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Minireview

20 years of leptin: Role of leptin in cardiomyocyte physiology and physiopathology

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ABSTRACT

Since the discovery of leptin in 1994 by Zhang et al., there have been a number of reports showing its implication in the development of a wide range of cardiovascular diseases. However, there exists some controversy about how leptin can induce or preserve cardiovascular function, as different authors have found contradictory results about leptin beneficial or detrimental effects in leptin deficient/resistant murine models and in wild type tissue and cardiomyocytes. Here, we will focus on the main discoveries about the leptin functions at cardiac level within the last two decades, focusing on its role in cardiac metabolism, remodeling and contractile function.

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Introduction

For many years the human being has adapted its body physiology to the lack of nourishment in order to survive [10]. Nowadays, overeating and sedentary lifestyle are increasing the prevalence of obesity worldwide reaching pandemic proportions and becoming an important public health problem [10]. As the prevalence

of obesity increases so does the burden of its associated comorbidities (Fig. 1), which includes type II diabetes, metabolic syndrome, or cardiovascular diseases such as myocardial infarction, angina pectoris, congestive heart failure, stroke, hypertension, and atrial fibrillation [76].

Many scientists have studied the mechanisms of the energy balance at cellular, tissue, organ and whole body levels in order to achieve a better knowledge about how to treat or prevent the incidence of obesity and its co-morbidities. One of the most important and widely studied players in the control of energy balance is the hormone leptin [30,71], discovered 20 years ago by Zang et al. [111]. Leptin is a 16 kDa protein mostly secreted from

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Fig. 1. Morbidities related to obesity [89].

adipose tissue which has a critical role regulating body weight and energy homeostasis [73,87]. Leptin mediates its effects by binding to specific leptin receptors (LepRs) expressed in the brain and in peripheral tissues [45]. In the hypothalamus leptin acts as an anorexigenic hormone regulating the melanocortine/neuropeptide Y system to reduce food intake, increase energy expenditure, and decrease body weight [7,71]. However, circulating leptin levels are increased in obese humans [42], suggesting that obesity may be either a result or a cause of leptin resistance [19,29,83]. In fact, local effects of leptin can be governed by deregulation of its receptor expression or downstream signaling components, in particular proteins known to suppress cytokine, and leptin signaling [38].

In an effort to better understand the pathophysiology of human obesity and its co-morbidities, several rodent models of obesity have been developed and implemented including high fat diet feeding and spontaneous mutants of leptin or its receptor such as *ob/ob* (mutant for leptin gene, leptin deficient) and *db/db* (loss-of function mutation in the leptin receptor, leptin resistant) mice or Zucker *fa/fa* rats (loss-of function mutation in the leptin receptor, leptin resistant) [57]. These animals have the common feature of compromised cardiac contractile function [85] and in humans circulating leptin levels are elevated in vascular and coronary heart diseases [84], favoring a contemporary perception of hyperleptinemia as an independent risk factor for the development of cardiovascular diseases.

Although the adipose tissue is the main source of leptin, it is also produced by other peripheral tissues, such as the liver, the skeletal muscle or the kidneys [53,100,102]. Within the heart, leptin and its receptor are abundantly expressed in cardiomyocytes [62,75] where it can regulate the baseline physiology of the heart, including cardiomyocyte contractility, hypertrophy, apoptosis, and metabolism [63]. In this review we will summarize the main discoveries about the leptin functions at cardiac level within the last two decades, focusing on its actions on cardiac tissue and cultured cardiomyocytes.

Leptin signaling and cardiomyocyte metabolism

Leptin and glucose and fatty acid metabolism

The constant pumping activity of the heart requires a permanent supply of energy [55]. It is widely accepted that fatty acids are the predominant energy substrates used in the normal adult myocardium, providing ~70% of adenosine triphosphate (ATP) necessary for the heart to maintain contractile function [55]. However, the cardiac metabolic network is highly flexible in using other substrates when they become abundantly available [47]. Thus, depending on the energetic context, the heart is capable of using different substrates (including carbohydrates, lipids, amino acids, and ketone bodies) for ATP production in the mitochondria (Fig. 2.A), a concept known as metabolic flexibility of the heart [47]. In a normal heart, mitochondria are largely fuelled by acyl-coenzyme A (CoA) and pyruvate, which are the primary

metabolites of fatty acids and carbohydrates, respectively [47]. Energy production from fatty acids requires oxygen consumption, whereas carbohydrate-derived ATP is produced by both glycolysis (oxygen independent) and glucose oxidation [69]. So that, although glucose represents a small component of total myocardial energy source, it is the most efficient means of energy production, particularly in conditions of ischemia/hypoxia [69]. During exercise lactate becomes the predominant energy substrate [33], and prolonged fasting or a ketogenic diet increases circulating levels of ketone bodies resulting in an enhanced use by the heart [104]. The ability of the myocardium to switch from one energy substrate to another (or to use multiple substrates simultaneously) is lost in obesity and diabetes, a state of metabolic inflexibility in which glucose transport, glycolysis, and glucose oxidation in cardiomyocytes decrease, while fatty acid uptake and oxidation increase [20,35,47].

Despite the fact that there exist a number of studies regarding leptin function in modulating systemic and skeletal muscle metabolism, little is known about its implication in regulating cardiomyocyte metabolism. Some groups have shown that *ob/ob* and *db/db* mice, and *fa/fa* rats show a metabolic profile in which carbohydrate uptake and utilization are reduced both in cardiac tissue [6,17,27,32,61,92,99] and in cultured cardiomyocyte [27,61] by diminishing glucose transporters GLUT4 translocation to plasma membrane or its protein and mRNA levels, and by reducing pyruvate dehydrogenase and oxoglutarate dehydrogenase activity. In contrast, fatty acid uptake rates are increased in these leptin deficient or resistant animal models through a mechanism that involves the increase in the expression and membrane localization of the fatty acid translocase (FAT)/CD36 and the stimulation of peroxisome proliferator-activated receptor α (PPAR α) signaling (Fig. 2.B) [1,12–14,61,69]. While in leptin deficient/resistant mice the increase in fatty acid uptake is accompanied by an increase in fatty acid oxidation, in *fa/fa* rats the increase on fatty acid uptake is uncoupled with the oxidation and yields to lipotoxicity [113]. These findings suggest a role for the disruption of leptin signaling in the development of the metabolic inflexibility observed in cardiac metabolism under pathological conditions, favoring fatty acid utilization and diminishing cardiac efficiency.

In vitro experiments with cardiac cells have shown that short-term (1 h) leptin treatment has no effect on glucose uptake and oxidation in HL-1 cardiomyocytes and in perfused rat hearts, while fatty acid uptake and oxidation are increased [1,69]. Long term leptin treatment (24 h) also has no effect on glucose uptake and oxidation in HL-1 cardiomyocytes and increases fatty acid uptake, however long term treatment induces a decrease in fatty acid oxidation leading to intracellular lipid accumulation [69], confirming the results obtained by Zhou et al. in *fa/fa* rats [113].

Leptin and cardiomyocyte autophagy

Autophagy is an evolutionarily conserved lysosome-mediated catabolic pathway that maintains cellular homeostasis through the renewal/recycling of cytoplasmic materials and organelles (such as

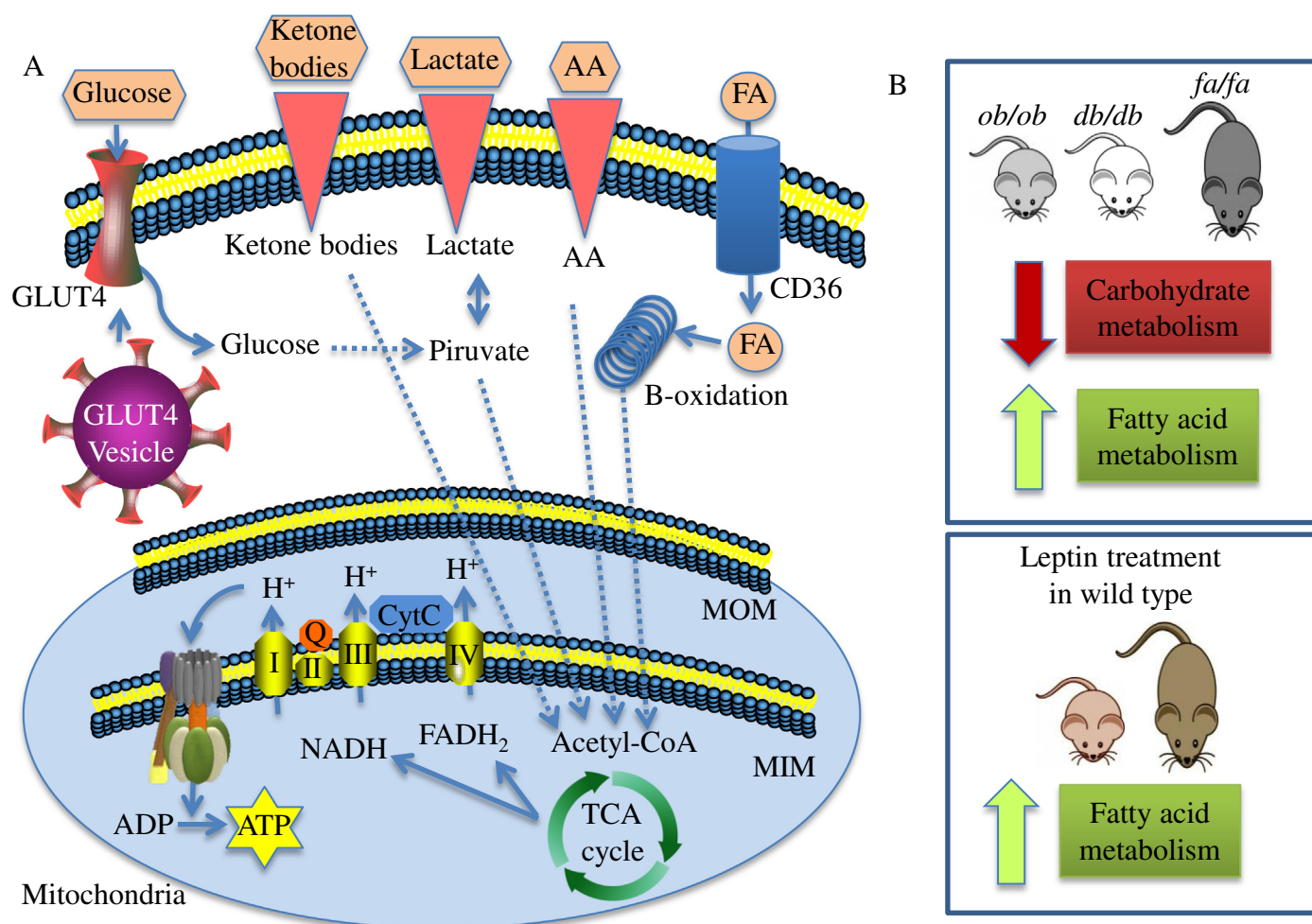


Fig. 2. Metabolic network in cardiomyocytes. A: catabolism of the different energetic substrates to produce ATP [47]. B: effect of leptin dysfunction (in deficient/resistant murine models) and treatment (in wild type) on cardiac metabolism. GLUT4: glucose transporter 4, CD36: fatty acid translocase, AA: aminoacid, FA: fatty acid, MOM: mitochondrial outer membrane, MIM: mitochondrial inner membrane, CytC: cytochrome C.

mitochondria) and protein aggregates that could become toxic to the cell [49]. Such cellular refreshing is particularly important in quiescent and terminally differentiated cells, like cardiomyocytes, in which damaged components are not diluted by cell replication, and in which aging increases its accumulation, making the function of the cells less efficient and decreasing their adaptability [96]. The breakdown products derived from autophagy have a dual role, providing substrates for both biosynthesis and energy generation, and being of special importance to assure substrate availability and energetic maintenance under stress [54]. Autophagy can be activated by a number of diverse stimuli including caloric restriction, oxidative stress, hormones, or other developmental signals [41], and it is typically measured by the stimulation of a post-translational modification of microtubule-associated protein 1 light chain 3 (LC3I) that increases its electrophoretic mobility due to proteolysis and lipidation (LC3II), the increase in AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation, the mammalian target of rapamycin (mTOR) dephosphorylation, the increase in proteins derived from autophagy-related genes (Atg) 5 and 7, and the reduction of the autophagic substrate p62 [59]. Deregulation of autophagy in cardiomyocytes is associated with various cardiac diseases, such as ischemic heart disease, hypertensive heart disease, arrhythmia, ischemia/reperfusion injury or heart failure [88]. Particularly, cardiomyocytes have extremely high mitochondrial density compared with other tissues due to its continuous high demand for energy. And, because of that, they

need a continuous basal-level autophagic turnover of mitochondria (mitophagy) to assure the proper functioning of the heart [41].

In leptin deficient or resistant mice, it has been shown an increase in autophagy in peripheral tissues such as the liver or the skeletal muscle, measured by the increase in LC3II levels and the diminution of p62 [59]. These results could indicate that leptin signaling disruption may induce autophagy; however, external application of leptin also stimulates autophagy in those tissues [59]. According to this, leptin has been shown to have a pro-autophagic effect in different cell types, including human cervical carcinoma HeLa cells, human HCT116 colorectal cancer cells, human U2OS osteosarcoma cells, mouse embryonic fibroblast, rat bone marrow-derived mesenchymal stem cells and piglet intestinal epithelial cells [59,93,101,103]. Nevertheless, leptin treatment also seems to protect from autophagy progression in human T cells [16].

With respect to leptin effect on cardiac autophagy, it has been reported that *db/db* mice show increased levels of myocardial autophagy (increased levels of LC3II and decreased of p62) [59], and leptin injection induces a higher increase of autophagy in myocardium, effect also observed in liver and skeletal muscle [59]. Moreover, leptin injection in C57BL/6 mice also induce an increase in autophagy in cardiac tissue, similar to the autophagy inducers spermidine, resveratrol or rapamycin [59], as well as leptin treatment of left ventricle cultured cardiomyocytes also induces autophagy [43]. Taking all together, those results suggest a complex relationship between autophagy and leptin signaling,

where any deviation from leptin homeostasis may affect autophagy progression.

Leptin signaling and cardiac remodeling

Leptin and cardiomyocyte hypertrophy

Cardiac hypertrophy is one of the main ways in which cardiomyocytes respond to mechanical and neurohormonal stimuli to increase their work output and improve cardiac pump function [15]. However, this compensatory mechanism can become overwhelmed by biomechanical stress, thereby resulting in the development of cardiac diseases such as ischemic heart disease, hypertension, heart failure or valve disease [15]. For instance, chronic excessive accumulation of body fat causes adaptations of the cardiovascular system to maintain whole body homeostasis such as increased cardiac output and a decrease in peripheral resistance [5]. Stroke volume, the major determinant in the increased cardiac output in the obese patient, increases due to the augmentation of circulating blood volume, induces an enlargement of the cardiac cavities and increases wall tension, leading to left ventricular hypertrophy (LVH) [5].

Deregulation of the leptin signaling pathway within the heart has been suggested to be one of the mechanisms that cause LVH, however there exist contradictory studies about leptin role in the development of LVH. A number of publications have shown a pro-hypertrophic effect of leptin treatment in neonatal rat cardiomyocytes [31,37,39,44,79–81, 106,108–110] and in human pediatric ventricular myocytes [58]. Also, it has been shown that the neutralization of LepR using antibodies abrogates hypertrophy in surviving myocardium after coronary artery ligation in rats [77]. According to this, clinical studies demonstrated a positive correlation between serum leptin levels and left ventricular mass or wall thickness in obese and in insulin resistant patients [70,74]. The mechanisms described through which leptin can induce cardiomyocyte hypertrophy seem to be quite different: induction of mTOR signaling [108], calcineurin activation and nuclear factor of activated T-cells (NFAT) nuclear translocation [80], activation of PPAR α signaling [37], mitogen-activated protein kinase 14 (p38) activation and translocation into the nuclei [79,109], activation of Rho and actin dynamics [110] or increased intracellular levels of reactive oxygen species [39,106]. However, it has also been shown that leptin treatment of neonatal rat and HL-1 cardiomyocytes has no effect on the development of hypertrophy [75].

On the contrary, mice lacking leptin (*ob/ob*) or its receptor (*db/db*) develop LVH when they become morbidly obese [3,4,50,82], while leptin repletion in *ob/ob* mice restores left ventricle normal thickness independently of body weight [4]. In hearts from diet induced obese mice showing hyperleptinemia, LepR continue to respond to elevated circulating or cardiac leptin, which seems to protect from cardiac hypertrophy via LepR-induced signal transducer and activator of transcription 3 (STAT3) activation compared to LepR mutant or *db/db* mice [50].

In summary, it is unclear whether cardiac hypertrophy is the consequence of pro-hypertrophic effects of leptin or rather the result of a resistance toward leptin's preventive effects on hypertrophic cardiac remodeling.

Leptin and cardiomyocyte apoptosis

The myocardium is comprised of terminally differentiated cardiomyocytes that are responsible for contractile function and that have a limited capacity to efficiently regenerate [68]. Maintenance of cardiac homeostasis depends on cardiomyocyte death and renewal, and excessive loss of cardiomyocytes has been implicated in cardiovascular diseases such as myocardial ischemia/reperfusion injury

and congestive heart failure, situations where, with fewer myocytes, the heart is unable to sustain efficient contraction [68]. Among the main mechanisms that promote loss of cells in the heart is apoptosis, a highly regulated process by which activation of specific signaling cascades ultimately leads to cell death [68]. An apoptotic cell undergoes structural changes including cell shrinkage, plasma membrane blebbing, nuclear condensation, and fragmentation of the DNA and nucleus, followed by fragmentation into apoptotic bodies that are quickly removed by phagocytes [68]. Apoptosis is mediated by two central pathways: the extrinsic or death receptor pathway, that is induced via activation of a death domain-containing receptor located at the plasma membrane, and the intrinsic or mitochondrial pathway, which is activated by intracellular stress signals such as loss of growth factors, hypoxia, oxidative stress, or DNA damage (Fig. 3.A) [68]. The immediate objectives of apoptotic signaling are the activation of caspases and the disabling of mitochondrial function to induce the cell death [21].

In obese Zucker rats (*fa/fa*), cardiomyocytes show increased levels of apoptosis which involve both the extrinsic and the intrinsic apoptotic pathways, pointing to the existence of local and systemic stresses in the Zucker *fa/fa* hearts to induce cell death [48,56]. Similar to Zucker *fa/fa* rats, *db/db* and *ob/ob* mice have increased total cardiac triglyceride levels and increased cardiomyocyte apoptosis, however, it has been shown that the triglyceride accumulation and the high levels of cardiac apoptosis in *ob/ob* mice can be returned toward normal with leptin repletion [3], as well as in *db/db* mice the rescue of cardiac leptin receptors prevents myocardial triglyceride accumulation and improves cardiac function [34], providing a direct role for leptin in preventing excess of cardiac lipid accumulation and in ameliorating cardiac function. These findings suggest that increased cell death in these obese models is not just a reflection of senescence or injury, but rather specific pathways directly related with leptin signaling disruption (Fig. 3.B). According to this, the generation of a cardiomyocyte-specific leptin receptor knock-out mouse showed that leptin signaling disruption exacerbates cardiac injury in the post-myocardial infarction failing heart by acting directly on cardiomyocytes to increase cardiac hypertrophy, apoptosis, and inflammation, as well as deleterious changes in cardiac structure, function, and glycolytic metabolism [63].

One of the mechanisms that induce apoptosis in the heart of the leptin resistant Zucker rats is the lipoapoptosis. Triacylglycerol content in nonadipose cells is normally quite low and is maintained within a narrow range, whereas in adipocytes it can vary considerably depending on the composition and quantity of food intake [97]. This reveals a limited ability of nonadipose tissue to accommodate excess of triglycerides and a strict regulation of triglyceride levels in both adipose and nonadipose cells [97]. In this line, in the Zucker *fa/fa* hearts a sharp increase in lipid accumulation that leads to cardiomyocyte apoptosis and cardiac dysfunction occurs (Fig. 3.B) [113]. Although triglycerides are themselves harmless, they are hydrolyzed to fatty acyl-CoA, providing increased substrate for synthesis of the pro-apoptotic sphingolipid ceramide, which triggers the intrinsic apoptosis pathway in cardiomyocytes [11,72]. In *fa/fa* Zucker cardiomyocytes the increase of triglyceride accumulation is correlated with increased ceramide levels and increased DNA damage, leading to lipoapoptosis [113].

Therefore, it could seem clear that at cardiac level leptin must have an anti-apoptotic effect. In several studies it has been shown that leptin treatment protects cultured cardiomyocytes from apoptosis in different ways (Fig. 3.B) [22,28,91,94,95,107,112]. Leptin has been shown to inhibit the mitochondrial permeability transition channel pore (mPTP) opening in murine cardiomyocytes [22,94,95], which is one of the mechanisms that triggers apoptosis due to the release of cytochrome C into the cytoplasm [8]. In the subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue (H9c2), leptin treatment exerts a protective effect against H₂O₂-induced apoptosis by preventing the activation of components of the intrinsic pathway of

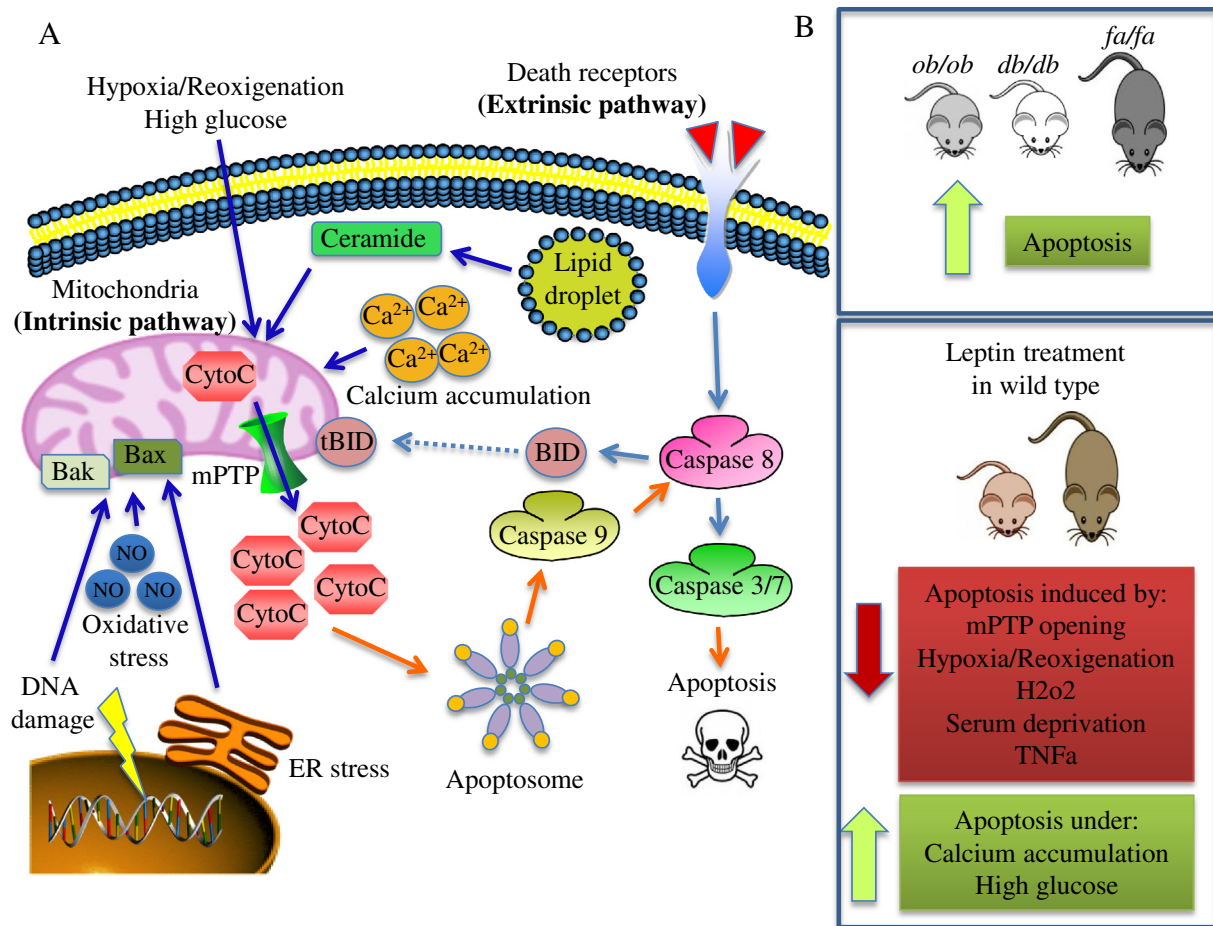


Fig. 3. Extrinsic and intrinsic apoptosis pathways. A: extrinsic and intrinsic apoptosis pathways and some of the mechanism that triggers them in cardiomyocytes [67,68]. B: effect of leptin dysfunction (in deficient/resistant murine models) and treatment (in wild type) on cardiac apoptosis. BID: BH3 interacting domain death agonist, tBID: truncated BH3 interacting domain death agonist, CytoC: cytochrome C, Bax: BCL2-associated X protein, Bak: BCL2-antagonist/killer, mPTP: mitochondrial permeability transition pore, NO: nitric oxide, ER: endoplasmic reticulum.

apoptosis by reducing Bax integration in the mitochondrial membrane and cytochrome C release from mitochondria [28] and it also attenuates hypoxia/reoxygenation-induced activation of the intrinsic pathway of apoptosis [91]. In neonatal rat cardiomyocytes leptin exerts a direct anti-apoptotic effect in serum-deprived cardiomyocytes by relieving oxidative stress and inactivating the intrinsic apoptotic pathway [112], and it also abrogates tumor necrosis factor α (TNF α)-induced apoptosis by blocking both the intrinsic mitochondrial pathway of apoptosis and the extrinsic apoptotic pathway upregulated by TNF α [107].

In the opposite, other research groups have suggested a pro-apoptotic function of leptin at cardiac level under damaging conditions (Fig. 3.B). In the presence of excessive intracellular calcium accumulation, leptin may contribute to mitochondrial dysfunction by inducing the mPTP opening and the development of cardiomyocyte apoptosis [60]; also, under high glucose conditions, the inhibition of leptin signaling protects H9c2 cells from mPTP opening [18] suggesting that, depending on the cellular context, leptin signaling may exert protective or detrimental actions on cardiomyocytes viability.

Leptin signaling and cardiomyocyte contractility

In the heart, Ca^{2+} influx acts as a multi-functional signal that triggers muscle contraction, controls action potential duration, and regulates gene expression [90]. The sarcoplasmic reticulum (SR) in striated muscle is a highly specialized form of endoplasmic reticulum which surrounds the myofilaments and operates in collaboration with deep invaginations of

the plasma membrane (or sarcolemma), called transverse (t)-tubules, to regulate the release of calcium from the SR lumen into the cytoplasm, where it regulates myocyte contraction [26]. During cardiac excitation-contraction coupling, β -adrenergic signaling induces the activation of $\text{Na}^+/\text{Ca}^{2+}$ exchanger channels through PKA signaling, leading to sarcolemma depolarization [9]. This depolarization stimulates the opening of high-voltage-activated L-type calcium channels (LTCC) in the sarcolemma, generating a Ca^{2+} current into the cytosol, which induces Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptor (RyR) channels to initiate myocyte contraction (Fig. 4.A) [9]. On the contrary, sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2) and phospholamban activation (PLN) have a key role in sarcoplasmic reticulum Ca^{2+} sequestration from the cytosol and myocyte relaxation [46]. Abnormalities in SR Ca^{2+} cycling are hallmarks of heart diseases such as heart failure or atrial fibrillation and contribute to the pathophysiology and progression of these diseases [40,46].

Ob/ob and *db/db* mice, and *fa/fa* rats develop cardiac contractile dysfunction, showing slowed intracellular Ca^{2+} decay rate and reduced contractile capacity in myocytes (Fig. 4.B) [24,25,51,65,85,86]. SERCA2 and $\text{Na}^+/\text{Ca}^{2+}$ exchanger channel activity are depressed in leptin deficient mice [23], while leptin treatment of cardiomyocytes from *ob/ob* mice improves β -adrenergic responsiveness with increased protein expression of the stimulatory guanine nucleotide-binding protein α subunit ($\text{Gs}\alpha$), enhanced PKA activity and enhanced phosphorylation of PLN [65]. These data provide a convincing link between adequate leptin signaling and cardiac function and suggest a mechanism by which leptin deficiency may lead to

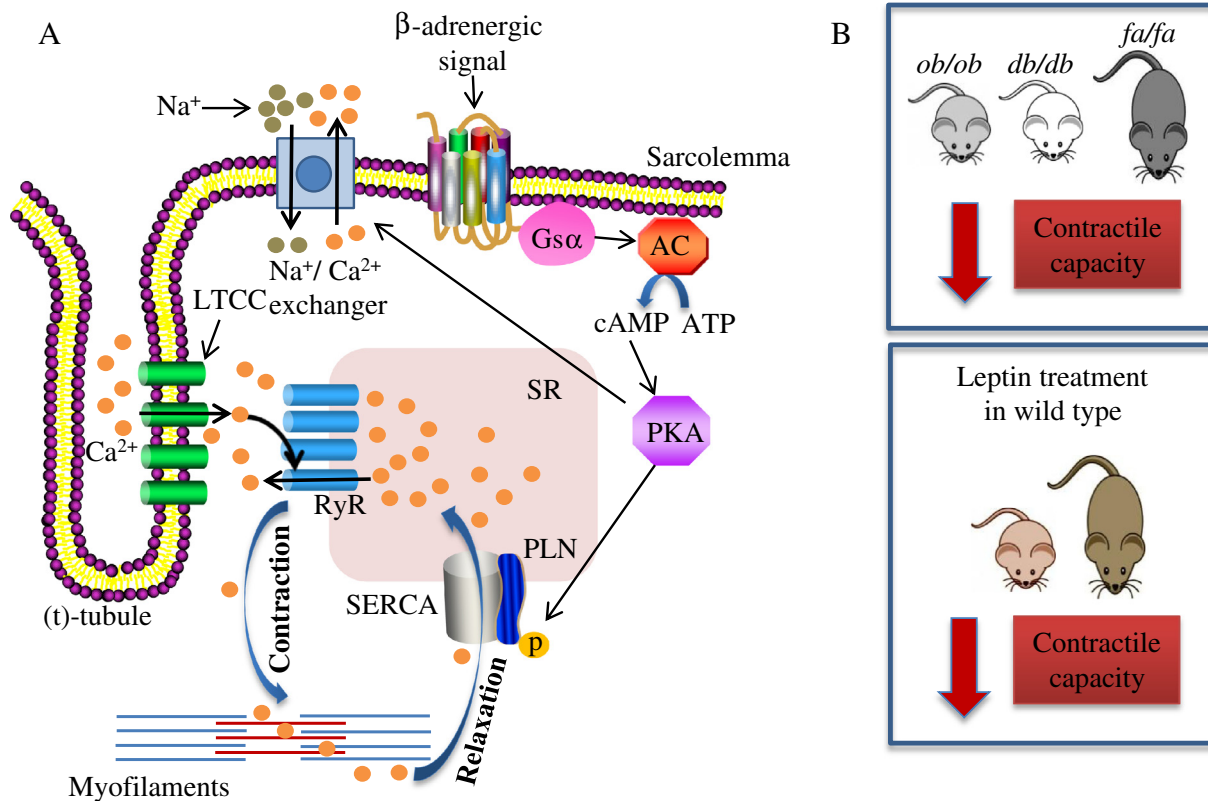


Fig. 4. Excitation-contraction coupling in cardiomyocytes. A: calcium cycling in the cardiomyocyte and regulation of excitation-contraction coupling [52]. B: effect of leptin dysfunction (in deficient/resistant murine models) and treatment (in wild type) on cardiac contractile capacity.

cardiac dysfunction [24,25]. However, it has been shown that leptin treatment of rat adult ventricular cardiomyocytes also depresses contractile function through different pathways (Fig. 4.B), including endothelin-1 receptor-NADPH oxidase pathway [24,25], the increase of NO production [66], the Jak/STAT pathway [105], interleukin 1 β signaling [78] or inducing autophagy [43]. Recent evidence shows that autophagy is involved in controlling contractile capacity in vascular smooth muscle cells, in which defective autophagy leads to an imbalance between calcium release/influx and calcium re-uptake/extrusion, resulting in higher basal calcium concentrations and significant effects on vascular contractility [64]. Therefore, autophagy activation could be an important mechanism through which leptin induces contractile dysfunction in cardiomyocytes.

Conclusion

Since the discovery of leptin in 1994 by Zhang et al., there have been a number of reports showing its implication in the development of a wide range of cardiovascular diseases [2,36,98,101,103]. However, there exists some controversy about how leptin can induce or preserve cardiovascular function, as different authors have found contradictory results about leptin beneficial or detrimental effects in leptin deficient/resistant murine models and in wild type tissue and cardiomyocytes. It seems clear that deregulation of leptin signaling may have important implications in heart physiology, but further investigation is needed to achieve a better understanding, as it is not clear whether a leptin resistance or its interaction with other cellular pathways could be involved.

Conflict of interest

The authors declare no conflict of interest.

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