8) combined with a low-sugar diet on the management of non-alcoholic fatty liver disease: A randomized controlled trial. Nutrition. 2023; 105:111847.

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