**Asprosin, a novel pleiotropic adipokine implicated in fasting and obesity-related cardio-metabolic disease: comprehensive review of preclinical and clinical evidence**

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**Abstract**

White adipose tissue is a dynamic endocrine organ that releases an array of adipokines, which play a key role in regulating metabolic homeostasis and multiple other physiological processes. An altered adipokine secretion profile from adipose tissue depots frequently characterizes obesity and related cardio-metabolic diseases. Asprosin is a recently discovered adipokine that is released in response to fasting. Following secretion, asprosin acts - via a G-protein coupled receptor and potentially via other unknown receptor(s) - on hepatocytes and agouti-related peptide-expressing neurons in the central nervous system to stimulate glucose secretion and promote appetite, respectively. A growing body of both i*n vitro* and *in vivo* studies have shown asprosin to exert a number of effects on different metabolic tissues. Indeed, asprosin can attenuate inulin signalling and promote insulin resistance in skeletal muscle by increasing inflammation and endoplasmic reticulum stress. Interestingly, asprosin may also play a protective role in cardiomyocytes that are exposed to hypoxic conditions. Moreover, clinical studies have reported elevated circulating asprosin levels in obesity, type 2 diabetes and other obesity-related cardio-metabolic diseases, with significant associations to clinically relevant parameters. Understanding the spectrum of the effects of this novel adipokine is essential in order to determine its physiologic role and its significance as a potential therapeutic target and/or a biomarker of cardio-metabolic disease. The present review offers a comprehensive overview of the published literature on asprosin, including both clinical and preclinical studies, focusing on its role in metabolism and cardio-metabolic disease.

**Keywords:** Asprosin, adipose tissue, adipokines, obesity, diabetes, cardiovascular disease

## Introduction

Over the past half century, obesity has become one of the most prevalent chronic non-communicable diseases worldwide [1,2]. This marked increase in the global obesity prevalence is attributed to a number of factors which collectively promote an obesogenic lifestyle and broader environment, including lifestyle westernisation (*e.g.* energy/fat-rich diet and sedentary lifestyle), socio-economic determinants (*e.g.* urbanization, low socio-economic status and deprivation), and changes in food processing and marketing (*e.g.* processed red meat and aggressive marketing of sugar-sweetened drinks) [1,3]. These factors facilitate the accumulation of excess adipose tissue due to a chronic positive balance between energy intake and energy expenditure, resulting in developing and sustaining obesity over time (often from a young age) [1,3]. It is now well-established that the pathophysiologic changes of adipose tissue in the course of obesity mediate the development of the cardio-metabolic complications which characterize chronic obesity and are frequently manifested as a constellation in the context of the metabolic syndrome [4].

Indeed, adipose tissue is now considered a vital, dynamic endocrine organ and not just a passive storage depot of excess energy [4,5]. This endocrine function of adipose tissue is mediated by a broad range of adipose-derived factors (*i.e.* adipokines), which are directly or indirectly implicated in the regulation of multiple physiological processes, including energy homeostasis and appetite, as well as immune and cardio-metabolic functions (*e.g.* regulation of blood pressure) [6,7]. The number of identified adipokines has been rapidly increasing during the past two decades [8,9], and a growing body of *in vitro*, *in vivo* and clinical data indicate that these factors play distinct roles in the pathophysiology of obesity-related cardio-metabolic diseases [10,11]. Asprosin represents one of the recently identified adipokines [12], with emerging data suggesting that it may exert various effects implicated in a number of cardio-metabolic diseases, including type 2 diabetes mellitus (T2DM), polycystic ovary syndrome (PCOS), non-alcoholic fatty liver disease (NAFLD), and heart disease. As such, the present review focuses on this novel adipokine and offers a comprehensive overview of

the available evidence on the effects of asprosin in metabolism and inflammation, including both preclinical and clinical data.

## Fibrillin 1 (*FBN1*) gene and asprosin

Asprosin is produced together with fibrillin-1 (a large, extracellular matrix glycoprotein) by furin-mediated c-terminal cleavage of the pro-protein (profibrillin) encoded by the *Fibrillin 1 (FBN1)* gene [12,13]. Indeed, the amino acid sequence of asprosin is encoded by the last two exons of *FBN1*, namely exon 65 and 66 [12]. Mammalian asprosin is approximately 30 kDa and consists of 140 amino acids. Interestingly, a recent study that was able to form a strain of *Pichia pastoris* which expresses asprosin showed three glycosylation sites (threonine 8, 9 and 10) in two amino acid sequences of asprosin [14].

Asprosin was first described by Romere *et al*. (2016) who investigated the sequencing of genomic DNA of patients with neonatal progeroid syndrome (NPS) and reported that mutations at the 3' end of the *FBN1* gene were present in two of these patients. These mutations translated to a truncated form of profibrillin [12], and were in accord to similar mutations in the *FBN1* gene of patients with NPS that had been previously reported by other studies [15–18]. Romere *et al.* (2016) confirmed that these mutations were around the c-terminal cleavage site, resulting in the absence of asprosin [12]. As such, these patients with NPS had significantly reduced circulating levels of asprosin compared to control subjects [12].

In both humans and mice, the greatest *FBN1* expression is observed in the white adipose tissue, as well as in the lungs and heart [12]. Although *FBN1* expression has been found to decrease during adipocyte differentiation, asprosin is readily secreted by mature adipocytes, thus representing an adipokine [12,13,19]. Accordingly, genetic ablation of adipose tissue in mice resulted in significantly decreased circulating asprosin levels compared to wild-type mice [12]. Notably, patients with NPS

lack subcutaneous adipose tissue, which contributes to appearing older [17,18], and may also explain the low levels of circulating asprosin in these patients.

**Glucogenic and orexigenic effects of asprosin**

Asprosin directly acts on the liver, as well as on appetite-regulating neurons of the brain [12,20]. To date, asprosin has been shown to strongly bind to the membrane of hepatocytes and promote glucose production via a cyclic-AMP (cAMP)-protein kinase A (PKA) pathway [12]. Indeed, in the initial study by Romere *et al.*, asprosin was reported to elicit its effects through a G-protein coupled receptor (GPCR), which was later identified as the olfactory receptor OLFR734 in mice [12,21]. *Olfr734* mRNA is highly expressed in the olfactory epithelium and olfactory bulb, as well as in the liver, kidneys and testis of mice. Using a liver-specific *Olfr734*-knockdown mouse model, Li *et al.* (2019) showed that asprosin treatment significantly reduces the hepatic expression of gluconeogenic genes and the circulating glucose levels in these knockdown mice compared to wild-type counterparts [21]. The latter showed an increase in circulating asprosin levels in response to fasting, with a corresponding significant increase of hepatic cAMP levels and glucose production, whereas these effects were significantly reduced in *Olfr734*-knockout mice [21]. These findings further validate the initial findings of Romere *et al.* (2016), indicating that asprosin is a glucogenic adipose-derived hormone that acts through the OLFR734 receptor. It should be highlighted that the glucose production in asprosin-treated *Olfr734*-knockdown mice was reduced, but was not completely abolished; thus, asprosin may be utilising additional receptors on hepatocytes which are yet to be identified. In addition, the human orthologue for OLFR734, *i.e.* OR4M1, remains to be investigated in relation to asprosin effects.

Furthermore, by creating a mouse model that is representative of NPS at a molecular level ($Fbn1^{NPS/+}$) and investigating the effects of asprosin on appetite, Duerrschmid *et al.* (2017) reported asprosin as an orexigenic hormone [20]. Similar to patients with NPS, $Fbn1^{NPS/+}$ mice were extremely lean,

consumed fewer calories due to low appetite (hypophagia) and had reduced plasma asprosin levels compared to wild-type mice. Notably, asprosin is able to cross the blood brain barrier and activate agouti-related peptide-expressing ($AgRP^+$) neurons (neurons that produce agouti-related protein to promote food intake and reduce energy expenditure) via a G-protein ($G\alpha_s$)-cAMP-PKA pathway [20,22]. As such, asprosin was shown to restore the firing frequency and resting membrane potential of $AgRP^+$ neurons in *Fbn1*$^{NPS/+}$ mice. Accordingly, asprosin-treated wild-type mice consumed more calories than non-treated mice, whilst ablation of $AgRP^+$ neurons abolished the appetite-stimulating effects of asprosin [20]. Moreover, asprosin reduced the firing frequency and resting membrane potential of neurons that produce pro-opiomelanocortin (POMC) to promote satiety ($POMC^+$ neurons) [20,22]. In line with these effects, decreased food intake was observed in mice with *Olfr734* knockdown after overnight fasting compared to wild-type mice [23]. Further investigation found that OLFR734 is expressed in $AgRP^+$ neurons, and that the proportion of active $AgRP^+$ neurons is significantly decreased in *Olfr734*$^{-/-}$ mice [23]. Interestingly, in accord with the findings of Duerrschmid *et al.* (2017), asprosin administration in wild-type mice promoted activation of $AgRP^+$ neurons; however, this effect was not observed in *Olfr734*$^{-/-}$ mice [20,23]. These findings suggest that the glucogenic and orexigenic effects of asprosin in mice are elicited through similar pathways via OLFR734 in hepatocytes and $AgRP^+$ neurons, respectively. Of note, Liu *et al.* (2020) also found that asprosin was able to enhance the olfactory performance of mice fed a high-fat diet (HFD) which was otherwise impaired [23]. As OLFR734 is highly expressed in the olfactory bulb, it is important to further explore the role of asprosin in olfaction, particularly since the sense of smell is increasingly linked to the regulation of appetite and palatability [24].

**The role of asprosin in inflammation and insulin action**

Obesity is characterized by the development of a chronic, generalized, low-grade inflammatory state due to increased production of pro-inflammatory mediators from the accumulated adipose tissue

which are secreted locally and enter the circulation [4,25,26]. In insulin-sensitive tissues (*e.g.* in skeletal muscle and adipose tissue) obesity-related inflammation can impact on insulin signalling, leading to insulin resistance [27]. Recently, a study by Jung *et al.* (2019) showed that asprosin can bind to myocytes with high affinity and promote insulin resistance. Moreover, asprosin also reduced insulin-stimulated glucose uptake in both a mouse skeletal muscle cell line (C2C12) and human primary skeletal muscle cells [28]. This was achieved by inhibiting the phosphorylation of insulin receptor substrate-1 (IRS-1; a key signalling protein for insulin action) and protein kinase B (Akt; a serine/threonine-specific protein kinase) [28]. In addition, *in vivo* experiments revealed that asprosin treatment led to glucose intolerance and insulin resistance in mice, as well as increased endoplasmic reticulum (ER) stress and circulating levels of pro-inflammatory cytokines, such as macrophage chemoattractant protein 1 (MCP-1), tumour necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) [28]. These effects of asprosin were found to be mediated through the protein kinase C delta (PKCδ)-sarco/endoplasmic reticulum Ca2+-ATPase 2 (SERCA2) pathway. PKCδ has been previously found to directly inhibit tyrosine phosphorylation of IRS-1 by phosphorylating IRS-1 at multiple sites in rat hepatocytes [29], whilst another study had found that deletion of PKCδ from muscle improved whole body insulin-sensitivity and insulin signalling in mice [30]. In addition, a PKC-dependent pathway is involved in pro-inflammatory cytokine production [31]. Therefore, PKCδ silencing in the study by Jung *et al.* (2019) reversed the asprosin-induced effects on nuclear translocation of nuclear factor kappa B (NF-κB), ER stress, IRS-1 and Akt phosphorylation [28].

In contrast to the findings by Jung *et al.* (2019), a more recent study found that asprosin treatment significantly upregulated the gene and protein expression of glucose transporter type 4 (GLUT4; a key glucose transporter that is regulated by insulin) and augmented AMPK phosphorylation, resulting in increased glucose uptake by C2C12 muscle cells [14]. This study also reported that asprosin did not affect Akt phosphorylation [14]. As these findings are conflicting, further experimental work is clearly required to elucidate the exact effects of asprosin on skeletal muscle.

Palmitate is a saturated fatty acid that has been found to promote oxidative and ER stress, as well as inflammation and apoptosis in pancreatic β-cells, ultimately impairing insulin secretion [32,33]. Lee *et al*. (2019) showed that palmitate increased asprosin secretion from pancreatic β-cells, and that silencing of asprosin ameliorated the detrimental effects of palmitate, indicating that asprosin mediates these effects of palmitate in an autocrine manner [33]. In this study, asprosin increased intracellular toll-like receptor 4 (TLR4) expression, which consequently increased c-Jun N-terminal kinase (JNK) phosphorylation. Contrary, silencing of TLR4 and JNK suppressed the effects of asprosin on the expression of pro-inflammatory cytokines (TNFα and MCP-1) and apoptosis, whist also improved cell viability and insulin release [33]. These findings indicate that the TLR4-JNK pathway is responsible for asprosin-induced pancreatic β-cell dysfunction. However, the receptor responsible for mediating these effects of asprosin on pancreatic β-cells, as well as skeletal muscle cells, remains to be clarified.

**Asprosin in obesity**

Circulating asprosin levels are significantly elevated in individuals with increased body weight (overweight or obesity) [12,34–37]. In addition, Ugur and Aydin (2019) reported that the concentration of asprosin in both the serum and saliva increased with body mass index (BMI), with the highest asprosin levels being observed in patients with class III obesity (BMI >45 kg/m$^2$) [35]. In this study, asprosin concentrations were approximately 4-fold and 6-fold higher in the serum and saliva, respectively, of patients with class III obesity compared to the control group, and exhibited significant positive correlations not only with BMI, but also with glucose and low-density lipoprotein (LDL) levels [35]. Asprosin levels in saliva, but not in serum, also correlated positively with circulating triglyceride concentrations. Such correlations between asprosin and glycemic and/or lipidemic parameters were not previously detected in an earlier study by Wang *et al.* (2019) which also reported significantly higher circulating asprosin levels in patients with obesity [34,35]. The

latter study, however, showed that circulating asprosin levels significantly decreases 6 months after bariatric surgery [34]. Notably the existing data on circulating asprosin levels in children with obesity are conflicting. Long *et al.* (2019) have reported significantly lower fasting asprosin serum levels in children with obesity compared to a normal-weight control group, with lower asprosin concentrations observed in boys compared to girls in the group with obesity [38]. Similar findings have recently been reported by Corica *et al.* (2021). However, Silistre and Hatipoğl (2020) found significantly higher concentrations of asprosin in the serum of children with obesity compared to normal-weight counterparts, with no gender-related differences [37].

Interestingly, plasma asprosin levels are significantly elevated in patients with anorexia nervosa compared to healthy controls [40]. When comparing between the restricting type and binge-eating subtype of anorexia nervosa, no significant difference in circulating asprosin levels was observed [40]. Of note, in this study circulating glucose levels were not significantly different between anorexia nervosa patients and control subjects, and no correlation was observed between plasma asprosin and glucose levels. As asprosin release is induced by fasting [12], this increase in circulating asprosin in patients with anorexia nervosa may be a response to starvation. However, asprosin levels were negatively correlated with duration of illness in these patients, suggesting that in the long-term circulating asprosin is likely to decline possibly due to reduction in fat [40].

**Asprosin in T2DM**

A number of studies have investigated the levels of circulating asprosin in patients with T2DM. Indeed, Zhang *et al.* (2019) reported elevated levels of circulating asprosin in patients with T2DM compared to healthy controls, which further independently predicted both fasting blood glucose and triglyceride levels in T2DM [41]. These findings are in accord to those of Li *et al.* (2018) who also found increased plasma asprosin levels in a T2DM cohort and a significantly positive correlation between plasma asprosin and fasting blood glucose levels, although no correlation with plasma

triglyceride levels was present in this study [42]. Another study with similar findings further reported that 120 minutes after a 75-gram oral glucose tolerance test, circulating asprosin levels decreased in participants with normal glucose tolerance, but not in patients with T2DM [43]. These findings suggest an impairment of asprosin response to glucose fluctuations in patients with T2DM [43]. Moreover, higher circulating asprosin levels have also been noted in patients with newly diagnosed T2DM compared to healthy controls, with significant positive correlations to BMI, the homeostatic model assessment of insulin resistance (HOMA-IR) and both fasting blood glucose and triglyceride levels [44]. In addition, plasma asprosin concentrations appear to be even higher in patients with impaired glucose regulation (IGT) compared to patients with newly diagnosed T2DM [45]. This study also revealed significant positive correlations between circulating asprosin levels and HOMA-IR, fasting blood glucose and triglyceride levels, whereas a negative correlation with β-cell function were also noted [45]. Collectively, these findings suggest that asprosin may be an early indicator of glucose and lipid dysregulation and insulin resistance, which could potentially be used as a surrogate biomarker for the risk of developing T2DM. Interestingly, a recent study reported that metformin treatment (2000 mg/day for three months) in patients with T2DM resulted in decreased serum and saliva asprosin levels, which were no longer statistically different to the those of healthy controls [46].

As diabetic nephropathy with impaired kidney function is a key long-term complication in patients with diabetes [47,48], a recent study by Deng *et al.* (2020) compared serum asprosin levels in patients with T2DM and normo-albuminuria, micro-albuminuria, or macro-albuminuria, showing that these were significantly elevated in the two latter groups, with even higher levels in the patients with macro-albuminuria [49]. This study further reported a number of correlations between circulating asprosin and markers of kidney function, including a significantly positive correlation with urine albumin-to-creatinine ratio and a negative correlation with the estimated glomerular filtration rate [49]. These data suggest that circulating asprosin levels may be related to the progression of kidney disease in patients with T2DM; however, further studies are required to clarify such potential associations.

**Asprosin in gestational diabetes mellitus**

Circulating levels of multiple adipokines are known to exhibit significant changes in women with gestational diabetes mellitus (GDM) [50,51]. Recently a number of studies have reported significantly increased circulating levels of asprosin in GDM [52–54]. Baykus *et al*. (2019) were the first to show increased circulating asprosin levels in women with GDM, as well as in women with pre-eclampsia (especially those with severe pre-eclampsia and macroscopic foetuses) compared to healthy pregnant women [52]. In this study, asprosin levels were also reported to be elevated in neonatal venous and arterial cord blood from women with GDM and pre-eclampsia [52]. These findings were also confirmed by Zhong *et al*. (2020) who further reported significantly higher circulating asprosin levels in women with GDM at both the second trimester (week 18-20) of pregnancy and before delivery compared with healthy pregnant women [53]. Interestingly, in this study circulating asprosin levels between the $24^{th}$ and $28^{th}$ gestational week in women with GDM were not statistically different to the control group [53]. This study also reported higher asprosin levels in the umbilical cords of neonates born to mothers with GDM [53]. Furthermore, data from Rezk *et al.* (2020), using a rat model of GDM by injecting rats with 2% streptozotocin at day 3 of pregnancy, showed that plasma asprosin levels were higher in pregnant compared to non-pregnant rats and that rats with GDM exhibited significantly elevated plasma asprosin levels compared to the healthy counterpart [54]. Notably, plasma asprosin levels were significantly reduced in insulin-treated GDM rats and were higher on day-20 than day-7 in all groups, although changes between groups were seen as early as day-7 [54]. In this animal study, plasma asprosin levels showed positive correlations with BMI, food intake, HOMA-IR, blood glucose and LDL levels, whereas correlated negatively with β-cell function and HDL levels [54]. Taken together, the results from these studies suggest that circulating asprosin levels may rise early in the course pregnancy and could be augmenting glucose production and promoting insulin resistance in GDM.

**Asprosin and non-alcoholic fatty liver disease**

Non-alcoholic fatty liver disease (NAFLD) describes the spectrum of metabolic-associated liver diseases that are not related to alcohol consumption and arise due to excess hepatic accumulation of lipids (steatosis) [55]. Excess lipids in the liver can promote inflammation (NASH; non-alcohol steatohepatitis), which can further progress to liver fibrosis and then liver cirrhosis [55,56]. A recent study by Ke *et al.* (2020) showed that serum asprosin levels were significantly elevated in patients with non-treated NAFLD compared to a healthy control group, whereas the serum levels of the anti-inflammatory adipokine adiponectin were significantly decreased [57]. In the NAFLD group of this study, positive correlations were noted between serum asprosin level and HOMA-IR, albumin, fasting glucose, and triglyceride levels [57]. Of note, in this study asprosin was independently related to both fasting glucose and triglyceride levels [57]. Given the effects of both asprosin and adiponectin on insulin signalling [28,58], the data by Ke *et al.* suggest that the changes in the levels of these two adipokines may be a contributing factor to the development of insulin resistance in NAFLD. However, recently reported data from a study in 97 patients with NASH showed that serum and saliva levels of asprosin were significantly lower in these patients compared to a control group [59]. It is plausible that circulating asprosin levels may change as NAFLD progresses from simple hepatic steatosis to NASH; however, further investigations are required to explore the role of asprosin in such metabolic-related liver diseases.

**Asprosin and heart disease**

Dilated cardiomyopathy is a common form of heart disease, which is mainly characterised by impaired left ventricular function due to dilation of the ventricle, resulting in systolic dysfunction [60]. Wen *et al.* (2020) reported that patients with dilated cardiomyopathy had significantly higher circulating asprosin levels compared to a healthy control group [61]. This study also reported that the patients with lower plasma asprosin levels had a greater risk of an adverse cardiovascular event over

a 5-year follow-up period compared to those with high plasma asprosin levels [61]. *In vitro* data from this study further showed that asprosin improved cell viability of cardiomyoblasts in a dose-dependent manner under hypoxic conditions [61]. Indeed, a significant reduction in mitochondrial respiration was observed in cardiomyoblasts treated with hydrogen peroxide ($H_2O_2$); however, the addition of asprosin significantly improved the mitochondrial function in $H_2O_2$-treated cells. Interestingly, no difference was observed between control cells without $H_2O_2$ and cells treated with asprosin alone, suggesting that asprosin primarily helps restore mitochondrial function under hypoxic conditions [61]. As one of the underlying mechanisms implicated in dilated cardiomyopathy is mitochondrial dysfunction [60], these findings suggest a potential relevant therapeutic role for asprosin. Moreover, the protective effects of asprosin appear to extend to mesenchymal stromal cells. A study using mesenchymal stromal cells pre-treated with asprosin showed that injecting such cells into infarcted mice hearts improved both the survival of mesenchymal stromal cells and the left ventricular ejection fraction compared to controls, whereas it reduced cardiac fibrosis [62]. This study further reported that asprosin treatment protected mesenchymal stromal cells from $H_2O_2$-induced reactive oxygen species (ROS) production and apoptosis via upregulation of superoxide dismutase 2 (SOD2) and activation of the ERK 1/ 2 (extracellular signal-regulated kinases) pathway [62]. Although further relevant research is still needed, the emerging evidence for the potentially protective role of asprosin in heart tissue is promising.

**Asprosin and polycystic ovary syndrome (PCOS)**

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, which is characterised by a combination of manifestations/symptoms, including anovulation, irregular menstrual cycles, hyperandrogenism and presence of polycystic cysts [63–65]. Notably, PCOS is closely associated with obesity (particularly visceral/central) and metabolic dysregulation *(e.g.* insulin resistance and dyslipidemia) that increase the risk of developing obesity-related

metabolic disorders including T2DM and cardiovascular disease [63,65]. Indeed, the majority of women with PCOS have increased body weight (overweight or obesity) and this increased adiposity (particularly visceral/central) can exacerbate a vicious cycle between insulin resistance and hyperandrogenism [65–67]. Emerging clinical data show that circulating asprosin levels appear to be higher in women with PCOS compared to respective controls [42,68]. Interestingly, when Li *et al*. (2018) further sub-categorized the studied women with PCOS based on BMI, there was no difference in the plasma asprosin levels between those with normal weight and overweight/obesity, potentially due to the small sample size of the groups (12 and 29 women with PCOS, respectively) [42]. In the women with PCOS of this study, after adjusting for age, plasma asprosin levels correlated positively to testosterone levels, as well as to a number of metabolic parameters, including HOMA-IR, fasting glucose and LDL levels [42]. Similar findings have been reported by Alan *et al*. (2018) who showed elevated levels of circulating asprosin in women with PCOS, which also related to insulin resistance [68]. Given that asprosin can promote insulin resistance in skeletal muscle [28], it is plausible that increased circulating asprosin levels can contribute to the development of insulin resistance in PCOS. However, a larger study in Taiwan which included 444 women with PCOS that were recruited over a 7-year period found no difference in circulating asprosin levels compared to a healthy control group [69]. Moreover, a recent study found that circulating asprosin levels were lower in healthy women on oral contraception compared to those not using oral contraception, with the highest concentrations noted in the early follicular phase and the mid-luteal phase of the menstrual cycle of women that used or not used oral contraception, respectively [70]. This study suggests that oral contraception use and menstrual cycle phase should be controlled for in studies investigating circulating asprosin concentrations in women. This is particularly relevant to studies in PCOS, since women with PCOS frequently exhibit menstrual disorders and/or are on treatment with oral contraceptives. Of note, two of the three studies mentioned above did not recruit women who were taking oral contraception [68,69]. Overall, the potential role of asprosin in PCOS merits further research, particularly in women with different phenotypes of PCOS and of different ethnic backgrounds.

## Asprosin and male fertility

Male fertility is dependent on the quality of sperm, including sperm quantity and viability, as well as sperm motility [71]. Interestingly, the mRNA of the known mouse receptor for asprosin, OLFR734, is shown to be highly expressed in mice testes [21,72]. A study by Wei *et al.* (2019) showed no difference in spermiogenesis between 10-week-old male *Olfr734*$^{-/-}$ and wild-type mice, which also had similar testis weight, morphology and histology, as well as body weight and blood glucose level [72]. However, the ability of the sperm to move in one clear direction was significantly impaired in the *Olfr734*$^{-/-}$ mice of this study [72]. In addition, *Olfr734*$^{-/-}$ mice exhibited a greater proportion of sperm with slow motility and reduced ATP levels, and lacked fertilisation potential compared to wild-type mice [72]. Asprosin treatment had no effect on spermiogenesis; however, it almost restored sperm ATP and cAMP levels, which are essential for the spermatozoon movement in the female reproductive tract, and the fertilisation potential in 40-week-old wild-type mice to that of 10-week-old mice [72]. Notably, these effects were not observed in *Olfr734*$^{-/-}$ mice [72]. Collectively, these findings indicate that asprosin may be implicated in male fertility, although potential associations between asprosin and sperm quality in men are yet to be investigated.

## Asprosin and exercise

It is well-documented that significant changes in the levels of circulating adipokines/cytokines can occur as a result of exercise [73,74]. Accordingly, a number of recent studies have reported changes in circulating asprosin levels in response to aerobic and anaerobic exercise. An animal study by Ko *et al.* (2019) investigated the effects of 8 weeks of aerobic exercise (4 days per week) on hepatic asprosin levels in a rat model of streptozotocin-induced type 1 diabetes mellitus (T1DM). In this study, asprosin protein levels in the liver were significantly higher in T1DM rats compared to the control group [75]. However, hepatic asprosin levels significantly decreased in the T1DM rats that

underwent aerobic exercise training, which became similar to those the control group [75]. The levels of hepatic PKA and transforming growth factor beta (TGF-β) also significantly decreased, while AMPK levels increased after this exercise intervention [75]. Studies have shown that hepatic TGFβ1 knockdown and increased hepatic AMPK activity can lower blood glucose levels, by suppressing glucose production, and promote insulin sensitivity [76,77]. As such, the findings from Ko *et al.* (2019) suggest that aerobic exercise lowers asprosin levels, which, in turn, attenuates glucose production via the PKA pathway through TGF-β inhibition and increased AMPK activity [75]. In line with these findings, another study in rats reported that, although there was no difference between the two training modes, continuous and interval swimming training (5 days/week for 8 weeks) significantly reduced serum asprosin levels in rats with metabolic syndrome [78]. In these rats, a significant decrease was also observed in circulating triglyceride, LDL, glucose and total cholesterol levels [78]. Asprosin only positively correlated with glucose levels in the rats with metabolic syndrome, indicating that exercise is effective in lowering circulating asprosin levels and ultimately improving glucose homeostasis [78].

More recently, the acute effects of aerobic exercise (30 minutes of moderately intense aerobic exercise in the morning and the evening) on circulating asprosin levels were examined in adult men with normal weight and overweight/obesity [36]. A significant decrease in serum asprosin levels was reported after exercise in both groups, with a greater decrease being observed in the evening in men with overweight/obesity [36]. Interestingly, these participants also consumed significantly less calories compared to those in the normal weight group [36]. Moreover, an acute effort of anaerobic exercise (a single bicycle sprint effort) has been shown to increase circulating levels of asprosin at 15, 30 and 60 min, and 24 hours following the exercise in women, but not in men [79]. In this study, serum leptin levels decreased at 3, 15, and 30 min after the exercise in women, whilst an increase in IL-6 levels was noted at 3, 15, 30, and 60 min after the exercise in men and at 15, 30, 60, and 24 hours following the exercise in women, with a decrease in IL-1β in men at 15, 30, and 60 min after the exercise, and at 15 and 30 min after the exercise in women [79]. Interestingly, another study by

the same group investigated the effects of 20 whole-body cryotherapy sessions (-130 °C for 3 minutes) on circulating asprosin levels in menopausal women with metabolic syndrome and hyperglycemia. Whole-body cryotherapy has been reported to induce improve lipid and adipokine profiles and exert anti-inflammatory and anti-oxidant effects [80,81]. In the study by Wiecek *et al.* (2019), circulating asprosin levels at baseline were significantly higher in women with hyperglycemia compared to those with normoglycemia, while no significantly different circulating asprosin levels were noted between women with metabolic syndrome and healthy menopausal women [82]. As expected, blood glucose levels were also higher in the metabolic syndrome and hyperglycemia groups compared to the controls [82]. Following the whole-body cryotherapy sessions, circulating asprosin levels decreased significantly in healthy menopausal women, menopausal women with metabolic syndrome and menopausal women with hyperglycemia [82]. Accordingly, blood glucose concentrations also decreased significantly after the whole-body cryotherapy, but only in menopausal women with hyperglycemia [82]. In addition, blood glucose concentrations decreased slightly, but not significantly, in the women with metabolic syndrome in response to whole-body cryotherapy and were no longer significantly different to those in healthy menopausal women. Whole-body cryotherapy did not alter the circulating levels of leptin, adiponectin or irisin in any of the study groups [82]. These findings are in accord with those of previous studies which indicate that asprosin plays a role in glucose regulation.

**Future perspectives and conclusion**

Adipokines play a crucial endocrine role mediating the dynamic communication between adipose tissue and other organs/tissues in order to regulate metabolic homeostasis, as well as multiple other functions of the body. Asprosin is a novel adipokine which appears to play a key role in maintaining normoglycemia during the fasted state by promoting hepatic glucose production and stimulating appetite by acting on $AgRP^{+}$ neurons of the brain [12,20]. Indeed, an increasing body of evidence from both clinical (Table 1) and preclinical (Table 2) studies indicate that this adipokine may play a

significant role in metabolism, obesity and related cardio-metabolic diseases. As such, clinical data have revealed elevated circulating levels of asprosin in a number of cardio-metabolic diseases including obesity, T2DM, GDM, PCOS and NAFLD (Table 1). Notably, acute aerobic exercise appears effective in lowering circulating asprosin levels, indicating that relevant lifestyle interventions could impact on asprosin secretion/regulation. Interestingly, animal data also indicate that asprosin may be implicated in male fertility by affecting sperm motility [72], thus suggesting that this recently identified adipokine may exert physiologic effects that extend beyond metabolism. Moreover, *in vitro* studies have found asprosin to elicit pro-inflammatory effects and attenuate insulin secretion and signalling in skeletal muscle and pancreatic β-cells, whereas it has been shown to protect and improve survival of cardiomyoblasts exposed to hypoxic conditions, as well as mesenchymal stromal cells transplanted into infarcted mice hearts (Table 2). To date, asprosin appears to exert its effects by acting on various signalling pathways via a G-protein coupled receptor, namely olfactory receptor OLFR734 in mice (OR4M1 is the human orthologue of OLFR734) and potentially via other receptors, such as TLR4 or other unknown receptors (Figure 1). Overall, the available evidence so far suggests that this novel adipokine may exert pleiotropic physiologic effects (Figure 2). Given this growing body of evidence, further preclinical and clinical research is required in order to fully elucidate the role of asprosin in health and disease, and explore whether it may constitute a potential therapeutic target and/or a biomarker for obesity and related cardio-metabolic diseases.

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**Table 1. Summary of key findings from clinical studies investigating circulating asprosin levels.**

| Reference | Cohort details | Main findings |
|---|---|---|
| **Neonatal progeroid syndrome (NPS)** | | |
| Romere *et al.* (2016) [12] | Healthy control group (*n*=2)<br>Patients with heterozygous *FBN1* frameshift mutation with proximal truncation (*n*=2)<br>Patients with NPS with distal truncation (*n*=2) | Patients with NPS showed the greatest reduction in plasma asprosin compared to healthy controls. |
| **Obesity** | | |
| Romere *et al.* (2016) [12] | Healthy control group (*n*=7)<br>Patients with obesity & insulin resistance (*n*=8) | Plasma asprosin levels were significantly increased in patients with obesity and insulin resistance. |
| Ugur and Aydin (2019) [35] | Participants with;<br>Low weight (*n*=8)<br>Normal weight (*n*=44)<br>Overweight (*n=19*)<br>Obesity: Class I (*n*=10); Class II (*n*=13); Class III (*n*=22) | Serum and saliva asprosin levels increased with body mass index (BMI), with the lowest levels found in the low weight group and the highest in patients with class III obesity. |
| Wang *et al.*, (2019) [34] | Healthy control group (*n*=57)<br>Patients with obesity (*n*=117) | Significantly higher plasma asprosin levels in patients with obesity, and significantly decreased plasma asprosin levels 6 months post-bariatric surgery. |
| Long *et al.* (2019) [38] | Normal-weight children (*n*=40)<br>Children with obesity (*n*=47) | Circulating asprosin levels were lower in children with obesity compared to healthy controls. Boys with obesity had lower circulating asprosin levels than girls, but this difference was not observed in the control group. |
| Corica *et al.* (2021) [39] | Normal-weight children (n=24)<br>Children with obesity (*n*=43) | Fasting asprosin was significantly lower in children with obesity. When comparing the entire participant cohort, boys had lower fasting asprosin levels than girls. |
| Silistre and Hatipoğl (2020) [37] | Normal-weight children (*n*=60)<br>Overweight children (*n*=54)<br>Children with obesity (*n*=44) | Serum asprosin was significantly higher in overweight children and more so in children with obesity, compared to normal-weight controls. |

| | | |
|---|---|---|
| **Anorexia nervosa** | | |
| Hu *et al.* (2020) [40] | Healthy control group (*n*=47)<br>Patients with anorexia nervosa (*n*=46) | Plasma asprosin levels increased in patients with anorexia nervosa compared to the healthy control group. |
| **Type 2 diabetes mellitus (T2DM)** | | |
| Wang *et al.* (2018) [45] | Participants with;<br>Normal glucose regulation (*n*=52)<br>Impaired glucose regulation (IGR) (*n*=40)<br>T2DM (*n*=51) | Patients with IGR and T2DM had significantly elevated levels of plasma asprosin compared to those with normal glucose regulation. Highest plasma asprosin levels was observed in the IGR group. |
| Li *et al.* (2018) [42] | Healthy control group (*n*=66)<br>Patients with PCOS (*n*=41)<br>Patients with T2DM (*n*=53) | Plasma asprosin levels were higher in patients with T2DM compared to the healthy control group. |
| Zhang *et al.* (2019) [41] | Healthy control group (*n*=86)<br>Patients with T2DM (*n*=84) | Patients with T2DM had significantly higher serum asprosin levels than the healthy control group. |
| Zhang *et al.* (2019) [43] | Normal glucose tolerance (NGT) (*n*=60)<br>Patients with T2DM (*n*=60) | Fasting and postprandial asprosin levels were significantly elevated in patients with T2DM compared to participants with NGT. Only in participants with NGT circulating asprosin levels decreased in response to a 75-gram oral glucose tolerance test. |
| Naiemian *et al.* (2020) [44] | Healthy control group (*n*=97)<br>Patients with new-onset T2DM (*n*=97) | Patients with new-onset T2DM had significantly higher serum asprosin levels than the healthy control group. |
| Gozel and Kilinc (2020) [46] | Healthy control group (*n*=30)<br>Patients with newly diagnosed T2DM (*n*=30) | Asprosin levels in the saliva and plasma were significantly higher in patients with newly diagnosed with T2DM compared to the healthy control group. However, after 3 months of metformin treatment, saliva and plasma asprosin levels decreased and were no longer significantly different compared to those in the healthy control group. |
| Deng *et al.* (2020) [49] | T2DM patients with;<br>Normoalbuminuria (*n*=107)<br>Microalbuminuria (*n*=80)<br>Macroalbuminuria (*n*=20) | Serum asprosin levels were significantly increased in T2DM patients with microalbuminuria, and more so in patients with macroalbuminuria. |

| | | |
|---|---|---|
| **Gestational diabetes mellitus (GDM)** | | |
| Baykus *et al.* (2019) [52] | Healthy pregnant women (*n*=30)<br>Pregnant women with;<br>GDM *n*=30)<br>pre-eclampsia (*n*=30)<br>severe pre-eclampsia (*n*=30)<br>intrauterine growth retardation (*n*=30)<br>macrosomic foetuses (*n*=29) | Maternal blood asprosin levels were significantly elevated in pregnant women with GDM, pre-eclampsia, severe pre-eclampsia and macrosomic foetuses compared to healthy pregnant women. However, blood asprosin was significantly lower in women with intrauterine growth retardation compared to healthy pregnant women. A similar trend was also observed in arterial and venous cord blood of the studied newborns. |
| Zhong *et al.* (2020) [53] | Pregnant women with;<br>Normal glucose tolerance (*n*=40)<br>GDM (*n*=40) | Compared to pregnant women with normal glucose tolerance, women with GDM had significantly higher blood asprosin levels at 18-20 week of gestation and before delivery. No difference was observed in the blood asprosin levels at 24-28 weeks of gestation. Asprosin levels in the umbilical plasma of women with GDM was also higher compared to the control group. |
| **Polycystic ovary syndrome (PCOS)** | | |
| Alan *et al*. (2018) [68] | Healthy women (*n*=78)<br>Women with PCOS (*n*=78) | Circulating asprosin levels were elevated in women with PCOS compared to the healthy control group. |
| Li *et al.* (2018) [42] | Healthy women (*n*=66)<br>Women with PCOS (*n*=41)<br>Women with T2DM (*n*=53) | Plasma asprosin levels in women with PCOS were significantly higher compared to healthy controls. No difference was observed between normal-weight women with PCOS and women with PCOS and overweight/obesity. |
| Chang *et al.* (2019) [69] | Healthy women (*n*=156)<br>Women with PCOS (*n*=444) | No difference in circulating asprosin levels were observed in women with PCOS compared to healthy controls. |
| **Oral contraceptive (OC) use** | | |
| Leonard *et al*. (2020) [70] | Trained OC users (*n*=8)<br>Trained non-OC users (*n*=6)<br>Untrained OC users (*n*=10)<br>Untrained non-OC users (*n*=8) | Plasma asprosin levels were lower in OC users compared to non-OC users. The highest concentrations of asprosin were noted in the early follicular phase and the mid-luteal phase of the menstrual cycle of OC users and non-OC users, respectively. Significant changes in plasma asprosin were observed during the menstrual cycle of untrained, but not trained, women. |

| | | |
|---|---|---|
| **Metabolic associated liver disease** | | |
| Ke *et al*. (2020) [57] | Healthy control group (*n*=50)<br>Patients with non-alcoholic fatty liver disease (NAFLD) (*n*=43) | Serum asprosin levels were significantly elevated in patients with NAFLD compared to healthy controls. |
| Cosar *et al.* (2020) [59] | Healthy control group (*n*=30)<br>Patients with non-alcoholic steatohepatitis (NASH); stage 1 (*n*=30); stage 2 (*n*=30); stage 3 (*n*=30); stage 4 (*n*=22) | Serum and salivary asprosin levels of patients with NAFLD were significantly lower than those of the healthy control group. |
| **Dilated cardiomyopathy** | | |
| Wen *et al.* (2020)[61] | Healthy control subjects (*n*=50)<br>Patients with dilated cardiomyopathy (*n*=50) | Serum asprosin levels of patients with dilated cardiomyopathy were higher compared to healthy control subjects. |
| **Effects of exercise & whole-body cryotherapy sessions** | | |
| Wiecek *et al.* (2018) [79] | Adults undergoing a single acute anaerobic exercise: Men (*n*=10); Women (*n*=10) | Circulating asprosin levels were only increased in women 15, 30, 60 minutes and 24 hours after an acute anaerobic workout. |
| Ceylan *et al.* (2020) [36] | Adults undergoing a single acute aerobic exercise session in the morning and evening:<br>Normal weight group (*n*=10)<br>Patients with overweight/obesity (*n*=10) | Significant decreases serum asprosin levels after exercise in both groups, with a greater decrease in the evening in men with overweight/obesity. |
| Wiecek *et al.* (2019) [82] | Healthy menopausal women (*n*=18)<br>Menopausal women with:<br>metabolic syndrome (*n*=19)<br>hyperglycemia (*n*=15)<br>normoglycemia (*n*=22) | At baseline, significantly higher circulating asprosin levels in women with hyperglycemia compared to those with normoglycemia, but no difference between women with metabolic syndrome and healthy menopausal women. After 20 whole-body cryotherapy sessions, circulating asprosin levels decreased significantly in healthy menopausal women, menopausal women with metabolic syndrome and menopausal women with hyperglycemia. |

**Table 2. Summary of key findings from preclinical studies investigating asprosin.**

| Reference | Experimental model(s) | Main findings |
|---|---|---|
| Romere *et al.* (2016) [12] | Mouse; *in vitro* | Asprosin is secreted by white adipose tissue and targets the liver by binding to a G-protein coupled receptor on the surface of hepatocytes. Asprosin activate a G protein-cAMP-PKA pathway resulting in increased secretion of glucose by hepatocytes. |
| Duerrschmid *et al.* (2017) [20] | Mouse | Asprosin can cross the blood-brain barrier and activate $AgRP^{+}$ neurons in the brain by binding to a G-protein coupled receptor and activating a G-protein-cAMP-PKA pathway. Asprosin can also inhibit the activation of $POMC^{+}$ neurons via a GABA-dependent pathway. Thus, asprosin can increase appetite. |
| Jung *et al*. (2019) [28] | Mouse; *in vitro* | Asprosin expression is increased in the visceral adipose of mice fed a high fat diet. Treatment of skeletal muscle cells with asprosin increased expression of endoplasmic reticulum stress and pro-inflammatory markers. Asprosin treatment decreased insulin receptor substrate-1 and protein kinase B phosphorylation, resulting in attenuated insulin signalling. These effects of asprosin were mediated through the PKCδ pathway. |
| Li *et al.* (2019) [83] | Mouse; *in vitro* | Asprosin promotes hepatic glucose production through the OLFR734 receptor in mice. The effects of asprosin on cAMP levels and hepatic glucose production are significantly reduced in mice with liver-specific Olfr734 knockdown. |
| Lee *et al*. (2019) [33] | *In vitro* | Increased expression and secretion of asprosin in palmitate-treated pancreatic β-cells. The effects of palmitate on inflammation, cell viability and insulin secretion are mediated by asprosin via the TLR4/JNK pathway. |
| Wei *et al.* (2019) [72] | Mouse | Sperm from *Olfr734*$^{-/-}$ mice have slow motility and significantly reduced fertilisation potential.<br>Asprosin treatment can enhance sperm motility and fertilisation potential of 40-week old mice to that of 10-week old mice, but these effects are diminished in *Olfr734*$^{-/-}$ mice. |
| Ko *et al.* (2019) [75] | Rat | Hepatic asprosin levels were higher in T1DM rats but significantly decreased in response to aerobic exercise. Hepatic PKA and TGFβ levels decreased but AMPK increased after |

| | | |
|---|---|---|
| | | aerobic exercise. These findings suggest that aerobic exercise lowers hepatic asprosin, which in turn attenuates hepatic glucose production via a PKA pathway and TGFβ inhibition. |
| Nakhaei *et al.* (2019) [78] | Rat | Continuous and interval swimming training, for 8 weeks, significantly reduced serum asprosin levels in rats with metabolic syndrome. No difference noted between the two training modes. |
| Zhang *et al.* (2019) [62] | Mouse; *in vitro* | Pre-treatment of mesenchymal stromal cells with asprosin significantly improved cardiac function and reduced cardiac fibrosis when implanted into infarcted mice hearts.<br>Asprosin treatment also protected mesenchymal stromal cells from $H_2O_2$-induced oxidative stress and apoptosis via the SOD2-ERK1/2 pathway. |
| Liu *et al.* (2020) [23] | Mouse | OLFR734 is expressed in $AgRP^+$ neurons and asprosin acts via this receptor to activate these neurons and promote food intake in fasting mice.<br>Asprosin also enhances olfactory performance via the OLFR734 receptor. |
| Wen *et al.* (2020) [61] | *In vitro* | Asprosin protected cardiomyoblasts from $H_2O_2$-induced cell death and improved mitochondrial respiration. |
| Rezk *et al.* (2020) [54] | Rat | Circulating asprosin levels were higher in pregnant compared to non-pregnant rats.<br>Rats with GDM exhibited significantly elevated plasma asprosin levels compared to the healthy counterpart, and these levels were reduced after insulin treatment. |
| Zhang *et al.* (2021) [14] | Mouse; *in vitro* | Asprosin, expressed by a strain of *Pichia pastoris*, showed three glycosylation sites (threonine 8, 9 and 10) in two amino acid sequences of asprosin.<br>Treatment of skeletal muscle cells with asprosin increased GLUT4 expression and AMPK phosphorylation, resulting in increased glucose uptake. |

AgRP; agouti-related protein, AMPK; 5' adenosine monophosphate-activated protein kinase, cAMP; cyclic adenosine monophosphate, ERK; extracellular signal-regulated kinases, GABA; gamma-aminobutyric acid, GLUT4; glucose transporter type 4, $H_2O_2$; hydrogen peroxide, JNK; c-Jun N-terminal kinases, PKA; protein kinase A, PKCδ; protein kinase C delta, POMC; pro-opiomelanocortin, SOD2; superoxide dismutase 2, T1DM; type 1 diabetes mellitus, TGFβ; transforming growth factor beta, TLR4; toll-like receptor 4

**Figure 1. Signalling pathways known to mediate the effects of asprosin.** Asprosin exerts its effects by acting mainly via a G-protein coupled receptor, namely olfactory receptor OLFR734 in mice (OR4M1 is the human orthologue of OLFR734), and potentially via other receptors, such as toll-like receptor 4 (TLR4) or other unknown receptors. Akt; protein kinase B, cAMP; cyclic adenosine monophosphate, ERK; extracellular signal-regulated kinases, ER stress; endoplasmic reticulum stress, IRS-1; insulin receptor substrate-1, JNK; c-Jun N-terminal kinases, NFκB; nuclear factor kappa-light-chain-enhancer of activated B cells, PKA; protein kinase A, PKCδ; protein kinase C delta; SERCA; sarco/endoplasmic reticulum Ca2+-ATPase, SOD2; superoxide dismutase 2.

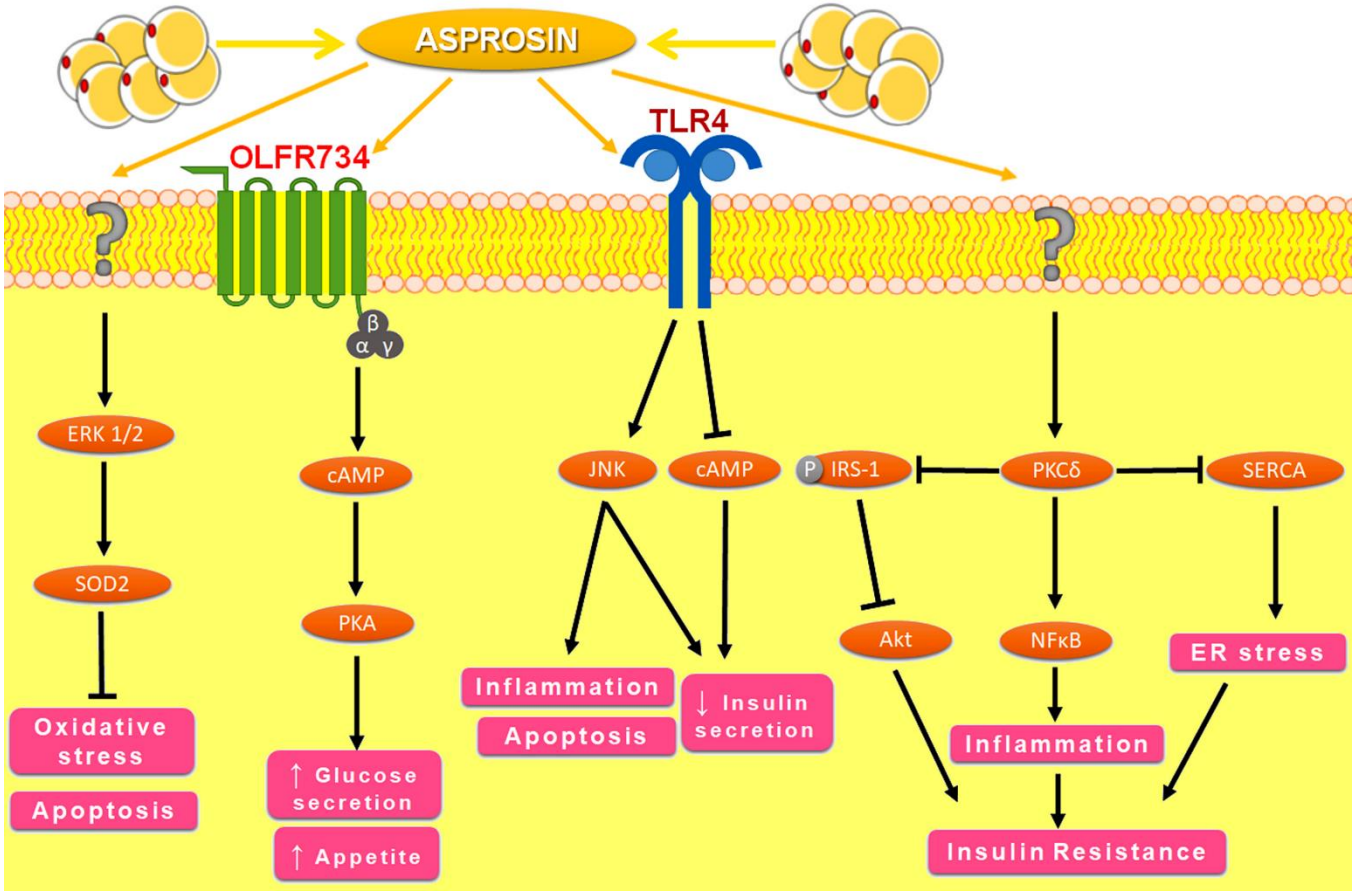

**Figure 2. Schematic representation of the effects of asprosin in different tissues/cells based on *in vitro* and *in vivo* data.** AgRP$^{+}$: agouti-related protein expressing neurons in the central nervous system; ER: endoplasmic reticulum.