

ASPROSIN: A NOVEL BIOMARKER OF TYPE 2 DIABETES MELLITUS

Asmaa Elnagar^{1*}, Hussein I. El-Belbasi¹, Ibrahim F. Rehan², Khalifa El-Dawy^{1*}

¹Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, 44511 – Zagazig, ²Department of Animal Behavior and Husbandry, Faculty of Veterinary Medicine, South Valley University, Qena, 83523, Egypt

*Corresponding authors, E-mail: dr_smsma21488@yahoo.com

Abstract: Type 2 diabetes mellitus has a deadly toll on human health. Therefore, more attention needs to be paid for the creation of biomarker to indicate the newly diagnostic type 2 diabetes and effective therapy. It well known that adipose tissue of mammals can store energy and secrete such hormones. Therefore, obesity is associated with hyperinsulinemia and insulin resistance. Recently, newly identified glucogenic hormone named “asprosin” has been developed in white adipose tissue, encoded by the gene *Fibrillin 1 (Fbn1)*. *Fbn1* is a 230-kb gene with 65 coding exons. These exons encode a 2,871-amino-acid long proprotein called *proFibrillin* which is proteolytically cleaved near its C-terminus by the enzyme furin convertase to give *Fbn1*, a member of the *fibrillin* family, in addition the 140-amino-acid long protein hormone asprosin. It has a metabolic role during fasting condition as it induces liver to secrete glucose for maintaining homeostasis as well as centrally stimulates appetite. It was reported that plasma asprosin concentrations increased in human/mice with type 2 diabetes compared with controls. However, it was significantly reduced in patients with neonatal progeroid syndrome who had characteristic features of low appetite and extreme leanness. This syndrome is due to the truncated mutation in *Fbn1*. Although the great biological role of asprosin *in vivo*, it still limited in research, particularly in the therapy of type 2 diabetes. This study aimed to provide an overview of asprosin and its possibility to be used as a novel biomarker of type 2 diabetes and obesity.

Key words: Asprosin; type 2 diabetes mellitus; *Fbn1* gene

Introduction

It was recorded that the prevalence of diabetes is expected to rise to 10.5% after twenty years (1). It is well known that adipose tissue can store energy and secrete bioactive adipokines (2-8). So, obesity is associated to hyperinsulinemia and insulin resistance (9,10). Several adipokines as adiponectin, resistin, and leptin are involved in the inflammation progress, homeostasis of glucose, and insulin sensitivity (2,3). Recently, asprosin has been

discovered as a novel hormone found in white adipose tissue (WAT) of mammals (11-15). It is named asprosin, a Greek word for white “aspros”. It is well known that WAT is the original source of plasma asprosin (11). It is secreted from WAT after fasting (12,15,16) for maintaining thermal energy standards among daily meals (13). In addition it has recently been known as a glucogenic hormone that regulates glucose homeostasis (11). Moreover, asprosin has a genetic background as encoded by *Fibrillin1* gene (*Fbn1*), the precursor of a newly

described glucogenic hormone (16-19). After analysis of *Fbn1* mRNA expression, the previous researchers confirmed the highest expression levels in WAT of humans and/or mice, suggesting the important function of *Fbn1* in adipogenesis (11,17). Therefore, asprosin is considered as a highly sensitive biomarker of type 2 diabetes mellitus (T2DM) and obesity (11,13,15).

Role of adipose tissue in glucose homeostasis

Adipose tissues can centrally control energy homeostasis. The secretion of adipokines can modulate storage of energy and essential for dissipation. New adipokines sourced from WAT, brown adipose tissue (BAT) and beige adipocytes have been detected (11). Detection of the sensitive receptors for adipokines is highly important for confirming hypothesis of the fat-derived signaling pathways that associated with energy homeostasis.

WAT represents nearly 27% of human body weight, is a key tool of endocrine system. Therefore, leptin therapy for patients with common lipodystrophy who regularly progress chronic hepatic steatosis, insulin resistance and DM has been permitted by Food and Drug Administration, 2014 (20). Even though generalized lipodystrophy is regularly linked to insulin resistance. Moreover, neonatal progeria syndrome (NPS)-patients developed unknown symptoms of partial lipodystrophy as they kept insulin sensitive and euglycaemic. In 2016, the team discovered mutations in distinguished gene that encodes *proFbn*, named *Fbn1*, in NPS-patients. This genetic mutation was related to the asprosin levels and the C-terminal cleavage product of *proFbn2* (11).

Asprosin is not only secreted by WAT but also secreted by other tissues (11). For instance, mammals also have BAT which can dissipate the energy in the form of heat in order to get rid of hyperthermia and obesity. BAT has a smaller mass compared to WAT inside the body of human (e.g., ~60 g of BAT in the adulthood period of human) (21). It could be contributed as an additional endocrine organ to whole-body homeostasis. On the contrary, it was recorded that secretory molecules from BAT, named

'batokines' commonly include fibroblast growth factor 21 (FGF21), vascular endothelial growth factor A (VEGFA), neuregulin 4 (NRG4), and bone morphogenetic protein 8B (BMP8B). Altogether, is confirming the physiological importance of BAT in endocrinology (22).

It was also noticed that "beige adipocytes" is the inducible form of thermo-genic adipocytes in adult humans (23). The differentiation of this kind of adipocytes can be stimulated by exposure to cold, severe exercise, bariatric surgery or cancer cachexia; moreover, the biological importance of these adipocytes was to modulate the energy of whole-body and glucose homeostasis (24). We therefore might understand that batokines can partially mediate the metabolic improvements as well as obtained by increasing beige fat mass.

Moreover, by 2016 the researchers supported the aforementioned hypothesis. They obtained beige adipocytes from the capillaries of subcutaneous WAT of human. Then, mice with diet-induced obesity were implanted with adipocytes (25). They showed that implantation of mice with beige adipocytes of human was a reason of lower levels of fasting blood glucose, higher glucose tolerance and lower hepatic steatosis, 50 days after implantation than controls whose were implanted with matrigel as a vehicle. The improved glucose tolerance after implantation of beige adipocytes was positively correlated with the upregulation of glucose turnover rate and the secretion of adiponectin from the transplanted human beige adipocytes.

The highest *Fbn1* mRNA expression level was found in adipose tissue due to increasing asprosin levels. Moreover, the genetic ablation of adipose tissue in mice (*Bscl2*^{-/-} mice) cause a two-fold reduction in circulating asprosin in comparable to wild-type mice (11). Furthermore, mature adipocytes in culture were able to secrete asprosin. All these findings concluded that the adipose tissue was the original source of asprosin. It is an important addition for the discovery of newly signaling molecules from adipocytes which can modulate energy homeostasis.

Definitely, detection of particular receptors of asprosin might be a new gate in biochemical

area of research to enable us identifying their target tissues/cells and to understand the mechanisms of action of asprosin. Therefore, adipose tissues can centrally control energy homeostasis due to secretion of adipokines. Meanwhile, it modulates energy storage and has the responsibility for dissipation.

Chemical structure of asprosin

Asprosin consists of 140-amino-acids protein (11,15,16). It is considered long C-terminal cleavage product of *proFbn* via the activation of the protease furin (26,27). Moreover, mature *Fbn1* acts as the extracellular matrix component. Meanwhile, asprosin is encoded by the two exons (65-, 66-encoded amino acids) of *Fbn1*. For instance, the exon 65 encodes 11-amino acids, whereas exon 66 encodes 129-amino acids. Together, those two exons showed an ideal model of higher vertebrate evolutionary maintenance score compared with the other *proFbns* coding sequence. In 2016, the researchers did their extensive efforts and created an asprosin-specific monoclonal antibody (mAb) and confirmed its sensitivity using *Fbn1* in wild-

type and null cells (11). Combination of immunoblotting of human plasma and the mAb indicated that a single protein ran on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 30 kDa, whereas bacterially produced recombinant asprosin (r-asprosin) ran at 17 kDa, (Figure 1) (11). Asprosin is anticipated to have three N-glycosylation sites as post-translational protein modifications. However, that was limited in bacteria. This clearly explained the molecular weight difference between mammalian and bacteria-produced asprosin. Therefore, the expressed asprosin from mammalian cells can form a protein which was secreted into the media and therefore ran on SDS-PAGE at 30 kDa (same molecular weight) (19). After the SDS-PAGE analysis, the researchers detected asprosin in human serum/plasma, cell lysates, media of mouse embryonic fibroblasts, and cell/tissue lysates of cultured adipocytes in WAT of mice. We suggest that the structural complexity of asprosin is a challenge for researchers to continue their experiments and therefore makes it limited in research.

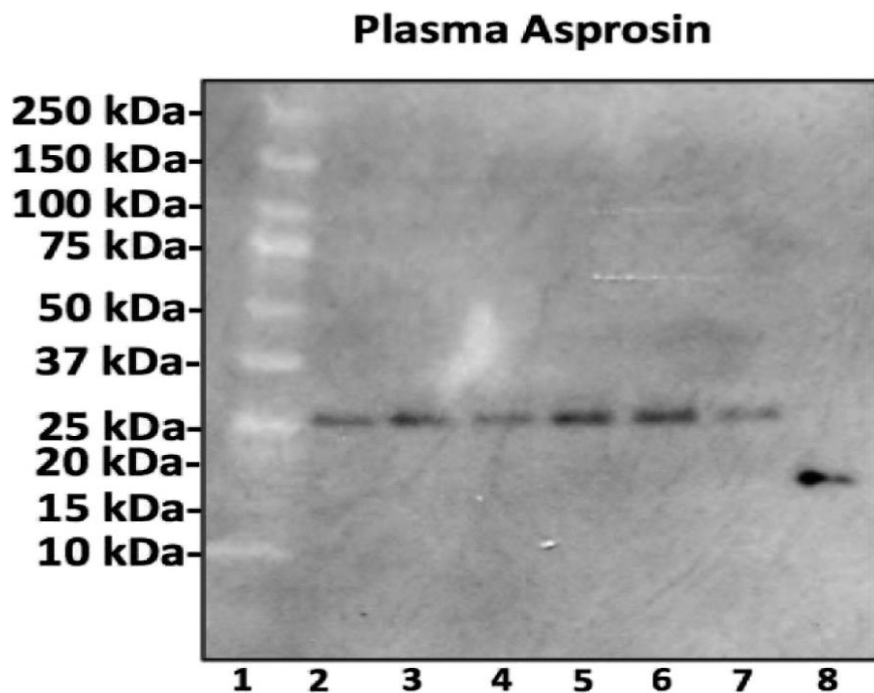


Figure 1: Asprosin immunoblotting on six individual human plasma samples (lanes 1-7), whereas bacterially produced recombinant asprosin was used as a positive control (lane 8), and the molecular weight marker (lane 1). As referenced by Romere et al. (11)

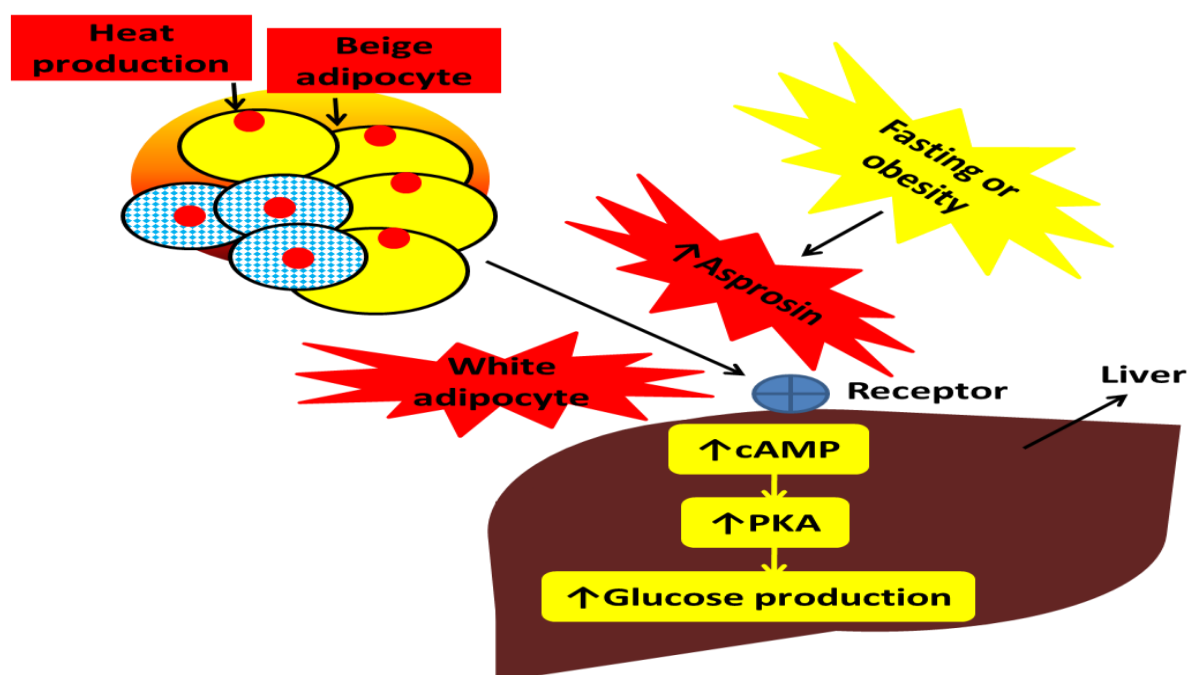


Figure 2: Schematic cartoon shows the modulation of plasma glucose homeostasis and insulin sensitivity through adipokines. The figure is drawn by the authors of this manuscript. cAMP: cyclic adenosine monophosphate receptor and PKA: protein kinase A

Mechanism of action of asprosin

Asprosin binds to the hepatocyte surface to initiate hepatocyte glucose release into the bloodstream (11,18) by crossing the blood brain barrier to activate orexigenic agouti-related neuropeptides (AgRP⁺ neuron) via a cyclic adenosine monophosphate (cAMP)-dependent pathway (16,28). This mechanism inhibits releasing of anorexigenic proopiomelanocortin (POMC)-positive neurons in a gamma-aminobutyric acid (GABA)-dependent manner in order to stimulate appetite and to store adiposity (14). Asprosin initiates the functional role of the liver during fasting time and is critical for normal neural function among daily meals. Therefore, there is a systematic coordination among the fasting condition, appetite stimulation and hepatic glucose release via asprosin, through distinct mechanisms in both liver and hypothalamus (11). For better understanding, see our schematic cartoon in Figure 2.

Metabolic effect of asprosin

It was reported that levels of asprosin increased in the blood under fasting "a base line of glucose condition" in healthy humans and/or rodents while decreased by feeding "a high glucose condition" (11,15,16,29). Hence insulin resistance is a direct cause of T2DM, asprosin occurs with excess adiposity because WAT can modulate glucose metabolism and maintain energy homeostasis, regardless enhancing or impairing insulin action (2,4). Our schematic cartoon in Figure (3) showed the direct effect of asprosin on liver glucose level and the indirect effect on insulin release. In addition, it seems likely that obesity is dramatically associated to a constellation of T2DM and metabolic syndromes (30-32). Therefore, asprosin has a great role in glucose metabolism as well it has a negative feedback-loop by suppressing circulating asprosin (11,14).

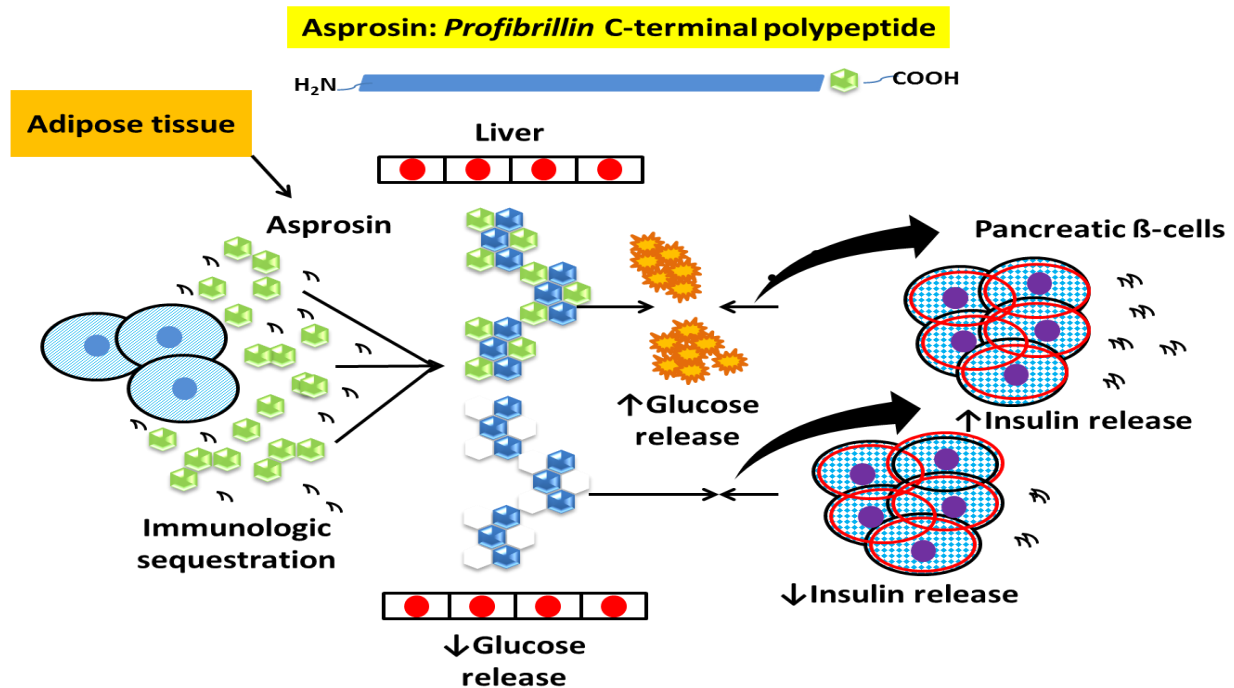


Figure 3: Schematic cartoon shows the role of asprosin in control hepatic glucose release to indirectly influence the insulin release. The figure is drawn by the authors of this manuscript

To evaluate circulating asprosin concentrations in daily basis, mice were kept at 12hr light/12hr dark program for one week to provide the optimum environmental condition, and further were put in a continuous darkness. Plasma was then isolated from these mice in 4hr intervals and then subjected to asprosin enzyme-linked immunosorbent assay (ELISA) analysis. The researchers at that time noticed that circulating asprosin showed circadian oscillation with a sharp decrease in levels equivalent with feeding time (11). In the contrary, fasting overnight in humans, mice and rats led to increase plasma asprosin. In the same study, they found a direct influence of asprosin on liver glucose while a slight influence on insulin compensatory mechanism. Moreover, inflammations are conveyed to contribute to the pathogenesis of DM (11,33). For instance, previous study indicated that asprosin stimulated liver glucose release by using cAMP as a second messenger, which was also included in the inflammatory responses (34-36). *Fbn1* exhibited a 70% down regulation in plasma asprosin in mR hypomorphic mice. Thus pro-inflammatory cytokines were altered and

contributed to the formation of inflammatory diseases, like aortic aneurysms (37). It was wondering whether circulating asprosin controlled glucose metabolism through inflammation. The high sensitivity C-reactive protein (hsCRP) levels dramatically increased among normal glucose regulation (NGR), impaired glucose regulation (IGR), and newly identified T2DM (nT2DM) groups and reached statistical significance in nT2DM-patients (14). However, a single dose of r-asprosin was administrated to mice giving a significant upregulation in blood glucose levels by stimulating hepatic glucose release (11). Therefore, they wondered whether the changed asprosin might be a real cause of glucose dysregulation, because the asprosin was an adipokine secreted by the adipose tissue. Moreover, T2DM is usually linked to the malfunction of adipose tissue which may then lead to unsuitable adipokine secretion. Although the researchers at that time did their great efforts, they had some limitations. First, they cannot consider the alteration of asprosin as a real cause of T2DM. Second, the influence of population characteristics and environmental factors on the secretory platform of adipokines

gave a negative impact to be applied on other populations. Third, not the all parameters correlated to the plasma asprosin levels were analyzed in their study. Finally, the circulating concentration of asprosin may also be associated with its catabolism, which was not evaluated in their study. Therefore, we suggest that future studies are required to assure correlation of asprosin and its potential mechanism, the insulin resistance and β -cell function for more clarification.

Relationship between asprosin and fasting glucose and triacylglycerol

High level of adiposity indicates a dysfunction of adipokines and considered as an evidence of metabolic diseases (5,6). It was reported that there was a positive correlations between circulating asprosin and adiposity-related parameters in T2DM-patients, including body mass index (BMI), waist circumference (WC), and waist-hip ratio (WHR) (15). This means increasing asprosin concentrations, BMI, WC, and WHR would be increased accordingly. However, several stepwise regression analysis represented that these adiposity indexes had no relation to asprosin concentrations. It was represented that the change of serum adiponectin levels and chemerin concentrations in T2DM was clearly independent of obesity (38). Moreover, it was found that the adipokine level inside the tissue is probably imbalanced to the amount entered the bloodstream (39). Therefore, it was reported that circulating asprosin had a potential influence on lipids metabolism.

Asprosin concentrations were positively correlated with triacylglycerol (TAG) regardless in healthy or in T2DM groups. Likewise, a study reported that a dyslipidemia was a common status in patients with high releasing of hepatic glucose in the insulin-resistant liver (40,41). Considering the liver as the major target organ of asprosin and therefore insulin resistance can direct us to the evidence of dyslipidemia. Therefore, asprosin was associated to the pathogenesis of lipids disorder. However, it was reported that the crude glucose area under the curve (AUC) of the rate of change (ROC) curve indicated that

asprosin was not the only parameter for analyzing T2DM of different age and sex (15). To avoid the limitations of previous studies, the accuracy of asprosin for T2DM diagnosis should be done in a large-scale analysis of prospective cohort study *in vitro* as well as *in vivo*. Also it should include a comparison among NGR, IGR, nT2DM and pre-diabetes-patients, in addition to underlay the relationship between serum asprosin and the physiopathologic mechanism of T2DM to be more elucidated. Meanwhile, serum asprosin concentrations were not sexually dimorphic in contrast to other adipokines like leptin and adiponectin (42,43). It well indicated that asprosin concentrations might not be influenced by male/female sex hormone. Altogether, is confirming the biological role of plasma asprosin in the improvement of T2DM. In addition, serum asprosin was positively linked to adiposity-dependent parameters in T2DM-patients.

Deficiency and increase of asprosin levels in the bloodstream

Asprosin was detected into the plasma/serum of humans, mice and rats. It is significantly reduced in NPS-patients due to the truncated mutation in *Fbn1* gene (i.e., loss of the C-terminal cleavage product of *proFbn*), giving a critical role of asprosin (11,44). Despite NPS-patients had insulin sensitivity, they had lipodystrophy with a low appetite and extreme leanness. This case was identical by mice having similar mutations and was completely rescued by asprosin compared with controls (11). It was reported that plasma asprosin concentration is decreased in [NPS-patients of genetic depletion, homozygous MgR mice of genetic depletion and immunologic sequestration in mice] while increased in [adenovirus-mediated overexpression, injection of r-asprosin and nT2DM]. By this, there was a correlated change in circulating glucose and insulin (11,15). In mice, plasma asprosin stimulated circadian oscillation which rises during overnight fasting and are impaired with the beginning of feeding time (11). In the same study, subjecting mice to streptozotocin therapy ablated pancreatic β -

cells as well as impaired circulating asprosin compared with controls.

A standard curve had been established using r-asprosin to estimate plasma as well as media levels. A high specificity of the asprosin sandwich ELISA had been displayed using media from wild-type mice and *Fbn1*^{-/-} cells. Asprosin was found in plasma of humans, mice and rats at regular nanomolar levels (11). Surprisingly, in the same study, NPS-patients showed a superior down regulation in plasma asprosin concentrations than those of heterozygous genotype (either wild-type mice as control subjects or patients that had heterozygous truncations of *proFbn* linked *N*-terminal) to undertake mRNA nonsense-mediated decay. Therefore, the mutation of *proFbn* NPS-patients cells has a negative reflection on asprosin release from the wild-type mice allele. Then the overexpression of the truncated mutation of *proFbn* in wild-type mice cells was examined. They found that there was an interference with the capability of those cells to release asprosin to the media in to the overexpression of other protein, like green fluorescent protein (GFP).

Therefore, circulating asprosin was clearly reduced in NPS-patient due to the truncated mutation in *Fbn1*.

To detect plasma asprosin concentration in T2DM-patients and controls, fasting overnight of 11 control subjects and 23 T2DM-patients was done and then examined 2-hs meal tolerance test in the early morning (12). The meal included 460 Kcal (e.g., 56.5g of carbohydrates, 18 g of protein and 18 g of fat). Collection of blood had been done prior and 2-hrs consequently after eating meals. To detect the level of plasma asprosin in T2DM-patient, glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG) and other biomarkers. The result in that study indicated that fasting asprosin concentrations in controls were significantly lower than those in T2DM-patients ($p < 0.05$). Moreover, in T2DM-patients, postprandial had significant reduction of asprosin levels with a negative correlation to fasting insulin levels ($p < 0.05$). However, asprosin levels and other parameters including FPG showed no significance. Therefore, we

concluded that plasma asprosin concentrations increased in human/mice of type 2 diabetes compared with controls.

Liver is the confirmatory main target of asprosin to increase circulating glucose

After applying cell autonomous glucose and examining insulin tolerance tests, mice had been treated with a single dose of r-asprosin. The mice clearly indicated a slight alteration of glucose uptake as a positive feedback of insulin in peripheral organs, like fat or muscle (11). However, it indicated a great alteration of glucose levels for re-implicating the liver. Performing the hyperinsulinemic-euglycemic clamp confirmed that the liver was the major location of asprosin function. This test uniquely represented that plasma asprosin levels were elevated which led to increase hepatic glucose release. However, peripheral organs had no ability to upregulate glucose as a feedback response to insulin. Then the researchers exposed the mouse hepatocytes to high concentrations of r-asprosin or GFP for 2hr. As their results, the media from cells exposed to asprosin showed an increase in glucose levels in a dose-dependent manner, suggesting a potential influence of asprosin on hepatocytes. In conclusion, liver was found to be the main and direct target of asprosin to increase plasma glucose.

Labeled r-asprosin with iodine-125 (I125) was prepared, and then intravenously injected in mice, consequently after a single-photon emission computerized tomography (SPECT) scans in order to detect the sites of accumulation. A typical amount of free I125 or I125-asprosin was used as a control. That amount was boiled for 5 min in order to stimulate the wastage of the asprosin tertiary structure. After analysis, data represented that I125-asprosin trafficked initially to the liver. In addition the tertiary structure of asprosin was necessary for the liver function (11). By this, liver trafficking, gamma counting of blood and viscera demonstrated that plasma r-asprosin levels were down regulated in parallel to the upregulation of liver glucose levels. To test plasma half-life after subcutaneous injection, scientists of the same study above followed the

protocol of a sandwich ELISA-analysis pointing the *N*-terminal histidine (His)-tag to the r-asprosin protein at the following time interval, 15, 30, 60, and 120 min. After the data analysis, plasma His-tagged asprosin indicated a half-life of maximally 20 min and a peak level of 50 nM for 20 min after injection in case of infusion of I125-asprosin. Moreover, to test the specific binding of asprosin to hepatocytes, mouse hepatocytes were incubated with the excess amount of asprosin-biotin conjugates, and then washed with PBS to finally examine the relative level of biotin at the hepatocyte surface. Asprosin connected to the hepatocyte surface in a dose-responsive manner. Repeating the same protocol in the presence of a 100-fold excess unconjugated asprosin ended the effect, indicating a kind of competition and high sensitivity for potential receptor-binding sites. Altogether, is confirming the quick response of asprosin receptors with a high affinity, to bind the hepatocyte surface.

As a result, bacterially produced r-asprosin held the metabolic activity which exhibited by the endogenous produced counterpart. Meanwhile, the alteration of plasma asprosin was enough to raise blood glucose and insulin concentrations. Mice were observed to deny eating along the experiment. It is well known that this single asprosin dose resulted in an immediate signal in plasma glucose levels. However, the real cause was compensatory hyperinsulinemia at the 15 min, which normalized the plasma glucose concentrations by 60 min after injection. Likewise, this hypothesis was found in mice which were exposed to an overnight fasting although the level of the consequential plasma glucose curve was dramatically decreased. It was probably due to fasting-induced reduction of glucogenic substrates. Therefore, liver was the main target organ of asprosin because of its primary role to store glucose (e.g., glycogen) and quickly upregulated into the bloodstream during fasting. Interestingly, asprosin gave no influence on plasma concentrations of catabolic hormones (e.g., glucagon, catecholamines and glucocorticoids) in order to stimulate liver glucose release.

Asprosin upregulates cAMP and activates protein kinase A in the liver

It was reported that mice exposing to a single dose of 30-mg r-asprosin for 20 min – 50 nM peak level, upregulated the cAMP and activated protein kinase A (PKA) in the liver (11), as illustrated in a schematic Figure 2. Similarly, that was done after incubation of mouse hepatocytes with r-asprosin for 10 min. Moreover, hepatocyte PKA activity was upregulated due to the binding of r-asprosin, as what they had noticed the same story in liver glucose production. Interestingly, the physiological role of asprosin on either hepatocyte glucose release or PKA activation was ended by suramin, a general heterotrimeric G protein inhibitor. Moreover, asprosin-mediated hepatocyte glucose release might be ended using cAMPS-Rp which acts as a competitive antagonist of cAMP binding to PKA. Glucagon and catecholamines also engaged to the identical intracellular signaling axis, thus the researchers in the same study wondered what is the reason for inhibition of the glucagon receptor as well as the β -adrenergic receptor on the stimulation of hepatocyte glucose release through asprosin?. They understood that the corresponding inhibitors clearly ended the influences of glucagon as well as epinephrine. However, they had no evidence of the ability of asprosin to stop hepatocyte glucose release. It comes by the fact that asprosin used such cell-surface receptor system which is distinctly different from those used by glucagon and/or catecholamines. Due to insulin induces a reduction in intracellular cAMP through stimulation of the Gai pathway, the same researchers examined whether insulin might interfere with asprosin effect on hepatocyte PKA activity and glucose release. The researchers reached to the answer through an increase in intracellular cAMP. In conclusion, it was confirmed that asprosin upregulates hepatocyte glucose release by binding the G protein-cAMP-PKA axis *in vitro* as well as *in vivo*. Moreover, it was noticed that insulin suppressed asprosin-mediated hepatocyte PKA activation and led to glucose release.

The immunological role of asprosin in metabolic-syndrome-associated hyperinsulinism

It was reported that circulating asprosin concentrations were altered in controls with insulin resistance (11). Likewise, alterations were shown in two mouse models of insulin resistance (either fed diet-induced obesity or with obese gene (OB) mutation). Interestingly, a single dose of an intraperitoneal injection of asprosin as a specific mAb was enough to reduce circulating asprosin concentrations. The effect started 3 and 6 hr post injection, with a back recovery within 24 hr. Both *ad-libitum*-fed (following 2hr fasting for making a synchronization program) of mouse insulin resistance represented a sharp reduction in circulating insulin along with euglycemia). That was immediately with decreasing circulating asprosin. To examine the influence of loss of asprosin on liver glucose release regardless the possible insulin compensatory mechanism, mouse hepatocytes was treated with the asprosin-specific Ab before the incubation with asprosin. As anticipated, the asprosin-specific Ab ended asprosin-mediated liver glucose release, whereas a nonspecific control Ab had no influence. To confirm immunologic sequestration as a legitimate dysfunction, *Fbn1* was examined in homozygous MgR mice (which had 20% of the wild-type *Fbn1*) (45). However, MgR mice had a 70% downregulation in circulating asprosin. It displayed a two-fold deficit in plasma insulin upon 2-hrs fasting, whereas keeping euglycemia (as the findings observed with immunologic sequestration of asprosin in *ad-libitum*-fed mice). However, upon a full-day fasting time, a physiologic situation was limited insulin release from mice. Moreover, MgR mice demonstrated fasting hypoglycemia, indicating that insulin's buffering mechanism requires to be blocked through a long fasting duration to reveal the downregulation in circulating glucose stimulated by asprosin dysfunction. Therefore, to confirm this theory, the scientists performed a hyperinsulinemic-euglycemic clamp research study on MgR mice. Those mice had been fasted for 18hr. It

was reported that a higher acute deficit in liver glucose release in MgR than wild-type mice (11). This result was similar to clamp results indicating an upregulate in hepatic glucose production upon the efficiency of asprosin. As anticipated, clamp study could not demonstrate a change in whole-body glucose disposal, indicating that, the influence of asprosin on glucose homeostasis was poor to be served as hepatic glucose release stimulation. Therefore, a single subcutaneous injection of asprosin after overnight fasted MgR mice was enough to totally reduce the insulin. Moreover, the insulin reduction displayed in MgR mice is due to a reduction in circulating asprosin not due to the expression levels of functional *Fbn1* protein. However, any alteration of circulating insulin concentrations is indirectly reflected on the change in hepatic glucose production.

*The ability of asprosin to release key phenotypes of *Fbn1NPS*⁺ mice*

To entirely release the hypophagia phenotype of *Fbn1NPS*⁺ mice, a single subcutaneous dose of r-asprosin was injected. It indicated that NPS-linked hypophagia is due to a lower circulating asprosin and not due to genetic mutation of *Fbn1* (16). In the same study, a single intracerebroventricular (ICV) injection of r-asprosin in *Fbn1NPS*⁺ mice mainly had the ability to restore the firing level of AgRP⁺ neurons. Moreover, it mainly restored the resting membrane potential to the AgRP⁺ neurons of recombinant GFP (rGFP)-injected wild-type mice. To confirm, the mouse-NPS-linked down regulated in AgRP⁺ neuron membrane potential as well as firing regularly were fully released by a simple incubation of the hypothalamic slices with r-asprosin but not with rGFP. In conclusion, we thought that NPS-linked hypophagia and depressed AgRP⁺ neuron activities are related to a reduction in asprosin. The asprosin concentration in the blood is essential to keep regular appetite feeling and AgRP⁺ neuron function.

Crossing asprosin via blood–brain barrier of human/mice and initiating the appetite

To evaluate asprosin levels in rat, cerebrospinal fluid (CSF) was prepared, followed by asprosin-specific sandwich ELISA analysis (16). Asprosin was found in CSF at concentrations up to four-to five-folds which then decreased below that was previously reported (11). Likewise, asprosin concentration in the CSF was upregulated by overnight fasting. To evaluate whether circulating asprosin might cross the blood brain barrier and enter the CSF, rats were intravenously injected with r-asprosin. This r-asprosin had *N*-terminal His-tag and examined for the evidence of the His-tag in the CSF using ELISA technique. Thus one hour post-intravenously injection, a strong signal for the His-tag in the CSF which was fitted by six-folds upregulated in asprosin of CSF. Then, to determine whether asprosin stimulates appetite, a single dose of bacterially/mammalian-expressed r-asprosin or rGFP was subcutaneously injected to wild-type mice. Asprosin-injected mice revealed a higher food intake than GFP-injected mice over the next-day hours regardless r-asprosin preparation was used. Importantly, mammalian-produced asprosin was twice the molecular weight of bacterially produced asprosin, and as anticipated previously (11). This alteration was mainly because of the glycosylation of the mammalian-produced protein. The plasma half-life of the mammalian-produced asprosin was up to 145 min (in comparison to 20 min for the bacterially produced asprosin), with a 60- μ g subcutaneous dose producing a peak plasma levels of 40 nM. Remarkably, there was a hidden phase of a few hours after injection of asprosin and prior to the orexigenic influence was noticed, which distinguished the influences of asprosin from those of more acute-acting orexigenic subjects like ghrelin (46). Dependable on the observation of the influence of subcutaneous injection of asprosin, an orexigenic influence has been found on asprosin after entering it to the CSF through ICV-injection. To realize the chronic responses, wild-type mice were treated with subcutaneous doses of r-asprosin for 10-days interval.

Moreover, the result suggested a hyperphagia, and no significance in energy, triacylglycerol content in feces, or body weight. A slightly downregulated in lean mass was observed as a result of the stress of daily injections. A significant upregulation in adiposity was also observed, which confirmed an orientation of the energy homeostasis equation in approval of increased energy intake. After using adenovirus-mediated hepatic human *Fbn1*, it was noticed its overexpression which caused by two-fold upregulation in plasma asprosin (11). Furthermore, it showed the same hyperphagic response and increasing in body weight. However, there were no significant values in energy expenditure or in lean mass. Surprisingly, there was a high level in adiposity once the mice were fed normally, and this influence was clearly abundant when subjecting the mice to a high-fat diet. Therefore, plasma asprosin can enter into the CSF through blood brain barrier to initiate appetite.

It was reported that NPS-patients of congenital lipodystrophy can affect the face and extremities (11). A novel heterozygous 3' mutations in the gene encoding *Fbn1* in those patients had been identified after whole-exome sequencing. By which, expression of a truncated *proFbn* protein had been detected as well as loss of the *proFbn* carboxy-terminal cleavage products. Moreover, in another study, a virtual phenocopy of NPS with hypophagia was the real cause of the presence of a heterozygous *Fbn1*-NPS mutation in mice and further low adipose mass and body weight were identified (16). Furthermore, the concentration of orexigenic agouti-related neuropeptides (AgRP⁺ neuron) activity was decreased in mice (28). It was reported that circulating asprosin concentration was significantly higher in IGR and nT2DM-patients compared with those in NGR human, especially in IGR group (14). Meanwhile, in the same study plasma asprosin levels were significantly correlated with parameters of glucose metabolism, obesity, lipid profiles, insulin resistance, and β -cell function, (Table 1). However, in the same study, the trends in the alteration of first phase of glucose stimulated asprosin secretion were greatest differences of insulin secretion among

three groups. Meanwhile, researchers revealed that the plasma asprosin levels in IGR group were the highest one among all (11). That confirmed the presence of a strong protein biomarker which will predict prediabetes. For more understanding, previous study demonstrated that the plasma asprosin was pathologically elevated in human as well as in both diet-and genetic-induced animals of insulin resistance. Consistently, other researchers found a strong positive relationship between plasma asprosin concentrations and insulin resistance. Therefore, the asprosin-related glucose dysregulation might be targeted to the role of asprosin in insulin resistance. In addition, pancreatic β -cell malfunction was

considered as another important pathway in the development of T2DM (47,48). Moreover, several adipokines have been reported to interact with the function, proliferation, death and failure of β -cell (49). However, the pattern of first-phase glucose-stimulated asprosin secretion was not correspondent with insulin secretion. By which, the glucose regulation role of asprosin might be independent of its impact on the first-phase insulin secretion. Therefore, the plasma asprosin might also contribute to β -cell malfunction and cause the glucose intolerance. In conclusion, plasma asprosin levels are increased in nT2DM, suggesting the critical roles of adipokines in the pathogenesis of T2DM.

Table 1: General clinical and laboratory parameters in people of NGR, IGR and nT2DM according to Wang et al. (14)

No.	Parameter	NGR	IGR	nT2DM
1	Sex (M/F)	52 (17/35)	40 (15/25)	51 (27/24)
2	Age (year)	54.73 $\pm$ 11.90	54.00 $\pm$ 9.62	53.73 $\pm$ 10.06
3	BMI (kg/m ²)	22.76 $\pm$ 3.61	23.86 $\pm$ 3.08	24.73 $\pm$ 3.55 ^b
4	Wc (cm)	81.08 $\pm$ 9.05	88.18 $\pm$ 6.48 ^b	87.71 $\pm$ 9.36 ^b
5	WHR	0.86 $\pm$ 0.06	0.87 $\pm$ 0.07	0.90 $\pm$ 0.06 ^{bd}
6	SBP (mmHg)	120.98 $\pm$ 14.04	125.03 $\pm$ 12.76	126.75 $\pm$ 13.71 ^a
7	DBP (mmHg)	75.42 $\pm$ 9.36	75.75 $\pm$ 9.14	79.10 $\pm$ 11.51 ^a
8	FPG (mmol/l)	5.19 $\pm$ 0.34	6.68 $\pm$ 0.25 ^b	8.27 $\pm$ 1.92 ^{bd}
9	2hPG (mmol/l)	4.70 $\pm$ 0.60	6.90 $\pm$ 1.17 ^b	11.79 $\pm$ 3.50 ^{bd}
10	HbA1c (%/mmol/mol)	5.55 $\pm$ 0.32/37	6.01 $\pm$ 0.41/42	7.78 $\pm$ 1.89/62 ^{bd}
11	FINS (mU/l)	5.92 $\pm$ 3.59	7.18 $\pm$ 3.79	8.25 $\pm$ 4.14 ^b
12	HOMA-IR	1.38 $\pm$ 0.88	2.14 $\pm$ 1.16 ^b	3.02 $\pm$ 1.64 ^{bd}
13	HOMA- β	71.03 $\pm$ 41.79	44.83 $\pm$ 22.80 ^b	38.92 $\pm$ 22.97 ^b
14	AUC	498.95 $\pm$ 481.04	165.86 $\pm$ 109.59 ^b	100.96 $\pm$ 113.99 ^b
15	AIR	62.89 $\pm$ 61.81	17.17 $\pm$ 11.69 ^b	10.93 $\pm$ 13.07 ^b
16	GDI	1.61 $\pm$ 0.45	1.30 $\pm$ 0.31 ^a	0.97 $\pm$ 0.26 ^{bc}
17	TC (mmol/l)	4.41 $\pm$ 0.98	4.73 $\pm$ 1.02	4.79 $\pm$ 1.08
18	TAG (mmol/l)	1.20 $\pm$ 0.50	1.98 $\pm$ 1.40 ^b	1.90 $\pm$ 1.06 ^b
19	HDL-C (mmol/l)	1.52 $\pm$ 0.52	1.32 $\pm$ 0.31 ^a	1.27 $\pm$ 0.31 ^b
20	LDL-C (mmol/l)	2.45 $\pm$ 0.95	2.84 $\pm$ 0.76 ^a	2.83 $\pm$ 1.00 ^a
21	hsCRP (mg/l)	1.37 $\pm$ 3.36	0.94 $\pm$ 0.78	2.31 $\pm$ 1.01 ^{ad}
22	Asprosin (ng/ml)	16.22 $\pm$ 9.27	82.40 $\pm$ 91.06 ^b	73.25 $\pm$ 91.69 ^b

2hPG: 2 h postchallenge plasma glucose; AUC: area under the curve of the first-phase (0–10 min) insulin secretion; AIR: acute insulin response; BMI: body mass index; DBP: diastolic blood pressure; FINS: fasting serum insulin; FPG: fasting plasma glucose; GDI: glucose disposition index; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; HOMA- β : homeostasis model assessment for beta-cell function; hsCRP: hypersensitive C-reactive protein, IGR: impaired glucose regulation; LDL-C, low-density lipoprotein cholesterol; NGR: normal glucose regulation; nT2DM: newly diagnosed type 2 diabetes; SBP: systolic blood pressure; TC: total cholesterol; TAG: triacylglycerol; Wc: waist circumference and WHR: waist hip ratio. Data are reported in (14), means $\pm$ SD. ^a P < 0.05 in comparable to NGT; ^b P < 0.01 in comparable to NGT; ^c P < 0.05 in comparable to pre-DM; ^d P < 0.01 in comparable to pre-DM.

Trials to use asprosin for treatment of T2DM

The circulating asprosin might be a predictor of early diagnosis in DM. Moreover, it has a good possibility to be used as a potential therapeutic target for pre-diabetes and T2DM. However, few trials have been done to discuss in this regard. For instance, to make a replenishment of circulating asprosin *in vivo*, a single subcutaneous injection was totally rescued hypophagia in *Fbn1*-NPS/+ mice. That demonstrated the binding of NPS-associated hypophagia to the reduction of circulating asprosin (11). Moreover, it was mentioned that a single subcutaneous dose of r-asprosin induced an immediate curve in plasma glucose concentration of fasted mice that was resulted in a compensatory hyperinsulinemia mechanism to normalize blood glucose concentrations within 60 min. Therefore, balancing of asprosin in the plasma with a mAb reduced appetite and body weight in obese mice in order to normalizing the plasma glucose level (16). Remarkably, circulating glucose and insulin were increased within 30 min after a single injection of r-asprosin to wild-type mice (18). Moreover, asprosin can cross the blood–brain barrier after intravenous injection to activate the hypothalamic feeding circuitry and initiate appetite stimulation. In a long term, this therapy will maintain adiposity (16). On the other hand, neutralizing specific mAb against asprosin or genetic deletion of *Fbn1* can block asprosin action through reduced circulating concentrations of insulin and hepatic glucose production *in vivo*. It is important to say that asprosin is a fasting-induced adipokine which controls liver glucose release and insulin sensitivity (11,18). As shown in Table 1, plasma asprosin levels were significantly correlated with several parameters of glucose and lipid metabolic disorders like glucose metabolism, obesity, lipid profiles, insulin resistance, and β -cell function, etc (14). By which, it was reported that plasma asprosin level was positively correlated with waist circumference, FPG, TAG, 2hrs-post-challenge plasma glucose (2hPG), and homeostasis mechanism led to insulin resistance. However, it was significantly negatively correlated with homeostasis model

to evaluate the β -cell function, AIR, AUC (0–10 min) insulin secretion, and glucose disposition index (GDI), ($P < 0.05$, each). It was mentioned that fasting serum asprosin levels in control humans were significantly lower than those who had T2DM, ($P < 0.05$). Moreover, in T2DM-patients, reduction of asprosin concentrations after eating meals showed a significant negative correlation with fasting insulin concentrations, ($P < 0.05$). However, there was no significant correlation between asprosin levels and other parameters like FPG (49). In other report, plasma asprosin levels were abundantly increased, ($P < 0.001$) in the T2DM-adult tertiles compared to controls (15), and concluded that fasting glucose and TAG were greatly associated with plasma asprosin in T2DM. On the other side, the remaining asprosin levels among obese of the both sex (19.9 ± 36.7 and 7.1 ± 7.5 , male and female respectively) were relatively the same comparable to non-obese (9.2 ± 9.5 and 12.4 ± 10.4). These similarities of asprosin levels resulted from individuals' experiences of sever exercise with the high inter-individual distributions. However, the low intra-individual spreading provided to obese as well as lean subjects (13). Altogether, confirmed the biological importance of asprosin *in vivo*, and its possibility *in vitro* to treat T2DM.

Conclusion

Eventually and after extensive effort done by scientists, asprosin is newly identified hormone would contribute greatly to the improvement of health. Asprosin found in white adipose tissue in mammals. It is positively correlated with T2DM and obesity due to the direct stimulation of hepatic glucose and appetite. However, it is negatively correlated with NPS-patients due to a genetic mutation of *Fbn1*. Our approach provides an overview of asprosin and its possibility to be used as a biomarker for early diagnosis of T2DM. Based on the recent view of literatures, asprosin antibody is a safe and efficient therapy of T2DM in a very short onset. Therefore, it is important to recommend physicians, chemists and also DM-patients to

use antibody of asprosin as a novel and effective therapy of T2DM.

Conflict of interest

None of the authors have any conflict of interest to declare.

Acknowledgments

The authors would like to express sincere thanks to Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Egypt for supporting this work.

References

1. Ogurtsova K, da Rocha Fernandes, JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract.* 2017;128: 40–50.
2. Andrade-Oliveira V, Câmara NO, Moraes-Vieira PM. Adipokines as drug targets in diabetes and underlying disturbances. *J Diabetes Res* 2015;2015: 681612.
3. Ahima RS. Adipose tissue as an endocrine organ. *Obesity* 2006;14 (S8): 242–9.
4. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue: an endocrine organ playing a role in metabolic regulation. *Horm Mol Biol Clin Investig* 2016; 26 (1): 25–42.
5. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. *Nat Med* 2017;23 (7): 804–14.
6. Clark M, Hoenig M. Metabolic effects of obesity and its interaction with endocrine diseases. *Vet Clin North Am Small Anim Pract* 2016;46 (5): 797–815.
7. Zhang L, Fu Y, Zhou N, Cheng X, Chen C. Circulating neuregulin 4 concentrations in patients with newly diagnosed type 2 diabetes: a cross-sectional study. *Endocrine* 2017; 57 (3): 535–8.
8. Sun L, Qi Q, Zong G, Ye X, Li H, Liu X, Zheng H, Hu F.B, Liu Y, Lin X. Elevated plasma retinol-binding protein 4 is associated with increased risk of type 2 diabetes in middle-aged and elderly Chinese adults–3. *J Nutr* 2014;144 (5): 722–8.
9. Conte C, Fabbri E, Kars M, Mittendorfer B, Patterson BW, Klein S. Multiorgan insulin sensitivity in lean and obese subjects. *Diabetes Care* 2012; 35 (6): 1316–21.
10. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP. Quantification of the relationship between insulin sensitivity and β -cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 1993; 42 (11): 1663–72.
11. Romere C, Duerschmid C, Bournat J, Constable P, Jain M, Xia F, Saha PK, Del Solar M, Zhu B, York B, Sarkar P, Rendon DA, Gaber MW, LeMaire SA, Coselli JS, Milewicz DM, Sutton VR, Butte NF, Moore DD, Chopra AR. Asprosin, a fasting-induced glucogenic protein hormone. *Cell* 2016; 165 (3): 566–79.
12. Tokumoto S, Okamura E, Abe M, Honjo S, Hamasaki A. Inverse association between fasting insulin levels and postprandial changes of plasma asprosin concentration in patients with type 2 diabetes. *Diabetologia* 2017; (60): 270.
13. Schumann U, Qiu S, Enders K, Bosnyák E, Laszlo R, Machus K, Trájer E, Jaganathan S, Zügel M, Steinacker JM. Asprosin, A newly identified fasting-induced hormone is not elevated in obesity and is insensitive to acute exercise. *Med Sci Sports Exerc* 2017;49 (5): 1023.
14. Wang Y, Qu H, Xiong X, Qiu Y, Liao Y, Chen Y, Zheng Y, Zheng H. Plasma asprosin concentrations are increased in individuals with glucose dysregulation and correlated with insulin resistance and first-phase insulin secretion. *Mediators of Inflamm* 2018; 2018: 9471583. doi: 10.1155/2018/9471583.
15. Zhang L, Chen C, Zhou N, Fu Y, Cheng X. Circulating asprosin concentrations are increased in type 2 diabetes mellitus and independently associated with fasting glucose and triacylglycerol. *Clin Chimica Acta* 2017.
16. Duerschmid C, He Y, Wang C, Li C, Bournat JC, Romere C, Saha PK, Lee ME, Phillips KJ, Jain M, Jia P. Asprosin is a centrally acting orexigenic. *Nat Med* 2017;23 (12): 1444.
17. Davis MR, Arner E, Duffy CR, De Sousa PA, Dahlman I, Arner P, Summers KM. Datasets of genes coexpressed with *Fbn1* in mouse adipose tissue and during human adipogenesis. *Data Brief* 2016; 8: 851–857.
18. Kajimura S. Adipose tissue in 2016: Advances in the understanding of adipose tissue biology. *Nat Rev Endocrinol* 2017;13 (2): 69–70.
19. Lönnqvist L, Reinhardt D, Sakai L, Peltonen L. Evidence for furin-type activity-mediated C-terminal processing of *proFibrillin-1* and interference in the processing by certain mutations. *Hum Mol Genet* 1998;7 (13): 2039–44.

20. Sinha G. Leptin therapy gains FDA approval. *Nat Biotechnol* 2014;32 (4): 300–2.
21. Virtanen KA, Lidell ME, Orava J, Heglund M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009; 360 (15): 1518–25.
22. Villarroya F, Cereijo R, Villarroya J, Giralt M. Brown adipose tissue as a secretory organ. *Nat Rev Endocrinol* 2016;13 (1): 26–35.
23. Shinoda K, Luijten IH, Hasegawa Y, Hong H, Sonne SB, Kim M, Xue R, Chondronikola M, Cypess AM, Tseng YH, Nedergaard J. Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nat Med* 2015;21 (4): 389–94.
24. Kajimura S, Spiegelman BM, Seale P. Brown and beige fat: physiological roles beyond heat generation. *Cell Metab* 2015;22 (4): 546–59.
25. Min SY, Kady J, Nam M, Rojas-Rodriguez R, Berkenwald A, Kim JH, Noh HL, Kim JK, Cooper MP, Fitzgibbons T, Brehm MA. Human 'brite/beige' adipocytes develop from capillary networks, and their implantation improves metabolic homeostasis in mice. *Nat Med* 2016;22 (3): 312–8.
26. Milewicz DM, Grossfield J, Cao SN, Kielty C, Covitz W, Jewett T. A mutation in *Fbn1* disrupts *proFibrillin* processing and results in isolated skeletal features of the Marfan syndrome. *J Clin Invest* 1995; 95 (5): 2373–8.
27. Birrell MA, Maher SA, Dekkak B, Jones V, Wong S, Brook P, Belvisi MG. Anti-inflammatory effects of PGE2 in the lung: role of the EP4 receptor subtype. *Thorax* 2015; 70 (8): 740–7.
28. Julliard A, Al Koborssy D, Fadool DA, Palouzier-Paulignan B. Nutrient sensing: another chemosensitivity of the olfactory system. *Front Physiol* 2017;8: 468.
29. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose metabolism and regulation: beyond insulin and 2004. *Diabetes Spectr* 2004; 17 (3): 183–90.
30. Sauvanet JP. Congress of the international diabetes federation (IDF-Paris 2003). *Press Med* 2003;32 (39): 1864–8.
31. Garvey WT, Garber AJ, Mechanick JJ, Bray GA, Dagogo-Jack S, Einhorn D, Grunberger G, Handelsman Y, Hennekens CH, Hurley DL, McGill J. The AACE Obesity Scientific, American association of clinical endocrinologists and American college of endocrinology position statement on the advanced framework for a new diagnosis of obesity as a chronic disease. *Endocr Pract* 2014;20 (9): 977–89.
32. O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obesity Rev* 2015;16 (1): 1–12.
33. Hotamisligil GS. Inflammation and metabolic disorders. *Nat* 2006;444 (7121): 860–7.
34. Jenei-Lanzl Z, Zwingenberg J, Lowin T, Anders S, Straub RH. Proinflammatory receptor switch from Gas to Gai signaling by β -arrestin-mediated PDE4 recruitment in mixed RA synovial cells. *Brain Behav Immun* 2015;50: 266–74.
35. Jiang J, Dingledine R. Prostaglandin receptor EP2 in the crosshairs of anti-inflammation, anti-cancer, and neuroprotection. *Trends Pharmacol Sci* 2013; 34 (7): 413–23.
36. Zimmerman NP, Kumar SN, Turner JR, Dwinell MB. Cyclic AMP dysregulates intestinal epithelial cell restitution through PKA and RhoA. *Inflamm Bowel Dis* 2012; 18 (6): 1081–91.
37. Ju X, Ijaz T, Sun H, LeJeune W, Vargas G, Shilagard T, Recinos III A, Milewicz DM, Brasier AR, Tilton RG. IL-6 regulates extracellular matrix remodeling associated with aortic dilation in a *Fibrillin-1* hypomorphic mgR/mgR mouse model of severe Marfan syndrome. *J Am Heart Assoc* 2014;3 (1): e000476.
38. Andersson DP, Laurencikiene J, Acosta JR, Ryden M, Arner P. Circulating and adipose levels of adipokines associated with insulin sensitivity in nonobese subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2016;101 (10): 3765–71.
39. Kovacs D, Lovaszi M, Poliska S, Olah A, Biro T, Veres I, Zouboulis CC, Stahle M, Ruhl R, Remenyik E, Torocsik D. Sebocytes differentially express and secrete adipokines. *Exp Dermatol* 2016;25 (3): 194–9.
40. Titchenell PM, Lazar MA, Birnbaum MJ. Unraveling the regulation of hepatic metabolism by insulin. *Trends Endocrinol Metab* 2007; 18 (7): 497–505.
41. Wu L, Parhofer KG. Diabetic dyslipidemia. *Metab* 2014;63 (12): 1469–79.
42. Combs TP, Berg AH, Rajala MW, Klebanov S, Iyengar P, Jimenez-Chillaron JC, Patti ME, Klein SL, Weinstein RS, Scherer PE. Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. *Diabetes* 2003;52 (2): 268–76.
43. Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, Jinagouda SD, el-Tawil K, Rude RK, Kamdar V. Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* 1997; 82 (2): 579–84.

44. O'Neill B, Simha V, Kotha V, Garg A. Body fat distribution and metabolic variables in patients with neonatal progeroid syndrome. *Am J Med Genet A* 2007;143A (13): 1421–30.
45. Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, Biery NJ, Dietz HC, Sakai LY, Ramirez F. Pathogenetic sequence for aneurysm revealed in mice underexpressing *Fibrillin-1*. *Proc Natl Acad Sci USA* 1999; 96 (7): 3819–3.
46. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nat* 2001;409 (6817): 194–8.
47. Del Prato S, Tiengo A. The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. *Diabetes metab Res Rev* 2001;17 (3): 164–74.
48. Bunt JC, Krakoff J, Ortega E, Knowler WC, Bogardus C. Acute insulin response is an independent predictor of type 2 diabetes mellitus in individuals with both normal fasting and 2-h plasma glucose concentrations. *Diabetes Metabolism Res Rev* 2007;23 (4): 304–10.
49. Dunmore SJ, Brown JE. The role of adipokines in beta-cell failure of type 2 diabetes. *J Endocrinol* 2013; 216 (1): 37–45.