INTRODUCTION

Malnutrition in children is a global public health problem with wide implications. Malnourished children have increased risk of dying from infectious diseases, and it is estimated that malnutrition is the underlying cause of 45% of global deaths in children below 5 years of age. It is also increases susceptibility to infections while infections aggravate malnutrition by decreasing appetite, inducing catabolism, and increasing demand for nutrients ***(Rytter et al., 2014).***

In response to infection, the immune system first executes innate and then subsequently acquired host defense functions of high diversity. Both processes involve activation and propagation of immune cells and synthesis of an array of molecules requiring DNA replication, RNA expression, and protein synthesis and secretion, and therefore consume additional anabolic energy. Mediators of inflammation further increase the catabolic response ***(Schaible et al., 2007)***

Several mechanisms may lead to oxidative stress in malnourished children. The most important one is the subnormal intake of nutrients such as carbohydrates, proteins and vitamins, leading eventually to accumulation of ROSs. Reduced concentrations of vitamin A and of the anti-oxidant vitamins C and E together with deficiency of trace elements (selenium) were previously reported in children with malnutrition ***(Boşnak et al., 2010).***

Leptin, the product of the *ob* gene, is a single-chain proteohormone produced by adipose tissue, but also by placenta (syncytiotrophoblasts), ovaries, skeletal muscle, stomach, mammary epithelial cells, bone marrow, pituitary, and liver, with multiple functions through various receptors Located centrally and peripherally. Centrally, leptin acts particularly on the hypothalamus to suppress food intake and stimulate energy expenditure ***(Soliman et al., 2016).***

In addition to playing a role in energy regulation, leptin also regulates endocrine and immune functions. Both the structure of leptin and that of its receptor suggest that leptin might be classified as a cytokine. The secondary structure of leptin has similarities to the long-chain helical cytokines family, which includes interleukin 6 (IL-6), IL-11, CNTF, and LIF, and the leptin receptor is homologous to the gp-130 signal-transducing subunit of the IL-6-type cytokine receptors ***(Faggioni et al., 2001).***

Leptin plays a role in innate and acquired immunity. Leptin levels increase acutely during infection and inflammation, and may represent a protective component of the host response to inflammation. More important, leptin deficiency increases susceptibility to infectious and inflammatory stimuli and is associated with dysregulation of cytokine production ***(Faggioni et al., 2001).***

Leptin deficiency also causes a defect in hematopoiesis. Leptin regulates T cells responses, polarizing Th cells toward a Th1 phenotype. Low leptin levels occurring during starvation mediate the neuroendocrine and immune dysfunction of starvation ***(Faggioni et al., 2001).***

In the study of ***Freemark (2015)*** showing that the major biochemical factor predicting mortality in malnourished children was low leptin. This study speculates that hypoleptinemic children can generate and oxidize free fatty acids acutely but deplete their adipose reserves under continuing stress. The depletion of white adipose stores is postulated to limit the ability of a child to sustain energy production during the acute phase of the illness; this would increase that child's risk of death from cardiopulmonary failure ***(Freemark, 2015).***

In two studies on infants and children with mild and severe forms of protein energy malnutrition (PEM), leptin concentrations were significantly decreased and positively correlated with triceps, scapular, and abdominal fat thickness. In severe PEM cases, concentrations of IGF-I are significantly low, whereas basal cortisol and GH concentrations are significantly high versus normal children. The BMI is correlated significantly with leptin, insulin and IGF-I. These findings suggest that during prolonged nutritional deprivation, the decreased energy intake, diminished fat mass, and declining

insulin (and possibly IGF-I) concentrations suppress leptin production ***(Soliman et al., 2016).***

The decrease of energy intake and adipose tissue and serum IGF-1 levels in children with PEM may result in decrease of leptin secretion. Decrease in serum leptin levels may initiate food intake by increasing appetite and stimulating the secretion of cortisol and GH that might increase energy expenditure through an autocrine mechanism. Moreover, serum leptin level may be an important signal to reflect the metabolism of children with PEM ***(Kilic et al., 2004).***

On the other hand, during recovery from malnutrition, leptin concentrations increase in relation to fat mass. During recovery from severe PEM, an increase in leptin concentration was observed only in children who showed catch-up growth. More interestingly, malnourished children with catch-up growth had higher serum leptin concentrations compared to healthy children. This suggested that leptin affects nutritional status during catch-up growth as a dynamic process, rather than merely being an index of body fat content ***(Soliman et al., 2016).***

Honey is a natural substance with a lot of benefits for nutrition and health. Among honey benefits are its anti-inflammatory, anti-oxidant and anti-microbial effects and knowing the pathogenesis of PEM such patients are expected to benefit from honey ***(Shaaban et al., 2010).***

Other important effects of honey on human digestion have been linked to oligosaccharides. These honey constituents have prebiotic effects, similar to that of fructo-oligosaccharides. The oligosaccharide panose was the most active oligosaccharide. The oligosaccharides cause an increase of bifidobacteria and lactobacilli and exert the prebiotic effect in a synergistic mode of action. In another study honey increased both *in vivo* (small and large intestines of rats) and *in vitro* the building of *Lactobacillus acidophilus* and *Lactobacillus plantarum,* while sucrose had no effect ***(Bogdanov et al., 2008).***

The gut microbiota plays an important role in the regulation of the host's metabolism and in the extraction of energy from ingested food. It is important to note that the development and functions of the gut immunity depends of the microbiota establishment some recently reports suggest a direct correlation between gut microbiota and the appetite-regulating hormones. Authors demonstrated that the increases of serum leptin levels were related negatively with the Bacteroides, Clostridium and Prevotella quantity. Also, other study demonstrated that a Prevotella genus was identified in obese and diabetic db/db mice ***(Gabriela et al., 2016).***

AIM OF THE WORK

To evaluate the effect of honey supplementation on malnourished infants and children regarding anthropometric measurements and serum leptin level.

LEPTIN

Introduction:

Leptin "thin", the "satiety hormone", is approximately ~16 kDa in mass and encoded by the obese (ob) gene. This hormone is made by fat cells which regulate the amount of fat stored in the body ***(Neill, 2010).***

It does this by adjusting both the sensation of hunger, and adjusting energy expenditures ***(Brennan and Mantzoros, 2006).***

Location of gene

The *Ob(Lep)* gene (Ob for obese, Lep for leptin) is located on long arm of chromosome 7 in humans (7q31) ***(GreGreen et al., 1995).***

Leptin is produced primarily in fat cells and also in the placenta, where it is regulated by estradiol ***(Gambino et al., 2010).***

It is also produced by the stomach (the lower part of the fundic glands) where it is released into the intestine and then absorbed, mammary epithelial cells, bone marrow, pituitary, liver and gastric chief cells ***(Cammisotto and Bendayan, 2012).***

Structure of hormone

The 167 amino acid leptin has a 21 amino acid N-terminal signal sequence that is cleaved during leptin

maturation. This sequence suggests that leptin is an important protein required for regulation of fat ***(Hong et al., 2010).***

Vertebrate leptins have two conserved cysteine residues (Cys117 and Cys167 in mouse leptin) that form an intramolecular disulfide bond between the C-terminus and the beginning of the CD loop ***(Procaccini et al., 2012).***

The function of the disulfide bond has been debated. Initial studies reported that the disulfide bond was essential for folding and receptor binding ***(Coppari and Bjorbaek, 2012).***

Leptin Secretion & Blood levels:

Physiologic variation:

Leptin circulates in blood in free form and bound to proteins. Leptin levels are pulsatile and follow a circadian rhythm, with highest levels between midnight and early morning and lowest levels in the early to mid afternoon, being maximal around 2.00 am and minimal around 8.00 am ***(Bluher et al., 2009).***

In specific conditions:

- In humans, many instances are seen where Leptin dissociates from the strict role of communicating nutritional status between body and brain and no longer correlates with body fat levels ***(Ahmed, 2012).***
- Leptin plays a critical role in the adaptive response to starvation ***(Friedman, 2009).***

- Leptin levels exhibit significant changes during progressive pubertal stages, with a distinct dimorphism between boys and girls. In boys, there is a pre-pubertal peak of serum leptin levels preceding the rise of free testosterone, growth hormone (GH), and insulin-like growth factor (IGF-1). Thereafter, about 3 years after the rise in serum testosterone levels, leptin levels fall to baseline concentrations ***(Soliman et al., 2016).***

- Girls have higher serum leptin levels than males and their leptin levels rise throughout puberty, concomitant with the rise in estrogen levels ***(Soliman et al., 2016).***

Leptin level is decreased:

After short term fasting (24–72 hours), even when changes in fat mass are not observed ***(Chan et al., 2003).***

- Sleep deprivation ***(Seaborg, 2007).***

- Leptin level is chronically reduced by physical exercise training ***(De Salles et al., 2010).***

Leptin level is increased by:

- Perceived emotional stress ***(Otsuka et al., 2006).***
- Dexamethasone ***(Considine et al., 1997).***
- Insulin ***(Kolaczynski et al., 1996).***
- Obesity ***(Caro et al., 1996).***

Leptin Elimination

The main elimination route for leptin is via the kidney ***(Cumin et al., 1997).***

Leptin Concentration

Leptin concentration reflects the amount of energy stored in body fat. Circulating leptin levels are directly proportional to the amount of body fat and fluctuate with acute changes in caloric intake. This is especially sensitive to energy deprivation ***(Chan et al., 2003).***

Females tend to have higher leptin levels than males. This sexual dimorphism is largely independent of body mass index (BMI), and is due in part to differences in sex hormones, fatk mass, and body fat distribution ***(Keiss et al., 2008).***

Leptin Receptors

Leptin binds to leptin receptors (*ObRs*) located throughout the central nervous system and several peripheral tissues. At least six variations or isoforms of the leptin receptor have been identified (*ObRa, ObRb, ObRc, ObRd, ObRe*, and *ObRf).* The short isoforms *ObRa* and *ObRc* are thought to play important roles in transporting leptin across the blood brain barrier (BBB) ***(Price et al., 2010).***

The long leptin receptor isoform *ObRb* is located in areas where leptin is thought to act, including hypothalamic and other brain stem nuclei. There is also a circulating form of the leptin receptors that binds leptin and may modulate its action ***(Meier and Gressner, 2004).***

Mechanism of Action

Leptin Signaling:

Primary site, known mechanisms of action and clinical results as shown in table (1).

Table (1): Leptin Signaling ***(Dardeno et al., 2010).***

Signaling Pathway	Primary Site of Action	Known Mechanisms of Action	Clinical Results
JAK-STAT3	Hypothalamus	Stimulates transcription of POMC and suppresses transcription of NPY.	Regulates appetite and, thus, body weight. May also contribute to neuro-endocrine function as neural-specific STAT3 deletion results in decreased linear growth and infertility.
P13K	Hypothalamus	Stimulates POMC neurons. Inhibits FOXO1, an inhibitor of POMC transcription, to increase POMC expression.	Regulates appetite and body weight. May contribute to leptin resistance in obesity, given the overlapping pathway with insulin. May mediate the stimulation of sympathetic outflow.
MAPK	Hypothalamus, liver, pancreas, adipose tissue, and myocytes	Stimulates POMC neurons and inhibits AgRP/NPY neurons.	Regulates appetite and body weight. Increases sympathetic activity to brown adipose tissue. Increases fatty acid oxidation in peripheral tissues. Promotes cardiomyocyte hypertrophy.
AMPK	Hypothalamus, muscle	Stimulates ACC activity in the hypothalamus to regulate food intake and weight. Inhibits ACC activity in muscle.	Regulates appetite and weight. Stimulates fatty-acid oxidation in muscle and may sensitize muscle to insulin.
mTOR	Hypothalamus	Induces phosphorylation of S6K1 to regulate protein synthesis.	Regulates appetite and weight.

Abbreviations: ACC, acetyl coenzyme A carboxylase; **AMPK**, 5'adenosine monophosphate-activated protein kinase; **ATP**, adenosine triphosphate; **FOXO1**, forkhead box O1; **JAK-STAT3**, janus kinase-signal transducers and activator of transcription 3; **K+**, potassium; **MAPK**, mitogen-activated protein kinase; **mTOR**, mammalian target of rapamycin; **NPY**, neuropeptide Y; **POMC**, pro-opiomelanocortin; **P13K**, phosphatidylinositol 3-kinase; **S6K1**, S6 Kinase 1 ***(Dardeno et al., 2010).***

The physiological roles of leptin

1- The Role of Leptin in Energy Homeostasis:

Leptin regulates overall metabolism, including food intake, energy balance and body temperature by signaling satiety and decreasing the sensation of hunger at the hypothalamic level ***(Khan et al., 2012).***

At the peripheral level, the hormone stimulates the process of oxidation of fatty acids in muscles, inhibits the accumulation of triglycerides in hepatic cells ***(Meier and Gressner, 2004).***

It has potent lipid-lowering effects in peripheral tissues and plasma that are thought to be essential for the prevention of cellular lipotoxicity and insulin resistance ***(Mostyn et al., 2001).***

Basic functions, such as thirst and hunger-mediated ingestive behavior, are programmed *in utero,* and leptin seems to be an essential regulator in this process through its neurotrophic actions at the hypothalamic level ***(McMillen et al., 2004).***

There is some evidence from experimental studies, that in early neonatal life, leptin may promote hyperphagia as an adaptive response to overcome the physiological weight loss observed during postnatal days, and may also be a signal that initiates the enteric feeding ***(El-Haddad et al., 2004).***

In two studies on infants and children with mild and severe forms of protein energy malnutrition (PEM), leptin concentrations are significantly decreased and positively correlated with triceps, scapular, and abdominal fat thickness. In severe PEM cases, concentrations of IGF-I are significantly low, whereas basal cortisol and GH concentrations are significantly high versus normal children. The BMI is correlated significantly with leptin, insulin and IGF-I ***(Soliman et al., 2016).***

On the other hand, during recovery from malnutrition, leptin concentrations increase in relation to fat mass. During recovery from severe PEM, an increase in leptin concentration was observed only in children who showed catch-up growth. More interestingly, malnourished children with catch-up growth had higher serum leptin concentrations compared to healthy children. This suggested that leptin affects nutritional status during catch-up growth as a dynamic process, rather than merely being an index of body fat content ***(Soliman et al., 2016).***

2- The Role of Leptin in Brain Development and Synaptic Connection Plasticity:

Leptin is thought to be related to brain development, because leptin receptors are widely expressed in the brain ***(Udagawa et al., 2006).***

Another interesting finding of a recent report indicates a possible action of leptin as a cognitive enhancer in the hippocampus and on excitatory synaptic strength ***(Harvey et al., 2005).***

Leptin may also rearrange synaptic connections, or stimulate synaptic plasticity, in the hypothalamus, and this may contribute to leptin's regulation of energy homeostasis ***(Abiazid et al., 2006).***

3- The role of Leptin in Neuroendocrine Physiology and Pathophysiology:

a. Leptin in relation to the hypothalamic-pituitary-gonadal axis:

Several neurons involved in energy homeostasis are anatomically associated with GnRH neurons (e.g. AgRP/NPY and POMC neurons) and may be the link between changes in energy balance and subsequent alterations in reproductive function ***(Hill et al., 2008).***

Leptin may mediate the reproductive axis through regulation of kisspeptins, products of the Kiss1 gene, as well as dynorphin and neurokinin B ***(Shahab et al., 2005).***

Human mutations in the gene encoding neurokinin B or its receptor leads to defective GnRH release and subsequent hypogonadism ***(Lehman et al., 2010).***

Leptin receptors have been identified in the hypothalamus, the gonadotrope cells of the anterior pituitary, and gonads of higher primates and human, Leptin administration increased serum concentrations of luteinizing hormone (LH) and growth hormone (GH) in the rodent. Intra cerebroventricular (ICV) administration of leptin suppressed feed intake, increased serum GH concentrations, and stimulated hypothalamic gonadotropin releasing hormone (GnRH) release in the rat ***(Soliman et al., 2016).***

b. Leptin in relation to the hypothalamic-pituitary-thyroid axis:

Leptin influences the thyroidal axis by regulating the expression of thyrotropin releasing hormone (TRH) ***(Ghamari-Langroudi et al., 2010).***

In healthy humans, thyroid-stimulating hormone (TSH) is secreted in a pulsatile fashion similar to that of leptin, reaching a peak in the early morning hours and nadir in late morning. Individuals with congenital leptin deficiency have a highly disorganized TSH secretion pattern, suggesting that leptin may regulate TSH pulsatility and circadian rhythm ***(Paz-Filho et al., 2009).***