Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 09, pp.73319-73323, September, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ASPROSIN: A RISK FACTOR FOR OBESITY AND METABOLIC DISTURBANCE

[1,2*]Husam M. Edrees and [1,3]Enas N. Morgan

[1]Faculty of Medicine-Department of Physiology- Zagazig-University, Egypt
[2]College of Public Health and Health Informatics, Qassim University, Saudi Arabia
[3]College of Medical Rehabilitation, Qassim University, Saudi Arabia

ARTICLE INFO

Article History:
Received 10th June, 2018
Received in revised form
27th July, 2018
Accepted 25th August, 2018
Published online 30th September, 2018

Key Words:
Obesity,
Asprosin, Insulin resistance,
Hepatic glucose Output, Adipose tissue.

List of Abbreviations

BMI: Body Mass Index
GLUT2: Glucose Transporter 2
G6P: Gucose-6-Phosphate
cAMP: Cyclic Adenosine Monophosphate
FBN1: Fibrillin-1 gene
TGFβ: Transforming Growth Factor beta
Fbn1NPS: FBN1 Neo¬natal Progeroid Syndrome
AgRP: Agouti-related protein

ABSTRACT

Obesity is a major health problem worldwide. The change in nutritional behaviour is the cheif cause of obesity. The asprosin is a newly discovered hormone which is induced by fasting and stimulates glucose production from the liver. Any decrease or increase in the circulating asprosin concentration results in a consistent alteration of appetite and adiposity. **Objective:** The aim of the present study is to demonstrate the role of asprosin in inducing obesity and causing metabolic disturbance in terms of measuring the BMI and other biochemical parameters. **Methods:** this study was carried out using 20 adult and healthy male albino rats, divided into two groups: Control group (Group I) and asprosin treated group (Group II) to determine the effect of subcutaneous asprosin injection (30 µg daily for 10 days) on body weight, BMI, serum glucose, insulin, insulin resistance and lipid profile. **Results:** The subcutaneous asprosin injection resulted in an increase in the body weight and body mass index with a significant dyslipidemia and hyperglycemia along with insulin resistance. **Conclusion:** The results suggest that the hormone asprosin is responsible for the observed metabolic disturbances and increased BMI in the rats.

Citation: Husam M. Edrees and Enas N. Morgan. **2018.** "Asprosin: A risk factor for obesity and metabolic disturbance", *International Journal of Current Research*, 10, (09), 73319-73323.

INTRODUCTION

Obesity is a principal global cause of death. With the changing life pattern the rate of obesity is increasing in adults and children. It affects women more than men. According to the specialists vision, it will be one of the gravest problems affecting public health in the 21st century (Dibaise, 2013). Obesity is a medical problem characterized by accumulation of excess body fatdue to a disturbance in energy homeostasis as a result of imbalance between energy input and outputresulting in harm full effects on overall health. The etiology of obesity is most generally due to extreme food intake, deficiency of physical activity, and genetic susceptibility (Yazdi *et al*., 2015). In addition, the endocrine disorders, drugs, or mental disorder may also be responsible (Bleich *et al*., 2008).

Corresponding author: **Husam M. Edrees,**
Faculty of Medicine-Department of Physiology- Zagazig-University, Egypt.
DOI: https://doi.org/10.24941/ijcr.32193.09.2018

Fasting in mammals induces cascade of interrelated processes synchronized by an array of hormones. Initial stage of fasting stimulates appetite and hepatic glucose production and release into the circulation to keep the brain nourished (Duerrschmid *et al*., 2017). This is vital for brain function and survival through a set of hormones that specifically control plasma glucose levels (Levine, 1986). The liver is the manager of energy metabolism as it acts as the center of metabolic connection between various tissues including skeletal muscle and adipose tissue. In postprandial state, the liver transforms glucose into glycogen and/or fatty acids or amino acids (Rui, 2014).The entrance of blood glucose into the hepatic cells occurs by a plasma membrane glucose transporter GLUT2.Deletion of GLUT2 in thehepatocytes prevents the hepatic glucose uptake, however, this deletion has no effect in the fasting state (Seyer *et al*., 2013). In the fed state, glucose-6-phosphate (G6P) is metabolized to synthesize glycogen and to generate pyruvate through glycolysis (She *et al*., 2008).

The plasma glucose concentration is detected by the balance between the rate of glucose release into the circulation (glucose appearance) and the rate of glucose uptake from the circulation (glucose disappearance). Glucose in the circulation results from postprandial intestinal absorption, glycogenolysis, and gluconeogenesis. Gastric emptying determines the rate of postprandial glucose appearance in the circulation (Stephen *et al*., 2004). The adipose tissue is thought to be an active endocrine tissue which secretes group of bioactive substances (adipokines). These adipokines signal to many organs specially those involved in metabolism resulting in modifying lipid and glucose metabolism in addition to haemostasis and blood pressure (Rabe *et al*., 2008). Asprosin, a protein hormone produced by white adipose fatty tissues in the mammals, stimulates cyclic adenosine monophosphate (cAMP) dependent pathway in the liver inducing rapid glucose production. The initial identification of asprosin was done through cleavage of the C-terminal product of profibrillin (encoded by FBN1 gene). In humans, it was found that fibrillin-1 is a large glycoprotein encoded by the FBN1 gene. It functions to strengthen the connective tissues and regulates the bioavailability of growth factor TGFβ. Up-regulation of FBN1 mRNA was seen in adipose tissues of the obese women more than the non-obese with a rise in adipocyte size (Warnick, 1983). Romere *et al.* (2016) demonstrated that intravenous administration of asprosin activates the hypothalamic feeding circuit by crossing the blood-brain barrier (BBB) leading to appetite induction. They also showed that single injection of recombinant asprosin subcutaneously was satisfactory to manage the hypophagia of Fbn1NPS/+ mice (ablated asprosin-coding region in mice), demonstrating that hypophagia associated with neonatal progeroid syndrome is due to decrease in the level of plasma asprosin. The plasmaasprosin level rises acutely with fasting and decreases with refeeding exhibiting circadian rhythm in organization with the state of nutrition.

In two patients with congenital progeroid look and partial lipodystrophy, FBN1 gene mutation was discovered. Deduction of the FBN1 protein in these patients resulted in a mutant fibrillin protein with reduced asprosin level in the blood and decreased mass of subcutaneous adipose tissue. Absence of FBN1 gene product profibrillin produces metabolic dysregulation in the form of partial lipodystrophy and low plasma insulin level with euglycemia (Greenhill, 2016). In some patients with Marfansyndrome, mutations in FBN1 were found to produce decrease in or abnormal adipose tissue causing lack of subcutaneous adipose tissue (Davis *et al*., 2016). Studies have suggested that decrease of circulating asprosin level due to genetic reduction either in the patients with neonatal progeroid syndrome or mice with Fbn1NPS/+ or severe elimination by asprosin monoclonal antibody in mice results in corresponding alteration in appetite and adiposity. These studies suggested that asprosin is essential for normal functions of AgRP+ neurons. However, mice with eradicated AgRP+ neuron was able to respond to asprosin after administration of high fat diet indicates that asprosin exerts its appetite-inducing effect through manyorexigenic neurons besides the AgRP+ neurons (Romere *et al*., 2016). To the best of our knowledge, asprosin is recently discovered and its role in inducing obesity and metabolic disturbance is still under investigation. Due to the increased risk of obesity and possible role for asprosin in regulating appetite and adiposity, the current study was designed to demonstrate the role of asprosin in inducing obesity and metabolic disturbance.

MATERIALS AND METHODS

Experimental Animal: 20 healthy adult male albino rats aging 8 –10 weeks and weighing 190-220 grams were used and were well-kept in cages (5 rats/cage), at room temperature and on a twelve hours day/night cycles. Two weeks before starting the experimentations, the rats were accommodated to laboratory circumstances and received received standard chow. All procedures in this study were implemented according to strategies of the ethical committee of the Faculty of Medicine, Zagazig University. Rats were randomly classified into:

Group I (Control Group): Ten rats received standard foodfor two weeks (Ahren and Scheurink, 1998).

Group II (Asprosin Treated Group): Ten rats received standard food for 2 weeks. The rats received a dose of 30 µg recombinant asprosin (Human recombinant Asprosin (2732-2871) dissolved in Tris-Buffered Saline(TBS) Solution, 1L (50 mMTris-Cl, pH 7.6; 150 mMNaCl) daily for 10 days by subcutaneous injection (Duerrschmid *et al*., 2017).For both groups, body weights and rat lengths were measured at the beginning and at the end of the experiment. After completion of the experiment, the rats were fasted overnight, and were anaesthetized with sodium thiopental (50 mg/kg body weight intra peritoneal). The livers were rapidly removed and the blood samples were collected, allowed to clot, centrifuged to obtain serum and stored at -20° C to be used for the assessment of the metabolic parameters.

Chemicals

Human recombinant Asprosin: Human Asprosin, amino acids, AVISCERA BIOSCIENCE, INC. 2348 Walsh Ave, Suite C, Santa Clara, CA 95051, California, U.S.A.

Assessment of the metabolic parameters

Serum glucose level: according to the method described by **Trinder** (1969).

Serum insulin level: using the method described by **Starr *et al.* (1978)**.

Evaluationof Insulin Resistance: using homeostasis model assessment (where HOMA-IR= (fasting insulin (µIU/ml) x fasting plasma glucose (mmol/L) /22.5 (Matthews *et al*., 1985).

Total Serum cholesterol (TC) levels: Using Allain *et al*. (**1974**) method.

Serum triglycerides (TG) levels: Using Naitomethod (Naito, 1989).

Serum high density lipoproteins (HDL) levels: Using Warnick *et al*. (**1983**) method.

Low density lipoprotein cholesterol: Using Friedewald *et al*. (**1972**) method.

Estimation of Body Mass Index (BMI): Body mass index (BMI) was estimated according to the following equation: body weight (gm) / length^2 (cm^2), this index can be used as an indicator of obesity where the cutoff value of obesity BMI is more than 0.68 gm/cm^2 (Saldanha *et al*., 2012).

Measurement of food intake: Equal amounts of food (30 g/day/rat) were provided to every rat in a separate compartment in the cage and the amount consumed by each rat was assessed (Reinehr, 2010).

Isolation of liver slices and their incubation: Sodium thiopental (intra peritoneal injection of 50 mg/kg bw) was used to anaesthetize rats. Wide opening of abdominal cavity using sharp clean scissor was done and the liver was removed. Using a microtome, Liver was sliced (0.05 mm thickness) according to McKee *et al* (1988). The liver slices were preserved in formerly weighed flasks containing3ml of carbogen gassed buffer solution (pH 7.42±0.01for five minutes with tight closure of flasks. Two control flasks containing 3 ml of buffer solution were incubated besides the experimental flasks in a metabolic shaker (120 RPM) at 37°C. After 1 hour of incubation, cooling of all flasks under tap water was done and the buffer samples were collected from each flask to determine the glucose content. The buffer solution used to preserve liver slices is Krebs-Ringer bicarbonate (pH 7.4). It consisted of NaCl 0.9 g/100ml, KCl 1.15 g/100ml, CaCl 1.22 g/100ml, KH2PO4 2.11 g/100ml, MgSO4 3.82 g/100ml, and Glucose 100 mg/100ml. Bovine albumin was added to act as a carrier for released non-esterified fatty acids(Haidara *et al*., 2006).

Calculation of glucose uptake: Glucose uptake was valuated using the following formula:

$$\text{Glucose uptake} = \frac{C \times V \times 1000}{W \times 100}$$

Where,

C = glucose concentration in control flasks − glucose concentration in the tissue flasks.
W = Wet weight of tissue in mg.
V = Volume in ml of incubation medium in the flasks.

Glucose concentration was assessed by enzymatic method using Coobas Integra 400 plus (Roche Diagnostic, Germany) (Trinder, 1969).

Statistical Analysis: The results are presented as mean ± standard error of the mean. Statistical Package for the Social Sciences (SPSS), version 19.0. P values was measured by unpaired, two-tailed Student's *t*-test followed by appropriate *post hoc* tests. P value <0.05 was considered to be statistically significant. To distinguish differences in statistics between the groups, Analysis of variance (ANOVA) Test was applied.

RESULTS

Body weight and body mass index (BMI): The effect of as pros in injection on body weight is shown in table 1. Initial body weights were 201.4±7.85 and 207.8±7.84 gm in group I and group II respectively. Asprosin injection significantly (p <0.001) increased body weight (351.1±25.12 gm) and body mass index (0.71± 0.1) in group II in comparison with the control group.

Food Intake: The effect of asprosin injection on food intake is shown in table 1. The average of food intake is significantly increased (p <0.001) with subcutaneous asprosin injection (94.51±4.21 gm/kg BW/day) when compared with control group (69.39± 3.06 gm/kg BW/day).

Serum glucose level: The effect of asprosin injection on serum glucose level is presented in table 1. Serum glucose level was found to be 4.33±0.15 and 13.82±0.34mmol/L in group I and group II respectively. Serum glucose level was significantly increased with subcutaneous asprosin injection when compared with control group (p <0.001).

Serum insulin level: The effect of asprosin injection on serum insulin level as shown in table 1. Serum insulin level was found to be 16.82±0.19and 36.92±1.71 µIU/ml in group I and group II respectively. We found that serum insulin level was increased significantly in asprosin injected group when compared with the control group (p <0.001).

Determination of Insulin Resistance (HOMA-IR): The effect of asprosin injection on serum insulin level (Table 1). HOMA-IR was found to be 3.24±0.15 and 22.7±1.6 in group I and group II respectively. It was found that HOMA-IR was significantly increased in asprosin injected group in comparison with control group (p <0.001).

Hepatic glucose output: As presented in Table 1, the mean values of hepatic glucose output was 6.76±0.62 mg/g wet weight/hour in group II (asprosin treated group). These values were significantly higher (P<0.001) when compared with that of group I (4.83±0.45mg/g wet weight/hour).

Serum cholesterol (TC) level: The effect of asprosin injection on serum cholesterol (TC) level (Table 1). The serum cholesterol level was found to be 137.7±10.51and 201.1±11.28 mg/dl in group I and group II respectively. in comparing with control group, asprosin injection significantly increased serum cholesterol level (p <0.001).

Serum triglycerides (TG) level: Serum triglycerides level (Table 1) was found to be 56.6±6.63and 187.3±9.48 mg/dl in group I and group II respectively (Table 1). It was found that serum triglycerides level was significantly (p <0.001)elevated in asprosin injected group as compared to control group.

Serum high density lipoproteins (HDL) level: The effect of asprosin injection on serum high density lipoproteins (HDL) level (Table 1). It was found that level of serum HDL was significantly (p <0.001) deccreased in asprosin injected group (35.9±2.03 mg/dl) in comparison to the control group (65.6±7.95 mg/dl).

Low density lipoprotein cholesterol (LDL-Choesterol) level: Serum LDL level was found to be 44.6±4.41 and 121.8±3.77 mg/dl in group I and group II respectively (Table 1). It was found that serum LDL level was significantly increased by asprosin injection in comparing to the control group (p <0.001).

DISCUSSION

The novel asprosin is a recently discovered hormone inviting good attention towards its role in modifying hepatic glucose output. Asprosin is discovered to be secreted by white adipose tissue then transported to the liver, where it triggers the G protein-cAMP-PKA pathway, leading to the fast release of glucose into the circulation.

Table 1. Body Weight and metabolic parameters in group I (Control) and group II (Asprosin Treated)

Parameter	Group I (Control Group)	Group III (AT Group)
Initial BW (gm)	201.4± 7.85	207.8±7.84
Final BW (gm)	206.9±7.13	351.1±25.12*
Final Body Mass Insex (BMI)	0.42± 0.06	0.71± 0.10*
Food Intake (gm/kg BW/day)	69.39± 3.06	94.51±4.21*
Serum Glucose (mmol/L)	4.33±0.15	13.82±0.34*
Serum Insulin (μIU/ml)	16.82±0.19	36.92±1.71*
HOMA IR	3.24±0.15	22.7±1.6*
Hepatic glucose output (mg/g wet weight/hour)	4.83±0.45	6.76±0.62*
TC (mg/dl)	137.7±10.51	201.1±11.28*
TG (mg/dl)	56.6±6.63	187.3±9.48*
HDL (mg/dl)	65.6±7.95	35.9±2.03*
LDL (mg/dl)	44.6±4.41	121.8±3.77*

Data are presented as Mean ± SD.
*P < 0.001 versus group I

It has been claimed that asprosin may be helpfull in treating type II diabetes (Dipali, 2016). Some authors found pathologically elevated levels of plasma asprosin in human with resistance to insulin, in diet-induced obesity and Ob mutation (independent mouse models of insulin resistance) (Greenhill, 2016). In this study, it was found that subcutaneous asprosin injection in rats significantly increased body weight, body mass index and food intake in group II (Asprosin Treated Group) in comparison to the control group suggesting the role of asprosin in increasing appetite of the rats. These results are consistent with Duerrschmid *et al*. (2017) who theorized that neonatal progeroid syndrome accompanying slimness could in part be clarified by asprosinlack and so, asprosin is essential for normal appetite in humans. They demonstrated that prolonged immunological repeal of asprosin in two autonomous models of obesity in mice (high fat diet-induced obesity and Leprdb/dbmutation) decreased daily food consumption and body weight. Jacquinet *et al*. (2014) briefed that genetic representation of mutations accompanying neonatal progeroid syndrome in mice led to heterozygous removal of the coding region of asprosin.

In this study, it was found that subcutaneous asprosin injection significantly elevated serum glucose levels in group II (Asprosin Treated Group) in comparison to the control group. This result is compatible with Romere *et al*. (2016) who stated that basal glucose level infasting increases the asprosin levels in circulation, while, high glucose level in feeding decreased circulating asprosin. They assumed that glucose acts as an inhibitor of plasma asprosin in a negative-feedback loop. They found that high blood glucose level in wild type mice treated with streptozotocin decreased plasma asprosin level more than was found in mice with normal blood glucose. These results support the concept that glucose negatively affect plasma asprosin levels in a negative-feedback loop. In this study, it was found serum insulin level and insulin resistance were significantly elevated in group II (Asprosin Treated Group) in comparison to the control group. These results are consistent with the findings of Romere *et al*. (2016) who reported that insulin resistance is accompanied with high asprosin level in white and brown adipose tissues and skeletal muscle, these tissues are commonly concerned with developing insulin resistance. Accordingly, asprosin reduction may signify an important beneficial treatment policy against type II diabetes. In contrast, O'Neill *et al*. (2007) found that deducting mutations in the FBN1 gene1 displayed a decrease in plasma asprosin that is accompanied with severe thinness, decreased subcutaneous adipose tissue and conservation of insulin sensitivity even with partial lipodystrophy.

Hepatic glucose output was found to be increased significantly in group II (Asprosin Treated Group) in comparison to the control group in our study. The same results were found by Duerrschmid *et al*. (2017) who reported that high levels of asprosin in adipose tissue induced hepatic glucose release. Acoordination between hepatic glucose release and asprosin level has been found. Furhter, Romere *et al*. (2016) identified a fasting-induced protein which is the C-terminal splittedproduct of profibrillin, and they named it asprosin. They reported that white adipose cells secrete asprosin which is transported to the liver, where it activates the G protein-cAMP-PKA pathway, leading to quick glucose release into the circulation. The current study revealed that in group II (Asprosin Treated Group), there was significant increase in the levels of TC, TG and LDL and significant decrease in the level of HDL in comparison to the control group. Jacquinet *et al*. (2014) discovered that lack of the C-terminal splitted product of profibrillinoutcomes in an exceptional way of metabolic abnormality that includes partial lipodystrophy. They reported that asprosin is highly presented in adipose tissue in a rare genetic disorder in humans, neonatal progeroid syndrome ((NPS; also known as Marfan lipodystrophy syndrome).

Conclusion

The current study suggests that the newly discovered hormone, asprosin, has diabetogenic and obesogenic effet causing metabolic disturbances, so, immunologic neutralization of asprosin may introduce a hopeful strategy to control and treat both diabetes and metabolic syndrome.

Acknowledgments

The authors would like to acknowledge the technicians in the Department of Physiology, Zagazig University for their helpful support in the experimental work. This research was not granted from any funding agency in the public commercial or not-for-profit sectors.

Conflicts of interest: None.

Funding: This research was not granted from any funding agency in the public commercial or not-for-profit sectors.

REFFERENCES

Ahren B. and Scheurink A.J.W. 1998. Marked hyperleptinemia after high fat diet associated with severe glucose intolerance in mice. Eur J Endocrinol; 139:461-7.

Allain C., Poon L.S., Chan C.S., Richmond. W., Fu PC. 1974. Enzymatic determination of total serum cholesterol. ClinChem; 20:470- 5.

Bleich S., Cutler D., Murray C. and Adams A. 2008. Why is the developed world obese?". Annu Rev Public Health (Research Support); 29: 273–95.

Davis M. R., Arner E., Duffy C.R.E., De Sousa P.A. and *et al*. 2016. Expression of FBN1 during adipogenesis: Relevance to the lipodystrophy phenotype in Marfan syndrome and related conditions. Molecular Genetics and Metabolism; 119: 174–185.

Dibaise J.K. and Foxx-Orenstein A.E. 2013. Role of the gastroenterologist in managing obesity". Expert Review of Gastroenterology and Hepatology (Review); 7 (5): 439–51.

Dipali P. 2016. "Discovery of Asprosin, New Hormone Could Have Potential Implications in Treatment of Diabetes". Houston, TX: Baylor College of Medicine.

Duerrschmid C., He Y., Wang C., Li C. and *et al*. 2017. Asprosin is a centrally acting orexigenic hormone. Nature Medicine; 23: 1444–1453.

Friedwald W.T., Levy R.I. and Fredrickson D.S. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. ClinChem; 18:499-502.

Greenhill C. 2016. "Liver: Asprosin - new hormone involved in hepatic glucose release". Nat Rev Endocrinol; 12 (6): 312.

Haidara M.A., Yassin H.Z., Rateb M.A., Ibrahim I.M. 2006. The Potential Protective Effects of Vitamin C on Glucose Homeostasis and Muscle Function in STZ – Induced Diabetic Rats, In Vitamin C: New Research; Nova Science Publishers, Inc. Editor Thomas Peel, pp. 41-56.

Jacquinet A., Verloes A., Callewaert B., Coremans C. and *et al*. 2014. Neonatal progeroid variant of Marfan syndrome with congenital lipodystrophy results from mutations at the 3′ end of FBN1 gene. Eur. J. Med.Genet. ; 57: 230–234.

Levine R. 1986. "Monosaccharides in Health and Disease". Annu. Rev. Nutr.; 6: 211–224.

Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A. and *et al*. 1985. "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man". Diabetologia; 28 (7): 412–9.

Naito H.K. 1989. Triglycerides in clinical chemistry:theory, analysis and correlation. Second edition by KaplanLA and Pesce AJ. (U.S.A.), P. 997.

Mckee R.L., Hruby V.J., Trivedi D.B., Johnson D.G. and *et al*. 1988. Perfused Precision-cut liver Slice System for the Study of Hormone-Regulated Hepatic Glucose Metabolism. Journal of Pharmacological Methods; 19: 339-354.

O'Neill, B., Simha, V., Kotha, V. and Garg, A. 2007. Body fat distribution and metabolic variables in patients with neonatal progeroid syndrome. *Am. J. Med. Genet*., A. 143A, 1421–1430.

Rabe K., lehrke M., parhofer K.G. and Broedl U. 2008. Adipokines and insulin resistance. Mol Med; 14:741-51.

Reinehr T. 2010. Obesity and thyroid function. Molecular and Cellular Endocrinology; 316:165–171.

Romere, C., Duerrschmid, C., Bournat, J., Constable, P. and *et al*. 2016. Asprosin, a Fasting-Induced Glucogenic Protein Hormone. Cell; 165: 566–579.

Rui L. 2014. Energy Metabolism in the Liver. ComprPhysiol; 4(1): 177–197.

Saldanha J.F., Carrero J.J., Lobo J.C., Stockler-Pinto M.B. and *et al*. 2012. The newly identified anorexigenicadipokine nesfatin-1 in hemodialysis patients: Are there associations with food intake, body composition and inflammation? Regulatory Peptides; 173(1- 3):82-5.

Seyer P., Vallois D., Poitry-Yamate C., Schutz F. and *et al*. 2013. Hepatic glucose sensing is required to preserve beta cell glucose competence. J Clin Invest; 123:1662–1676.

She P., Shiota M., Shelton K.D., Chalkley R. and *et al*., 2000. Phosphoenolpyruvate carboxykinase is necessary for the integration of hepatic energy metabolism. Mol Cell Biol; 20:6508–6517.

Starr J.I., Mako M.E., Juhn D., and Rubenstein A.H. 1978. Measurement of serum pro-insulin–like material: cross reactivity of porcine and human proinsulin. J. Lab. Clin. Med.; 91: 691-692.

Stephen L., Berkowitz K. and Shreiner B. 2004. Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. Diabetes Spectrum; 17(3): 183-190.

Trinder, P. 1969. Enzymatic determination of glucose. An. Clin. Bioch; 6: 24-27.

Warnick G.R., Benderson V. and Albers N. 1983. Selected methods. ClinChem; 10: 91-9.

Warnick G.R., Benderson V. and Albers N. 1983. Selected methods. ClinChem; 10:91-9.

Yazdi F.T., Clee S.M. and Meyre D. 2015. Obesity genetics in mouse and human: back and forth, and back again. PeerJ; 3: e856.

****