STUDYING THE ROLE OF LEPTIN AND ADIPONECTIN HORMONES AND THEIR RELATION WITH SOME REPRODUCTIVE HORMONES IN BREASTFEEDING WOMEN

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ABSTRACT
This study aims to find out the role of the hormones leptin and adiponectin and their relationship with some reproductive hormones (Prolactin, FSH and LH) among suckling women. The whole sample is about 120 women (aged 25-35 years), included 60 suckling mothers and 60 non-suckling women. Each of these both suckling and non-suckling women divided into three groups (20 women/group) according to the periods of suckling as the following: first group (six months), second group (twelve months), third group (eighteen months). Results revealed: Leptin levels increased significantly (except first and second groups) (p ≤ 0.05) in the non-suckling women in comparison with the suckling women for different groups. Adiponectin hormone levels increased significantly (except third group) (p ≤ 0.05) in the non-suckling women in comparison with the suckling women for different groups. Prolactin hormone levels decreased significantly (p ≤ 0.05) in non-suckling women in comparison with the suckling women for different groups. Follicle-stimulating hormone (FSH) levels increased significantly (p ≤ 0.05) in non-suckling women in comparison with the suckling women for different groups. Luteinizing hormone (LH) levels increased significantly (p ≤ 0.05) in non-suckling women in comparison with the suckling women for different groups. The physiological impacts for these changes be discussed according to the role of suckling and their positive effects on those women related with these current hormones.

Key words: leptin, adiponectin, prolactin, FSH and LH.

I. INTRODUCTION
Suckling is a natural and direct outcome of pregnancy and birth, and it is a vital part of the reproductive process that benefits both the mother and the infant (Kent, 2006). Moreover, suckling is an important part of a woman's reproductive process, and it has long-term effects on metabolism, as well as the ability to reduce adverse metabolic risk factor changes associated with pregnancy, awoman's body undergoes several dramatic changes in metabolism during pregnancy, which may predispose her to some health disparities if not reversed (Hyatt et al., 2017).

Stuebe and Rich-Edwards (2009) stated that suckling plays a critical and effective role in mobilizing fat stores accumulated during pregnancy and "resetting" maternal metabolism, lowering the mother's risk of metabolic diseases and improving maternal health in the future. Conversely, when a woman does not suckling, adverse metabolic changes last longer, potentially predisposing her to certain health inequalities, dysregulation of metabolism has been linked to health disparities (such as obesity and type 2 diabetes) (Hyatt et al., 2017).

Adipokines, also known as adipocytokines, are a group of cytokines (cell signaling proteins) that include (leptin, adiponectin, resistin, chemerin, visfatin, etc.) adipose tissue secretes (Ryu, 2018), which modulate various biological functions (Lecke et al., 2011; Recinella et al., 2020), these adipokines regulate homeostasis of total body energy and serve as a biomarker for the metabolic state of the body (Ranjan, 2017).

Leptin and adiponectin play a role in controlling glucose and lipid metabolism, energy consumption, inflammatory reaction, immunity, reproduction, and cardiovascular action in the endocrine system (Lecke et al., 2011), these endocrine markers are associated with subsequent metabolic disease risk (Schwartz and Niswender, 2004; Stuebe et al., 2011), whereas high leptin levels are linked to poor metabolic profiles, and
high adiponectin levels are linked to a reduced risk of diabetes and metabolic disease (Moschos et al., 2002; Oh et al., 2007; Stuebe et al., 2011). Many studies demonstrated the direct and indirect roles of leptin during pregnancy and suckling. The leptin has myriad effects on tissues and endocrine systems that ultimately lead to the coordination of whole-body energy metabolism (Houseknecht et al., 1998), that leptin and metabolic hormones regulating food intake and body weight are key players in promoting neural and physiological adaptations that occur during pregnancy, lactation, and postnatal growth (Boyle and Le Foll, 2019).

The presence of adiponectin in cerebrospinal fluid and the expression of its receptor AdipoR1 in the arcuate nucleus of the hypothalamus support the hypothesis that adiponectin has central activity in the regulation of energy homeostasis, stimulating food intake and decreasing energy expenditure (Kubota et al., 2007; Savino et al., 2012).

Many studies demonstrated the direct and indirect roles of adiponectin during pregnancy and suckling, in order to provide sufficient nutrition to the fetus and infant, the maternal metabolism is dynamically altered, however, gestational insulin resistance occurs from mid-pregnancy, facilitating attempts to provide energy substrate to the fetus, which preferentially uses glucose as an energy source (Asai-sato et al., 2006).

The main function of prolactin is during pregnancy and lactation in the development of mammary glands, milk synthesis and maintenance of milk secretion (Saleem et al., 2018), where prolactin plays a major role in driving many of the maternal body's adaptations to enable the mother to meet the physiological requirements of both pregnancy and lactation, including the developing fetus's high energy requirements followed by milk output to sustain offspring after birth (Lopez-Vicchi et al., 2020). During pregnancy, serum prolactin levels rise rapidly due to an increase in the size and number of lactotrophs, during lactation, suckling causes rapid prolactin secretion through a neuroendocrine reflex pathway (Saleem et al., 2018).

GnRH secretion is stimulated by leptin, and the pituitary gland releases LH as a result (Tena-Sempere et al., 2000; Popovic et al., 2001), there is mounting evidence that kisspeptin neurons in the anteroventral periventricular nucleus mediate the nutritional signal that connects leptin and GnRH. Kisspeptin neurons express the leptin receptor, and leptin therapy restores suppressed KISS-1 expression in rodents with gonadotropin deficiency and low leptin, such as the ob/ob mouse and the streptozotocin-induced diabetic rat (Tena-Sempere et al., 2000), a leptin-induced increase could mediate the increase in GnRH pulsatility that occurs after recombinant leptin is administered to low-weight women with hypothalamic amenorrhea (Welt et al., 2004). In vitro and in vivo evidence suggests that adiponectin could be one of the hormones regulating the relationship between energy balance and fertility in many mammals, including humans. Its two receptors, AdipoR1 and AdipoR2, are expressed in the hypothalamic–pituitary–gonadal axis, and their activation controls KISS-1, GnRH, and gonadotropin expression and/or secretion (Barbeet et al., 2019). Continuing to breastfeed increases prolactin levels, which inhibits gonadotropin-releasing hormone (GnRH), which inhibits luteinizing hormone (LH) and follicle-stimulating hormone (FSH), resulting in the absence of ovulation and menopause during the variable-term lactation period (Milenković et al., 1994; Levine and Muneyyirci-Delale, 2018). In view of foregoing, we tried to shed some light on the role of leptin, adiponectin and some reproductive hormones.

II. MATERIALS AND METHODS

The sample of this study included (120 women) aged 25-35 years, included sixty suckling women and sixty non-suckling women. Each these both suckling and non-suckling women divided into three groups (20 women/group) according to the periods of suckling as the following: First group (six months), Second group (twelve women), Third group (eighteen months). The blood samples prepared by the usual procedure in order to measure Leptin, Adiponectin, Prolactin, FSH and LH. Determination of Leptin and Adiponectin Principle of the Assay was based on sandwich enzyme-linked immune-sorbent assay technology, was performed using Human Leptin ELISA Kit and Human Adiponectin Kit (Elabscience / USA) respectively, according to the manufacturer's instructions. Prolactin, FSH and LH measured via Sandwich principle, was used by using the hormones kits (Roch /Germany). The results are expressed as mean ± standard division (SD). Statistical analysis was performed by IBM SPSS statistics, version 26 (IBM Co., Armonk, NY, USA). The statistical analysis was performed by t-test at (p-value ≤ 0.05) significant level.

III. RESULTS

Hormonal parameters

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Leptin hormone
First Group
Results revealed : that the leptin levels in the non - suckling women (6.827 ±0.308 ng/ml) increased (not significantly) in comparison with the suckling women (6.705 ±0.374 ng/ml). Figure (1).

Second Group
Results revealed : that the leptin levels in the non - suckling women (6.870 ±0.266 ng/ml) increased (not significantly) in comparison with the suckling women (6.700 ±0.390 ng/ml). Figure (1).

Third Group
Results revealed : that the leptin levels in the non - suckling women (6.911 ±0.218 ng/ml) increased significantly ($p \leq 0.05$) in comparison with suckling women (6.666 ±0.311 ng/ml). Figure (1).

Adiponectin hormone
First Group
Results revealed : that the adiponectin levels in the non - suckling women (283.470 ±23.467 ng/ml) increased significantly ($p \leq 0.05$) in comparison with the suckling women (255.070 ±26.886 ng/ml). Figure (2).

Second Group
Results revealed : that the adiponectin levels in the non - suckling mothers (277.110 ±24.968 ng/ml) increased significantly ($p \leq 0.05$) in comparison with the suckling women (255.712 ±28.873 ng/ml). Figure (2).

Third Group
Results revealed that the adiponectin levels in the non - suckling women (255.440 ±18.791 ng/ml) increased (not significant) in comparison with the suckling women (249.630 ±15.897 ng/ml). Figure (2).

Prolactin hormone
First Group
Results revealed : that the prolactin levels in the non - suckling women (25.875 ±4.568 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (89.958 ±8.844 ±0.374 ng/ml). Figure (3).

Second Group
Results revealed : that the prolactin levels in the non - suckling women (24.609 ±5.239 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (75.282 ±9.802 ng/ml). Figure (3).

Third Group
Results revealed : that the prolactin levels in the non - suckling women (18.464 ±3.719 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (52.679 ±8.645 ng/ml). Figure (3).

FSH
First Group
Results revealed : FSH hormone levels in the non - suckling women (7.810 ±0.560 mIU/mL) increased significantly ($p \leq 0.05$) in comparison with the suckling women (6.949 ±0.923 mIU/mL). Figure (4).

Second Group
Results revealed : FSH hormone levels in the non - suckling women (8.083±0.864 mIU/mL) increased significantly ($p \leq 0.05$) in comparison with the suckling women (7.403 ±0.778 mIU/mL). Figure (4).

Third Group
Results revealed that the FSH hormone levels in the non-suckling women (8.602 ± 0.885 mIU/mL) increased significantly (p ≤ 0.05) in comparison with the suckling women (7.713 ± 0.965 mIU/mL). Figure (4).

**LH**

First Group

Results revealed: LH hormone levels in the non-suckling women (7.276 ± 0.929 mIU/mL) increased significantly (p ≤ 0.05) in comparison with the suckling women (5.944 ± 0.974 mIU/mL). Figure (5).

Second Group

Results revealed: LH hormone levels in the non-suckling women (8.188 ± 0.998 mIU/mL) increased significantly (p ≤ 0.05) in comparison with the suckling women (6.551 ± 0.645 mIU/mL). Figure (5).

Third Group

Results revealed: LH hormone levels in the non-suckling women (8.643 ± 0.949 mIU/mL) increased significantly (p ≤ 0.05) in comparison with the suckling women (7.606 ± 0.840 mIU/mL). Figure (5).

Figure (1): Leptin levels in suckling and non-suckling women for different groups.

The values represent mean ± SD.
Figure (2): Adiponectin levels in suckling and non-suckling women for different groups.

The values represent mean ± SD.

Figure (3): Prolactin levels in suckling and non-suckling women for different groups.

The values represent mean ± SD.
IV. DISCUSSION:

The present results revealed that the leptin hormone levels increased significantly (except first and second group) in the non-suckling women in comparison with the suckling women for different groups. Figure (1).

Leptin is considered as one of the fatty hormones whose secretion increases with the high percentage of fat mass, therefore, the present results might be pointed out some high presence of fatty mass in non-suckling women groups (especially the third group), while the present lipid profile in non-suckling women groups indicated a high presence of fatty parameters in these women. On the other hand, the present reduction of leptin hormone in suckling women groups (especially the third group) might be pointed out a high reduction in fatty parameters due to the depletion of those fat in suckling requirements and milk production.

Circulating leptin levels are positively correlated with the amount of body fat (Chan and Mantzoros, 2005; Savino et al., 2010). Plasma leptin was found to be strongly associated with body mass index (BMI) in rodents and in lean and obese humans, suggesting that it may play an important role in controlling body weight by signaling the size of adipose tissue mass (Maffei et al., 1995). Leptin is a hormone secreted by adipose tissue in

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Figure (4): FSH hormone levels in suckling and non-suckling women for different groups. The values represent mean ± SD.

Figure (5): LH hormone levels in suckling and non-suckling women for different groups. The values represent mean ± SD.
proportion to its mass, it circulates in the bloodstream and works on the brain to control food intake and energy consumption, when fat mass decreases, plasma leptin levels decrease, stimulating appetite and suppressing energy consumption until fat mass is restored, when fat mass rises, leptin levels rise, inhibiting appetite until weight is lost, this mechanism retains homeostatic regulation of adipose tissue mass (Friedman, 2011). Leptin appears to be a crucial link between adipose tissue and not only the hypothalamic centers that regulate energy homeostasis, but also the reproductive system, indicating whether sufficient energy reserves are present for normal reproductive function (Moschos et al., 2002; Gale et al., 2004).

On the other hand, the conditions of milk synthesis and release create a state of negative energy balance in the suckled mother and, in laboratory rodents, are followed by a dramatic hyperphagia (Crowley, 2011).

Furthermore, the decrease in leptin secretion, a hallmark of negative energy balance, may be a significant endocrine signal to hypothalamic systems that integrate lactation-related food intake with neuroendocrine systems (Crowley, 2011).

The present decreased adiponectin levels in suckling women groups (figure 2) may be attributed to the high levels of prolactin hormone that inhibited the adiponectin production during the suckling period, meanwhile, the high levels of prolactin in suckling women groups and a low levels of prolactin in non-suckling women groups in current study may be explained the high and low levels of adiponectin in non-suckling and suckling women groups, respectively.

Maternal adiponectin levels are suppressed during the lactation period compared to levels during pregnancy or those for non-pregnant and non-breastfeeding women under normal reproductive cycles (Asai-Sato et al. 2006; Fuglsang et al., 2010; Anderson et al., 2016).

According to Asai-Sato and colleagues (2006), prolactin influences the regulation of maternal metabolism by suppressing adiponectin, it has also been suggested that adiponectin is involved in the alterations of maternal metabolism during lactation under the influence of prolactin. PRL suppresses lipid accumulation as well as the release of adipokines including adiponectin, interleukin-6, and possibly leptin, according to several lines of evidence. PRL has also been linked to the regulation of adipogenesis (Brandebourg et al., 2007).

Adiponectin prevents lipolysis in humans and mice (Qiao et al., 2011; Wedellova et al., 2011; Singh et al., 2014), as a result, lower adiponectin concentrations promote the rate of lipolysis, in addition, the effect of adiponectin on insulin sensitivity and glucose and fatty acid metabolism leads to nutrient partitioning, which may affect nutrient availability in the mammary gland for milk production, furthermore, some pathological factors such as inflammation and endocrine hormones may affect adiponectin expression in adipose tissue (Singh et al., 2014).

The high levels of prolactin in suckling women (figure 3) be necessary to help the continuous production of milk via the suckling response, suckling stimulates the synthesis of prolactin hormone, while, the non-suckling women (without suckling response) have a less levels of prolactin.

Prolactin (PRL) is the main hormonal signal that stimulates milk composition in the mammary glands (Crowley, 2011). During suckling, PRL sends out a thorough signal that encourages the synthesis and excretion of milk components, as well as the survival of the alveolar cell, the amount of milk produced is determined by the removal of milk from the gland, a function that is dependent on the posterior pituitary gland's secretion of the hormone oxytocin and the contraction of muscle epithelial cells to push the milk out of the alveoli (Neville, 2006).

While milk secretion is a continuous operation, the amount of milk produced is controlled by infant demand, which means it is dependent on the suckling response. Suckling must begin within 3–4 days of delivery to keep milk secretion going (galactopoiesis) (Moreno-Villares et al., 2019).

The development of mammary glands, milk synthesis, and milk secretion maintenance are the main functions of PRL during pregnancy and lactation (Saleem et al., 2018), where PRL plays a significant role in driving many of the maternal body's adaptations to enable the mother to meet the physiological requirements of both gestation and breastfeeding, including the high energy requirements of the developing fetus followed by milk production to sustain offspring after childbirth (Lopez-Vicchi et al., 2020).
Prolactin levels will not stay elevated after delivery; PRL levels will only spike during times of nipple stimulation, allowing for control over milk production; as long as suckling is sustained, PRL levels will remain elevated; if the mother does not nurse her son, PRL levels will fall to non-pregnant levels after 1 to 2 weeks (Freeman et al., 2000; Al-Chalabi et al., 2020).

However, Neville and Morton, 2001 showed the levels of PRL in postpartum are similar in both suckling and non-suckling women, so that the basic process occurs regardless of whether breastfeeding is initiated.

Elevated prolactin may have an inhibitory action on the gonadotropin (FSH and LH), therefore, the reduction in FSH and LH hormones levels during the different groups in suckling women (figure 5) may be attributed to the present high concentrations of prolactin hormone in suckling women, whereas, the increase in the levels of FSH and LH hormones during different groups in non-suckling women may be attributed to present reduction in prolactin hormone concentrations in non-suckling women.

Grattan and colleagues (2007) discovered evidence of PRL-R mRNA expression in a similar small subpopulation of GnRH neurons, and their findings support the hypothesis that PRL inhibits LH secretion by action that is at least partially mediated by direct suppression from the GnRH neuron.

Since prolactin inhibits the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, a lack of GnRH causes a lack of pulsatile stimulation of gonadotrophic cells, resulting in the loss of FSH and LH from the anterior pituitary, as FSH and LH are the primary hormones required to control menstruation, lactating females will experience a phase of transient amenorrhea before breastfeeding is discontinued (Koike et al., 1991; Grattan et al., 2007; Al-Chalabi et al., 2020).

The pulsatile secretion of luteinizing hormone (LH), which reflects hypothalamic gonadotropin-releasing hormone (GnRH) release, is irregular and much slower during this time of breast-feeding-induced amenorrhea, and is much slower than the one pulse per hour needed in the normal follicular phase of the menstrual cycle to drive follicle development (McNeilly, 2001) when the suckling stimulus falls below a certain level, fairly organized pulsatile LH secretion resumes, which is correlated with follicle growth and some steroid secretion (McNeilly, 2001). According to the above finding, we can concluded that the leptin and adiponectin may be interfere with prolactin, FSH and LH in breastfeeding women, reflecting the positive role of breastfeeding.

REFERENCES


