# Caloric excess or restriction mediated modulation of metabolic enzyme acetylation - proposed effects on cardiac growth and function

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## Abstract

Caloric excess has been postulated to disrupt cardiac function via: i) the generation of toxic intermediates; ii) via protein glycosylation and iii) through the generation of reactive oxygen species. It is now increasingly being recognized the nutrient intermediates themselves may modulate metabolic pathways through the post-translational modifications of metabolic enzymes. In light of the high energy demand of the heart, these nutrient mediated modulations in metabolic pathway functioning may play an important role in cardiac function and in the capacity of the heart to adapt to biomechanical stressors. In this review the role of protein acetylation and deacetylation in the control of metabolic programs are explored. Although, not extensively investigated directly in the heart, the emerging data support that these nutrient mediated post-translational regulatory events modulate: i) cardiac metabolic pathways; ii) integrate nutrient flux mediated post-translational effects with cardiac function and iii) may be important in the development of cardiac pathology. Areas of investigation that need to be explored are highlighted.

## 1. Introduction

The modern scourges of obesity and type 2 diabetes mellitus (T2DM) have multiple disease consequences including the potential to reverse decades of medical therapeutics and interventions that have diminished cardiac morbidity and mortality [1]. The cardiac health effects of nutrient excess is epitomized by the strong correlation between body mass index and the development of cardiac hypertrophy [2,3] and heart failure [4]. However, whether caloric excess directly perturbs myocardial contractile function and hypertrophy, independent of the systemic effects of obesity and diabetes such as endothelial dysfunction, hypertension and hyperlipidemia, is fiercely contested, and has been difficult to delineate in epidemiologic studies.

If caloric excess does perturbs myocardial growth and function, sequelae would be expected to manifest as direct perturbations in cellular metabolic pathway biology in parallel with

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alterations in cardiac pump functioning and/or in the cardiac growth program. A reductionist approach can be employed to investigate the role of nutrient perturbations on metabolic pathways and to link these to effects to cardiomyocyte function. The focus of this review is on the effects of nutrient intermediates $NAD^+/NADH$ and acetyl-CoA and their role on post-translational modifications in linking metabolism, mitochondrial biology and myocardial function. The hypothesis explored contends that these intermediates indirectly and directly modulate metabolism and mitochondrial function, which in turn, may orchestrate changes in cardiac muscle function and growth. The objective of this manuscript is to review the current knowledge pertaining to: i) the modulation in cardiac function in response to changes in caloric load; ii) the role of $NAD^+$-linked biology to changes in metabolic and mitochondrial function; iii) the role of acetyl-CoA as a signaling intermediate to modulate metabolic biology and iv) an overview of evidence how these metabolic pathway mediated regulatory effects alter cardiac hypertrophy and function. An additional important energetic-dependent system that modulates cardiac biology is the AMPK signaling system. The role of AMPK in nutrient sensing and as a signaling modulator has recently been extensively reviewed [5] and is not a primary focus in this manuscript. Additional mechanisms that orchestrating 'nutrient' mediated perturbations in cardiac and skeletal muscle metabolism and mitochondrial function include lipotoxicity [6,7], the consequences of protein glycosylation [8], and via caloric-excess mediated augmentation of reactive oxygen species (ROS) [9,10] with subsequent detrimental sequelae. These mechanisms are similarly not the focus of this review.

## 2. Modulation in cardiac function with changes in dietary load

In order to establish whether 'nutrients' themselves modulate cardiac contractile function, proof of concept studies have been performed at the extreme limits of nutrient levels. Experimental and human studies are discussed here to show that at the macronutrient level, substrate alterations can modify numerous aspects of cardiac metabolism and function.

Changes in fuel substrate levels appear to initially affect cardiac relaxation or diastole. Diastolic dysfunction is defined as abnormalities in the relaxation phase of the cardiac mechanical cycle, that delays ventricular filling and can result in symptoms and signs of heart failure [11]. Although diastolic dysfunction was initially proposed to occur independently of systolic contractile dysfunction it is now recognized that a relative degree of contractile dysfunction accompanies filling/relaxation deficits [11]. The effects of nutrient biology on the continuum of diastolic and systolic function and cardiac hypertrophy are reviewed.

### 2.1. Caloric restriction

The acute restriction in caloric intake provokes adipose tissue lipolysis resulting in elevated levels of circulating non-esterified fatty acids [12]. This metabolic intervention results in increased cardiac exposure to fatty acids where incorporation of triglyceride content into the myocardium can be measured by $^1H$-magnetic resonance spectroscopy ($^1H$-MRS). Human studies have been performed using $^1H$-MRS in parallel with cardiac functional imaging to correlate changes in cardiac triglyceride content and ventricular pump function. Fasting up to 3 days, or restriction of calories over the same period, result in progressive uptake of triglycerides into the myocardium with a triglyceride 'dose-dependent' associated attenuation in diastolic relaxation [13,14]. Interestingly this acute triglyceride-level dependent attenuation in cardiac relaxation appears to be independent of alterations in high-energy phosphate levels [13].

Longer term caloric restriction results in a reduction in circulating free fatty acid and triglyceride levels [15,16]. This dietary intervention results in both a reduction in cardiac

triglyceride content and an improvement in diastolic function in obese type 2 diabetic subjects [16]. In otherwise healthy individuals long-term caloric restriction additionally protects against age-associated decline in diastolic function [17]. Unfortunately in this latter study, myocardial triglyceride content was not measured and the contribution of changes in triglyceride levels to this ameliorative phenotype was not shown. Systemic changes including a reduction in blood pressure may have also contributed to the preservation of cardiac relaxation in this study. The validity of change in intracardiac triglyceride and the modification in cardiac function is further supported by a human study showing a strong correlation between the age-associated increase in myocardial triglyceride content and impaired diastolic relaxation [18].

## 2.2. The cardiac clock and metabolic and functional changes with diurnal rhythm

A continuous flux in substrate availability to the heart naturally occurs during the feeding and fasting states associated with the sleep-wake cycle and circadian rhythm. A link between the circadian clock, cardiac metabolism and contractile function has recently begun to be explored [19]. Here, it is intriguing to note, that in rats cardiac contractile function, glucose oxidation and oxygen consumption are significantly higher in the middle of the dark cycle compared to the middle of the light cycle [20]. This period coincides with the feeding period and correlates with the lowest circadian levels of circulating non-esterified fatty acid levels [20].

## 2.3. Caloric overload

Chronic nutrient overload results in the development of obesity and predisposes to insulin resistance and/or T2DM. These clinical conditions predispose to cardiac dysfunction and animal models have been interrogated to facilitate the investigation of the temporal effects of obesity and T2DM on myocardial functioning and metabolism [9].

The leptin deficient ob/ob mouse exhibits insulin resistance and diabetes in association with profound obesity. At 11 weeks of age, ob/ob mice demonstrate increase neutral lipid accumulation in the heart with echocardiographic parameters consistent with diastolic dysfunction [21] Furthermore, these mice have impaired mitochondrial respiratory capacity in association with diminished pyruvate dehydrogenase activity and a restricted glucose-dependent oxidative capacity response to increased workload [22]. In response to the direct addition of fat as the fuel substrate, oxygen consumption is enhanced, but at the expense of cardiac efficiency suggesting that the cardiac mitochondria in ob/ob mice are susceptible to lipid-mediated mitochondrial uncoupling [22]. Indeed, direct measurement of mitochondrial proton leak in the db/db (leptin receptor loss-of function mutation) mice supports the existence of fatty acid induced mitochondrial uncoupling with perturbed leptin signaling [23]. Further characterization of the db/db mice using an isolated working heart model system shows classical features of diastolic dysfunction as measured by an increased in the left ventricular end diastolic pressure and increased myocardial stiffness [24]. These perturbations are proposed to result from the diminution in myocardial efficiency as measured by the ratio between cardiac output (work) and oxygen consumed ($MVO_2$). This excess in $MVO_2$ was found to be exacerbated with higher levels of fat as the substrate for cardiac metabolism. db/db mice additionally show diminished glucose uptake and an early thickening of the myocardium in young mice, with progressive systolic dysfunction with aging [25]. The metabolic underpinning of these deficits is supported in that overexpression of the glucose transporter GLUT4 with concomitant increase in glucose oxidation rescues cardiac contractile function in db/db mice [26,27].

Additional animal models that support caloric overload mediated disruption of cardiac function include the Otsuka Long-Evans Tokushima Fatty rats which show disrupted left

ventricular diastolic filling [28] and evidence that cardiac contractile dysfunction is accelerated and mortality increased in hypertensive rats when they are subject to a high carbohydrate-diet [29]. Moreover, high-fat feeding of Wistar rats also evokes cardiac contractile dysfunction, which appears to result from enhanced fatty acid uptake through CD36 and intramyocellular triacylglycerol accumulation [30]. This observation is further supported by a study showing that obese Zucker rats fed a high fat 'western diet' exhibit increased cardiac triglyceride content within a week of this dietary intervention in parallel with reduced cardiac contractile functioning. Longer term exposure to this diet promotes fatty acid oxidation which correlates with the prevention of further deterioration in cardiac function or in progressive cardiac triglyceride accumulation [31]. Interestingly, a role for genetic susceptibility to developing this dietary response is inferred in that the Zucker lean control rats show a greater resistance to cardiac triglyceride accumulation and no adverse effects on the maintenance of cardiac power on this same diet [31].

Human subjects with diabetes or glucose intolerance similarly have increased cardiac steatosis as evident by $^1$H-MRS [32]. Furthermore, the measurement of high-energy phosphates in diabetic and non-diabetic subjects without clinical coronary artery disease and normal echocardiographic studies indirectly suggest that diabetic subjects can develop diminished cardiac energetics prior to evidence of measureable cardiac dysfunction [33]. Moreover, obesity is associated with evidence of cardiac hypertrophy and chamber dilatation, although dissecting out the relative contribution of nutrient excess from obesity associated effects on blood pressure and leptin homeostasis is difficult to delineate [34].

## 2.4. Caloric flux and modification in protein lysine-residue acetylation

The studies discussed above show that the modulation in cardiac substrate exposure can have measureable effects on cardiac function and structure. Additionally, calorie excess associated disease processes associated with obesity and T2DM can similarly affect cardiac function, although it should be reiterated that the relative contribution of 'nutrient' mediated effects in this biology is complicated by the comorbidities associated with these systemic disorders.

Nutrient-intermediate mediated changes in metabolic pathway biology are actively being explored and appear to function, in part, via post-translational modification of protein lysine-residues by acetylation [35]. The majority of investigation into this arena has occurred in the liver, where changes in protein acetylation status have been explored comparing fasting versus fed conditions. These studies demonstrate that the lysine-residues of enzymes controlling the spectrum of metabolic pathways employed to catabolize nutrients for energy production are modified by acetylation [35,36] in a carbon source dependent fashion [35]. Interestingly, increasing glucose and fatty acid levels resulted in the increased acetylation in mitochondrial metabolic pathway enzymes [35], and conversely fasting results in the global deacetylation of mitochondrial proteins [36].

As the activities of cytosolic and mitochondrial metabolic enzymes are altered by the change in acetylation status [35,37], the regulatory enzymes orchestrating these post-translational modifications may be important in modulating nutrient handling and cardiac function. Interestingly, the most characterized 'nutrient' dependent alteration in this program is caloric restriction induced protein lysine-residue deacetylation via the activation of the sirtuin family of deacetylase enzymes. The biochemistry and biology of this enzymatic system has recently been extensively reviewed [38,39] but will be briefly discussed in the context of cardiac metabolism, growth and contractile function.

## 3. Sirtuin enzyme mediated metabolic protein deacetylation and cardiac functional consequences

### 3.1. The sirtuin family of enzymes

In contrast to the class I, II and IV histone deacetylases, which are zinc dependent, the sirtuins are designated as class III deacetylases and are $NAD^+$-dependent enzymes. The founding member of these enzymes is yeast Sir2, which was found to silences chromatin via deacetylation of histones [40]. Sir2 enzymes have been shown to mediate lifespan extension in yeast, worms and flies and are postulated to function, in part, via the modulation of mitochondrial function [41]. Mammals have 7 sirtuin enzymes designated as SIRT1 through SIRT7. The mammalian sirtuins are phylogenetically divided into five subclasses based on the homology of their 250 amino acid core domain [42]. The mitochondrial enriched SIRT3 clusters with SIRT1 and SIRT2 in subclass I. These three enzymes show closest homology to yeast Sir2 and exhibit the most robust deacetylase activity. The additional mitochondrial enriched sirtuins SIRT4 and SIRT5 are assigned to subclasses II and III, and exhibit predominant ADP-ribosyltransferase and weak deacetylase activity respectively [43,44] and SIRT6 and SIRT7 are classified as subclass IV enzymes [42].

Furthermore, the sirtuin isoforms have distinct tissue distributions and subcellular localizations [45]. With the exception of SIRT4 and SIRT7, all other family members are robustly expressed in the heart [46]. The subcellular localization of the sirtuins is probably a central feature in dictating their biological targets. Furthermore, their locations are not exclusive and may be dynamic under specific conditions. For example, SIRT1 is exclusively nuclear during cardiac embryogenesis and then displays both nuclear and cytoplasm postnatal localization [47]. Similarly, the subcellular localization of SIRT3 is predominantly in the mitochondrial matrix [48–50], although some studies suggest that SIRT3 is exclusively mitochondrial [51] while others show nuclear and cytosolic locations in whole tissue preparations [52] and following overexpression [46,53,54]. Whether changes in the subcellular localization of SIRT3 are associated with biological stressors, are tissue specific [50,52] and/or result from the genetic manipulation studies is not completely resolved [55]. SIRT2, appears to be exclusively cytoplasmic, SIRT5 in the inner mitochondrial membrane or matrix and SIRT6 and SIRT7 in the nucleus [44,45,56].

### 3.2. NAD biochemistry

Sirtuin activation is directly linked to the energetic and redox status of the cell as measured by the ratio of $NAD^+$:NADH, by the absolute levels of NAD and by the $NAD^+$ catabolite nicotinamide [57–59]. $NAD^+$ is a cofactor in the deacetylation reaction whereby the nicotinamide ribose is cleaved at its glycosidic bond to yield nicotinamide and the ribose accepts the acetyl group from the sirtuin substrate to produce O-acetyl-ADP-ribose. The biochemistry of this metabolic intermediate is not the focus of this review, but is beginning to be explored [60]. Interestingly, the nicotinamide generated in the deacetylation reaction itself inhibits sirtuin activity and nicotinamide-depletion during $NAD^+$ biosynthesis inversely activates sirtuins [61].

In vertebrates *de novo* $NAD^+$ biosynthesis is the minor $NAD^+$ generation pathway, and uses tryptophan and nicotinic acid as metabolic precursors. This pathway does, however, appear to be induced by exercise and following the administration of peroxisome proliferator activated receptor alpha (PPARα) agonists [62]. The major pathway to generate $NAD^+$ involves the salvage of $NAD^+$ using nicotinamide as the precursor. In mammals there are two intermediary steps in $NAD^+$ salvage, initiated by the conversion of nicotinamide to nicotinamide mononucleotide (NMN) via the nicotinamide phosphoribosyltransferase (NAMPT) enzyme. Nicotinamide/nicotinic acid mononucleotide adenylyltransferase

(NMNAT) then converts NMN to $NAD^+$. These biochemical pathways are most well characterized in the nucleus, and are pivotal for the activity of SIRT1 [63]. Moreover, NAMPT has been identified as the rate-controlling step in $NAD^+$ biosynthesis in that overexpression of Nampt but not Nmnat increased cellular $NAD^+$ levels [63].

The investigation into the biology of $NAD^+$ in the mitochondria has begun to be explored, and the identification of a mitochondrial-enriched NMNAT isoform implicates that subcellular compartment specific functioning of NAD biosynthesis may be operational [64]. This is further supported in that the metabolic stress of fasting increases mitochondrial NAMPT with the concomitantly increase in mitochondrial $NAD^+$ levels [65].

The regulation and role of this metabolic pathway in controlling cardiac metabolism and contractile function have not been extensively explored, although the transcript and protein levels of NAMPT are downregulated in the murine heart in response to pressure overload-induced cardiac hypertrophy and the overexpression of NAMPT in cardiomyocytes increased cellular $NAD^+$ and ATP levels [66]. Taken together these data would suggest an ameliorative role of this regulatory pathway and of sirtuin activation in cardiac metabolism and in the adaptation to pathologic hypertrophic growth. Further support of this concept is that the administration of $NAD^+$ to mice or primary cardiomyocytes exposed to hypertrophic agonist simulation, ameliorates the hypertrophic phenotype in parallel with the maintenance of cellular ATP levels [67]. The activation of this $NAD^+$-mediated anti-hypertrophic program appears to function via SIRT3 mediated activation of the AMPK program, and the attenuation of redox stress [67]. The direct metabolic consequences of this and their effect, if any, on the hypertrophic program have not been ascertained. No data to date appears to have explored the manipulation of the $NAD^+$ metabolic pathway and its effects on cardiac contractile functioning.

### 3.3. Sirtuin control of substrate metabolism and cardiac muscle function

It has been well established that cardiac substrate utilization is altered with the development of cardiac hypertrophy and the transition to heart failure [68,69]. This metabolic remodeling results in the diminution in fatty acid oxidation as a reducing equivalent source and an augmentation in the use of glucose to generate energy. The role of this metabolic remodeling in this progressive pathophysiology is uncertain. Interestingly, the supplementation of fatty acids as substrates can delay the onset of pressure-overload induced cardiac hypertrophy and possibly delay the progression to heart failure [70,71], in parallel with the maintenance of fatty acid oxidation [72]. Whether sirtuin biology regulates these programs is not known, although a role of sirtuins in regulating substrate utilization is increasingly becoming apparent.

Although, the role of SIRT1 in regulating fat metabolism has been quite extensively investigated, it has not directly been studied in the heart. In skeletal muscle, under low nutrient conditions SIRT1 increases fatty acid oxidation and leads to a metabolic switch from glucose oxidation to fatty acid oxidation [73]. In this nutrient limited environment AMPK enhances SIRT1 activity by increasing cellular $NAD^+$ level, resulting in the deacetylation and modulation of cognate substrates including PGC-1α, FOXO1 and FOXO3a to regulate skeletal muscle energy metabolism [74]. In an apparent perpetuating cycle, the deacetylation of AMPK kinase LKB1 by SIRT1 concordantly increases AMPK activity [75,76]. Additionally, in a caloric excess environment SIRT1 is downregulated in skeletal muscle in parallel with the suppression of mitochondrial respiratory function [77]. In the postnatal heart fatty acids make up the major carbon source for ATP production [69]. From a nutrient perspective it is interesting to note that SIRT1 overexpression protects cardiomyocytes from serum starvation induced cell death [78]. Furthermore, the modest induction of SIRT1 in the heart in transgenic mice prevents aging associated cardiac

dysfunction and protects against paraquat-induced oxidative stress [79]. This ameliorative effect is 'gene-dose dependent' as a very high copy number of the SIRT1 transgene induces cardiomyopathy and increases oxidative stress [79]. Whether the ameliorative effects of SIRT1 in the heart function, in part, through the modulation of fat metabolism has not been determined.

SIRT3 has recently been reported to promote mitochondrial fatty-acid oxidation, in the heart and other tissues, through the deacetylation and activation of long chain acyl-CoA dehydrogenase (LCAD) [80]. A direct link between SIRT3 controlled fatty acid oxidation, cardiac hypertrophy and heart failure has not been established, however, mice with genetic depletion of SIRT3, possess greater susceptibility to angiotensin II induced cardiac hypertrophy compared to wildtype mice and an anti-hypertrophic effect of SIRT3 overexpression functions, in part, via its reactive oxygen species modulatory effects [81].

Although not directly related to cardiac metabolism or cardiac contractile function several mitochondrial proteins have been identified as SIRT3 targets which link SIRT3 to various metabolic pathways. Acetyl-CoA synthetase 2 (AceCS2) was the first mitochondrial proteins found to be deacetylated and activated by SIRT3 [82,83]. AceCS2 converts free acetate, which is generated from endogenous cellular reactions or absorbed from the gut, into an active metabolite acetyl-CoA for energy production through the TCA cycle. Interestingly, AceCS2 activation plays an important role in acetate conversion under ketogenic conditions such as diabetes. A second enzyme regulating ketone body production, i.e. mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 is similarly activated by SIRT3 to facilitate energy production under fasting conditions [84]. In addition, other two mitochondrial matrix proteins, GDH, an enzyme important for amino acid metabolism, and isocitrate dehydrogenase 2 (IDH2), a key regulation enzyme for TCA cycle are also deacetylated and activated by SIRT3 [50,56]. Interestingly, several components of the electron transfer chain (ETC) have found to be regulated by SIRT3. For example, NDUFA9, a subunit for ETC complex I [85], succinate dehydrogenase flavoprotein (SdhA), the complex II subunit [86], and ATP synthase alpha and beta subunits [87,88] were shown to interact with SIRT3. Interaction with SIRT3 increases both complex I and complex II activities [85,86], implying that SIRT3 is a central player in energy metabolism. Consistent with this, SIRT3 is found to regulate and maintain tissue basal ATP levels in vivo [85]. SIRT3 activation additionally modulates reactive oxygen species levels by activation of MnSOD [89] and by increasing levels of reduced glutathione [90]. These effects on reactive oxygen species level modulation, may, hypothetically have cardioprotective effects, although this too has not been directly explored. One laboratory has shown that the absence of SIRT3 predisposes to the spontaneous development of cardiac hypertrophy [81]. However, in general SIRT3 knockout mice have shown no obvious basal metabolic abnormalities [50], suggesting that the regulatory role of SIRT3 is most likely operational under certain 'nutrient 'stress conditions, such as during caloric restriction and in response to caloric excess. The mitochondrial metabolic pathways regulated by SIRT3 are schematized in figure 1.

Again, although the metabolic component of their function has not been directly ascertained, a role of SIRT2 and SIRT7 appear to be operational in the cardiac derived cells and the heart respectively. Although not directly explored in the intact heart, the cytosolic sirtuin SIRT2 modulates mitochondrial function via the modulation of mitochondrial pro-apoptotic proteins and via mitochondrial antioxidant enzyme regulation [91,92]. SIRT7 resides in the nucleoli and following its genetic depletion mice develop and die from cardiac hypertrophy and an inflammatory cardiomyopathy [93]. The absence of SIRT7 in primary murine cardiomyocytes enhances p53 acetylation, additionally leading to increased apoptosis and increased susceptibility to oxidative and genotoxic stressors [93].

## 4. Metabolic protein acetylation

A counter-regulatory program to counterbalance sirtuin deacetylase function is less well established. However, in much the same way as histone acetyltransferases (HAT's) counterbalance the histone deacetylases (HDAC's), it is expected that sirtuin acetyltransferases should exist. With respect to HAT's and sirtuin acetyltransferase enzymes, acetyl-CoA would be expected to be the essential cofactor for protein acetylation [94]. Calorie mediated control of this process has been succinctly demonstrated where excess glucose availability has been shown to be result in the ATP-citrate lyase dependent generation of nuclear acetyl-CoA from citrate with the subsequent acetylation of histones and the induction of genes encoding for glucose uptake and metabolic enzymes [95].

The first functional characterization of a sirtuin deacetylase demonstrated that acetyl transferase general control of amino-acid synthesis (GCN5) acetylates PGC-1β to inhibit its activity to counter the deacetylation function of SIRT1 [96]. The inhibition of the predominant cardiac PGC isoform, PGC-1α has similarly been shown to be acetylated and inhibited by the nuclear transcriptional coactivator and acetyltransferase p300 [97]. Although not directly studied in the heart, one could propose that these or other acetyltransferases could function to dampen the PGC regulated mitochondrial biogenesis and β-oxidation enzymes programs central to cardiac contractile functioning and stress resilience [98,99].

As acetyl-CoA is a predominant intermediate of both pyruvate dehydrogenase and the mitochondrial fatty acid β-oxidation cycle it would not be unreasonable that this metabolite may function within mitochondria as a 'sensor' of caloric excess. Indeed, dynamic regulation of mitochondrial acetyl-CoA levels are suggested by its accumulation in liver mitochondria following high-fat feeding and diabetes [100], and excess glucose and fatty acids have been shown to increase the acetylation of mitochondrial metabolic cycle proteins with a concomitant modulation of enzyme activities [35]. However, to date no mitochondrial enriched acetyl transferase has been identified to employ acetyl-CoA for protein acetylation and/or to counterregulate the deacetylase actions of SIRT3. These proteins need to be identified and characterized before this putative nutrient regulated program could be investigated in the regulation mitochondrial function in general and more specifically in the heart in response to nutrient load mediated alterations in cardiac growth and function.

## 5. Conclusions

There is increasing recognition that 'nutrient' intermediates can play an important role in regulating metabolic pathways via the modulation of the acetylation status of metabolic enzymes. This review highlights new areas of research and as importantly, significant deficits in our knowledge of the role of caloric-load biology in the modulation of cardiac substrate utilization and as to whether these regulatory programs play a role in cardiac adaptation and maladaptation. The schematic figure 2 shows proposed functioning of these programs in the modulation of cardiac contractile function and in response to hypertrophic stressors. The calorie excess scenario in this schematic additionally highlights the consequences of excess nutrient availability as an innate cardiac 'toxin'. It is also important to recognize that the metabolic regulatory effects and the resulting cardiac consequences of the change in metabolic enzyme acetylation status probably functions to fine-tune these biomechanical and bioenergetic interactions, versus operating in a binary fashion displaying 'all or nothing' effects. This is best illustrated where the knockout of SIRT3 has a subtle phenotype with metabolic effects most apparent under maximal activation of the deacetylase program [80]. Nevertheless, chronic alternations in 'fine-tuning' of metabolism that may be operational in caloric excess conditions such as obesity and T2DM probably still have

profound effects on the high-energy demand system orchestrating cardiac contractile function and it's adaptation to extra-cardiac biomechanical stressors. We look forward to exciting advances in the understanding of this regulation as additional targets of sirtuin biology are delineated. Finally, the identification and characterization of the sirtuin counter-regulatory programs, especially in the modulation of metabolic enzymes remains enigmatic and may be pivotal in our understanding of nutrient mediated modulation in cardiac function.

## Acknowledgments

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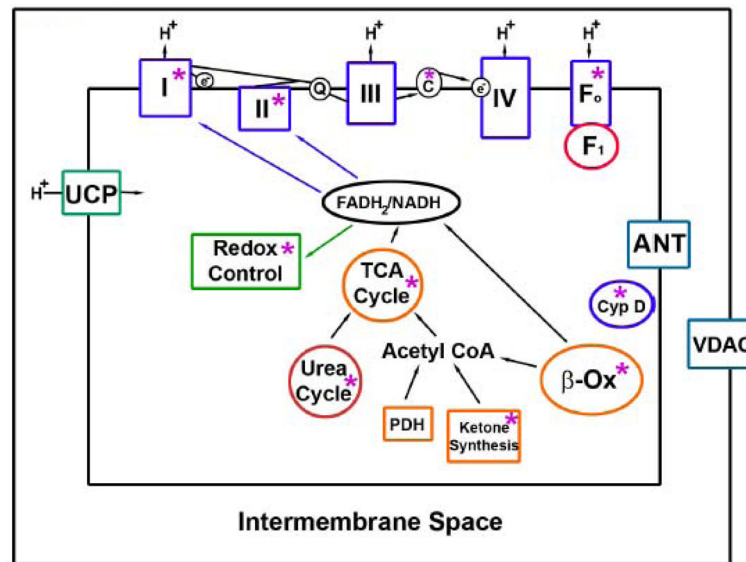
**Fig 1.**
Mitochondrial pathway targets of SIRT3. The metabolic pathways, protein complexes and individual proteins deacetylated by SIRT3 are highlighted by asterisks. In general, the deacetylation of these proteins results in increased protein/pathway activation. The exception is the deacetylation of cyclophilin D (Cyp D), as this modification inhibits this protein to attenuate susceptibility to increased mitochondrial permeability. Additional abbreviations: TCA – tricarboxylic acid; PDH – pyruvate dehydrogenase; I – IV – represent the complex of the election transfer chain; Fo and F1 are components of ATP synthase; ANT – adenine nucleotide translocase, UCP – uncoupling proteins, VDAC – voltage dependent anion channel.
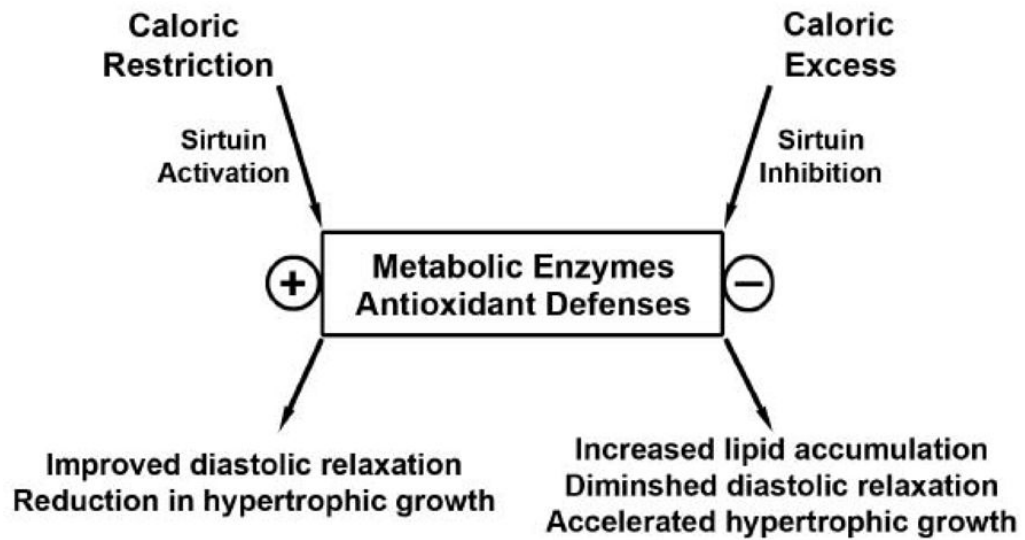
**Fig 2.**
Schematic of sirtuin mediated regulatory events under 'caloric load' control and proposed effects on cardiac function. The plus sign represents protein activation and the minus sign, protein inhibition.