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SYMPOSIUM REVIEW

Mitochondrially derived peptides as novel regulators of metabolism

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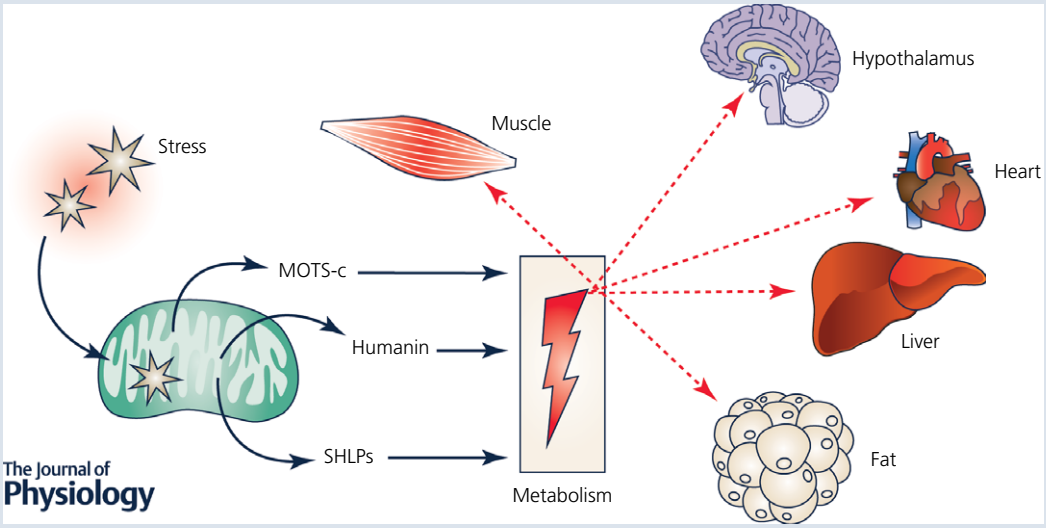

Abstract Mitochondrially derived peptides represent a new class of circulating signalling molecules. Humanin, the first member of this class, has been shown to have several metabolic effects such as reducing weight gain and visceral fat and increasing glucose-stimulated insulin release. The discovery of several other new members, such as MOTS-c and SHLP1–6, has further added to this group. These new peptides have also been found to affect metabolism with MOTS-c potently decreasing weight gain in mice on a high-fat diet. This review covers the basic biology of this class of peptides and discusses the relevance to organismal metabolism.

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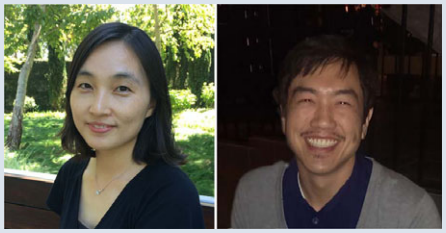
Su-Jeong Kim received her PhD in biology at Yonsei University. Her PhD thesis was focused on the cytoprotective roles of PARK7 during neuronal cell death in various Parkinson's disease models. She is currently a postdoctoral fellow in the laboratory of Pinchas Cohen at USC Leonard Davis School of Gerontology. She has identified humanin-mediated signalling pathways that regulate autophagy and cytoprotection. Her research focuses on understanding the roles of mitochondrially derived peptides including humanin in healthspan and lifespan. **Kelvin Yen** is a research assistant professor at the Leonard Davis School of Gerontology at the University of Southern California. He received his PhD at the Icahn School of Medicine at Mount Sinai, New York, in the lab of Charles Mobbs. He has spent nearly 20 years researching the biology of ageing, with his most recent research focusing on the physiological role of mitochondrially derived peptides and how they affect ageing and age-related diseases.



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Abstract figure legend Mitochondrially derived peptides regulate metabolism. Mitochondrial stress leads to changes in MOTS-c, humanin, and SHLP levels. This in turn plays a protective role in metabolism and leads to tissue specific effects.

Abbreviations AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, AMP-activated protein kinase; CNTFR, ciliary neurotrophic factor receptor; ERK, extracellular signal-regulated kinases; HFD, high-fat diet; HNG, S14G humanin; IGFBP3, insulin-like growth factor binding protein 3; JAK, Janus kinase; MCP-1, monocyte chemoattractant protein-1; MDP, mitochondrially derived peptide; MOTS-c, mitochondrial open reading frame of the 12S rRNA-c; RANKL, receptor activator of nuclear factor-κB ligand; SHLP, small humanin-like peptide; STAT, signal transducer and activator of transcription.

The discovery of humanin nearly two decades ago has ushered in a new interest in mitochondrial biology that has combined several nascent fields of research (small open reading frame and alternative reading frame biology) with the more established field of mitochondrial biology (Yen *et al.* 2013; Xiao *et al.* 2016). More recently we have discovered that humanin is but the first of several mitochondrially derived peptides that are found within small, alternative reading frames of the mitochondrial genome (Fig. 1; Hashimoto *et al.* 2001*b*; Guo *et al.* 2003; Ikonen *et al.* 2003). Humanin has a number of different cytoprotective and metaboloprotective effects while the recently discovered small humanin-like peptides (SHLP) 1–6 have similar but distinct properties compared with humanin (Cobb *et al.* 2016). The discovery of another mitochondrially derived peptide (MDP), namely mitochondrial open reading frame of the 12S rRNA-c (MOTS-c), as an exercise mimetic and activator of AMP-activated protein kinase (AMPK) suggests that these peptides will have an important role in metabolism and could be used as future therapeutics (Lee *et al.* 2015).

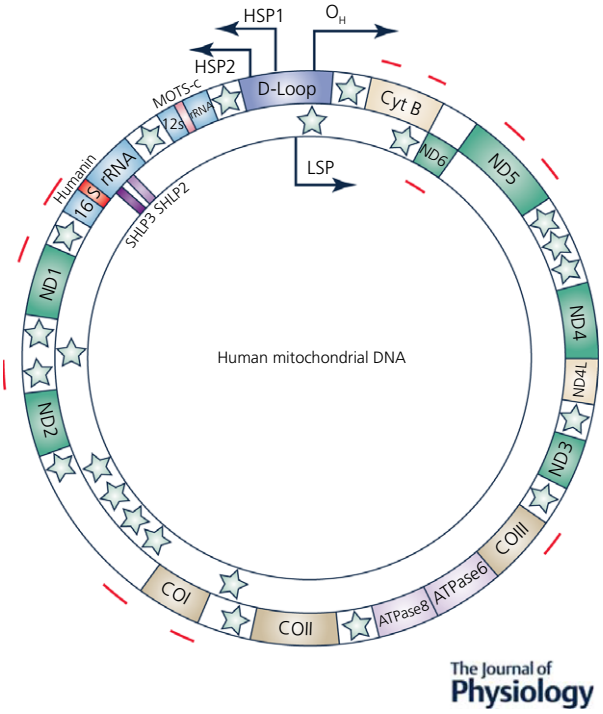

Figure 1. The human mitochondrial genome
Relative locations of genes are represented as coloured blocks including humanin (red), MOTS-c (yellow), SHLP2 and SHLP3 (pink and magenta, respectively). Twenty-two tRNAs encoded from mitochondria are represented as green stars. Potential epigenetic modulation CpG sites in the mtDNA are marked in brown and the coordinates were obtained from Yu *et al*. 2017.

Humanin

As the first MDP to be discovered, humanin has been found to play a diverse role in a number of different processes. It was first discovered in a screen for proteins that could confer a protective effect from amyloid-β, a possible cause of Alzheimer's disease. It was later independently found to have both an anti-apoptotic effect and the ability to bind insulin-like growth factor binding protein 3 (IGFBP3; Guo *et al.* 2003; Ikonen *et al.* 2003). As will be discussed in more detail below, humanin and its analogues have effects on metabolism, increasing glucose-stimulated insulin release and decreasing body weight gain and visceral fat (Kuliawat *et al.* 2013; Gong *et al.* 2015).

In the past year, several new papers have further established the positive effects of humanin in several different domains. Thummasorn *et al.* have confirmed that humanin treatment can protect against ischaemia–reperfusion injury and shown that this may be due to a decrease in reactive oxygen species generation (Muzumdar *et al.* 2010; Zhao *et al.* 2012; Thummasorn *et al.* 2016). Two other papers have shown the importance of humanin in neurocognition by showing that it can prevent diazepam-induced memory dysfunction as well as act as an anxiolytic agent (Murakami *et al.* 2016; Zhao *et al.* 2016). Gidlund *et al.* (2016) discovered that muscle humanin levels were increased during resistance training compared to control and aerobic exercise (Nordic walking), but circulating levels were not affected. The authors believed that this change could be due to humanin's role in glucose metabolism. Looking at the cardiovascular aspects of humanin, Nikolakopoulos *et al.* (2017) examined a population of pre-eclampsia patients where it was found that humanin levels were elevated; they suggested that this could be in response to cardiovascular stress.

Because of the small size of humanin, thorough investigation into the importance of each amino acid residue has already been conducted (reviewed in Yen *et al.* 2013). Both phenylalanine-6 and lysine-21 are required for humanin binding to IGFBP3 (Ikonen *et al.* 2003). Changing serine-14 to a glycine (S14G humanin; HNG) increases the potency of the peptide although the mechanism is still unknown (Hashimoto *et al.* 2001*a*). Other residues have been found to be required for secretion of the peptide into the extracellular matrix (Yamagishi *et al.* 2003). Upon secretion, humanin is believed to activate two different receptors. The first receptor described was the ciliary neurotrophic factor receptor (CNTFR)–gp130 (IL6ST)–the interleukin 27 receptor subunit alpha (WSX1) tripartite receptor that then activates Janus kinase (JAK), signal transducer and activator of transcription (STAT), AKT and extracellular signal-regulated kinases (ERK) (Hashimoto *et al.* 2009; Kim *et al.* 2016; Fig. 2). The formyl peptide receptor like (FPRL) 1 and FPRL2 receptors have also been shown to be activated by humanin and they also signal through ERK (Harada *et al.* 2004; Ying *et al.* 2004). As the first MDP discovered, humanin has been the most comprehensively investigated and both structural and functional aspects have been described. Its function as a cyto-protective, anti-apoptotic peptide has been thoroughly investigated, while its function in cognition is still being examined.

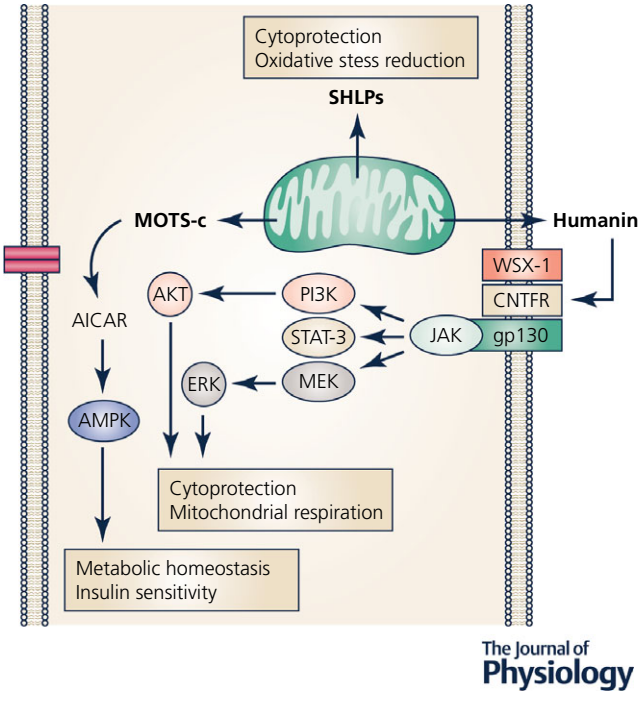

Figure 2. MDP signalling
Schematic illustration of humanin-, MOTS-c- and SHLP-mediated signalling pathways and biological functions. MEK, mitogen-activated protein kinase kinase; PI3K, phosphoinositide 3-kinase.

Newly discovered mitochondrially derived peptides: MOTS-c and SHLPs

In addition to humanin, an *in silico* search of the mitochondrial genome revealed several additional potential MDPs. The mitochondrially derived peptide MOTS-c that was recently discovered by Lee *et al.* (2015, 2016) is a 16 amino acid peptide located in the 12S rRNA gene. It regulates both insulin sensitivity and metabolic homeostasis via AMPK, increases 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) levels and activates AMPK in HEK293 cells (Fig. 2; Lee *et al.* 2015). Reduction of this AMPK activation by chemical compounds or siRNA abolishes the enhanced glucose-stimulated glycolytic response (Lee *et al.* 2015). *In vivo* MOTS-c infusion significantly increased glucose clearance and insulin-stimulated glucose disposal rate in glucose-tolerance test and clamp studies. MOTS-c further prevents high fat diet (HFD)-induced obesity and insulin resistance in CD-1 mice, as well as preventing HFD-induced obesity independent of caloric intake in C57BL/6J mice (Lee *et al.* 2015). Supporting the fact that MOTS-c activates AMPK, Ming *et al.* (2016) showed that MOTS-c inhibits receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclast formation via AMPK activation *in vitro* and suppresses ovariectomy-induced bone loss in mice (Ming *et al.* 2016). More recently, the m.1382A>C polymorphism, which is unique to the Northeast Asian population and causes a Lys14Gln replacement, was found to be correlated to longevity in Japanese people through its putative endocrine action, though further studies will be needed to elucidate the actual mechanism (Fuku *et al.* 2015).

Cobb *et al.* (2016) recently reported another six small humanin-like peptides, small humanin-like peptides (SHLP) 1–6, within the same 16S rRNA gene in which humanin is located (Fig. 1). SHLP2 and SHLP3 demonstrated similar protective effects as humanin and both improved mitochondrial metabolism by increasing oxygen consumption rate and reducing apoptosis and reactive oxygen species (ROS) in NIT-1 and 22RV1 cells. Just as humanin increases mitochondrial biogenesis, SHLP2 and SHLP3 may also increase both mitochondrial biogenesis and oxygen consumption rate. Alternatively, this increase in oxygen consumption rate could be due to increased uncoupling. Further studies on whether MDPs modulate mitochondrial uncoupling will give us a better understanding of the cause of this increase in oxygen consumption rate. Because mitochondrial oxygen consumption is coupled to ATP production, the increase in energy production and its TCA cycle metabolites may enhance mitochondrial metabolism. Intracerebral

infusion of SHLP2 increases glucose uptake and suppresses hepatic glucose production, suggesting that it functions as an insulin sensitizer both peripherally and centrally (Cobb *et al.* 2016). Further supporting their role as insulin sensitizers, both SHLP2 and SHLP3 also enhance 3T3-L1 pre-adipocyte differentiation. Another *in vivo* effect of SHLP2 injection is to increases leptin levels but with no effect on the inflammatory markers interleukin 6 and monocyte chemoattractant protein-1 (MCP-1). On the other hand, SHLP3 elevated both metabolic and inflammatory biomarkers (Cobb *et al.* 2016). Similar to humanin, the circulating levels of MOTS-c and SHLP2 decline with age (Lee *et al.* 2015; Cobb *et al.* 2016), indicating that they are potential regulators of ageing.

With the addition of MOTS-c and SHLP1–6, this class of peptides continues to grow exponentially. Unsurprisingly, because these peptides come from the mitochondrial genome, they are involved in common processes such as apoptosis and metabolism, but even though there is some overlapping function, clearly each MDP also has a unique signalling signature leading to an individual response. Future studies have yet to be conducted to determine the signalling pathways involved.

Mitochondria, mitochondrial peptides and metabolism

Cellular bioenergetics. Mitochondria are the primary energy source for all cellular functions. Mitochondria couple the oxidation of nutritional substrates to ATP synthesis. Carbohydrates, fats and proteins are broken down to glucose, free fatty acids and amino acids that can be utilized by mitochondria to produce ATP. Glycolysis generates 2 ATP whereas mitochondrial oxidation of pyruvate derived from glucose and palmitate derived from fatty acids generates 31.5 and 113 ATP, respectively (Mookerjee *et al.* 2015). These metabolic intermediates are translocated into the mitochondrial matrix and then enter the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. The TCA cycle generates NADH and $FADH_2$ that are fed into the electron transport chain at complex I and II, respectively, to provide electrons. The electron transport chain complexes transfer electrons to oxygen and concomitantly pump protons across the inner mitochondrial membrane to generate an electrochemical proton gradient. This proton-motive force is then utilized for ATP synthesis and active transport processes in the mitochondria. As mitochondria are the primary source of cellular ATP, mitochondrial quality control mechanisms are required for cellular fitness. For example, mitophagy removes damaged mitochondria that lose their membrane potential (Ashrafi & Schwarz, 2013). In addition to mitochondrial turnover, mitochondrial peptides are produced to preserve essential functions related to energy production. Humanin directly affects mitochondrial bioenergetics by increasing basal oxygen consumption rate, maximum respiration, respiration capacity and ATP production in retinal pigment epithelial (hRPE) cells (Sreekumar *et al.* 2016). Thus, humanin protected hRPE cells from oxidative damage (*tert*-butyl hydroperoxide treatment) perhaps by inhibiting the alteration of mitochondrial bioenergetics by maintaining ATP production and mitochondrial reserve capacity. Increased mitochondrial biogenesis is one possible mechanism for how cells optimize mitochondrial bioenergetics when it is required, and humanin increases the copy number of mtDNA, the number of mitochondria and the expression level of mitochondrial transcription factors, suggesting that humanin increases mitochondrial biogenesis (Sreekumar *et al.* 2016). Although seemingly contradictory to the previous study, humanin *suppresses* the increase in mtDNA copy number in serum-deprived lymphocytes (Kariya *et al.* 2003). One possible explanation for this opposite result is that humanin treatment improved basal metabolic activity of the mitochondria in serum-deprived cells. Therefore, the compensatory increase in mtDNA copy number was offset by improved quality of mitochondria. Humanin-treated cells showed higher metabolic activity and mtDNA copy number could be decreased to maintain cellular homeostasis.

The humanin analogue HNG also increases ATP production and mitochondrial reserve capacity. In addition, HNG increases the mitochondrial membrane potential in H9c2 myoblast cells, and it rescues the loss of membrane potential in response to H_2O_2 treatment (Klein *et al.* 2013). Since increased mitochondrial membrane potential is linked to elevated cellular ATP production, in turn, the cellular ATP level is elevated in the presence of HNG in H9c2 cells. Substitution of alanine for Phe6 completely negated the interaction between humanin or HNG and IGFBP3 and generated an enhanced form of humanin (HNF6A or HNGF6A) (Ikonen *et al.* 2003). HNGF6A regulates glucose metabolism and energy production. HNGF6A promoted the glucose-induced GLUT2 transporter translocation to the plasma membrane, and increased glucose oxidation and ATP production in βTC3 cells (Kuliawat *et al.* 2013). HNGF6A also increased the mitochondrial membrane potential in the cells. Similar to humanin, both SHLP2 and SHLP3 increase mitochondrial respiration and ATP production (Cobb *et al.* 2016).

MOTS-c, which is encoded in the 12S rRNA region of mitochondria, has also been reported to influence mitochondrial metabolism. MOTS-c administration increased glucose uptake and glycolysis, whereas it suppressed mitochondrial respiration in cultured cells and skeletal muscle. This resembles a Crabtree effect, namely decreased mitochondrial oxygen consumption rate in response to high glucose uptake (Lee *et al.* 2015). Furthermore, AMPK and sirtuin 1 (SIRT1) siRNA reduce

the glucose-stimulated glycolytic response, which suggests that AMPK and SIRT1 play roles in MOTS-c actions on cellular bioenergetics.

Mitochondrially derived peptides are produced in the mitochondria and play an important role in energy production. Exogenously applied humanin localizes to the mitochondria, so it may directly modulate mitochondrial membrane potential to enhance mitochondrial respiration. MOTS-c may regulate mitochondrial respiration by activation of signalling pathways mediated by AMPK or SIRT1. However, the detailed mechanism concerning the role of MDPs in energy production still needs to be studied.

Amino acid, lipid, and nucleotide metabolism. In addition to producing ATP, mitochondria play important roles in amino acid, lipid and nucleotide metabolism. Thus, the TCA cycle metabolites are utilized for building macromolecules. For example, α-ketoglutarate and oxaloacetate can be transported into the cytosol and are utilized for *de novo* protein and nucleotide synthesis (Bohovych & Khalimonchuk, 2016). In addition, citrate can be transported into the cytosol and is utilized for protein acetylation as well as *de novo* fatty acid synthesis (Wellen *et al.* 2009; Buchakjian & Kornbluth, 2010). One of the mitochondrial peptides, MOTS-c, is closely associated with amino acid and lipid metabolism. MOTS-c modulates the one-carbon metabolism cycle and purine biosynthesis. It also activates AMPK by blocking *de novo* purine biosynthesis, resulting in an accumulation of endogenous AICAR. Moreover, MOTS-c affects fatty acid metabolism via the AICAR–AMPK pathway. As AMPK is the cellular signalling hub for balancing fuel usage and energy demand, MOTS-c stimulates carnitine shuttles, reduces levels of essential fatty acids and increases the β-oxidation intermediates (Lee *et al.* 2015). In addition, MOTS-c increases metabolite levels of NAD^+, glycolysis and the pentose phosphate pathway. Mitochondria modulate amino acid and lipid metabolism in response to cellular homeostatic alteration and metabolic demand. Understanding whether cellular energy demand and metabolic stress can equally regulate MDP expression is an avenue of research that will provide an insight into MDP biology.

Systemic glucose homeostasis and adiposity. Going from the biochemical and cellular level to the physiological level, ATP and metabolites generated from mitochondrial respiration modulate insulin secretion in pancreatic β-cells. In β-cells, glucose-stimulated ATP production increases the ATP/ADP ratio, resulting in closing of ATP-dependent K^+ channels in β-cells, leading to membrane depolarization and activation of the voltage-dependent calcium channel (Muoio & Newgard, 2008). The resulting increase in cytoplasmic calcium concentration leads to exocytosis of insulin in pancreatic β-cells.

Patients with mitochondrial DNA mutations exhibit impaired β-cell function, and inhibition of mitochondrial metabolism can inhibit glucose-stimulated insulin secretion (Suzuki *et al.* 1997; Maechler & Wollheim, 2001). In addition to ATP, metabolites from mitochondrial metabolism including malonyl-CoA, long-chain acyl-CoA and NADPH modulate insulin secretion by inhibiting ATP-dependent K^+ channels (Maechler *et al.* 1997). Additionally, glutamate is generated in the mitochondria from α-ketoglutarate and directly stimulates insulin exocytosis (Maechler & Wollheim, 1999).

Mitochondria are closely associated with insulin function as well as insulin secretion. For example, abnormal morphology, decreased mitochondrial number, decreased mitochondrial oxidative enzymes and lower ATP production were commonly found in insulin-resistant metabolic tissues including skeletal muscle, liver and adipose (Kim *et al.* 2008; Oropeza *et al.* 2015). Elevated circulating free fatty acids accumulating in these tissues will also decrease insulin-stimulated glucose disposal (Boden *et al.* 1994; Shah *et al.* 2002; Boden, 2005). This impaired insulin signalling is a major cause of insulin resistance because it not only affects insulin-stimulated glucose metabolism in skeletal muscle but also impairs other actions of insulin in diverse tissues including liver, adipose tissue and heart. Therefore, glucose and lipid metabolism in the mitochondria plays a critical role in insulin signalling and glucose homeostasis.

Mitochondrial bioenergetics and metabolism are closely associated with insulin signalling and glucose homeostasis (Pagel-Langenickel *et al.* 2008; Auger *et al.* 2015). As MDPs modulate cellular bioenergetics and metabolism *in vitro*, they also show systemic regulation of metabolism *in vivo*. Moving from *in vitro* to *in vivo systems*, the typical dosage administered to rodents is possibly supraphysiological. It should be noted that the half-life of humanin and probably most MDPs is in the minutes range and so after an hour, circulating levels of these MDPs would return to baseline (Chin *et al.* 2013). Multiple animal model studies administering humanin and its analogues support the crucial role of humanin in glucose homeostasis. Intracerebroventricular administration of humanin led to increased insulin sensitivity in the liver and muscle, causing a reduction of hepatic glucose production and increased insulin-mediated AKT signalling and fatty acid metabolism signalling (Muzumdar *et al.* 2009). These effects were modulated by humanin-mediated STAT-3 activation in the hypothalamus. Peripheral administration of humanin also enhanced peripheral glucose uptake and suppressed hepatic glucose production. HNGF6A, HNG and humanin, but not HNF6A, show insulin-sensitizing effects. HNGF6A increased glucose-stimulated insulin secretion in isolated islets from both normal and *db*/*db*

mice and in mouse pancreatic cells (βTC3) (Kuliawat *et al.* 2013). Elevated ATP production and the activity of aspartate aminotransferases of the malate–aspartate NADH shuttle are key mechanisms of HNGF6A regulation of insulin secretion. A hyperglycaemic clamp study found that HNGF6A enhanced glucose-stimulated insulin secretion in young Sprague–Dawley rats (Muzumdar *et al.* 2009). Additionally, HNGF6A significantly lowers blood glucose in Zucker diabetic fatty rats. The direct effect of HNGF6A on isolated islets and βTC3 cells suggests that HNGF6A mitigates some of the metabolic abnormalities present in islets in type 2 diabetes (Hoang *et al.* 2010; Kuliawat *et al.* 2013). Recently, a new role of humanin in lipid metabolism was revealed. Intraperitoneal administration of HNG decreased body weight gain, visceral fat and hepatic triglyceride accumulation in high fat diet-fed mice (Gong *et al.* 2015). Increased energy expenditure was examined in HNG-injected mice, partially explaining the decrease in body weight gain and visceral fat in these mice. The decrease in hepatic triglyceride accumulation is caused by increased activity of hepatic microsomal triglyceride transfer protein and increased hepatic triglyceride secretion. Vagotomy was performed on mice to investigate the role of the hypothalamus in hepatic triglyceride secretion. When these mice were injected with HNG, the surgery blocked humanin's effect of both intravenous and intracerebroventricular infusion on hepatic triglyceride secretion. These results suggest that the effect of humanin is mediated through the hypothalamus as the vagus nerve serves as an efferent connection from the hypothalamus to the liver, but not by a neuroendocrine signal.

SHLP2 and SHLP3 have insulin-sensitizing effects *in vitro* and *in vivo*. Both SHLP2 and SHLP3 accelerated 3T3-L1 cell (a murine pre-adipocyte cell line) differentiation in the presence of insulin (Cobb *et al.* 2016). This suggests that SHLP2 and 3 promote cellular differentiation and enhance insulin sensitivity in adipose tissue. Furthermore, SHLP2, but not SHLP3, enhances the insulin-sensitizing effect of hepatic glucose production suppression and increased glucose disposal in peripheral tissues. Both ATP and mitochondrial respiration metabolites are equally important for insulin secretion. Although both SHLP2 and SHLP3 enhance ATP production, their modulation of different metabolites could be the mechanism differentiating their distinct effects *in vivo*. Further investigation to address the mechanism is required.

MOTS-c enhances whole-body insulin sensitivity, acting primarily through the muscle. MOTS-c increases the insulin-stimulated glucose disposal rate, an indicator of enhanced skeletal muscle insulin sensitivity, but does not alter the rate of hepatic glucose production (Lee *et al.* 2015). Insulin-mediated AKT signalling is elevated in the muscle isolated from MOTS-c-injected C57BL/6J mice, and differentiated L6 rat myotubes overexpressing MOTS-c have accelerated glucose uptake and enhanced glucose-stimulated and maximum glycolytic rate. The role of MOTS-c in enhancing insulin sensitivity and glucose homeostasis has also been examined in high fat diet-fed CD-1 mice. MOTS-c-treated HFD-fed mice showed reduced weight gain but did not show any difference in food intake. This result suggests that MOTS-c may increase the metabolic rate of these mice and experiments using metabolic cages found that HFD-fed mice treated with MOTS-c showed increased respiratory exchange ratio, reflecting increased glucose utilization. This result suggests that MOTS-c may increase the metabolic rate of these mice and experiments using metabolic cages found that HFD-fed mice treated with MOTS-c showed increased energy expenditure and respiratory exchange ratio, reflecting increased glucose utilization. Hepatic lipid accumulation was dramatically reduced in HFD-fed mice treated with MOTS-c and MOTS-c prevented HFD-induced hyperinsulinaemia, indicating improved glucose homeostasis. Moreover, MOTS-c promoted AMPK activation and GLUT4 expression in the skeletal muscles of HFD-fed mice.

Humanin's physiological effects are well established and changes in glucose utilization and insulin sensitization have been found. Hypothalamic signalling is central to these effects as is STAT3 signalling. In contrast, the physiological effects of MOTS-c and the SHLPs have yet to be thoroughly established. While there are hints of a mechanism, much more research will be required to discover the signalling pathways activated by these MDPs.

Signalling centre. The two functions of mitochondria, to generate ATP and to support biosynthesis, are balanced to meet cellular needs. Clearly, mitochondria receive signals in response to stress and metabolic changes (anterograde signalling) (Quirós *et al.* 2016), but emerging data suggest that mitochondria are also actively sending signals back to the cytosol and nucleus (retrograde signalling) (Picard *et al.* 2013). The mitochondrial unfolded protein response is one of the retrograde signalling pathways that increase mitochondrially localized chaperones and proteases to recover mitochondrial protein homeostasis (Nargund *et al.* 2012; 2015). Interestingly, the mitochondrial unfolded response also modulates cellular metabolism including increased glycolysis and decreased expression of TCA cycle and oxidative phosphorylation genes, potentially to reduce mitochondrial stress and alter cellular metabolism to promote survival (Nargund *et al.* 2015; Quirós *et al.* 2016). In addition to the mitochondrial unfolded protein response, mitochondrially derived peptides are emerging as another retrograde signal in response to cellular stress (Lee *et al.* 2013). Although MDPs play important roles in the regulation of cellular

energetics and systemic metabolism, whether MDPs can modulates gene expression and whether there is epigenetic modification in the nucleus remain unexplored. Investigation into the role of MDPs in the nucleus will give us a more comprehensive understanding of MDP signalling.

Conclusions

Mitochondria are metabolic hubs within cells that alter cellular functions in response to cellular stress. Emerging evidence shows that mitochondrially derived peptides are retrograde signalling molecules. These peptides regulate mitochondrial bioenergetics and mitochondrial metabolism; subsequently, they modulate systemic insulin sensitivity and glucose homeostasis. In addition to their intracellular effects, MDPs are found in the circulation and represent a novel method for the mitochondria to signal to the CNS and other peripheral tissues. Although relatively recently compared to humanin, other MDPs are being discovered and their role in metabolism is still emerging. Many more studies will be required to elucidate the mechanism of action of each of these MDPs. Further translational studies will also be required to test how MDPs can be diagnostic markers and potent therapeutics for metabolic diseases including type 2 diabetes.

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Additional information

Competing interests

P.C. is a consultant and stockholder of CohBar Inc. K.Y. has consulted for CohBar Inc.

Author contributions

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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