The role of the autophagy-inducer spermidine in cardiovascular ageing

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Abstract

Due to progressive degeneration of tissues with age, ageing has been recognised as a significant risk factor for the development of most chronic diseases, including cardiovascular diseases. Interventions – such as the addition of the polyamine spermidine to the diet – have been suggested to extend lifespan, as seen in a mouse model. This has partly been attributed to slowing down the negative effects of the ageing process on the cardiovascular system. Spermidine acts as a calorie restriction mimic (CRM), which is naturally abundant in eukaryotic cells; however, the concentration of spermidine declines in cells with age. As spermidine is naturally found in several food sources, such as aged cheeses, fermented soybeans and wheatgerm, it is plausible to investigate the impact of dietary interventions on reducing the risk of age-related cardiovascular diseases. The data summarised in this paper indicates that the cardioprotective properties of spermidine can be partly attributed to its ability to promote autophagy in cardiac muscle, a vital process for the recycling of dysfunctional and potentially harmful cellular components, thus, preventing cardiomyopathies. Overall, the evidence presented in this review paper supports the use of spermidine as a promising candidate for delaying some of the age-related changes to the structure and function of the cardiovascular system.

Keywords: Spermidine, cardiac autophagy, increasing lifespan, slowing cardiovascular ageing, calorie restriction mimic, spermidine and longevity, spermidine and autophagy induction

Introduction

The world’s population is ageing, resulting in older persons making up a larger proportion of the population (United Nations, 2017). This has societal implications across a number of sectors including – but not limited to – goods and services, labour and healthcare (United Nations, 2017). The health challenges inherent in an ageing population will undoubtedly give rise to an increase in the rates of chronic diseases,
and thus the need to address this. Ageing is the main risk factor for the development of cardiovascular diseases (Niccoli and Partridge, 2012), equating to an increased risk in potential cases of myocardial infarction, stroke, heart failure and subsequent deaths (Niccoli and Partridge, 2012). The latest projections detailing the challenges of an ageing population within the United Kingdom show that there will be an additional 8.6 million people aged 65 and over by 2060 (Storey, 2018). This serves to place further strain on the publicly funded National Health Service (NHS; Storey, 2018), with cardiovascular diseases being the dominant cause of death (Majeed and Aylin, 2005).

A promising **pharmacological** candidate in addressing some of the problems associated with increased prevalence of cardiovascular diseases is the **polyamine** spermidine (Eisenberg *et al.*, 2016; Wang *et al.*, 2020). It has been suggested that decline in the levels of spermidine in cells is associated with an increased risk of the development of age-related cardiovascular diseases (Eisenberg *et al.*, 2009). Therefore, we aim to highlight some of the key physiological changes that occur as a result of ageing in the **cardiovascular** system and, more importantly, to collate the current research on the role and mechanism of the action of spermidine in delaying some of the observed age-associated cardiovascular diseases. We will also consider the potential of spermidine as a treatment and as a preventative tool for these challenges found in the ageing population.

**Spermidine and autophagy**

Spermidine is a member of the polyamine family and is naturally found in certain foods such as wheatgerm, fermented soybeans and aged cheeses (Madeo *et al.*, 2018a). Polyamines, such as spermidine, possess **cations** that interact with molecules such as lipids, proteins, DNA and RNA. These can have a negative charge, serving to stabilise them (Minois *et al.*, 2011). They therefore play various roles in cell proliferation, survival and growth by helping to mitigate DNA mutations and degradation along with delaying cell senescence and necrosis. This has implications for increasing longevity and reducing the incidence of age-related disease (Minois *et al.*, 2011).

Autophagy has been recognised as a vital process for lifespan extension and longevity (Rubinsztein *et al.*, 2011). Supplementation with spermidine has upregulated autophagy in yeast, worms, flies, human immune cells and mice, which subsequently led to lifespan extension (Eisenberg *et al.*, 2009). The same concept has been shown to hold true for cardiomyocytes, where spermidine promoted cardioprotective autophagy (Madeo *et al.*, 2018a; Eisenberg *et al.*, 2016). Autophagy plays a pivotal role in
maintaining optimal cellular environments by removing damaged organelles and toxic protein aggregates (Rubinsztein et al., 2011). During ageing, damaged, misfolded and dysfunctional proteins accumulate, disrupting cellular environments and increasing risk of apoptosis induction (Escobar et al., 2019). Therefore, discovering easily implementable methods of upregulating autophagy is of vital importance in tackling developments of cardiac pathophysiology.

**Ageing and the increased risk of cardiovascular dysfunction**

There are a number of molecular mechanisms contributing to the development of cardiac pathology during the ageing process (Chiao and Rabinovitch, 2015). These include mitochondrial reactive oxygen species (ROS) production and dysfunction, calcium homeostasis impairment, extracellular matrix (ECM) remodelling, neurohormonal signalling, stem cell ageing, miRNA deregulation and altered nutrient and growth signalling (Chiao and Rabinovitch, 2015). The physiological processes responsible for the development of cardiovascular dysfunction are numerous, including altered left-ventricular diastolic filling, left-ventricular hypertrophy and increased aortic root diameter (Gerstenblith et al., 1977). To compound this further, there are reductions in maximum heart rate and contractility along with a decline in cardioprotective and repair processes (Strait and Lakatta, 2012). Such repair processes are vital for protection against oxidative stress, the loss of proteostasis and mitochondrial dysfunction, which eventually culminate in cardiomyopathies (Strait and Lakatta, 2012). Spermidine has the potential to mitigate pathophysiological developments through inducing a number of cardiovascular changes (Figure 1).

Autophagy is the principle mechanism by which benefits are conferred due to the upregulation of oxidative stress protection, recycling of dysfunctional proteins and mitochondrial biogenesis (LaRocca et al., 2013; Minois et al., 2011; Wang et al., 2020).

Cardiac hypertrophy predominantly occurs due to the dysfunction of nutrient and growth signalling pathways, particularly involving mammalian target of rapamycin (mTOR) and insulin-like growth factor-1 (IGF-1) (Chiao and Rabinovitch, 2015). Studies in both Drosophila and mice have shown the importance of mTOR in cardiac-hypertrophy development with downregulation of mTOR signalling increasing resistance to cardiac ageing and upregulation impairing resistance to cardiac ageing (Chiao and Rabinovitch, 2015). Moreover, the insulin/IGF-1 pathway has been implicated in the development of cardiac disease with reduced activation of this pathway slowing cardiomyocyte dysfunction in mice and Drosophila (Li et al., 2008; Wessels et al., 2004). Spermidine works independently of the mTOR pathway to elicit
an anti-ageing effect via gene hypoacetylation as opposed to altering the phosphorylation status of mTOR (Minois, 2014). With regard to Insulin/IGF signalling, it has been demonstrated that reducing such signalling leads to glycine-N-methytransferase (Gnmt)-dependent spermidine level increase in Drosophila, resulting in increased lifespan (Tain et al., 2020).

Additionally, mice over-expressing catalase in the mitochondria (mCAT) have shown slowed cardiac ageing, decreased hypertrophy and improved diastolic function (Dai et al., 2009), whereas mice over-expressing mitochondrial polymerase – and hence increased mitochondrial mutation – have shortened lifespan and earlier onset of cardiac disease (Dai et al., 2010). Subsequent mCAT mutations in these mice demonstrated reduced mitochondrial damage and cardiomyopathy (Trifunovic et al., 2004; Dai et al., 2010). Defective mitochondria play a pivotal role in age-related decline and cardiac dysfunction but can be targeted by spermidine through mitophagy induction (Madeo et al., 2018a).

Changes in the ECM of cardiomyocytes as ageing progresses is also implicated in cardiac function decline. The cardiac ECM environment is comprised of collagen types I through to VI, elastin, laminin, fibrinogen and fibronectin, all generated by cardiac fibroblasts (DeQuach et al., 2010). The ECM is responsible for aligning the cardiac myocytes and structurally supporting the heart. However, ECM components such as metalloproteinases, along with the quantity and quality of collagen deposition, can also contribute to diastolic heart failure depending on the individual’s phenotype (Ouzounian et al., 2008). While spermidine does not appear to impact the degree of collagen deposition in the ECM of cardiomyocytes in aged mice, it directly impacts cardiomyocytes through increased mitochondrial volumes and decreased sarcoplasmic volume. These changes were shown to increase myocardial compliance (Eisenberg et al., 2016).

Although interventions in treating heart disease are relatively effective – mainly targeting cholesterol levels and blood pressure – they do not target the underlying mechanisms of cardiovascular disease development (Cabo and Navas, 2016). Spermidine, however, has demonstrated the potential to target these mechanisms in mouse models (Cabo and Navas, 2016). This is where the calorie restriction mimetic (CRM) spermidine may be extremely valuable in mitigating age-related cardiac pathologies.
Mechanism of spermidine in inducing autophagy

It has been shown that the beneficial effects of spermidine are due to its ability to induce autophagy (Eisenberg et al., 2009). Model organisms such as yeast, worms and flies, with deficient machinery to induce autophagy, acquired no life-extension benefits from the introduction of spermidine. Such studies suggest that effective machinery to induce autophagy is necessary for spermidine to be effective (Eisenberg et al., 2009). It has also been demonstrated that spermidine-induced paraquat resistance is markedly reduced in Drosophila that have defective machinery for autophagy compared to those without deficits (Minois et al., 2012). Spermidine inhibits the activity of certain histone acetyltransferases (HATs) resulting in H3 hypoacetylation (Eisenberg et al., 2009). This confers gene transcription regulation favouring the induction of autophagy and apoptosis as opposed to inflammatory necrotic cell death (Eisenberg et al., 2009).

Hypoacetylation of histones has been shown to promote longevity in yeast cells also, particularly through the activation of Sir2 and NAD+-dependent histone deacetylases.
and is therefore considered a potential key event in healthy ageing (Imai et al., 2000). This provides further evidence that hypoacetylation may be key in promoting longevity, which is a key mechanism of spermidine.

Additionally, spermidine acts as an acetyltransferase inhibitor in E1A-associated protein p300 (EP300) through competitive inhibition of the EP300 protein with Acetyl-CoA (Pietrocola et al., 2015). It has been demonstrated that when Acetyl-CoA levels are depleted in human and murine cell lines, deacetylation of cytoplasmic proteins occurs stimulating autophagy in heart and muscle cells (Mariño et al., 2014). However, when Acetyl-CoA levels are maintained during starvation, autophagy induction is inhibited (Mariño et al., 2014).

Another pathway in which the action of spermidine is critical involves the polyamine-eIF5A-hypusine axis (Puleston, Buck and Pearce, 2019). Spermidine promotes autophagy in B cells and macrophage activation through the hypusination of a conserved lysine residue in the eIF5A protein (Zhang, Alsaleh, Feltham and Sun, 2019) (Puleston et al., 2019). Activation of eIF5A subsequently triggers the expression of mitochondrial proteins involved in the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (Puleston et al., 2019). In B cells, post-translational modification of the eIF5A protein stimulates the production of autophagy transcription factor TFEB, which has the potential to reverse immune cell senescence (Zhang et al., 2019).

**Induction of autophagy for cardiac protection by spermidine**

Spermidine is synthesised *in vivo* via the conversion of arginine to ornithine, which is then converted to polyamines putrescine, spermidine and spermine mediated by ornithine decarboxylase (Figure 2) (Minois, 2014).
Spermidine is acquired in a number of ways including cellular biosynthesis, microbiota generation and through oral uptake (Madeo et al., 2018a). It is well established that spermidine levels decline in humans as we age with concentrations of cellular spermidine being determined by dietary intake, cellular biosynthesis, rates of catabolism and urinary excretion (Madeo et al., 2018a). However, increased dietary intake of polyamines has been shown to increase the blood concentration of polyamines in both mice and humans (Soda et al., 2009). Polyamine levels in this study were determined through centrifugation of blood samples and subsequent high-performance liquid chromatography analysis, which showed statistically significant blood concentration increases (Soda et al., 2009). The transport mechanism of polyamines into the intracellular environment is currently poorly understood with only three competing models, none of which fully explain the transport process (Nowotarski et al., 2013).

Recently discovered is the ability of spermidine to improve cardiac function in rats through activation of the AMPK/mTOR pathway and through slowing cardiac hypertrophy (Yan et al., 2019). Investigations in rat models fed a high salt diet with simultaneous spermidine administration show similar results (Eisenberg et al., 2016).
Delayed hypertension onset, decreased cardiac hypertrophy and increased diastolic functioning were shown while renal injury due to increases in blood pressure was reduced (Eisenberg et al., 2016). This has been demonstrated in both rats and mice with spermidine significantly improving markers of cardiac ageing such as left-ventricular hypertrophy and stiffness while mitigating diastolic dysfunction (Eisenberg et al., 2016). Expression of LC3-II – an autophagy marker – was reduced in aortas of older mice, whereas p62 – an autophagy substrate marker – was increased relative to younger mice (LaRocca et al., 2013). Addition of spermidine restored the LC3-II expression and reduced p62 in older mice, but did not have an effect in younger mice. Moreover, H3 histone acetylation was decreased and Atg3 – an autophagy protein – was upregulated in both old and young mice (LaRocca et al., 2013). It has been shown that the induction of autophagy in mice through exogenous supplementation with spermidine stimulates cholesterol efflux (Michiels et al., 2016). This inhibits lipid accumulation and necrotic core formation in vascular smooth muscle cells, hinting at a potential to prevent vascular disease (Michiels et al., 2016).

Expression of the Atg5 protein has also been observed and is vital for the induction of autophagy in cardiomyocytes as demonstrated in Atg5-deficient mice (Nakai et al., 2007). Loss of this protein was shown to induce cardiomyopathy in mice, producing hypertrophy, left-ventricular dilatation, contractile dysfunction and increased ubiquitination (Nakai et al., 2007). In addition, it was seen that contractile tissue was preserved, an improvement in mitochondrial function was noted along with improved inflammation suppression mechanisms (Nakai et al., 2007). The most likely explanation for this effect is the increased bioavailability of nitric oxide (NO) acting as a vasodilator, reducing arterial blood pressure (Van Faassen et al., 2009). In old mice, endothelial dilation functions are approximately 25 per cent lower compared to young mice and also approximately 20 per cent increase in arterial stiffness in old mice hypothesises to be due to decreased NO bioavailability (LaRocca et al., 2013). This poses the question of whether the NO pathways and autophagy are independent mechanisms or can only work in conjunction with each other (Madeo et al., 2018a).

**Conclusion**

Spermidine has demonstrated promising results for promoting longevity and improving health span in various research models, particularly through the polyamine’s ability to induce autophagy (Madeo et al., 2018a). Cardioprotective effects are also apparent through the induction of nitric-oxide synthesis by spermidine; however, this mechanism needs to be elucidated further to identify therapeutic
molecular targets (Madeo et al., 2018a). Although one survey-based study linked increased consumption of spermidine rich foods to lower rates of cardiovascular disease in humans (Kiechl et al., 2018), more research is needed to ascertain whether the effects seen in mice are translatable to humans with respect to longevity and the extent of benefits to cardiac health. For example, levels of blood spermidine concentrations needed to maintain optimal cardiovascular health in humans are not known. Nonetheless, noting that spermidine levels reduce in ageing individuals (Madeo et al., 2018a) and blood concentrations of the polyamine can be increased through dietary intake (Soda et al., 2009) provides a promising basis for the potential use of spermidine as a supplement.

These findings represent an important step in establishing treatment targets and discovering precise molecular pathways implementable in preventing age-related disease. However, minimum dosages will need to be determined that could be potentially therapeutic. It will also need to be determined whether the potential effects are translatable to the majority of the population (Madeo et al., 2018b). There have been conflicting results with respect to how supplementation of spermidine impacts blood levels of the compound, with some studies demonstrating an increase and others having no impact (Brodal et al., 1999. Soda et al., 2009; Schwarz et al., 2018). However, the differences observed in these studies are likely to be associated with the concentrations of spermidine administered. Spermidine is also being investigated for treatment surrounding intrauterine growth restriction in newborns and reducing the impact of oxidative stress caused by hypoxia on the heart (Chai et al., 2019).

Future research with spermidine is currently being centred around safety and tolerability of the compound with one study demonstrating this it is safe and well tolerated in mice and older adults with subjective cognitive decline (Schwarz et al., 2018) leading the way for longer exposure studies to be conducted. That being said, spermidine is naturally abundant in all eukaryotic cells so is unlikely to be poorly tolerated if acquired from exogenous food sources.

A larger number of studies will need to be completed to determine any possible side effects and utility of spermidine in human populations with more epidemiological studies taking into consideration any confounding factors. If successful, the compound has the potential clinical application of targeting specific mechanisms responsible for the onset of cardiovascular diseases.
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Figure 1. Cardioprotective effects of spermidine

Figure 2. A) Biosynthetic pathway of polyamine spermidine via ornithine decarboxylase. B) Chemical structure of spermidine.

References


Cabo, R. and P. Navas (2016), 'Spermidine to the rescue for an aging heart', *Nat Med*, 22 (12), 1389–90


Mariño, G., F. Pietrocola, T. Eisenberg, Y. Kong, S. Malik, A. Andryushkova, .. G Kroemer (2014), ‘Regulation of autophagy by cytosolic acetyl-coenzyme’, *A. Mol*


Schwarz, C., S. Stekovic, M. Wirth, G. Benson, P. Royer, S. Sigrist, ... C. Dammbrueck (2018), 'Safety and tolerability of spermidine supplementation in mice and older adults with subjective cognitive decline', Aging, 10 (1), 19–33


Wang, J., S. Li, J. Wang, F. Wu, Y. Chen, H. Zhang, ... Y. Zhao (2020), 'Spermidine alleviates cardiac aging by improving mitochondrial biogenesis and function', Aging, 12 (1), 650–71

Yan, J., J. Yan, Y. Wang, Y. Ling, X. Song, S. Wang, ... P. Yang (2019), 'Spermidine-enhanced autophagic flux improves cardiac dysfunction following myocardial infarction by targeting the AMPK/mTOR signalling pathway', *Br J Pharmacol*, 176 (17), 3126–42


**Glossary**

**Acetylation** Introduction of an acetyl (O=C-R) functional group into a chemical compound

**Acetyl CoA** A molecule involved in many biochemical reactions, mainly the tricarboxylic acid cycle for energy production

**Ageing** The occurrence of structural and functional changes that occur over time

**Apoptosis** Controlled cell death as part of an organism’s normal growth process

**Autophagy** Cell regulatory mechanism that removes dysfunctional or unnecessary components

**Biogenesis** Synthesis of substances by a microorganism

**Calorie restriction mimetic** Molecule mimicking the anti-ageing effects of calorie restriction

**Cardiovascular** Relating to the heart and blood vessels

**Cardiomyocytes** Cells comprising heart muscle

**Cardiomyopathy** Disease of the heart muscle

**Catabolism** The break-down of molecules through metabolic pathways

**Cations** Positively charged ions

**Cell necrosis** Death of cells due to injury, disease or lack of blood supply

**Chronic disease** A health condition lasting for longer than three months
**Compound** A substance formed when two or more chemical elements are combined

**Diastolic** Relaxation phase of the heart

**Eukaryotic cell** Cells found in animals, plants and fungi

**Exogenous** From an outside source

**Extracellular matrix** Network of proteoglycans, water, minerals and fibrous proteins that form structural and biochemical support to surrounding cells

**Fibroblasts** Principle active cell in connective tissue that synthesises the extracellular matrix and collagen

**Glycine-N-methyltransferase** Catalyses the methylation of glycine from S-adenosylmethionine

**Histone** Proteins in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes

**Histone acetyltransferases** Enzymes that transfer acetyl groups to acetylate lysine amino acids on histone proteins

**Homeostasis** Maintenance of an equilibrium

**Hypertension** Persistently raised arterial blood pressure not within healthy limits

**Hypertrophy** Enlargement of an organ or tissue due to increase in cellular size

**Hypoacetylation** Insufficient acetylation causing gene inactivation

**Hypoxia** A lack of oxygen supply

**Hypusination** Addition of the amino acid hypusine, which is only found in eukaryotic translation initiation factor 5A (eIF5A) and archaeabacteria

**In Vivo** Within a living organism

**Metalloproteinase** Any protease enzyme with a metal involved in its catalytic mechanism

**Microbiota** Communities of microorganisms
**MiRNA** Micro RNA functioning in RNA silencing and post-transcriptional regulation

**Mitochondria** An organelle responsible for respiration and energy production

**Mitochondrial Polymerase** Mitochondrial replication machinery

**Mitophagy** Degradation of mitochondria by autophagy

**mTOR** Mammalian Target of Rapamycin is a protein kinase involved in a number of cellular processes (ageing in this case).

**Oxidative phosphorylation** The process of ATP formation through the transfer of electrons from NADH or FADH$_2$ to Oxygen forming H$_2$O

**Paraquat** A chemical herbicide that is highly toxic

**Pathophysiology** Abnormal functioning associated with disease or injury

**Polyamine** A carbon containing compound comprising of more than two amino (-NH$_2$) groups

**Pharmacological** Uses, effects and modes of action of drugs

**Proteostasis** Biological pathways in cells that regulate biogenesis, folding, trafficking and protein degradation

**Sarcoplasmic volume** Amount of cytoplasm of striated muscle cells

**Senescence** Deterioration with age

**Systolic** Contraction phase of the heart

**Tricarboxylic acid cycle** The second stage of cellular respiration occurring in the mitochondria, involved in energy production.

**Ubiquitination** The process of adding a ubiquitin protein to a protein tagging the protein for degradation

**Vasodilator** Increasing the diameter of a blood vessel