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Ferroptosis and aerobic training in ageing: A review

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Abstract. Ferroptosis is a form of programmed cell death that plays a significant role in causing several diseases such as heart attack and heart failure, through alterations in fat, amino acid, and iron metabolism. Comprehending the regulatory mechanisms of ferroptosis signaling is critical because it has a considerable effect on the elderly's mortality. Conversely, age-related changes in substrate metabolism and metabolite levels are recognized to give rise to obesity. Furthermore, research has proposed that aging and obesity-related changes in substrate metabolism may aggravate ferroptosis. The suppression of ferroptosis holds potential as a successful therapeutic approach for managing different diseases, including sarcopenia, cardiovascular diseases, and central nervous system diseases. However, the pathologic and biological mechanisms behind the function of ferroptosis are not fully comprehended yet. Physical activity could affect lipid, amino acid, and iron metabolism to modulate ferroptosis. The aim of this study is to showcase the current understanding of the molecular mechanisms leading to ferroptosis and discuss the role of aging and physical activity in this phenomenon.

Keywords: Aerobic training, elderly, iron metabolism, amino acid metabolism, lipid metabolism

Abbreviations

DNA	Deoxyribonucleic acid
GPX4	glutathione peroxidase 4
TNF-α	Tumor necrosis factor alpha
NF-κB	Nuclear factor kappa B
ROS	reactive oxygen species
RSL3	RAS-selective lethal
PUFA	polyunsaturated fatty acyl phospholipids

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VDAC 2,3   voltage-dependent anion channels 2 and 3
DPI7   Diphenylene iodonium 7
COQ10   coenzyme Q10
Fino2   ferroptosis-inducing peroxide compound
FIN56   Ferroptosis inducer 56
PE   phosphatidylethanolamine
IKE   Imidazole ketone erastin
SAS   Sulfasalazine
BSO   Buthioninesulfoximine
AMSNs   arginine-rich manganese silicate nanobubbles
LDLDHA   Low-density lipoprotein docosahexaenoic acid
ZVI NPs   zero-valent-iron NPs
MONp53   polyethylenimine/p53 plasmid complex
PSAF NCs   PEGylated single-atom Fe-containing nanocatalysts
ACSL4   Acyl-CoA Synthetase Long Chain Family Member 4
Hmox-1   heme oxygenase-1
TFRC   the transferrin receptor
FTH1   ferritin heavy chain 1
IREB2   iron response element binding protein 2
SLC7A11   Ferroptosis inhibiting gene solute carrier family 7 member 11
SLC3A2   solute carrier family 3 member 2
Xc-system   a cystine/glutamate antiporter system
NO   Nitric oxide
NOS   nitric oxide synthase
PI3K   phosphatidylinositol 3-kinase
mTOR   mechanistic target of rapamycin
AKT   Akt kinase" (also called protein kinase B or PKB)
mTORC1   mechanistic target of rapamycin complex 1
NF-κB   nuclear factor kappa B
RNS   Reactive Nitrogen Species
ERR   Estrogen-related receptor
PPAR-α Peroxisome Proliferator-Activated Receptor Alpha
PGC1 α Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
NRF   Nuclear factor
FAO   fatty acid oxidation
FFA   Free fatty acids
MLKL   Mixed lineage kinase domain-like
RIPK1   Receptor-interacting protein kinase 1
RIPK 3   Receptor-interacting serine/threonine-protein kinase 3

1. Introduction

Traditionally, three types of cell death have been identified: apoptosis, autophagic cell death, and necrosis. This classification is mainly based on morphological features [1, 2]. Apoptosis, also known as type 1 cell death, was the first form of programmed cell death to be identified. It involves DNA fragmentation, chromatin compaction, cell shrinkage, plasma membrane blebbing, and apoptotic trunk formation, without loss of cell membrane integrity [3]. Type 2 cell death, also called autophagic

cell death, is characterized by cell demise without chromatin condensation, and is accompanied by large-scale formation of autophagic vacuoles in the cytoplasm [4]. Autophagy is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules [5]. Although autophagy is generally considered a critical survival mechanism, its dysregulation can be detrimental and result in cell death when stress is excessive. Necrotic cell death, known as type 3 cell death or necrosis, is characterized by an increase in cell volume (oncosis), organelle swelling, rupture of the plasma membrane, and extrusion of intracellular contents [6]. Programmed necrosis mainly comprises mitochondrial permeability transition-dependent necrosis, necroptosis, ferroptosis, and pyroptosis. An increasing number of studies have demonstrated that pyroptosis plays a role in various diseases [7]. Distinctions between apoptosis, necrosis, and autophagy entail differences in the mode of death and morphologic, biochemical, and molecular attributes [8, 9]. On the other hand, that neutrophil extracellular traps (NETs) and ferroptosis can cause epithelial injury. In addition, little attention has been given to how NETs affect microcirculation [10]. Also, consumption of some natural substances due to their anti-proliferative, pro-apoptotic, antioxidant and anti-angiogenic properties, immune modulating proteins, NF-κB, p52, TNF-α, etc. can be effective in delaying the aging process [11–13].

Currently, there are at least twelve distinct types of regulated cell death, including ferroptosis [14]. Ferroptosis is a type of programmed cell death that works in a regulated manner in normal cells. Its disruption is associated with the occurrence and progression of several diseases, such as cancer, neurological diseases, acute kidney injury, ischemia-perfusion, and other conditions [15, 16]. According to Green and Victor (2012), ferroptosis is a form of 'cell sabotage' where the cell's normal metabolic functions lead to its death. This differentiation sets ferroptosis apart from apoptosis and other forms of regulated cell death that could be characterized as 'cell suicide' [17]. Ferroptosis involves genetic, metabolic, protein regulators, triggers, and mechanisms of execution that differ from other forms of regulated cell death [18]. Morphologically, ferroptosis occurs mainly in cells where the mitochondrial volume decreases, bilayer membrane density increases, and mitochondrial cristae decrease or disappear [18, 19]; however, the cell membrane remains intact. The nucleus has a standard size, and no chromatin condensation occurs. Biochemically, ferroptosis is promoted by intracellular glutathione (GSH) depletion and glutathione peroxidase 4 (GPX4) activity reduction. The GPX4-catalyzed reduction reaction cannot metabolize lipid peroxides, allowing ferrous iron (Fe^{2+}) to oxidize lipids in a Fenton-like fashion, resulting in the production of large amounts of reactive oxygen species (ROS) [19, 20]. From a genetic point of view, ferroptosis is process regulated by several genes [21]. Lipophilic antioxidants, chelators, lipid peroxidation inhibitors, and reduced levels of polyunsaturated fatty acyl phospholipids (PUFA-PLs), the primary substrates of fatal lipid peroxidation, can inhibit ferroptosis [22, 23]. Ferroptosis is marked by a gradual increase in the ratio of iron (Fe) to sulfur (S). The main cause of oxidative stress is iron [24], and peptides (such as glutathione) and proteins (such as thioredoxin) provide the primary resistance to oxidative stress through S or sulfhydryls (SH-) groups [25]. Iron excess or sulfur deficiency contributes to ferroptosis. A key aspect of this mechanism is iron-catalyzed peroxidation of unsaturated fatty acids in phospholipids. Inactivation of cellular glutathione-dependent antioxidant defenses triggers ferroptosis, resulting in the accumulation of toxic lipid reactive oxygen species [18, 26]. GSH is a crucial ligand for cytosolic Fe^{2+} [27] and a vital substrate for GPX4. The peroxidase scavenges membrane lipid peroxides that lead to ferroptosis cell death [26, 28]. Studies have confirmed the positive effects of aerobic training on health in aging [29–32]. It has been reported that aerobic training can affect ferroptosis through different pathways [33, 34]. Iron, amino acid and lipid metabolism are disturbed in aging, and iron, amino acid and lipid metabolism are regulated by aerobic training (Fig. 1). Therefore, investigating the effects of aerobic training on the factors that affect ferroptosis in the elderly is considered important.
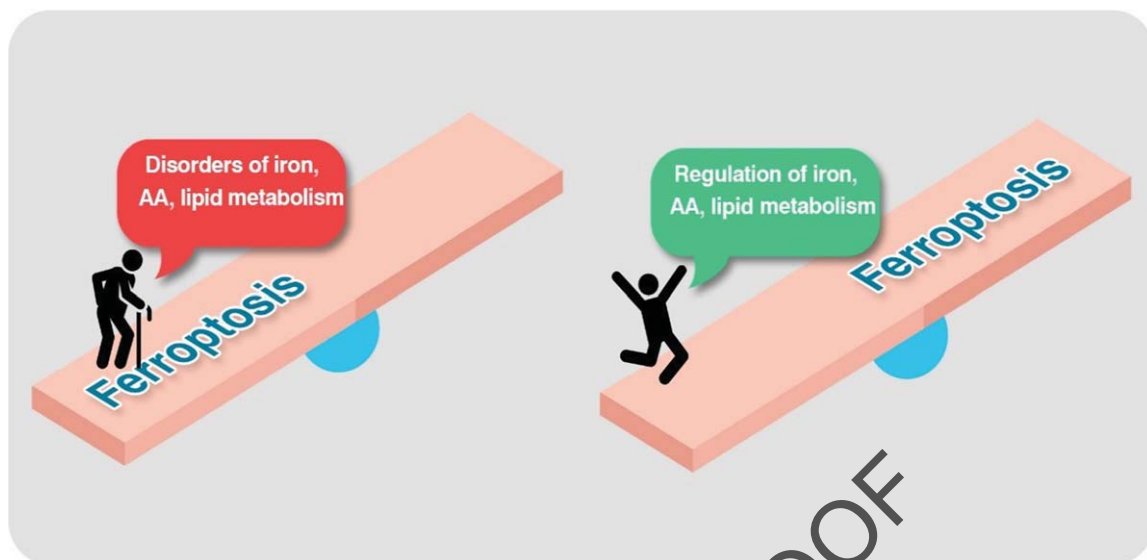
Fig. 1. Role of Iron, Amino Acid, and Lipid on Ferroptosis during training and Aging.

2. Inducers and inhibitors of ferroptosis

Four categories of ferroptosis inducers have been identified, each selectively involving specific signaling pathways (Table 1). The first category includes erastin, and is the primary model for ferroptosis induction and works by directly suppressing the cystine/glutamate antiporter (System X_c^-), thus reducing GSH levels. Erastin binds to and blocks voltage-dependent anion channels 2 and 3 (VDAC 2 and 3), which eventually results in mitochondrial dysfunction and cell death. Ferroptosis activation through erastin increases the levels of lysosomal-associated membrane protein 2A, thus promoting chaperone-mediated autophagy and leading to GPX4 degradation [35]. The second group of inducers, including RSL3 and DPI7, directly inhibit GPX4, resulting in increased lipid peroxidation and the induction of ferroptosis. The third category of inducers induces ferroptosis in two ways: First, FIN56 leads to the destruction of GPX4. Second, FIN56 binds to the enzyme squalene synthase, leading to a reduction in the endogenous antioxidant coenzyme Q10 (COQ10). This process increases cell sensitivity to ferroptosis induced by FIN56 [36]. The fourth class of compounds comprises FINO2, an organic peroxide that shares many properties with artemisinin. FINO2 induces ferroptosis by directly oxidizing unstable iron and inactivating GPX4 [36]. As research on the mechanism of ferroptosis has advanced, specific inhibitors of ferroptosis, like ferostatin-1 (FER-1), lipoxastatin-1, and vitamin E, have been identified in addition to iron scavengers. These substances prevent ferroptosis by blocking the formation of lipid peroxides [21]. Several nanoparticles have been developed to trigger ferroptosis. These particles are valuable tools for investigating the ferroptosis mechanism and may also have potential therapeutic applications in cancer treatment [37].

2.1. Ferroptosis inhibitors

Iron chelators can prevent ferroptosis-induced cell death, but their potential toxicity limits their use. Iron is a fundamental element for life and is widely used in society. As an alternative, non-chelating iron inhibitors may be of vital importance to researchers. Developing new strategies to protect cells from ferroptosis-induced damage is crucial for clinical applications. These inhibitors comprise:

Table 1
A summary of identified ferroptosis inducers and their precise mechanisms of action

Type	Form	Mechanism
Group 1	Erastin	Inhibition of SLC7A11 activity
	PE	Inhibition of SLC7A11 activity
	IKE	Inhibition of SLC7A11 activity
	SAS	Inhibition of SLC7A11 activity
	Sorafenib	Inhibition of SLC7A11 activity
	Cysteinase	Cysteine depletion
	Glutamate	Inhibition of SLC7A11 activity
	BSO	Glutathione depletion
	DPI2	Glutathione depletion
	Cisplatin	Glutathione depletion
Group 2	1S,3R-RSL3	Inhibition of GPX4 activity
	ML162	Inhibition of GPX4 activity
	ML210	Inhibition of GPX4 activity
	Altertamine	Inhibition of GPX4 activity
	Vitaferin A	Reduce and disable GPX4
Group 3	FIN56	Reduction of GPX4 and coenzyme Q10
	Statins (lovastatin, simvastatin, lovastatin)	Inhibition of squalene synthetase enzyme
Group 4	Ammonium ferric citrate	Iron entry
	Ferric chloride	Iron entry
	hemoglobin	Iron entry
	Lapatanib	Increased expression of transferrin receptor
	Salinomycin	Decrease of ferritin
	Artesunate	Increase of intracellular iron
	FINO2	Inhibition of GPX4 activity
Nanoparticles	AMSNs	Glutathione decrease
	LDLDHA	Entry of omega-3 fatty acid
	ZVI NPs	Iron entry
	MONp53	Iron entry – inhibition of SLC7A11 activity
	PSAF NCs	Increase of intracellular iron

Phosphatidylethanolamine (PE), Imidazole ketone erastin (IKE), Sulfasalazine (SAS), Buthion-inesulfoximine (BSO), Diphenylene iodonium 2 (DPI2), Ferroptosis inducer 56 (FIN56), Ferroptosis-inducing peroxide compound (FINO2), Arginine-rich manganese silicate nanobubbles (AMSNs), Low-density lipoprotein docosahexaenoic acid (LDLDHA), Zero-valent-iron NPs (ZVI NPs), Polyethylenimine/p53 plasmid complex (MONp53), PEGylated single-atom Fe-containing nanocatalysts (PSAF NCs)

2.1.1. Lipooxygenase inhibitors

In the case of ferroptosis, lipid peroxides accumulate to a lethal level inside cells. Various types of lipids such as fatty acids, phospholipids, cholesterols, cardiolipins, and sphingolipids are found in cells, it is difficult to determine exactly which of these lipids are involved in the process of ferroptosis or if they play a functional role in the lethal phenotype of ferroptosis. Tracking lipid oxidation events in ferroptosis could provide insight into how GPX4 is regulated. Oxylipins function as messenger

molecules in ferroptosis. Lipid peroxidation can occur through enzymatic and non-enzymatic pathways, and these pathways can be inhibited through lipid oxidation inhibitors (antioxidants that trap free radicals) and lipoxygenase inhibitors [38, 39]. Interestingly, Studies have shown that unsaturated fatty acids can act as inhibitors of ferroptosis. Magar et al. found that adding unsaturated fatty acids to cultured cells protects them against ferroptosis. Adding unsaturated fatty acids inactivates stearoyl coa dehydrogenase enzyme activity, thereby preventing the conversion of stearic acid to unsaturated fatty acids

2.1.2. Antioxidants

Radical scavenging antioxidants are molecules that react with chains of radicals to halt the oxidation chain reaction. Alpha-tocopherol, the biologically active form of vitamin E, is used as an antioxidant for numerous cardiovascular and neurodegenerative diseases. Multiple analogs of vitamin E have been explored as fat-soluble antioxidants. Of the synthetic compounds, tetrahydro naphthyridine (THNs) is the most promising for clinical application. This compound responds more than 30 times quicker to peroxyl radicals in organic solvents and liposomes compared to alpha-tocopherol [40]. Liproxstatin and frustatins are compounds with high inhibitory power against ferroptosis. Liproxstatin-1 is the first-member molecule of liproxstatins class, with nanomolar level suppression of ferroptosis, very suitable pharmacological properties, and a plasma half-life of 4.6 hours. The improvement in acute kidney injury shown by Liproxstatin-1 in a mouse model with a GPX4 defect indicates that this compound is an *in vivo* ferroptosis inhibitor [41]. The combination of liprostatin-1 and frustatin-1 leads to the preservation of tissue function in kidney and liver ischemia-reperfusion (I/R) injury [42]. Frustatin-1 has been found to exhibit antioxidant properties in some studies. However, its ability to act as an antioxidant in reaction with peroxyl radicals is lower than that of alpha tocopherol [43].

2.1.3. ACSL4 enzyme inhibitors

ACSL4 (Acyl-CoA Synthetase Long Chain Family Member 4) enzyme transfers arachidonic acid and adrenergic acid to phosphatidylethanolamine (PE) and oxidizes them. Thiazolidinediones, acting as specific inhibitors of ACSL4, may suppress ferroptosis. By inhibiting ACSL4 enzyme, thiazolidinediones act as insulin sensitizers and stimulants of peroxisome proliferator-activated receptors to regulate ferroptosis. Pharmacological inhibition of ACSL4 offers a novel approach to inhibit ferroptosis [44, 45].

3. Role of iron, amino acid and lipid metabolism on ferroptosis

3.1. The role of iron metabolism on ferroptosis

Autophagy has been demonstrated to regulate sensitivity to ferroptosis by modulating intracellular iron metabolism [46]. Also, Ferritin-selective autophagy enhances sensitivity to ferroptosis via the regulation of iron availability in cells [47]. Heat shock protein beta-1 (HSPB1) has been reported to decrease intracellular iron levels by inhibiting TRF1 expression, and overexpression of HSPB1 can significantly attenuate ferroptosis [48]. Additionally, ferritin comprises ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1). A significant increase in the expression of FTL and FTH1, resulting in the inhibition of erastin-induced ferroptosis, can be achieved by inhibiting the expression of iron response element binding protein 2 (IREB2), which is the major transcription factor of iron metabolism [49]. The expression of genes related to iron metabolism increases significantly upon suppressing IREB2 expression through RNAi. These genes include F-box, leucine-rich repeat protein 5, iron-sulfur cluster assembly enzyme, FTH1, and FTL. Moreover, this suppression also limits the

ferroptosis caused by erastin [18, 19]. The membrane protein ferroportin (also known as SLC11A3), functions as an iron efflux pump and mediates iron efflux. In addition to this, it can oxidize Fe^{2+} to Fe^{3+} [50]. Cells that are sensitive to ferroptosis and have a Ras mutation show an increase in transferrin receptor 1 (TFR1) and a decrease in the expression of ferritin (FTL and FTH1) compared to cells that are resistant to ferroptosis [19]. Increased iron absorption and decreased iron storage may contribute to iron overload during ferroptosis. It has been shown that iron scavengers such as deferoxamine, desferrioxamine mesylate, and ciclopirox olamine can reduce iron overload and inhibit erastin-induced ferroptosis. On the contrary, exogenous sources of iron such as ferric ammonium citrate, ferric citrate, and iron chloridehexahydrate can increase death caused by erastin [18, 19]. Thus, cellular mechanisms that participate in iron absorption and utilization are necessary for the occurrence of ferroptosis [50]. Many metabolic enzymes involved in redox reactions and lipid peroxidation are iron-dependent, which is an important point to note [51, 52]. However, Distinguishing the role of iron-dependent ROS production and iron-dependent enzyme activity in regulating ferroptosis remains challenging.

3.2. The role of amino acid metabolism on ferroptosis

Two cellular components, namely the xc- and GPX4 systems, were identified through mechanistic studies. These systems are respectively inhibited by erastin and RSL3 compounds and can lead to ferroptotic death [18, 26, 53].

Induction of ferroptosis by suppression of GPX4: The expression of GPX4 is controlled by the availability of cellular selenium as it is one of the 25 selenoproteins found in humans [54, 55]. GPX4 plays a central role in the occurrence of ferroptosis among the various members of the GPX family. It acts as the primary regulator by inhibiting the formation of lipid peroxides. GPX4 converts GSH to oxidized glutathione (GSSG) and reduces cytotoxic lipid peroxides (L-OOH) to their corresponding alcohols (L-OH). If GPX4 activity is inhibited, lipid peroxides can accumulate. This accumulation is a marker of ferroptosis [21]. GPX4 is a selenoprotein whose active site contains the amino acid selenocysteine. Cysteine is transported into the cell in a one-to-one ratio with intracellular glutamate by using the cystine/glutamate transporter system located on the plasma membrane (X-). This cysteine is then required for the production of glutathione. Preventing cysteine absorption ultimately results in the accumulation of hyperoxidation products. The X-system is inhibited and intracellular cysteine is depleted due to the high extracellular concentration of glutamate [56]. A study reported that cells with decreased GPX4 expression were more susceptible to ferroptosis, in contrast, an increase in GPX4 expression impeded ferroptosis [19]. RSL3 induces ferroptosis by inhibiting the activity of GPX4, which reduces the cells' antioxidant capacity and causes ROS accumulation leading to ferroptosis [26].

Induction of ferroptosis by suppression of System Xc-: The Xc- system is a widely distributed amino acid antiporter found in the phospholipid bilayer. SLC7A11 and SLC3A2 are two subunits that form a heterodimer, which is part of an essential antioxidant system inside cells. The Xc- system facilitates the exchange of cysteine and glutamate between the inside and outside of the cell, with equal amounts of both exchanged, as per a ratio of 1 : 1 [18]. If cysteine is removed from cells, its amount decreases and this can affect the synthesis of GSH. GSH can reduce ROS and reactive nitrogen through the action of glutathione peroxidases GPXs. Inhibiting the activity of the Xc- system by halting the absorption of cystine can affect GSH synthesis. This can lead to decreased GPX activity, cellular antioxidant capacity, and the accumulation of lipid ROS. In the end, oxidative damage and ferroptosis may occur. Moreover, P53 can also inhibit the uptake of cysteine Xc from the system by reducing the expression of SLC7A11. This can affect the activity of GPX4, leading to reduced cell antioxidant capacity and the accumulation of lipid ROS. Ultimately, ferroptosis can occur [57, 58]. The regulation of ferroptosis involves amino acid metabolism and glutamine synthesis [59]. Glutaminases (GLS1 and GLS2) can

convert glutamine to glutamate, thereby regulating the extracellular concentration of glutamate [60]. α-Ketoglutarate, which is produced by the intracellular metabolic pathway driven by glutamine, can serve as a substitute for glutamine in meeting the need for ferroptosis. Ferroptosis is triggered when the levels of glutamine and α-ketoglutarate are depleted, leading to the accumulation of lipid peroxides and ROS caused by the depletion of cysteine [61].

3.3. The role of lipid metabolism on ferroptosis

Fatty acids, including saturated fatty acids, monounsaturated fatty acids (MUFAs), and polyun-saturated fatty acids (PUFAs), perform multiple functions in cells. Polyunsaturated fatty acids, like arachidonic acid and adrenic acid, are the primary substrates of lipid peroxidation in the ferroptosis process, which can lead to damage in membrane structure and function. Unlike monounsaturated fatty acids (such as oleic acid and palmitoleic acid), PUFAs are prone to lipid peroxidation. The produc-tion of PUFA derivatives for ferroptosis relies on two crucial enzymes of PUFA biosynthesis, namely ACSL4 and LPCAT3, and also glutaminolysis. Lipid droplets can release fatty acids under stress conditions, leading to lipid peroxidation during ferroptosis, which is further promoted by autophagy activation. Adopting a low-fat diet can be a helpful and safe strategy to prevent diseases associated with ferroptosis [62]. Lipid peroxidation is another factor in the activation of ferroptosis. Phospho-lipids that contain PUFA are the primary substrates for lipid peroxidation in ferroptosis. Phospholipids are basic components of the biological membranes of cells, which undergo oxidation by lipid per-oxidases, particularly by lipid hydroperoxides. These include phosphatidylcholine, cardiolipin, and phosphatidylethanolamine [23]. Phospholipids always have an acyl residue attached to the PUFA binding site at the sn-2 position. Due to PUFA attached to the sn-2 position, phospholipids exhibit high sensitivity to oxidation. Esterification of unsaturated fatty acid acyl-CoA (PUFA-CoA) catalyzes the attachment of PUFA units to the sn-2 position of phospholipids. ASCL4, an enzyme that connects coenzyme A to long-chain PUFAs, is a member of Acyl-CoA synthase which catalyzes the formation of PUFA-CoA and hence affects ferroptosis. Perioxidation of PUFA-CoA results in the formation of arachidonyl (AA) and adrenyl (AdA) acids, leading to ferroptosis [63]. Research has demonstrated that inhibition of ACSL4 leads to a reduction in phospholipid PUFA. ACSL4-deficient cells show a significant increase in the level of unesterified PUFA compared to oxygen-esterified PUFAs. Inhibit-ing ACSL4 has a protective effect against ferroptosis in cells [45]. Hence, higher concentrations of PUFA and L-OOH can increase the amount of ferroptosis, which may lead to disease in organs and tissues. The process of PUFA oxidation that leads to ferroptosis can occur either enzymatically or non-enzymatically. The non-enzymatic mechanism occurs through the reactive oxygen species and hydroxyl radical pathways, which is known as the Fenton reaction. Lipoxygenases (LOX) catalyze the enzymatic oxidation of PUFA [64]. According to reports, LOX inhibitors like flavonoids and certain vitamin E derivatives are capable of preventing ferroptosis-mediated cell death under certain circumstances [65].

4. Iron, amino acid and lipid changes in aging

4.1. Iron changes in aging

Recently, iron has received considerable attention for its impact on accelerating the aging process and is expected to play a crucial role in this process. Aging leads to disruptions in iron metabolism. Iron deficiency anemia, low serum ferritin, and systemic iron deficiency in the elderly have negative effects, including cognitive impairment, increased frailty, cardiovascular disease, and mortality [66]. Iron accumulation in old age is associated with many age-related diseases. Through drugs or natural

products, it increases lifespan by blocking iron absorption. Many life-prolonging interventions, such as old plasma dilutions, caloric restriction, and rapamycin, can have effects on iron absorption, excretion, and metabolism. Keeping the low normal range of body iron stores low may be an important intervention that may lead to increased longevity [67]. Besides poor diet and drug use, elevated hepcidin levels (as a result of chronic inflammation) are likely one of the causative factors of systemic iron deficiency [68]. Tissue iron stores increase during aging. This is caused by a redox imbalance and can result in ferroptosis, cell viability disruption, and death [69]. High hepcidin levels may be associated with decreased systemic iron and increased intracellular iron [68]. Thus, it can be postulated that modification of iron dyshomeostasis and chronic inflammation may have some control over aging [70]. Certain chronic diseases can result in an overaccumulation of intracellular iron, which triggers ferroptosis [68, 69]. Iron deficiency during growth and development can cause defects in the main growth pathways. Likewise, excess iron retention can lead to accelerated aging in adulthood. Some possible causes of excess iron with age include (i) Menopause due to relative iron overload in women; (ii) Reducing the amount of hemoglobin, which contains 60% of the total iron; (iii) Reducing the metabolic rate and the need for iron by cofactor molecules. Accumulating excessive iron throughout life in human body tissue can lead to harmful effects on cellular function, aging, and increased mortality [69]. Age-related disruption of iron homeostasis and cellular dysfunction may result in cell death associated with iron metabolism. Maintaining the normal iron level is crucial for the survival of the cell, and its regulation can assist in controlling cellular energy. Conversely, uncontrolled iron can expedite aging, aggravate the pathogenesis of disease, and increase mortality. Abnormal levels of iron can result in cell toxicity and energy imbalance. Inhibitors of iron metabolism can delay aging and reduce the risk of chronic diseases [70].

4.2. Amino acid changes in aging

Various biological aspects related to branched-chain amino acids (BCAAs) are compromised as age increases. During aging, anabolic resistance becomes apparent, resulting in a decrease in the synthesis of muscle proteins when exposed to anabolic stimuli like physical activity, insulin, and leucine. This dependence is partly caused by the alteration of mTOR disorder, mitochondrial activity, and BCAA pharmacokinetics [71]. As one age, certain changes occur, including a reduction in muscle mass, protein intake in the diet, and an increase in muscle catabolism. BCAA metabolism changes play a crucial role in substantially decreasing the total body protein synthesis [72]. Studies have shown that BCAT (branched chain aminotransferases) expression significantly decreases during the aging process [73]. As a consequence, age-related changes lead to decreased blood levels of BCAAs in humans [74, 75]. Furthermore, a common indicator of aging in human studies is a decrease in blood branched-chain amino acids levels contrasting with increased citrulline [75]. mTOR is one of the primary targets of BCAAs. It regulates various indicators of cellular aging, including cell growth, autophagy, alterations in mitochondrial function, and apoptosis [76]. Nutritional interventions such as protein and caloric restriction are known to delay aging and increase lifespan in many species. These interventions are observed to correlate with reduced mTOR activity [77]. A decrease in branched-chain amino acid concentration, which is inversely associated with longevity, is caused by calorie restriction [78]. Rapamycin is considered the most potent pharmacological agent for delaying aging in laboratory animal models, which includes senescent mice [79]. Deletion of the downstream target of mTOR, S6K, increases lifespan in female mice, while knocking out mTOR is lethal [80]. However, Significant differences exist between the effects of rapamycin and calorie restriction. Rapamycin leads to insulin resistance, whereas caloric restriction enhances insulin sensitivity [81].

4.3. Lipid changes in aging

As age increases, the level of plasma triglycerides also increases concurrently with the increase of plasma lipoproteins. However, the rate of plasma triglyceride clearance decreases when lipoprotein lipase activity reduces [82, 83]. Mitochondria have a crucial role in lipid metabolism, and the aging process results in alterations in mitochondria [84]. Certain phospholipids also participate in cellular signaling pathways [85]. Phospholipid-like sphingolipids play an important role in maintaining the plasma lipid bilayer. Some sphingolipids, such as sphingosine, are crucial mediators of various physiological processes, including apoptosis, proliferation, stress response, necrosis, and inflammatory pathways [86]. Changes in lipid metabolism that regulate chromatin states may impact lifespan [87]. Changes in chromatin, including alterations in DNA and histones, are responsible for regulating gene expression, particularly in aging-related conditions [88]. In fact, Chromatin markers change with aging. Certain chromatin modifiers influence lifespan in various species [89]. Lipid metabolism is altered by chromatin regulation. Certain lipid metabolites or lipids have a direct impact on histone acylation and acetylation. As a cofactor, Acetyl-CoA adds acetyl groups to lysine residues [90]. Furthermore, chromatin modification and lipid metabolism share common precursors, such as S-adenosyl methionine (SAM). SAM is required for the production of phosphocholines in phospholipids as well as for histone methylation [91]. It has been suggested that the regulation of lipid-induced changes in signal transduction pathways is an indirect reflection of chromatin state. Pro-inflammatory lipid molecules, such as eicosanoids and lipopolysaccharides, have been suggested to be potent epigenetic modifiers [85, 92]. Phospholipid and free fatty acid derivatives are capable of binding to G protein-coupled receptors (GPCRs) and influencing the epigenetic landscape [93]. Chromatin modifications can have an impact on both lipid metabolism and lifespan. Epigenetic modifications associated with lipid metabolism are known to be mediated by methyltransferases. Inhibiting MUFA synthesis has been shown to increase lifespan while increasing MUFA synthesis has the opposite effect [94]. Sirtuin is one of the chromatin modifiers associated with aging and metabolism. The deacetylation of non-histone and histone proteins by sirtuins is responsible for regulating metabolic processes like lipid metabolism and longevity [95]. DNA methylation is an additional factor that affects lipid metabolism. Li et al. recently demonstrated that aging can cause lipid metabolism disorders that are associated with DNA methylation [96]. Disturbance in the metabolism of fat, amino acid and iron in aging leads to stimulation of ferroptosis (Fig. 2).

5. Effects of aerobic training on iron, amino acid and lipid metabolism in aging

5.1. The effect of aerobic training on iron metabolism in aging

In older adults, physical activity results in a reduction of iron stores in the body [97]. An excess of iron reserves is associated with diseases such as cancer, neurological disorders, arteriosclerosis, and certain heart diseases. For instance, an increase of 1% in blood ferritin levels is linked to a 4% increase in the likelihood of heart attack [90]. A reduction in iron levels or phlebotomy has been observed in older women after Nordic walking, which could be due to a decline in body iron levels with age [98]. On the other hand, the iron stores increase at a slow rate with age. Physical activity increases hepcidin in the blood, so it can be assumed that the decrease in body iron stores is related to the increase in hepcidin levels. Inflammatory cytokines and high iron levels increase hepcidin production, while hepcidin levels decrease over time [99–101]. There is a positive correlation between post-exercise hepcidin levels and blood ferritin [97]. Hemojuvenin (Hjv) is a protein found in the cell membrane, which is associated with glycosylphosphatidylinositol. It acts as a co-receptor for bone morphogenetic protein (BMP), which activates the BMP/SMAD signaling pathway and leads to the expression of hepcidin [102]. Soluble
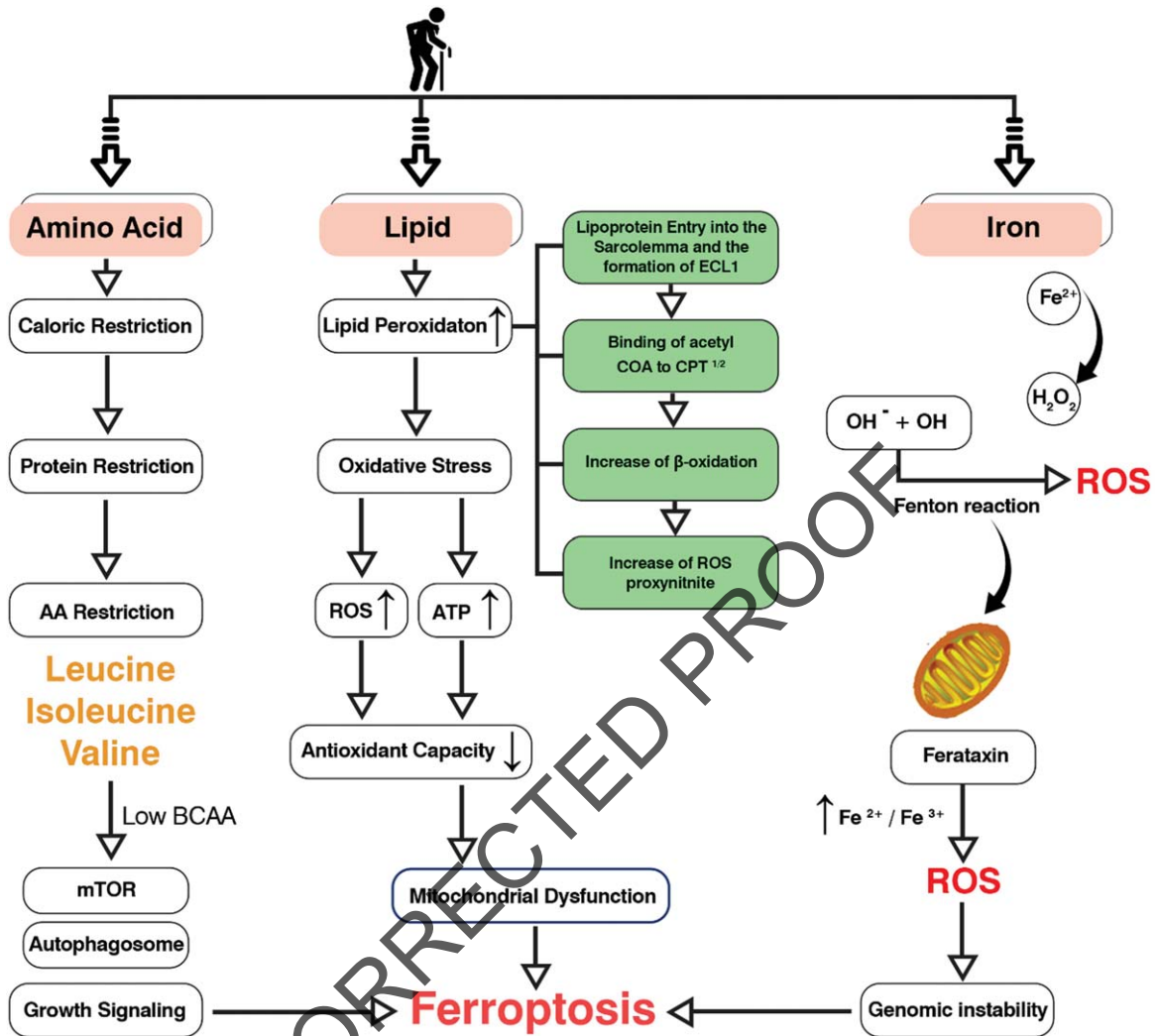
Fig. 2. Role of Aging on Ferroptosis; Amino Acid (AA), Branched-chain amino acid (BCAA), Mammalian target of rapamycin (mTOR), Reactive oxygen species (ROS), Carnitine palmitoyltransferase I (CPT I), Extracellular loop 1 (ECL1).

hemoglobin (sHjv) selectively binds to BMP ligands and inhibits endogenous hepcidin expression [103]. Inflammation is another essential factor that affects iron metabolism. Hepcidin Antimicrobial Peptide (HAMP) gene expression, which codes for hepcidin, is induced by proinflammatory cytokines [104]. There is a positive correlation between hepcidin and CRP after aerobic activity, indicating the involvement of inflammation in hepcidin changes. Stimulating inflammatory processes requires excess iron because the transcription factor NF-κB can be activated by the labile iron pool (LIP) and regulate the expression of pro-inflammatory cytokines [105]. There is interference between hepcidin and the absorption of iron. Consequently, the body's iron reserves decrease. Thus, it may function as an anti-inflammatory hormone. A positive correlation between CRP and blood ferritin exists before and after exercise. Therefore, reducing iron reserves can potentially decrease systemic inflammation in older adults [97]. The level of vitamin D may be another effective factor in reducing pro-inflammatory signals [106]. Kertas et al. found that increased sHjv after aerobic training was negatively correlated with 25OHD3 concentration in elderly women. Therefore, vitamin D could impact iron metabolism in elderly humans, despite the unknown biological cause [97].

5.2. The effect of aerobic training on amino acid metabolism in aging

Aerobic training impacts muscle protein metabolism. Acute aerobic training can enhance muscle protein synthesis in individuals who are fed or fasted [107–111]. Long-term aerobic training enhances muscle protein synthesis during periods of inactivity. [112, 113]. While Short-term aerobic training promotes the synthesis of mitochondrial proteins, it has limited effects on myofibrillar protein synthesis [114]. After a session of aerobic training, a study reported an increase in the synthesis of myofibrillar protein during feeding [115]. Aerobic training can increase muscle strength and size in older women [116], suggesting that aerobic training improves muscle protein balance and potentially minimizes the effects of sarcopenia. Nevertheless, the capacity of aerobic training to increase muscle mass in the elderly is dependent on the sensitivity of the muscles to the anabolic effects of insulin [117]. It seems that the activation of the mTORC1 pathway is responsible for regulating muscle protein metabolism after aerobic activity. The phosphorylation of mTORC1 (Ser2448) is acutely increased by aerobic activity, which leads to the activation of the mTORC1 pathway [118–120].

On the other hand, the expression of PGC1α and β mRNA increases after aerobic training [121, 122]. This increase in PGC1α and β mRNA expression signifies an acute adaptive response before mitochondrial biogenesis [123, 124]. PGC1α mRNA expression may increase in response to physical training (increased AMP: ATP ratio) and cellular stress due to phosphorylation by AMPK and/or p38 MAPK [124, 125].

Aerobic training stimulates the synthesis of plasma proteins, particularly fibrinogen, in both young and old individuals [111]. Fibrinogen is a crucial liver protein that can circulate rapidly [126]. Notably, certain studies indicate that age does not affect the response of leg blood flow to moderate-intensity aerobic training [111, 127]. Previous research has established that aerobic exercise increases albumin and fibrinogen levels [126, 128]. It is evident that high-intensity aerobic training is strongly linked to albumin synthesis when plasma volume and total albumin content increase [129, 130]. Post-exercise recovery may trigger an increase in fibrinogen synthesis as a compensatory response to physical training (i.e. stress, contraction). Additionally, rapid protein breakdown is the cause of the initial flow of N2 from the muscle [111].

5.3. The effect of aerobic training on lipid metabolism in aging

With aging, adipose tissue undergoes several changes, making it a significant and dynamic endocrine organ. Adipose tissue crucially contributes to insulin resistance, metabolic dysfunction, and inflammation [131, 132]. The proportion of total fat to body weight increases in both men and women with age. Moreover, the rate of obesity is higher in individuals over the age of 60 compared to young people [133]. Elderly individuals commonly experience changes in both subcutaneous and visceral fat. These alterations may contribute to developing metabolic syndrome and decreased insulin sensitivity as an individual age [134, 135]. Nevertheless, Physical activity has been shown to significantly reduce the risk of age-related metabolic disorders. The incidence of cardiovascular disease and type 2 diabetes can be reduced through physical activity and the reduction of visceral and subcutaneous fat [136]. Furthermore, physical activity can enhance the metabolic alterations associated with adipose tissue in elderly individuals. This issue may result from a reduction in the sensitivity of adipose tissue to beta-adrenergic stimulation as well as a decline in sympathoadrenal response to physical training [136, 137]. Endurance training improves fatty acid oxidation in older individuals without significantly altering the availability of fatty acids or lipolysis. This indicates a modification in muscle fatty acid metabolism, an increase in fatty acid oxidation due to exercise, and a change in adipose tissue lipolysis [138]. Aerobic training can improve the oxidation of fatty acids (both long and medium chain), strengthen muscle metabolism and modulate insulin sensitivity [139]. In summary, the impact of aerobic or resistance
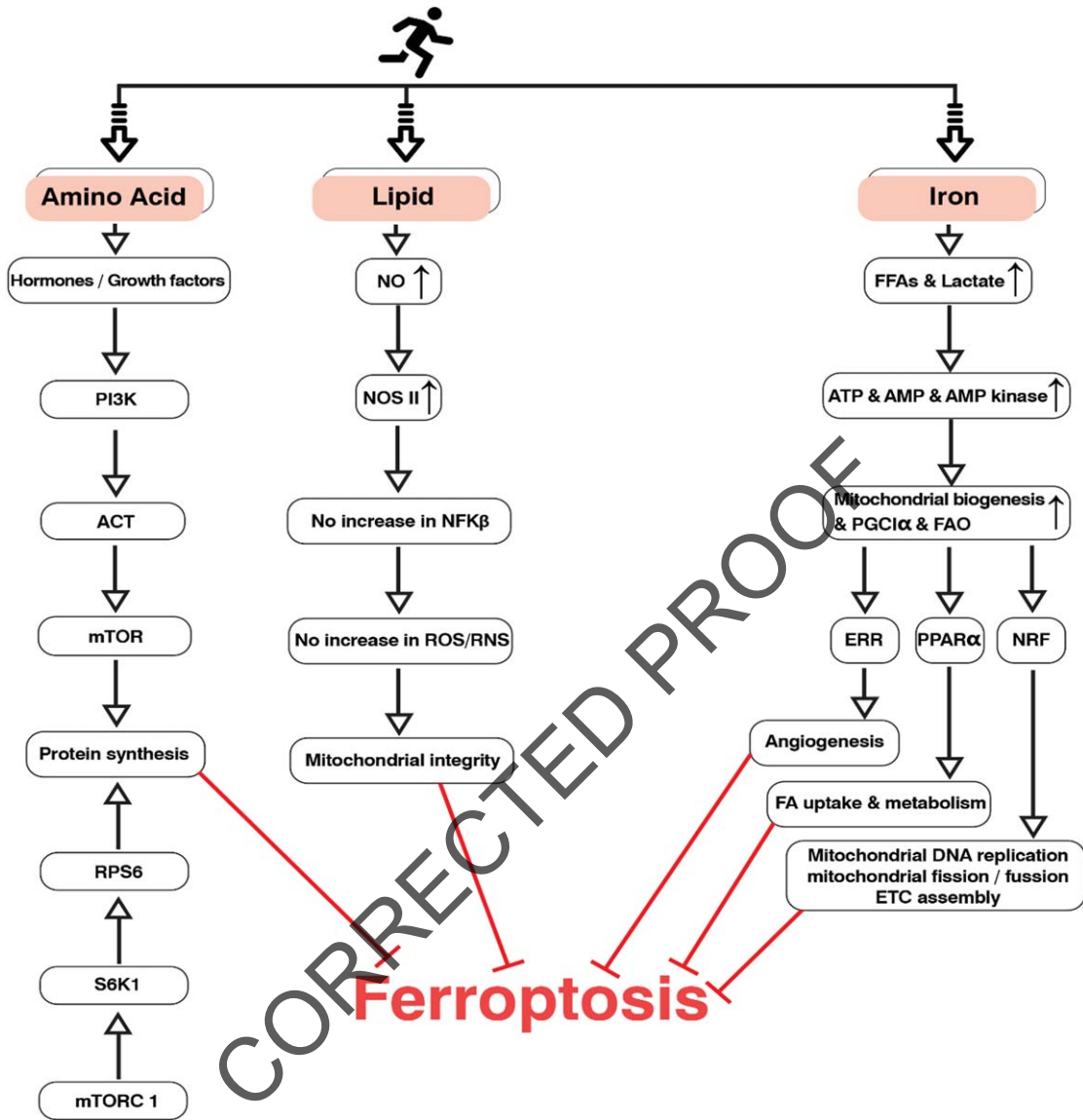
Fig. 3. Role of Aerobic training in Ferroptosis; Phosphatidylinositol 3-kinase (PI3K), Akt also known as Protein kinase B (PKB), Mammalian target of rapamycin (mTOR), Mechanistic target of rapamycin complex 1 (mTORC1), Ribosomal protein S6 kinase beta-1 (S6K1), Ribosomal protein S6 (rpS6), Nitric oxide (NO), Nitric oxide synthase-2 (NOS2), Nuclear factor kappa B (NF-κB), Reactive oxygen species (ROS), Reactive nitrogen species (RNS), Free fatty acids (FFAs), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1a), Fatty acid oxidation (FAO), Estrogen receptor-related receptor (ERR), Peroxisome proliferator-activated receptor alpha (PPAR-alpha), Nuclear respiratory factor (NRF).

training on obesity in the elderly remains unclear. Physical activity has been shown to reduce obesity and improve metabolic health in the elderly. Likely, the effects of aging on fat metabolism are only minimally impacted by physical activity. Menopause causes changes in fat reserves and metabolism in women [139]. However, studies indicate that older individuals who maintain regular physical activity show greater mitochondrial content in their adipose tissue [140]. Aerobic training produces inhibitory effects on ferroptosis by making changes in iron, amino acid and lipid (Fig. 3).

6. Conclusion

Studies have recently demonstrated that excessive iron levels mediate a significant impact on various diseases, such as cardiovascular disease and toxicity, in both animals and humans [141]. Numerous studies have indicated that ferroptosis is a crucial contributing factor across a range of diseases. Additionally, the use of ferroptosis inhibitors can effectively prevent the onset of these diseases. The inhibition of ferroptosis helps in the prevention of cell death. Ferroptosis-inhibiting agents, including frustatin-1 and dexrazoxane, are predicted to be efficacious therapies for several diseases [42, 142]. Clinically, ferroptosis inhibition is a promising therapeutic strategy for treating and preventing cell damage. Lipid peroxidation is a well-established mechanism of ferroptosis. Due to extensive knowledge of the chemistry of lipid oxidation, radical scavenging antioxidants have been proposed as potent inhibitors of ferroptosis. Moreover, the recently discovered ACSL4 enzyme inhibitor drugs are suggested to be regarded as a target of ferroptosis. Despite this, studies have shown that the suppression of ferroptosis can also be caused by inhibiting other cellular processes such as glutaminolysis, ferritinophagy, lysosomal function, and the MEK signaling pathway. Nevertheless, the potential of inhibitors of these pathways in preventing ferroptosis has not yet been adequately assessed [59]. Some modifiers, including p53, affect both ferroptosis and apoptosis, though ferroptosis operates independently of other pathways. Primary apoptotic factors, like BAX, BAK, and caspase, aren't involved in ferroptosis. Ferroptosis can occur even in the absence of early necrosis components, such as RIPK1, MLKL, and RIPK3 [41]. The pursuit of new molecular targets for ferroptosis has recently garnered attention. Preventing ferroptosis and cardiac cell death can serve as an effective therapeutic strategy for treating diseases, particularly in old age. It has been proven that aging affects fat, amino acid, and iron metabolism. It can be concluded that the onset of aging increases ferroptosis levels. Regular physical activity with appropriate intensity can affect the metabolism and lower the risk of diseases such as metabolic syndrome and cardiovascular diseases in the elderly. Physical activity can reduce the process of fruptosis by affecting the hormonal system, improving the antioxidant and inflammatory system, and changing cell reserves. Several studies have shown that long-term training changes some rheological functions and blood fluidity indicators, such as reducing fibrinogen concentration, plasma viscosity (ηp) and plasma renin activity (PRA). Therefore, long-term training can improve blood flow and thus microcirculation [143, 144]. Aerobic training at appropriate intensity is recommended for elderly people to reduce cell death and ferroptosis.

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