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The Assessment of Adiponectin, Leptin, Some Reproductive Hormones Levels, Some Biochemical Parameters and Antioxidants in suckling and Non-Suckling women in Maysan Province.

A thesis

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بسم الله الرحمن الرحيم

((وَالْوَالِدَاتُ يُرْضِعْنَ أَوْلَادَهُنَّ حَوْلَيْنِ كَامِلَيْنِ ۖ لِمَنْ أَرَادَ أَن يُتِمَّ الرَّضَاعَةَ ۚ))

صدق الله العلي العظيم

سورة البقرة _ الآية_٢٣٣

Supervisor's certificate

I certify that this thesis entitled " The Assessment of Adiponectin, Leptin, Some Reproductive Hormones Levels, Some Biochemical Parameters and Antioxidants in suckling and Non-suckling women in Maysan Province**.**"

"Has been prepared under my supervision at the college of science, university of Misan; as a partial fulfillment of the requirements for the degree of master of biology "

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Recommendation of head of biology department

In view of the available recommendations ; I forward this thesis to debate by the examining committee.

Signature

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Dedication

I Dedicate My Work To The Master Of The Age And Time, To The Rest Of Allah In His Land, To The Present Absent Imam, The Owner Of Time, The Expected Imam Al-Hujjah. I’m Dedicate Too My Word To My Family For Their Patient And Support.

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Summary

This research aims to study the physiological profile of adiponectin, leptin, some other reproductive hormones, oxidative stress, pro-inflammatory markers, and the lipid profile of suckling and non-suckling mothers in Maysan province, during the period from February - October 2020. The total sample of this study were 120 women their ages between (25-35 years) old, included 60 suckling mothers and 60 non-suckling mothers, who visited Al Sadder Teaching Hospital and some other healthy clinic centers. Each of these suckling and non-suckling mothers were divided into three groups (20 mothers / group) according to the periods of suckling as the following: First group (1-6months), Second group (7-12months) and Third group (13-18months).

Results revealed: Adiponectin hormone levels increased significantly (except third group) ($p \leq 0.05$) in the non - suckling mothers in comparison with the suckling mothers in different groups. Adiponectin levels decreased significantly ($p \leq 0.05$) (except second group in comparison with the first group) in non-suckling mothers for different groups. Adiponectin levels decreased slightly (except second group increased slightly in comparison with the first group) in suckling mothers for different groups.

Leptin levels increased significantly (except first and second groups) ($p \leq 0.05$) in the non - suckling mothers in comparison with the suckling mothers in different groups. Leptin levels increased and decreased slightly in non-suckling mothers and suckling mothers, respectively, for different groups.

Prolactin hormone levels decreased significantly ($p \leq 0.05$) in non - suckling mothers in comparison with the suckling mothers in different groups. Prolactin levels decreased significantly ($p \leq 0.05$) (except second group comparison with the first group) in non- suckling mothers for different groups. Prolactin levels decreased significantly ($p \leq 0.05$) in suckling mothers for different groups.

Follicle-stimulating hormone (FSH) levels increased significantly ($p \leq 0.05$) in non - suckling mothers in comparison with the suckling mothers in different groups. FSH levels increased significantly ($p \leq 0.05$) (except second

group in comparison with the first group) in non-suckling and suckling mothers for different groups.

Luteinizing hormone (LH)levels increased significantly ($p \leq 0.05$) in non - suckling mothers in comparison with the suckling mothers in different groups. LH levels increased significantly($p \leq 0.05$) (except third group in comparison with the second group) in non- suckling mothers for different groups. LH increased significantly ($p \leq 0.05$) in suckling mothers for different groups.

Malondialdehyde (MDA) levels increased significantly ($p \leq 0.05$) in non - suckling mothers in comparison with the suckling mothers in different groups. MDA levels increased significantly ($p \leq 0.05$) (except third group in comparison with the second group) in non- suckling mothers for different groups. MDA levels decreased significantly ($p \leq 0.05$) (except second group in comparison with first group) in suckling mothers for different groups.

Glutathione (GSH) and superoxide dismutase [Cu-Zn] (SOD1) levels decreased significantly ($p \leq 0.05$) in non - suckling mothers in comparison with the suckling mothers in different groups. GSH levels decreased significantly ($p \leq 0.05$) in different groups in non- suckling and in suckling mothers in different groups. SOD1 levels increased significantly ($p \leq 0.05$) in non- suckling and in suckling mothers for different groups.

Interleukin-6(IL-6) increased slightly in non-suckling mothers in comparison with the suckling mothers in different groups.IL-6 levels increased significantly ($p \leq 0.05$) (except IL-6 levels in the second group in comparison with the first group) in non-suckling mothers and suckling mothers for different groups.

C-reactive protein (CRP)increased significantly ($p \leq 0.05$) in non-suckling mothers in comparison with the suckling mothers in different groups. CRP levels decreased significantly ($p \leq 0.05$) for different groups in non -suckling mothers. CRP levels decreased slightly in suckling mothers for different groups.

Total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL- C), very low-density lipoprotein cholesterol (VLDL- C) increased

significantly ($p \leq 0.05$) and high-density lipoprotein (HDL- C) decreased significantly ($p \leq 0.05$) in non - suckling mothers in comparison with the suckling mothers in different groups.

Total cholesterol, TG, LDL- C, and VLDL- C decreased slightly in suckling mothers for different groups. HDL- C levels increased significantly ($p \leq 0.05$) (except third group in comparison with the second group) in suckling mothers for different groups .

The physiological impacts for these changes be discussed according to the role of suckling and their positive effects on current parameters, that reflect positively on health development for these mothers.

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List of abbreviations	
ADP	**Adiponectin**
ACRP30	**Adipocyte complement-related protein of 30 kDA**
AdipoQ	**Adiponectin , C1Q And Collagen Domain Containing**
AdipoR	**Adiponectin receptor**
APM1	**Adipose most abundant gene transcript 1.**
ACTH	**Adrenocorticotropic hormone**
ALT	**Alanine aminotransferase**
ALP	**Alkaline Phosphatase**
APPs	**Acute phase proteins**
ARH	**Arcuate hypothalamus**
AST	**Aspartate Aminotransferase**
AVPV	**Anteroventral periventricular nucleus**
BMI	**Body mass index**
C	**Ascorbic Acid**
C1q	**Complement factor**
CAT	**Catalase**
CNS	**Central nervous system**
CSF	**Cerebrospinal fluid**

CRP	**C-reactive protein**
DNA	**Deoxyribonucleic acid**
EC	**Extracellularly**
FALS	**Familial amyotrophic lateral sclerosis**
FSH	**Follicle-stimulating hormone**
GBP28	**Gelatin-binding protein 28**
GSH	**Glutathione**
GSSG	**Glutathione disulfide**
GSSG- rx	**Glutathione disulfide reductase**
GSTs	**Glutathione s-transferase**
GPX	**Glutathione peroxidase**
GnRH	**Gonadotropin-releasing hormone**
G-CSF	**Granulocyte colony-stimulating factor**
GH	**Growth hormone**
HDL	**High-density lipoprotein**
HMW	**High-molecular-weight**
HGH	**Human growth hormones**
HPL	**Human placental lactogen**
H_2O_2	**Hydrogen peroxide**
OH	**Hydroxyl**
AMPK	**Hypothalamic 5' adenosine monophosphate-activated protein kinase**
HPA	**Hypothalamic-pituitary-adrenal axis**
IR	**Insulin resistance**
IGF-1	**Insulin-like growth factor-1**
IL-6	**Interleukin-6**
L1β	**Interleukin-1β**
IL-12	**Interleukin-12**
IS	**Insulin sensitivity**
Kiss1	**Kisspeptin**

LEP	**Leptin**
LEPR	**Leptin receptor**
LPL	**Lipoprotein lipase**
LDL	**Low-density lipoprotein**
LH	**Luteinizing hormone**
LGI	**Low-grade inflammation**
MDA	**Malondialdehyde**
MetS	**Metabolic syndrome**
MCP-1	**Monocyte Chemoattractant Protein-1**
NADPH	**Nicotinamide adenine dinucleotide phosphate**
NO	**Nitric oxide**
NO2·-	**Nitrogen dioxide**
OSM	**Oncostatin M**
PVN	**Para ventricular nucleus**
OONO-	**Per oxynitrite**
ROO	**Peroxyl**
PHGPX	**Phospholipid hydroperoxide glutathione peroxidase**
POA	**Pre optic area**
PRL	**Prolactin hormone**
PRL-R	**Prolactin receptor**
RNS	**Reactive nitrogen species**
ROS	**Reactive oxygen species**
Se-GSHpx	**Selenium-dependent glutathione peroxidase**
SAA	**Serum amyloid A**
***O2**	**Superoxide anion radical**
ObRb	**ObRb: leptin receptor long - form b**
SOD	**Superoxide Dismutase**
SOD1	**Superoxide Dismutase [Cu-Zn]**
SOD2	**Superoxide Dismutase [Mn]**
SOD3	**Extracellular Cu-Zn SOD**

SON	**Supraoptic nucleus**
E	**Tocopherol**
TC	**Total Cholesterol**
TG	**Triglyceride**
TRH	**Thyrotropin-releasing hormone**
TNF-α	**Tumor necrosis factor alpha**
T2D	**Type 2 diabetes**
VLDL	**Very low-density lipoprotein**
WAT	**White adipose tissue**

Chapter One

Introduction

1. Introduction:

Suckling is a complicated physiological process which includes a variety of variables ranging from psychological factors to the secretory functioning of mammary epithelial cells, all of which lead to successful breastfeeding (Truchet and Honvo-Houéto , 2017), it is a characteristic of mammals that involves complex biochemical and neuroendocrine processes that result in the synthesis and secretion of milk from the mammary glands , these processes depend on coordinated activity of all of the body's physiological systems (Ciampo and Ciampo , 2018) , thus , "suckling is a natural and direct outcome of pregnancy and birth , it is a vital part of the reproductive process that benefits both the mother and the infant "(Kent , 2006).

The breast (mammary gland) undergoes a series of physiological changes in structure , shape , and size in preparation for breastfeeding , which occur at various stages of female development , including puberty , pregnancy , and lactation , these changes are vital to successful breastfeeding (Pillay and Davis , 2020) . During pregnancy, the mammary gland transforms from a simple ductal tree to a highly efficient external organ with vesicular-alveolar structures, allowing it to secrete milk (Servera *et al*., 2012) , where progesterone and prolactin levels rise to promote alveolar development and epithelial cell proliferation in the mammary gland (Neville *et al*., 2002; Arendt and Kuperwasser , 2015; Aranda-Gutierrez and Diaz-Perez , 2020) , , milk output , also known as lactogenesis , is divided into two phases: secretory differentiation and secretory activation (Pang and Hartmann , 2007) , secretory differentiation (lactogen 1 formation) of the mammary gland begins around mid-pregnancy as the hormones as the progesterone , PRL, estrogen, and some metabolic hormones such as insulin stimulate breast tissue to specialize into lactose cells which have an ability to produce milk components. Progesterone levels drop rapidly after birth, while PRL levels increase, allowing the start of the

secretory activation phase (formation of lactogen 2), which hormonal changes begin with the secretion of milk from the gland's epithelial cells in the breasts , this stage requires the presence of insulin and cortisol in addition to PRL, which is needed to control this stage (Pang and Hartmann , 2007; Berlato and Doppler , 2009 ; Truchetn and Honvo-Houéto, 2017).

In addition, suckling allows neuronal impulses to be transmitted to the hypothalamus, which induces the posterior pituitary to secrete oxytocin , leading to constriction of the mammary gland cells in the alveolar complex, enabling milk to flow out to the ducts in the nipple, making it accessible to the infant (Ballard and Morrow , 2013 ; Crowley , 2011 ; Feldman and Bakermans-Kranenbur , 2017) . Continuing to breastfeed increases PRL levels , which inhibits GnRH , which inhibits FSH and LH , resulting in the absence of ovulation and menopause during the lactation period (Milenković *et al.*, ,1994; Levine and Muneyyirci-Delale , 2018) .

Moreover , suckling is an important step in the reproductive process of women , and it has long-term effects on metabolism , as well as the ability to reduce unfavorable metabolic risk factors associated with gestation ,where a woman's body experiences several dramatic changes in metabolism during pregnancy , which may display her to some healthiness problems if not reversed (Hyatt *et al.*, 2017) , when a pregnancy begins there is an accumulation of visceral lipid , development insulin resistance(IR), and an increment in fat and triglyceride concentration (Stuebe and Rich-Edwards, 2009). When breastfeeding begins a metabolic shift occurs that changes resource allocation from storage to milk synthesis (Hyatt *et al.*, 2017) , because of the increased request for protein and energy required for the production of breastmilk , suckling represents the greatest physiological stress in a woman‘s life cycle (Makrides and Gibson , 2000) .

During the breastfeeding period , the maternal energy metabolism is characterized by an increase in fat mobilization from the mother's stores , as well as increased glucose production , to ensure sufficient milk production for child growth and development (Vila *et al.*, 2015).

On the other hand, in their hypothesis (the reset hypothesis), Stuebe and RichEdwards (2009) announced that breast feeding plays a critical and effective role in mobilizing fat stores accumulated during gestation and "resetting maternal metabolism ", thus reducing the mother's prospect of metabolic disease and enhancing maternal health in the future. In contrast, unfavorable metabolic alterations last longer in women who do not breastfeed , possibly predisposing them to specific health disparities , dysregulation of metabolism has been shown to play an implicit role in health inequalities (such as fatness and diabetes type two) (Hyatt *et al ., 2017).*

Furthermore, oxidative stress has also been related to the creation of these inequalities in health (Hyatt *et al* ., 2017), and "oxidative stress is defined as an imbalance in the production of potentially harmful reactive oxygen species and antioxidant defenses" (Christensen *et al*., 2015). There is mounting evidence that the relationship between oxidative damage and suckling is not linear (Valencak *et al.*, 2016).

At the same time , several studies have shown that breastfeeding causes a variety of physiological changes in the mother that has a direct positive impact on her health (Dieterich *et al.*, 2013) , Al-Zubeedy (2000) found that the activities of Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) enzymes decreased with the progressive period of suckling pointed an improvement physiological changes in these suckling mothers , moreover

, breastfeeding has been shown improves the positive effects on maternal blood lipids (Dieterich *et al*., ,2013) , as blood triglycerides decrease after birth (Stuebe and Rich-Edwards , 2009) , HDL cholesterol rises (Kallio *et al.*, 1992) and improves their metabolic condition after pregnancy (Stuebe and Rich-Edwards, 2009 ; Stuebe *et al*., 2011) , as well as , most studies have indicated an important relationship between breastfeeding and weight loss (Dewey *et al*., 1993) , protection against type 2 diabetes (T2D)(Gunderson , 2007) , and metabolic syndrome (MetS) (Ram *et al.*, 2008) and cardiovascular disease (Nguyen *et al.*, 2017) , as well , it has been announced that postpartum women within 4 to 6 weeks, especially those who are exclusively breastfed, have heightened and activated innate and adaptive immune defenses (Groer *et al*., 2005) , while another , study indicated the lack of evidence for pro-inflammatory changes during lactation (Kuzawa *et al.*, 2013) .

"Adipokines, also known as adipocytokines, are a group of cytokines (cell signaling proteins) that include (adiponectin ,leptin, resistin, chemerin, visfatin, etc.) secreted by adipose tissue" (Ryu, 2018), which modulate many biological functions (Lecke *et al*., 2011 ;Recinella *et al* ., 2020) , these adipokines regulate whole-body energy homeostasis and serve as a marker for the body's metabolic state (Ranjan , 2017). Leptin and adiponectin are contributes in the endocrine processes of regulating glucose and lipid metabolism, energy consumption, inflammatory response, immune system , reproduction, and cardiovascular function (Lecke *et al.*, 2011). These endocrine markers are linked to consequent metabolic illness risk (Schwartz and Niswender ,2004 ; Stuebe *et al* ., 2011) , whereas high concentrations of leptin were linked to poor metabolic profiles, elevated concentrations of adiponectin were linked to a reduced risk of diabetic and metabolic illness (Moschos *et al* ., 2002 ; Oh *et al.*, 2007 ; Stuebe *et al*., 2011) .

In addition , "adiponectin is an adipocyte-derived peptide hormone, which is inversely associated with adiposity and an active indicator of insulin sensitivity" (Kershaw and Flier 2004 ; Rose *et al* . , 2004) , adiponectin modulates a range of human physiological processes related to metabolism and inflammation(Ryo, 2004).

White adipose tissue (WAT) produces leptin in proportion to the amount of fat in the body (Martínez-Sánchez , 2020). This hormone controls feeding and bodyweight in humans and some mammals by functioning as an afferent satiety signal (Lecke *et al*., 2011) , the circulating leptin reduce food intake and increases energy consumption due to its effect on the hypothalamus (Baskin *et al.*, 1999) , as a result , it plays important role in the regulation of energy consumption as well as the metabolism of key tissues involved in energy storage and dissipation (Banks *et al.*, 2000) , thus leptin may plays a vital role in the regulation of metabolic adaptation of nutrient partitioning during the energy-consuming processes of gestation and suckling (Moschos *et al* ., 2002) .

The aim of the study

According to previous studies, this study was designed as an attempt to shed some light on the role of the hormones adiponectin and leptin, some other reproductive hormones, oxidative, inflammatory and biochemical parameters in suckling mothers in Maysan province. Therefore, the present research included studying the following parameters:

1. **Hormonal parameters:**
   a. Adiponectin hormone .
   b. Leptin hormone .
   c. Prolactin hormone (PRL) .
   d. Follicle-stimulating hormone (FSH).
   e. Luteinizing hormone (LH).
2. **Oxidative Stress And Antioxidant Parameters :**
   a. Malondialdehyde (MDA) .
   b. Glutathione (GSH).
   c. Superoxide Dismutase (SOD).
3. **3. Pro- inflammatory markers :**
   a. Interleukin-6(IL6) .
   b. C-reactive protein (CRP) .
4. **4. Lipid profile :**
   a. Total Cholesterol (TC).
   b. Triglyceride (TG).
   c. Low-density lipoprotein (LDL-C) .
   d. Very low-density lipoprotein (VLDL-C) .
   e. High-density lipoprotein (HDL-C).

Chapter TOW

Literature Review

2. Literature Review :

2.1 : Lactation: an overview

Lactation (suckling) is a dynamic process that has developed to create a complex biological fluid that supplies nutritive and non-nutritive factors to nursing offspring (Lee and Kelleher , 2016) . It's a special physiological process characterized by a set of behavioral , endocrine, and neural adaptations aimed at facilitating offspring nutritional support (Crowley, 2011) .

Successful lactation requires coordination of processes responsible for nutrient transport , milk output , and secretion from the mammary gland and driven by molecular, biochemical, and cellular events that are primarily regulated by reproductive hormones(Lee and Kelleher , 2016).

The proper amount and composition of breast milk for the growth, protection, and development of the baby is dependent on the full development of the breast (mammary gland) during lactation. Breast (mammary gland) development culminates during the pregnancy and lactation cycle, when the mammary gland undergoes complete remodeling, maturating into a functional milk-secretory organ (Hassiotou and Geddes ,2013).

Mammary glands , specialized milk-producing sweat glands that allow offspring to be fed , are a distinguishing feature of mammals (Cardiff and Wellings, 1999; Oakes *et al.*, 2008),that consists of a number of different cell types: epithelial cells (form the ductal network of the gland), adipocytes (constitute the fat pad which the ductal network is embedded), vascular endothelial cells (make up the blood vessels) , stromal cells (including fibroblasts , and a variety of immune cells) , moreover , mammary gland have two types of epithelium : luminal forms the ducts and the secretory alveoli , and basal consists essentially of myoepithelial cells ,

these two types of epithelium form a bi-layered structure of simple epithelium that is embedded within the fatty stroma (Watson and Khaled , 2008).

Throughout puberty, hormonal stimulation produced by the female gonads (ovaries) and pituitary gland coordinates the growth of the breast (mammary gland) (Oakes *et al.*, 2008) , the mammary glands develop during the mother's adulthood, when cyclic stimulation by estrogen and progesterone facilitates the development of the breast ducts. During gestation, estrogen, progesterone, PRL, insulin , cortisol , and thyroid hormones all contribute to the elaboration of glandular tissue (Pang and Hartmann , 2007;Stuebe , 2015).

Lactogenesis is the process through which the mammary gland develops the ability to secrete milk where lactogenesis includes all changes that transform the mammary gland from its undifferentiated condition in early pregnancy to its fully differentiated stage just after birth (Neville *et al.*, 2001; Moreno-Villares and Germán-Díaz , 2019) , lactogenesis takes place in two phases, secretory differentiation and secretory activation (Pillay and Davis,2020).

Lactogenesis phase I (secretory differentiation) occurs in the second half of gestation and defined by the differentiation of mammary alveolar epithelial cells into lactocytes, the specialized secretory cells, in response to hormones" (estrogen, progesterone, PRL),other hormones such as, placental lactogen and insulin hormones (Pang and Hartmann, 2007; Moreno-Villares and Germán-Díaz, 2019, Pillay and Davis, 2020).

Lactogenesis phase II (secretory activation), the initiation of profuse milk secretion in women, occurs during the first four days after birth ((Neville *et al* ., 2001; Pillay and Davis, 2020). In this stage, the levels of progesterone decrease dramatically (after delivery of the placenta), whereas elevated levels of PRL,

cortisol, and insulin are imperative for organization of this important stage, colostrum is often found for the first 3–5 days after birth, followed by transitional milk until about week 2–3 postpartum, after which phase III (Lactogenesis) is mature milk and replaces the transitional milk (Bryant and Thistle, 2020).

The synthesis of milk depends on the availability of substrate and on both endocrine and autocrine regulation. In early lactation, endocrine factors appear to predominate, PRL levels are highest in the early weeks of breastfeeding , T4, GH, cortisol, and insulin also contribute to normal milk synthesis (Lemay *et al.,* 2013; Stuebe, 2015).

Prolactin hormone (PRL) stimulates milk synthesis while milk secretions are triggered by oxytocin in the posterior pituitary (Stuebe, 2015). The oxytocin hormone is a tiny nine amino acid peptide that is involved in many physiologic and pathologic actions such as sexual activity, milk ejection, gestation, uterus contraction, maternity behavior, osteoporosis, diabetes, and cancers (Viero *et al*., 2010). Oxytocin is produced in the hypothalamic "supraoptic nucleus (SON)" and "paraventricular nucleus (PVN)" and is important during lactation (Uvnäs Moberg *et al*., 2020). Oxytocin, which is secreted into the bloodstream during suckling, induces milk outflow by contracting the myoepithelial cells circumjacent to the mammary gland alveoli and relaxing the milk duct sphincters (Uvnäs Moberg *et al.,* 2020).

In mammals, oxytocin is responsible for inducing the transfer of milk from the mammary glands' alveoli into the mouths of the suckled young. An efferent milk-ejection reflex limb is formed by intermittent secretions of large pulses of oxytocin from the posterior pituitary gland through lactation or as an imminent lactation

reflection. Milk cannot transmitted without oxytocin, as seen in oxytocin-deficient mice, and the young die (Leng and Russell , 2016).

Breastfeeding causes a decrease in the secretion of the gonadotropin-releasing hormones LH and FSH, leading to amenorrhea. It also lowered, lactation leads to the return of ovulation. Furthermore, during nursing, the menstrual cycle before 6 months , without ovulation usually, and fertility stays low. In addition to that, the postpartum infertility (lactational amenorrhea) method depends on three synchronous conditions , it is the child is under six months, the mother has no menstrual bleeding , and she exercises complete or quasi-complete suckling on request , day and night (Vekemans, 1997) .

Suckling is linked to central hypogonadotropic hypogonadism and the cessation of cyclic ovarian function, which is mediated by extensive crosstalk between the hypothalamic circuits that regulate appetite and gonadotropin-releasing hormone (GnRH) secretion (Smith *et al*., ,2010; Muroi and Ishii ., 2016; Sadovnikova *et al.*, 2020) , breastfeeding represses GnRH gene expression, as well as the pulsatile secretion of GnRH and LH , as a result , estrogen output is inhibited , and ovulation is prevented (Smith *et al* ., 2010 ; Grattan , 2015; ; Sadovnikova *et al*., 2020) .

Kisspeptin (Kiss1) "is a key regulator of GnRH secretion that is produced in the hypothalamus by neurons in the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus in rodents, and within the preoptic area (POA) and the infundibular nucleus in humans" (Smith *et al*., 2010; Marques *et al.*, 2018; Ieda *et al.*, 2020). Kiss1 signaling interacts with peripheral estrogen levels in complicated ways to control both basal GnRH pulsatility and the estrogen-induced ovulatory surge of GnRH/LH (Harter *et al*., 2018;Sadovnikova *et al*., 2020).

Many studies found that (Kiss1) release suppressed during lactation(Yamada *et al*., 2007 ; Marques *et al*., 2018; Voigt and Bennett , 2018) , and the suppression of Kiss1 may be the key factor in the inhibition of GnRH during suckling (Smith *et al., 2010).*

2.2 : Adiponectin : an overview related with suckling.

Adiponectin is" a secretory protein , it is the most abundant adipokine in human plasma" , that was independently discovered by different researchers in the middle1990s and named "adipocyte complement-related protein of 30 kDA" (ACRP30) , "gelatin-binding protein 28" (GBP28) , (AdipoQ) "Adiponectin , C1Q And Collagen Domain Containing" , and adipose most abundant gene transcript 1" (APM1) , this protein is encoded by the adiponectin "ADIPOQ , also known as APM1" gene (Wu *et al.*, ,2014) . the human adiponectin gene encodes a "244 amino acid , 30 kDa secreted protein , which contains a putative signal sequence , a collagen-like domain , and a globular domain" , adiponectin shares structural similarity with collagens VIII and X , TNF-α) , and complement factor C1q (Swarbrick and Havel , 2008) , because of its protective functions against various disease states associated with obesity, it is considered a guardian angel adipocytokine. (Parida *et al* ., 2019).

Adiponectin has insulin-sensitising, anti-inflammatory, and anti-atherogenic properties. Therefore, it plays a key role in the control of energy metabolism (Barbe *et al.*, 2019). It is primarily secreted from WAT (Choi *et al.*, 2020) , it is also secreted by other tissues such as human murine osteoblasts, liver, colon, and salivary gland parenchyma cells, skeletal and cardiac myocytes, endothelial cells, epithelial cells, brown adipose tissue, fetal tissue, and placental tissue. (Delaigle *et al*., 2004

; Fujimoto *et al.,* 2005 ; Katsiougiannis *et al.,* 2006 ; Brochu-Gaudreau *et al.*, 2010 ; Lee *et al* ., 2015; Gelsomino *et al*., 2019) .

Adiponectin exerts its action through its identified receptors, which include: adiponectin receptor1, also referred to as AdipoR1 (expressed ubiquitously and at a high level in skeletal muscle) , adiponectin receptor 2, also referred to as AdipoR2 (expressed predominantly in the liver) (Tanabe *et al.,* 2015; Vasiliauskaite-Brooks *et al.*, 2017), and receptor T-cadherin, "is predominantly expressed in endothelial cells and smooth muscle cells" (Sternberg *et al*., 2017). The majority of studies show the fundamental role of the association between adiponectin and T-cadherin is for the purpose of precaution against cardiovascular pathologies (Denzel *et al*., 2010; Parker-Duffen *et al.,* 2013; Fujishima *et al*., 2017).

It circulates in the blood in three different forms: "low-molecular-weight" (trimeric)form , medium-molecular-weight (hexameric)form , and "high-molecular-weight" (multimer)(HMW) form (Bohler *et al.,* 2010). The different forms have varying effects on target tissues, with the high-molecular-weight form affecting the liver and endothelial cells (Guerre-Millo,2008), furthermore, Savino and his colleagues (2012) proposed that HMW adiponectin is the most active type in terms of metabolic regulation.

The circulating plasma concentrations of adiponectin in humans range from 3 to 30 µg/mL (Brochu-Gaudreau *et al.,* 2010; Gelsomino *et al.,* 2019). Typically, in women , adiponectin levels are significantly higher than in men , with peaks of secretion in the morning and reduced production during the night (Cnop *et al*., 2003; Gelsomino *et al.,* 2019). Adiponectin has an inverse relationship with body fat mass and visceral obesity (Parida *et al*., 2019).

In addition, a lower level of adiponectin is associated with insulin resistance (IR), obesity, MetS, and cardiovascular diseases (Mantzoros *et al*., 1996; Han *et al*., 2007; Yosaee *et al*., 2019). Low level of circulating adiponectin may be used as a possible biomarker for MetS (Brooks *et al*., 2007; Yosaee *et al*., 2019).

Adiponectin levels are lowered during gestation, inflammation, and states of metabolic and oxidative stress, while adiponectin levels are increased following weight loss and in anorexia nervosa (Swarbrick *et al*., 2008).

Furthermore, positive associations of plasma adiponectin concentration with insulin sensitivity (IS) and inverse relationships with several components related to the decline in IS , such as serum low HDL-cholesterol, TG, and diastolic blood pressure, are observed in humans (Matsubara *et al*., 2002; Fernandez-Real *et al*., 2003).

Adiponectin promoted fatty acid oxidation and IS ,while also down-regulating hepatic glucose production and increasing skeletal muscle glucose uptake (Knights *et al*., 2014; Anderson *et al*., 2016).

Adiponectin reduces inflammatory cytokines and oxidative stress, which leads to an improvement of IR. Thus , adiponectin -induced improvement of IR and adiponectin itself reduce hepatic glucose production and increase the utilization of glucose and fatty acids by skeletal muscles , lowering blood glucose levels (Yanai and Yoshida , 2019).

Adiponectin regulates numerous biological processes in systemic organs, such as the brain, liver, skeletal muscle, heart, and endocrine glands (Lehr *et al*., 2012), and it physiologically governs glucose levels and lipid metabolism, and it is fundamental in the reproductive system (Gelsomino *et al*., 2019). In addition, it plays an important role in setting up the metabolism and improving the function of

various organs. Adiponectin in the liver prevents the accumulation of fat and free radicals that cause damage to liver cells and tissue. This adipokine, by preventing inflammatory processes, oxidative stress, obesity and IR , improves vascular function and prevents the development of atherosclerosis (Esmaili *et al.*, 2020).

Recent studies have shown that adiponectin could have a pro-inflammatory role in individuals with autoimmune diseases. Thus, adiponectin appears to have both pro-inflammatory and anti-inflammatory effects (Konturek *et al.*, 2008; Choi *et al*., 2020).

In addition to its peripheral actions, adiponectin has central activity in the regulation of energy homeostasis, stimulating food intake and reducing energy expenditure , this assumption is supported by the evidence of its presence in cerebrospinal fluid and the expression of its receptor AdipoR1 in the "arcuate nucleus of the hypothalamus (ARH") (Kubota *et al*., 2007; Savino *et al*., 2012).

On the other hand, studies have revealed broader regulatory effects of adiponectin on energy metabolism, where information strongly suggests that, as an adipocyte-derived starving hormone, adiponectin maintains energy homeostasis in favoring systemic energy conservation through improving energy efficiency in major metabolic tissues such as skeletal muscle and WAT (Lee and Shao, 2014).

Kubota and his colleagues (2007) suggested a hypothesis on the role of adiponectin in the regulation of food intake and energy homeostasis. Under fasting conditions, serum and cerebrospinal fluid (CSF) adiponectin levels and AdipoR1 expression in the ARH increase. Thus, hypothalamic AMP-activated protein kinase (AMPK) is activated, which stimulates food intake and suppresses energy expenditure, promoting fat storage. On the other hand, after refeeding, serum and CSF adiponectin levels and AdipoR1 expression in the ARH decrease, and,

consequently, hypothalamic AMPK activity decreases, resulting in reduced food intake and increased energy expenditure.

Both under fasting conditions and after refeeding, serum leptin levels are regulated inversely in relation to serum adiponectin levels. Leptin suppresses hypothalamic AMPK activity and food intake, as opposed to the action of adiponectin. Therefore, central adiponectin/leptin signals may represent the physiological pathway by which hypothalamic AMPK activity and food intake are stimulated during fasting and suppressed after refeeding (Kubota *et al.,* 2007).

In addition to this short-term regulation of food intake and energy expenditure by adiponectin and leptin. Adiponectin and leptin may also participate in the long-term regulation of energy homeostasis. The fundamental roles of leptin and adiponectin seem to be to preserve an adequate fat reserve : leptin acts as a satiety signal, and adiponectin acts as a starvation signal(Kubota *et al* ., 2007) .

Furthermore , circulating adiponectin levels are regulated by different physiological , environmental , and pharmacological factors such as hormonal production , inflammatory processes , genetic polymorphisms , nutritional status , and drug administration (Swarbrick and Havel , 2008),where adiponectin production is inhibited by a number of hormones, including testosterone, prolactin, glucocorticoids, and growth hormone, and by oxidative stress in adipose tissue (Swarbrick and Havel, 2008) , in addition, different studies addressed pro-inflammatory cytokines released from adipose tissue , such as tumor necrosis factor (TNF-α)and interleukin-6 (IL-6), as inhibitors of adiponectin synthesis (Tilg and Moschen., 2006; Gelsomino *et al.,* 2019), while weight loss , exercise , nutritional factors , anti-diabetic drugs , hypolipidemic drugs , and anti-hypertensive drugs have

been associated with an increase of serum adiponectin levels (Yanai and Yoshida , 2019).

Multiple studies have shown the protective role of adiponectin in obesity-associated diseases and cancer (Parida *et al* . , 2019 ; Yanai and Yoshida , 2019) .

Based on in vitro and in vivo evidence, that adiponectin could also be one of the hormones controlling the interaction between energy balance and fertility in several species, including humans. Indeed, its two receptors—AdipoR1 and AdipoR2—are expressed in the hypothalamic–pituitary–gonadal axis and their activation regulates kisspeptins, GnRH, and gonadotropin expression and/or secretion (Barbe *et al.*, 2019).

The maternal metabolism is dynamically altered in order to provide adequate nutrition to the fetus and newborn. Furthermore, gestational insulin resistance appears from mid-pregnancy, and this facilitates efforts to supply energy substrate to the fetus, which preferentially uses glucose as an energy source (Asai -Sato *et al.*, 2006).

After childbirth, immediately profound changes in the maternal metabolism occur, where insulin sensitivity rapidly reversed and fasting insulin levels reduced by 50–60% within 24 hours of placenta delivery (Fuglsang *et al.*, 2006 ; Fuglsang *et al.*, 2010 ; Anderson *et al*., 2016), while pregnancy glucose intolerance rapidly improves after childbirth, maternal carbohydrate and energy metabolism remain altered throughout breastfeeding in order to that satisfy the glucose demand in milk synthesis (Asai-Sato *et al* . , 2006) .

It was noted that the circulating levels of adiponectin are affected rapidly after childbirth and the removal of the placenta. In addition , it was found that the

composition of adiponectin multimers changes towards the non-pregnant state with the termination of gestation (Fuglsang *et al.*, 2010).

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

The advancement from gestation to breastfeeding is characterized by comprehensive metabolic and endocrine changes. Furthermore, fetal growth, lactogenesis, and galactopoiesis require the targeted partitioning of nutrients towards the placenta and the mammary gland, a process that is accomplished by decreasing insulin sensitivity in peripheral tissues, thus attenuating their uptake of glucose, amino acids, and fatty acids and facilitating lipolysis in adipose tissue (Block *et al.*, 2001; Singh, 2014).

Fuglsang and his colleagues (2006) reported serum adiponectin levels peaked in mid- pregnancy and the lowest levels were seen in late gestation. They also observed an inverse association with maternal BMI and hypothesized that the decline in plasma adiponectin levels postpartum might be due to an increase in maternal fat stores during gestation and lactation.

Nevertheless , Asai-sato and his colleagues (2006) found that plasma concentrations of adiponectin at the third trimester of pregnancy and breastfeeding declined, with no attachment to the degree of gestational weight gain. Thus, the gestational adipose store seems to have a little effect on the regulation of adiponectin secretion at a physiological level.

Combs and his colleagues (2003) showed that adiponectin levels are suppressed from mid-gestation until weaning ,of all the endocrine changes that peak

around pregnancy, placental lactogens and prolactin best fit the time course for the suppression of adiponectin. Placental prolactin-like molecules appear in the maternal circulation around mid-gestation, whereas pituitary prolactin levels increase gradually during gestation and remain elevated until suckling stops.

Earlier studies by Combs and his colleagues (2003) demonstrated that exogenous PRL inhibited adiponectin secretion in mice in vivo and the prolactin hormone also suppressed adiponectin secretion by cultured human adipose tissue and decreased the serum adiponectin level in PRL transgenic mice (Nilsson *et al*., 2005; Asai-Sato *et al*., 2006).

In vitro evidence suggests that hypodiponectinemia in lactating women is possibly induced by hyperprolactinemia. It has also been indicated that adiponectin is involved in the alterations of the maternal metabolism during lactation under the influence of PRL (Asai-Sato *et al*., 2006).

Many studies in some laboratory rats and farm animals demonstrated the role of the adiponectin hormone in different physiological conditions.

Qi and his colleagues (2004) indicated that adiponectin increased the basal energy expenditure and that a decrease in plasma adiponectin levels suppressed the basal metabolism , thus accelerating the energy storage in mice. Additionally, it is well known that PRL inhibits brown adipose tissue thermogenesis in rodents during suckling (Chan and Swaminathan ,1990) ,this results in a substantial reduction in the energy requirements of the mother, which is an energy sparing advantage for milk production .Therefore, it is probable that the decline in plasma adiponectin influences the energy metabolism of a suckling mother (Asai-Sato *et al.*, 2006).

The concentrations of circulated adiponectin in dairy cows decreased from day 21 before delivery, with adiponectin reaching a low at the time of birth and then

increasing gradually(Singh *et al.,* 2014). The transition period from late pregnancy to lactation is a period of physiological adaptations and overall metabolism to fulfill the mammary gland's nutritional demand for milk production, and since adiponectin is involved in regulating glucose and fatty acid metabolism (Yamauchi *et al.,* 2002; Singh *et al*., 2014), therefore, adiponectin concentrations are likely to change during this period of negative energy balance (Singh *et al*., 2014).

Late gestation is characterized by low-grade inflammatory situations in adipose tissue with elevated production of inflammatory cytokines such as TNF- α and IL-6 in both humans and animals, these cytokines, as well as monocyte chemoattractant protein-1(MCP-1), inhibit adiponectin manufacturing and release by fat cells (Radaelli *et al*., 2006;Chazenbalk *et al*., 2010;De Castro *et al*., 2011 ; Singh , 2014). Furthermore, hormonal changes associated with delivery, including as cortisol (Goff and Horst, 1997), estradiol-17, PRL , insulin, and GH (Oda *et al*., 1989; Bell, 1995), are likely to impact circulating adiponectin concentrations (together with the cytokines listed above) (Singh , 2014).

2.3 Leptin : an overview related with suckling .

Leptin is "a 167-amino acid peptide hormone discovered in 1994, primarily expressed in WAT , and found in a variety of tissues including the placenta, mammary gland, ovary, skeletal muscle, stomach, pituitary gland, brown adipose tissue, and lymphoid tissue" (Margetic *et al*., 2002; Anubhuti and Arora, 2008; Park and Ahima, 2015) .The word "leptin" comes from the Greek word "leptós," which means "thin." Friedman's team chose the name leptin after doing preliminary studies in which they injected leptin into mice and observed a significant reduction in body weight and obesity (Ramos-Lobo and Donato, 2017).

Leptin belong to the family of long-chain helical cytokines and has structural similarities with" IL-6 , PRL, GH , interleukin-12(IL-12) , interleukin- 15, granulocyte colony-stimulating factor (G-CSF)and oncostatin M (OSM)", so it is considered as a pro-inflammatory cytokine (Otero et al .,2005).Furthermore, leptin increased the release of pro-inflammatory cytokines, such as TNF-α ,IL-6, and IL-12, from mouse macrophages and activated human blood neutrophils (Chihara *et al* .,2020).

Leptin acts by binding to leptin receptors(LEPR) on the surface of cells, leptin receptors exist on neuronal, hepatic, pancreatic, cardiac , and perivascular intestinal tissue (Dornbush and Aeddula, 2020). Also, the leptin receptors belongto the glycoprotein130family of cytokine receptors and consist of six isoforms. Isoform-b (the long form) is the most characterized of these isoforms (Wasim *et al*., 2016; Dornbush and Aeddula, 2020). Leptin circulates in plasma as a free form or bound to leptin-binding proteins and interacts directly with the leptin receptor (Lammert *et al.,* 2001; Savino *et al.,* 2010).

Leptin concentrations in the blood positively correlate with the amount of fat in the body(Chihara *et al*., 2020),in humans, the leptin hormone secretion showed a circadian profile, with the highest levels at night and the lowest during the day (Saad *et al.,* 1997; Ramos-Lobo and Donato, 2017),women have higher circulating leptin levels than men (Hellström *et al.,* 2000).Additionally, serum leptin levels are increased in patients with obesity , T2D, MetS, and cardiovascular disease (Ghadge and Khaire, 2019; Chihara *et al.*, 2020).

Once secreted into the circulation , leptin reaches the central and peripheral nervous systems, where it regulates appetite and food intake, bone mass, basal metabolism , reproductive function, and insulin production by binding and activating the long form of the leptin receptor (Abella *et al* ., 2017).

The leptin hormone is a main regulator of energy and body fat balance and plays an important role in controlling glucose homeostasis, heat generation, the autonomic nervous system, and neuroendocrine axons (Ramos-Lobo and Donato, 2017). Furthermore, leptin represents the afferent loop informing the hypothalamus about the states of fat stores, with hypothalamic efferents regulating appetite and energy expenditure, indicating that leptin plays an important role as a metabolic adaptor in overweight and fasting cases (Leal-Cerro *et al.*, 2001). Leptin circulates in the blood and acts on the brain to regulate food intake and energy expenditure. When fat mass increases, plasma leptin levels rise, suppressing the appetite until weight is lost. When fat mass falls, leptin levels fall , stimulating appetite and suppressing energy expenditure until fat mass is restored. This system maintains the balanced control of fat tissue mass (Friedman , 2011).

Leptin acts as an energy reserve signal for hypothalamic regions that control feeding behaviour, metabolism , and endocrine function to maintain energy homeostasis (Chilliard *et al.*, 2001) .

Moreover, the concentration of circulating leptin serves as a gauge for energy reserves and directs the central nervous system to regulate food intake and energy expenditure. Therefore, leptin exerts immediate effects by acting on the brain to regulate appetite through obRb-receptor binding in the hypothalamus (Robertson *et al.*, 2008; Kelesidis *et al.*, 2010). Leptin controls food intake by stimulating hypothalamic anorexigenic pathways and inhibiting orexigenic ones (Proulx *et al.*, 2002).Outside of the hypothalamus, leptin interacts with the mesolimbic dopamine system, which is involved in motivation for and reward of feeding, and the nucleus of the solitary tract of the brainstem to contribute to satiety (Robertson *et al.*, 2008; Kelesidis *et al.*, 2010).

Park and Ahima (2015) concluded that the effect of leptin on the central nervous system (CNS) and peripheral tissues leads to its being a responsible agent for neuroendocrine function, metabolism, immune function and other systems.

Ahima and his coworkers (1996) proposed that leptin levels increase with increasing adiposity in rodents and humans. It's to act as a negative feedback "adipostatic signal" to brain centres controlling energy homeostasis , limiting obesity in times of nutritional abundance.

Furthermore , starvation causes reduction in plasma leptin concentrations , hypoleptinemia activates the hypothalamic-pituitary-adrenal (HPA) axis , increasing WAT lipolysis and mediating a shift from glucose to fat metabolism to maintain euglycemia in fasted rats (Perry and Shulman, 2018). During fasting, low leptin levels trigger metabolic and hormonal responses in mice and humans (Ahima *et al.,* 1996; Park and Ahima , 2015). This response includes decreasing reproductive hormone levels, which prevents pregnancy, decreasing thyroid hormone levels that slow metabolic rate, increasing GH levels that may mobilize energy stores, and decreasing insulin-like growth factor-1 (IGF-1) levels that may slow growth-related processes (Ahima *et al.*, 1996 , Chan *et al.,* 2008; Kelesidis *et al* . , 2010) .

Furthermore, leptin also plays an important role in the regulation of glucose homeostasis, independent of actions on food intake, energy expenditure, or body weight. Leptin improves IS in the liver and skeletal muscle and regulates pancreatic β-cell function (Marroquí *et al.,* 2012; Jung and Choi, 2014),whereas it impairs insulin signaling in murine adipocytes (Jung and Choi , 2014) .

Several studies have demonstrated that the disequilibrium in circulating levels and a dysregulation of leptin secretion by WAT, as well as by other peripheral

tissues , can impair immune function and the integrity of a correct immune response (Abella *et al* ., 2017).

Additionally , leptin production are regulated by several factors including insulin , glucocorticoids , catecholamine ,cytokines and sex steroids (Ahima and Osei, 2004 ; Park and Ahima , 2015) .

Leptin stimulates the secretion of GnRH and directly stimulates the pituitary to release LH (Yu *et al.*, 1997a, b). There is accumulating evidence that kisspeptin neurons in the anteroventral periventricular nucleus mediate the nutritional signal linking leptin to GnRH. Kisspeptin neurons express the leptin receptor, and leptin treatment normalizes suppressed KISS-1 expression in rodents with gonadotropin insufficiency and low leptin , including the ob/ob mouse and the streptozotocin-induced diabetic rat (Tena-Sempere *et al.*,2000; Bohler *et al.*,2010).An increase in GnRH pulsatility elicited by recombinant leptin injection in low-weight women with hypothalamic amenorrhea could be explained by a leptin -induced rise (Welt *et al*., 2004; ;Bohler *et al.*, 2010; Dardeno *et al.*, 2010).

Many studies have demonstrated the direct and indirect roles of leptin during pregnancy and suckling . 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 controlling food intake and body weight are key players supporting neural and physiological adaptations that occur during pregnancy, lactation, and postnatal development (Boyle and Le Foll, 2019).

The surge in circulating leptin is a natural result of increased fat deposition during pregnancy (Boyle and Le Foll, 2019). Leptin levels drop drastically below those of non-pregnant control women 24 hours after delivery, then gradually

increase over the next 6 postpartum months (Lage *et al.*, 1999). Because this rapid drop in leptin occurs before the reduction of maternal fat depots, it is likely that this drop reflects the loss of the placenta, which is also a primary source of leptin during pregnancy (Masuzaki *et al.*, 1997).

Furthermore, variation in blood leptin levels during pregnancy and lactation is indicative of a highly metabolic adaptive state of the body (Boyle and Le Foll, 2019). Leptin may be important in regulating maternal nutrition and the metabolic adaptation of nutrient partitioning during the energy-consuming processes of pregnancy and lactation (Moschos *et al.*, 2002; Feuermann *et al.*, 2006).

Butte and his colleagues (1997) proposed that in postpartum women within 3 and 6 months, normalization of leptin was associated with changes not only in weight and fat mass, but also in serum insulin. Furthermore, no major differences were observed in leptin levels between lactating and non-lactating women. They also proposed that in lactating women, adjusted for fat-free mass and fat mass, the rates of energy expenditure were not significantly correlated with leptin .

The demands of milk synthesis and release , produce a condition of negative energy balance in the suckled mother, and, in laboratory rodents, are accompanied by a dramatic hyperphagia. The reduction in secretion of the adipocyte hormone, leptin, a hallmark of negative energy balance, may be an important endocrine signal to hypothalamic systems that integrate lactation-associated food intake with neuroendocrine systems (Crowley, 2011).

Furthermore, in animals as well, during gestation , leptin levels remain high and decline rapidly towards parturition (Block *et al.*, 2001; Singh *et al.*, 2012). There are a number of factors that may contribute to a change in the energy balance of a nursing animal , one of them is leptin , which is known to act in part

as a satiety factor to reduce food intake (Brogan *et al.*,1999). In rats, energy reserves are increased during gestation by fat accumulation during breastfeeding by hyperphagia (Johnstone and Higuchi, 2001).However, despite the massive increase in food intake during lactation, plasma leptin levels continue to fall, suggesting that leptin release in response to food intake is suppressed during lactation (Johnstone and Higuchi, 2001) .

Singh and his colleagues (2012) indicate that a fall in circulating leptin levels towards and during lactation is due to the energetic costs of milk production , the suckling stimulus itself did not appear to influence the decrease in leptin concentration. Eliminating the energetic cost of lactation by preventing milk delivery in rats and cows could cause an increase in plasma leptin levels together with an increase in energy balance (Block *et al.,* 2001; Singh *et al* ., 2012).

Johnstone and Higuchi (2001) indicated decreased leptin production and action during late pregnancy and lactation will result in a decreased satiety effect , with up-regulation of orexigenic factors that produce hyperphagia , so allowing adequate energy intake for successful rearing of offspring .

Furthermore, Singh and his colleagues (2012) proposed that the onset of the negative energy balance is largely responsible for the declining leptin concentrations towards parturition and that the low leptin levels during lactation probably induce the hyperphagia of lactation.

Finally, leptin contributes in coordination with other regular hormones during pregnancy, such as (estrogen) , acting as a functional link between fat cells and epithelial cells in the mammary gland, providing information on the adequacy of energy stored in adipose tissue (Pérez-Pérez *et al.*, 2015).

Furthermore , it has been found the highest level of expression LEPR occurs when active growth of the mammary gland begins in the middle of pregnancy , which indicates that LEPR may be important in regulating the growth and development of the mammary gland during pregnancy and lactation (Laud *et al.*, 1999; Pérez-Pérez *et al.*, 2015). Thus, absence of leptin may result in failure of mammary gland growth and subsequent failure of lactation, as evidenced by the complete failure of lactation in ob/ob female mice after an otherwise normal delivery (Mounzih *et al.*, 1998; Pérez-Pérez *et al* ., 2015).

2.4 Prolactin

Prolactin (PRL) "is a hormone that is mostly synthesized and secreted by lactotrophs in the anterior pituitary gland." It is a 23 KD single-chain protein of 199 amino acids "(Freeman *et al*., 2000; Saleem *et al.*, 2018) , lactotrophs account for around 15–25 percent of the total number of cells in a healthy human pituitary gland; the number of lactotrophs is alike in both sexes and does not change considerably with aged , furthermore , lactotrophs secrete PRL, which triggers a variety of responses important for offspring feeding, such as (mammary epithelial cell proliferation and differentiation , as well as neurogenesis), which is required for maternal behavior in both mammalian and non-mammalian organisms (Bernard *et al* ., 2019).

Prolactin is also produced in the CNS, the immune system, the uterus and related pregnancy tissues, and even the mammary gland (Freeman *et al.*, 2000). It is "a member of the cytokine family of proteins", having "a three-dimensional structure consisting of four antiparallel helices and strong structural similarities to human growth hormones (HGH) and Human placental lactogen (HPL) " (Horseman and

Yu-Lee, 1994; Saleem *et al.*, 2018). This family of hormones is descended from a single ancestral gene (Al-Chalabi *et al*., 2020).

Prolactin is released into the bloodstream and acts on a variety of tissues throughout the body, including mammary glands and important tissues contributory in energy equilibrium regulation, like (the fat tissue, brain, pancreas, small intestine, and liver) (Aoki *et al*., 2019).

Prolactin signaling is regulated by the membrane-bound PRL-R, whose structure has been found to be similar to that of many biologically important receptors of the class 1 hematopoietic cytokine receptor family, such as the growth hormone receptor (Bole-Feysot, 1998; Bernard *et al.*, 2019).

The Prolactin -receptor is commonly expressed in numerous tissues and cell kinds throughout the body, suggesting that PRL has the ability to affect a wide variety of functions (Aoki *et al* ., 2019) , since there are so many tissues that express PRL-R, it's hard to determine the precise impact of PRL activity on whole-body metabolism (Lopez-Vicchi *et al*., 2020) .

While PRL is the only pituitary hormone whose release is regulated by hypothalamic inhibitory tone, other hormones, such as oestrogens, thyrotropin-releasing hormone (TRH), and vasoactive intestinal peptide, have been reported to stimulate PRL secretion (Gullu, 2008; Gargiulo, 2017).Additionally, dopamine is the primary inhibitor of PRL production under physiological settings, as it is produced by multiple distinct hypothalamic dopaminergic neuron populations that express PRL receptors (Voigt and Bennett , 2018) .

Lactation is the most powerful and better characterized physiological stimulus for PRL secretion, where the magnitude of the suckling-induced PRL rise is robust during early suckling but wanes thereafter. In addition, basal PRL levels

remain raised through the first 2–3 weeks after delivery in women who do not breastfeed and then decrease (Ben-Jonathan and Hnasko , 2001).

Prolactin's primary function during gestation and suckling is in the development of mammary glands, milk manufacture, and the keeping of milk secretion (Saleem *et al* ., 2018), where PRL plays a crucial part in driving many of the maternal body's adaptations to let the mother to meet the physiological requirements of both gestation and lactation, including the growing fetus's high energy demands, followed by milk production to help progeny after delivery (Lopez-Vicchi *et al.*, 2020). In addition, PRL's levels in the serum increase rapidly during gestation as the size and quantity of lactotrophs increase , during breastfeeding, suckling promotes fast PRL production via a neuroendocrine reflex route (Saleem *et al* ., 2018) .

Moreover, PRL also has an effect on other facets of human body functions, such as "osmoregulation, metabolism , immune regulation , and the central nervous system" (Ignacak *et al.*, 2012) , as well as contribution in metabolic balance including (control of body weight, adipose tissue, control of lactotrophs cell balance, maternal behavior, adrenal pressure response, pancreas, skin , hair follicles, and bones" (Bernard *et al.*, 2019) ,when PRL concentrations are rising during gestation and breastfeeding, the wide range of roles for PRL are thought to converge, and it is hypothesize that PRL plays a role not just in milk output but also as a signal to many of the body's systems , driving motherly adaptations to meet the requirements of these physiologically difficult conditions (Grattan,2001; Lopez-Vicchi *et al* ., 2020) .

In the non-gestation and non-suckling state, PRL concentrations are generally low , with females having somewhat higherprolactin concentrations than males

(Wadoo *et al*., 2017; Al-Chalabi *et al*., 2020) . Stress states, including "anesthesia , surgery, electric shock , strenuous exercise, and insulin-induced hypoglycemia", also stimulate PRL secretion in both males and females (Ben-Jonathan and Hnasko, 2001) .

However, there are many clinical disorders of elevated PRL ("hyperprolactinemia)"that exist, including such prolactin-secreting tumors (prolactinoma) and dopamine receptor antagonizing drug treatments, and these pathologically elevated PRL levels are likely to affect many different aspects of physiology and lead to metabolic disorders (Majumdar and Mangal , 2013; López *et al* .,2013) .

While sufficient levels of PRL are advantageous for fatty metabolism in humans, lowered levels of serum PRL have been connected to patients with glucose intolerance , T2D, and IR, as well as children with MetS and fatness, according to several cohort studies (Balbach *et al*., 2013; Wang *et al*., 2013 ; Ponce *et al*., 2020) . Furthermore , a high circulating total PRL concentration within physiologically normal limits was linked to a decrease danger of T2D (Li *et al*., 2018).

Furthermore, increasing evidence connects low PRL levels within the normal range to signs and results of metabolic dysfunction (Serri *et al* ., 2006 ;Wang *et al* ., 2013), a decreased expression of PRL-receptor mRNA was discovered in the visceral fatty tissue of subjects with IR compared to control subjects (Ponce et al., 2020), in some circumstances, both reduced PRL concentrations and reduced PRL sensitivity of these fat cells may contribute to a loss of PRL's beneficial effect on fatty metabolism (Lopez-Vicchi *et al* ., 2020).

Maintaining high blood PRL levels within the physiological range can increase IS and promote correct fat distribution , hence altering metabolic dysfunction (Wang *et al* ., 2013 ;Yang *et al* ., 2021).

A study on the role of PRL in driving appetite show that systemic administration of this hormone by a number of techniques, such as injections or ectopic pituitary transplants, increases food consumption in female rats (Lopez-Vicchi *et al*., 2020).

Prolactin, released from the rats' pituitary in response to suckling stimulation , acts centrally to stimulate food intake throughout suckling, independent of milk delivery and negative energy balance, in these rats (" where the galactophores are cut, which prevents milk delivery to the pups"), food intake is boosted above virgin rat levels in spite of no energy demands for milk output and this is attributed to increased PRL levels (Woodside , 2007) .

One mechanism by which PRL can enhance food intake is through influencing the satiety actions of the adipose-derived hormone leptin, wherein in the rat and humans, a physiological state of leptin insensitivity or resistance develops during gestation (Ladyman ,2008; Ladyman *et al*., 2010 ; Ladyman *et al.*, 2012) , recent data propose that PRL, and the related placental lactogen, might play a major role in the development of leptin resistance during gestation (Augustine and Grattan, 2008; Ladyman *et al* ., 2010).

Chronic injection of PRL in hypovirgin female rats can lead to a state of leptin insensitivity (Naef and Woodside, 2007). Also, even after a central injection of leptin, female rats given persistent PRL infusions into the cerebral ventricles showed no change in food consumption or body weight (Naef and Woodside, 2007).

While PRL has the ability to influence food intake by regulating central leptin sensitivity, the mechanisms behind this effect are still unknown. As the amount of co-localization of PRL and leptin -responsive cells differs within different feeding-related areas of the brain , PRL may act directly on some but not all leptin -responsive neuron populations to reduce leptin sensitivity during gestation (Ladyman *et al*., 2010, Nagaishi *et al*., 2014; Lopez-Vicchi *et al*., 2020).

Prolactin influences both white and brown adipose tissue, and the PRL-receptor is expressed in both white and brown adipose tissue in humans and rats (Ling *et al* .,2000; Ling *et al* ., 2003;Ben-Jonathan *et al* .,2008), where PRL is involved in adipogenesis and differentiation of adipocytes (McAveney *et al*.,1996; Fleenor *et al*., 2006 ; Grattan ,2015) .

Prolactin receptor-deficient mice show lower body weight and a lower percentage of fat mass compared with wild-type controls (Freemark *et al*.,2001), PRL receptor-deficient mice gave direct evidence that PRL signaling is contributory to the regulation of adipogenesis, influencing energy equilibrium and metabolic adaptability, particularly during development (Carré and Binart , 2014) , furthermore, PRL signaling has been shown to have a role in brown adipose tissue development and function (Carré and Binart , 2014) . Female mice overexpressing PRL have reduced retroperitoneal fatty tissue mass although having the the same body weight as control mice (Ling *et al* ., 2000) ,this proposes that PRL can have both positive and negative effects on adipose tissue mass, which is consistent with PRL 's predicted role in both fat mass accumulation during gestation and fat mass mobilization during breastfeeding (Lopez-Vicchi *et al*., 2020).

2.5 Oxidative Stress

Oxidative stress is "usually defined as an imbalance arising when the rate of production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defence and repair mechanisms, leading to oxidative damage to biomolecules". However, the definition can be extended to include the disruption of reduction and oxidation (redox) reactions involved in cellular signaling (Metcalfe and Alonso-Alvarez, 2010).

Oxidative stress is also known "as a phenomenon induced by an imbalance between the creation and accumulation of ROS in cells and tissues and a biological system's capacity to detoxicate these reactive products" (Pizzino *et al*., 20 17).As a result, an imbalance between ROS and antioxidant defences is a pathological situation that causes not only direct cellular damage but also an inflammatory cascade that increases the perpetuation of tissue injury (Charlton *et al.*, 2021), it is a harmful process that can negatively affect several cellular structures , such as membranes, lipids , proteins, lipoproteins , and DNA (Young and Woodside 2001 ; Droge , 2002 ; Pizzino *et al.*, 2017), furthermore, oxidative stress causes the activation of proinflammatory cytokines and subsequent inflammation, which promotes the formation of ROS, thereby damaging cells and tissues (Oguntibeju , 2019 ; Charlton *et al* ., 2021).

Oxidative stress occurs when there is a lack of equilibrium between the production of reactive species (ROS) in living organisms and the antioxidant power regulated by both antioxidant enzymes and the antioxidants involved in the living organisms, and when, consequently, oxidation becomes so predominant that it causes oxidative damage to the living organisms (Halliwell and Gutteridge , 2015; Kuramoto and Kitagawa ,2017).

Since the most biologically important free radicals are oxygen-centered, the free radicals of interest are commonly indicated to as ROS in most biological systems (Aprioku , 2013) , ROS were first discovered as byproducts of cellular activity, specifically mitochondrial respiration, and their high reactivity has been related to the disruption of macromolecules such as proteins, lipids, and DNA (Tauffenberger and Magistretti , 2021) , furthermore, "biotic foreign substances (such as anti-plastics drugs) and environmental stressors (such as ultraviolet radiation, ionizing radiation, pollutants, and heavy metals") contribute significantly to increasing reactive oxygen species output and thus create an unequilibrium that leads to harmed cells and tissue (oxidative stress) (Pizzino *et al* . , 20 17).

Biological free radicals are highly unstable molecules that are products of normal cellular metabolism. They have electrons that can react with various organic substrates such as lipids, proteins, and DNA (Oguntibeju, 2019), free radicals, which involve reactive oxygen species (ROS) and reactive nitrogen species (RNS), are byproducts of cellular metabolism and can have both negative and positive effects on living organisms (Valko *et al* ., 2006 ;Yuksel *et al* ., 2015) , in living systems, free radicals play a dual role: they are toxic byproducts of aerobic metabolism that cause oxidative damage and tissue dysfunction, and they also serve as molecular signals that activate beneficial stress responses (Di Meo and Venditti , 2020) .

The terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) are frequently used to describe free radicals and other non-radical reactive derivatives known as oxidants (Oguntibeju , 2019) , ROS are highly energetic and reactive tiny molecules produced from oxygen, such as superoxide (*O2), peroxyl (ROO), hydroxyl (OH), and hydrogen peroxide (H2O2) (Jha *et al.*, 2016;Charlton *et al.*, 2021) , while RNS include nitric oxide (•NO) , nitrogen dioxide (NO2•-) and peroxynitrite (OONO-) (Oguntibeju , 2019) .

Reactive oxygen species are freed as by-products of oxidative metabolism , primarily through mitochondrial respiration, or freed as part of a defense mechanism during cellular response to xenobiotics or cytokines (Starkov , 2008 ; Finkel , 2011;Tauffenbergerand and Magistretti , 2021) .

Reactive oxygen species and other radicals contribute to a variety of biological events, including carcinogenesis, mutation, degenerative and other diseases, aging, inflammation, and development (Kohen and Nyska, 2002).

Cellular homeostasis is critical to how an organism will develop and age , disruption of this delicate equilibrium is often associated with health degradation and, ultimately, death (Tauffenberger and Magistretti , 2021) .

Cellular functions depend on a variety of extracellular signals and intracellular signals that operate in concert to keep cells in a state of cellular homeostasis. Most cellular processes necessitate a significant amount of energy, and mitochondria are well-known for providing the quantities of energy required for cell growth and homeostasis. However, the creation of ROS as a byproduct of the electron transport chain in mitochondria is the dark side of energy production (Murphy , 2009; Tauffenberger and Magistretti , 2021) .

Different enzyme systems involved in redox balancing in vivo keep the high intracellular physiological quantities of ROS at low levels in normal states (Rahal *et al* ., 2014).Excessive ROS levels, on the other hand, are damaging to the human body, as they can lead to the accumulation of oxidative damage in specific subcellular compartments, which can be very toxic to DNA, proteins, and fats (Wang *et al* ., 2017).

Many studies show that oxidative stress exerts a role in the emergence and/or development of many diseases, to varying degrees of significance " such as cancer ,

diabetes , metabolic disorders , atherosclerosis , and cardiovascular disease " (Taniyama and Griendling , 2003; Pizzino *et al* .,2017) .

Furthermore, it is well recognized that living systems have not just adapted to coexisting with free-radicals , but have also developed strategies to transform these harmful compounds in their favor by using them in critical physiological processes (Di Meo and Venditti , 2020) , where organisms contain a variety of defense mechanisms that can protect them from oxidative stress (Garratt *et al* ., 2011).

Aerobic organisms have integrated antioxidant systems that include both enzymatic and nonenzymatic antioxidants that are usually effective in preventing the harmful effects of ROS (Birben *et al* ., 2012).

Lipid peroxidation is" one of the damages caused by oxidative stress conditions" . Lipid peroxides break down to generate a diversity of compounds such as "epoxides , hydrocarbons, and aldehydes" , among the aldehyde compounds produced is malondialdehyde (MDA) (Zainuri and Wanandi , 2012).

The lipid peroxidation process begins when the oxidizing compounds target the lipids . It is a chain reaction that produces multiple breakdown molecules , such as Malondialdehyde and , 4-hydroxy-2-nonenal. Among the many substrates, proteins and DNA are particularly vulnerable to modification caused by these aldehydes ,where , malondialdehyde and 4-hydroxy-2-nonenal approaches play important roles in multiple cellular processes and may contributein secondary adverse reactions (i.e. crosslinking) by supporting intra- molecular or inter- molecular protein/DNA cross-linking , which can result in profound changes in the biochemical properties of biomolecules , facilitating the development of different pathologic conditions (Ayala *et al* .,2014).

Malondialdehyde (MDA) " is a highly reactive three-carbon dialdehyde formed as a byproduct of the peroxidation of polyunsaturated fatty acids and the metabolism of arachidonic acid" (Dzoyem *et al* .,2014), it is frequently assessed as an index of oxidative stress damage because it is a highly reactive dialdehyde formed when polyunsaturated lipids are degraded by ROS (Landau *et al* ., 2013 ; Dzoyem *et al* ., 2014).

Malondialdehyde readily interacts with functional groups of proteins, lipoproteins, DNA, and RNA (Landau *et al*., 2013) . It has been reported that it contributes to the pathogenesis of diabetes mellitus, aging, brain ischemia, and other neurodegenerative illnesses (Lovell and Markesbery, 2007; Landau *et al* ., 2013).

Suckling is the most energetically demanding time of a female's life in mammals, and it is marked by a dramatic increase in the organism's energy and nutrient needs for milk production (Gutgesell *et al.,* 2009; Pichaud *et al.,* , 2013),during which both intake and spending to meet the high energy demands of progeny growth and somatic protection are enhanced (Zheng *et al*., ,2015) , thus providing a greater opportunity for ROS production. As a consequenced, it has been projected that oxidative stress will increased during this reproductive period. Many researchers believe that oxidative stress is a physiological cost of reproduction that has the possibility to affect future female reproductive function and longevity (Monaghan *et al.,* 2009; Dowling and Simmons , 2009).

Despite the fact that this theory has gained popularity, the findings of studies that have tested its validity have yielded ambiguous results (Speakman and Garratt , 2014). Zheng and colleagues (2015) revealed that antioxidant activity is physiologically regulated during the peak of lactation in response to elevated ROS production . Garratt and his colleagues (2011) concluded that physiological

regulation of antioxidant systems is an effective mechanism for maintaining antioxidant equilibrium and may thus play a main role in inhibiting macromolecule oxidation .

Pichaud and his colleagues (2013) reported that the mitochondrial adjustments in the liver of lactating female mice, compared to non-reproductive female mice, might help to spare substrates and therefore energy for milk output in the mammary gland, and they also proposed that these alterations led to an increment in ROS output that subsequently up-regulates antioxidant defence activity and lowers oxidative stress.

In case of oxidative stress and the anti - oxidative defense system of suckling mothers, the oxidative stress level was elevated and the antioxidant power level was lessen in the early puerperium , but the antioxidant power level then showed a clear propensity to recover and the oxidative stress gradually decreased in the first 3 months after delivery (Kuramoto and Kitagawa , 2017).

According to Garratt and his colleagues (2013), oxidative damage to proteins was lower in the livers of mice females with an eight-puppy litter than in mice females with two pups or non-reproductive control mice females ,in their experiment on mice during lactation ,they found that although protein oxidation decreased , activity levels of the antioxidant enzyme superoxide dismutase increased in the liver, suggesting that this might be one of the pathways used to protect against oxidative stress.

According to Hyatt and his colleagues (2017), lactating rats did not showed any lasting effects in oxidative damage or anti-oxidant concentrations. They also proposes that suckling goes beyond returned females to a non-reproductive baseline and improved metabolism conditions long after reproduction has ended , which is

consistent with the Stuebe and Rich-Edwards' reset hypothesis, which indicates "that suckling reverses many of the metabolic changes that occur during gestation".

Furthermore , Hyatt and his colleagues (2018) found that lactating rats had lower body mass, lower white adipose tissue mass , and changes in mitochondrial function and select indicators of metabolism and oxidative stress after breastfeeding stopped. Furthermore, a greater level of superoxide dismutase (Mn-SOD) was detected in the liver and in WAT. These animal findings may aid future research efforts in discovering putative physiological processes that underlie the long-term maternal metabolic health benefits conferred by breastfeeding in human populations. This topic remains an important clinical issue , as identifying these targets can be used to benefit mothers who are unable to breastfeed their young .

2.6 Antioxidants

Endogenous enzymatic and non-enzymatic antioxidants are part of the body's encircled a complex antioxidant protection grid. These molecules collectively act against free radicals to resist their damaging effects on vital biomolecules and, and ultimately body tissues (Ighodaro and Akinloye , 2018) .

They can be classified as first , second , third , or even fourth line defense antioxidants based on their responses to the general free radical invade. The first line of protection antioxidants, primarily "superoxide dismutase (SOD) , catalase (CAT) , and glutathione peroxidase (GPX) , play a significant and necessary role in the overall antioxidant defense strategy, particularly in relation to the superoxide anion radical (*O2)", which is constantly produced in normal body metabolism, especially via the mitochondrial energy production pathway (Ighodaro and Akinloye, 2018).

Antioxidants in defense systems work on a different level, including prevention, radical scavenging, repair and de novo, as well as the fourth line of

defense, adaptation. On the basis of line of defense, antioxidants can be classified as:

The first line defense

The preventive antioxidants are the first line of defense, as they inhibit the generation of free radicals. Although the precise mechanism and site of radical formation in vivo are not fully understood , the metal-induce decomposition of peroxols and H2O2 must be one of the important source .To prevent such reaction , some antioxidant first convert peroxols and H2O2 to alcohol and H2O, respectively, without produce free radicals, and some proteins sequester metal ions. It is well establish that (GPX) , glutathiones-transferase , phospholipid hydroperoxide glutathione peroxidase (PHGPX)"and peroxidase , breakdown lipid hydroperoxid to their corresponds alcohols. PHGPX is unique in its ability to decreased hydroperoxides of phospholipids embedded in bio membranes , while GPx and CAT degraded H2O2 to H2O (Niki, 1993; Lobo *et al.,* 2010).

The Second line defense

It is antioxidants that scavenge active radicals to inhibit chain start and/or break propagation reactions. There are several types of endogenous radical scavenging antioxidants: some are water-soluble, while others are lipid-soluble. Vitamin Ascorbic Acid (C), uric acid, albumin, and thiols are water-soluble antioxidants that scavenge free radicals, whereas vitamin tocopherol (E) and ubiquinol are lipid-soluble antioxidants that scavenge free radicals. Vitamin E is often regarded as the most powerful lipophilic antioxidant for scavenging free radicals (Niki, 1993; Lobo *et al.,* 2010).

The Third line defense

It is the repair and de novo antioxidants. Proteolytic enzymes, proteinases, proteases, and peptidases, found in the cytosol and mitochondria of mammalian cells, recognize, breakdown, and eliminate oxidatively changed proteins while preventing oxidized protein buildup. Furthermore, DNA repair systems play a crucial role in the overall defense against oxidative damage. Glycosylases and nucleases, for example, are enzymes that repair damaged DNA(Niki, 1993; Lobo *et al*., 2010).

The Fourth line defense

Another key role is adaptation, in which the signal for free radical production and reactions causes the creation and delivery of the appropriate antioxidant to the correct location (Niki, 1993; Lobo *et al*., 2010).

2.6.1 Glutathione

Glutathione (GSH) is a tripeptide, γ-l-glutamyl-l-cysteinyl-glycine, it is the most important low molecular weight antioxidant synthesized in cells, and particularly concentrated in the liver , it is synthesized by the sequential addition of cysteine to glutamate followed by the addition of glycine. GSH plays a critical role in protecting cells from oxidative damage and the toxicity of xenobiotic electrophiles and maintaining redox homeostasis (Forman et al ., 2009; Vairetti *et al* ., 20 21).

Glutathione, also known as "the master antioxidant," is involved in many metabolic processes in addition to antioxidant defense systems, and hence its importance cannot be overstated. GSH deficiency causes the risk of oxidative damage in cells, and thus, as expected, GSH imbalance is observed in a wide range of pathological conditions (Teskey *et al* ., 2018).

GSH has many important cellular and extracellular functions, of which the detoxification of ROS and of xenobiotics is especially important (Schmidt and Dringen, 2012) .

The antioxidant mechanism, in which GSH is a key player, counterbalances the ROS-generating processes. Under pathological conditions, however, the amount of ROS rises above the steady-state, resulting in oxidative stress (Lushchak , 2012 ;Vairetti *et al* ., 2021), the intermediates created, such as H2O2 and superoxide (O2•), can generate toxic oxygen radicals that trigger fat peroxidation and cell injury. To avoid this, the endogenously generated H2O2 is reduced by glutathione in the presence of selenium-dependent glutathione peroxidase (Se-GSH-px). In the process, glutathione is oxidized to glutathione disulfide (GSSG), which in turn is reduced back to glutathione by glutathione reductase (GSSG-rx) at the expense of NADPH, forming a redox cycle (GarciaRuiz and Fernández-Checa, 2006; Lu, 2009).

Furthermore , "glutathione peroxidase (GPx)" and "glutathione s-transferase (GSTs)"can both decrease organic peroxides. Catalase , which is only found in the peroxisome, can also reduce H2O2, but GSH is very important in the mitochondria because catalase is not present. In fact , mitochondrial GSH is critical in protecting from oxidative stress caused by both physiological and pathological processes (Garcia-Ruiz and Fernández-Checa, 2006 ; Lu, 2009) .

2.6.2: Superoxide dismutase (SOD).

Itis the primary antioxidant defense system against superoxide anion. It is one of the most powerful intracellular enzymatic antioxidants (Fukai and UshioFukai, 2011; Kurutas, 2015) which catalyzes the breakdown of two molecules of

superoxide anion (*O2) into hydrogen peroxide (H2O2) and molecular oxygen (O2), reducing the danger of the superoxide anion . Superoxide dismutase (SOD) requires a metal coenzyme for action and can be classified according to the metal ion attached to it (Morón and Castilla-Cortázar , 2012).

Superoxide dismutase is classified into three types based on the type of metal ion required as a cofactor for the enzyme. Superoxide dismutase is found in humans, all other mammals, and most chordates. Superoxide dismutase 1 (SOD1), or CuZn-SOD, is detected in the cytoplasm, SOD2, or Mn-SOD, is detected in the mitochondria, and SOD3, or EC-SOD, is detected extracellularly. SOD1 and SOD3 are dimers (two subunits), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc,whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre(Zelko *et al* .,2002 ; Pongsavee, 2019).

In humans, several genes encode different "isozymes" of SOD: Cu-Zn SOD "is encoded by the SOD1 gene on chromosome 21 (Levanon.,1985), MnSOD is encoded by the SOD2 gene on chromosome 6 (Miele *et al*., ,1995), and extracellular Cu-Zn SOD is encoded by the SOD3 gene on chromosome 4 (Rosen *et al* .,1993).

A substantial amount of SOD3 is found virtually in all human tissues , a number of tissues, including the heart, have been observed to possess the cellular resources to transcribe SOD3 mRNA from SOD DNA. This is of great importance since SOD3 is the major enzymatic antioxidant defense against vascular and cardiovascular diseases (neurological diseases, lung disease, atherosclerosis, diabetes , hypertension, inflammatory conditions, and ischemia reperfusion injury). An association between SOD deficiency and a number of pathologies has been observed in both animals and humans (Ighodaro and Akinloye, 2018).

In humans, decreased SOD2 activity has been linked to a number of chronic illnesses, including ovarian cancer and type I diabetes, whereas SOD2 overexpression appears to reduce malignancy in cultured cells (Lebovitz *et al* ., 1996). Zn-deficient wild-type and mutant human SOD1 have been linked to the neurodegenerative disease" familial amyotrophic lateral sclerosis (FALS)", which affects nerve cells in the spinal cord and brain (Roberts *et al*., 2007; Bakavayev *et al.*, 2019). Because SOD levels fall as people age and free radical production rises, it's been proposed that taking SOD supplements on a daily basis can protect the immune system, minimize illness risk, and slow down the aging process (Krishnamurthy and Wadhwani , 2012).

2.7 Inflammation

Inflammation "is a biological response of the immune system that can be triggered by a variety of factors such as pathogens, damaged cells, and toxic compounds. These factors may induce acute and/or chronic inflammatory responses in the heart , pancreas , liver, kidney , lung , brain , intestinal tract and reproductive system , potentially leading to tissue damage or disease (Chen *et al* ., 2018) ,it is part of the body's defense mechanism , which is the process by which the immune system recognizes and removes harmful and foreign stimuli , and begins the healing process (Pahwa *et al*., 2021).

The inflammatory process limits itself in normal circumstances, but in some disorders it becomes persistent, and chronic inflammatory diseases can develop later (Ferrero-Miliani *et al.*, 2007), There are five fundamental signs of inflammation that include heat , redness , swelling , pain, and loss of function. Inflammation can be classified into three types based on the time of the process responding to the injurious cause, acute inflammation that occurs immediately after infection and lasts for several days , chronic inflammation that may last for

months or even years when acute inflammation fails to stabilize, and subacute is a period of transformation from acute to chronic that lasts two to six weeks(Pahwa *et al.,* 2021).

During the acute phase of the inflammatory response, cells of the immune system migrate to the site of injury in a carefully orchestrated sequence of events that is facilitated by soluble mediators such as cytokines, chemokines, and acute-phase proteins. Depending on the degree of injury, this acute phase may be sufficient to resolve the damage and initiate healing processes (Germolec *et al.*, 2018).

A woman's many physiological reproductive events such as ovulation, menstruation, implantation and the initiation of labor show distinct signs of inflammation, which are regulated by specific molecular pathways that include a range of growth factors , cytokines, chemokines , and lipid mediators (Jabbour *et al* ., 2009) .

The female reproductive system has the particular advantage of being able to quickly resolve these inflammatory events to restore normal reproductive function. The resolution of the inflammation includes the removal of leukocytes and tissue debris as well as the restoration of mucosal and vascular function in the affected tissues. However, it has been seen as a response to tissue injury. In addition, there are specific anti-inflammatory and pro-resolution biochemical pathways that are activated, which facilitate the re-establishment of homeostasis in the affected tissues (Serhan *et al*., 2008;Jabbour *et al*., 2009).

Jabbour and his colleagues (2009) proposed that reproductive processes are regulated by inflammatory events, and thus tight control of the onset and resolution

of these inflammatory events ensures normal reproductive function. Exacerbated or premature activation of inflammation can contribute to disease.

Cytokines are small proteins secreted by cells of both innate and adaptive immune systems and can regulate diverse functions in the immune response , dysregulation of cytokine secretion and their consequent signaling networks is an important component of the pathogenesis of autoimmune disease (Moulton, 2016).

Interleukin-6 (IL-6) is a single-chain glycoprotein, produced by monocytes, endothelial cells, and adipose tissue" (Rattazzi *et al*., 2003; Van der Velde *et al*., 2015), it belongs to the class of four-helical cytokines , it acts via a cell surface-expressed Interleukin-6 receptor , this receptor, when complexed with interleukin-6 , associates with the signalling receptor glycoprotein 130 kDa (Rose-John , 2020).

Interlukin 6 is a pleiotropic cytokine that has important roles in the regulation of the immune response , inflammation , and hematopoiesis (Nishimoto and Kishimoto , 2006) , however IL-6 regulation disturbance may affect the immune response and thus lead to immune-mediated inflammatory diseases such as rheumatoid arthritis, idiopathic arthritis in children , Castleman disease, and Crohn's disease (Nishimoto and Kishimoto , 2006) .

Interlukin 6 is produced by a plethora of immune and stromal cells, including "monocytes , macrophages, endothelial cells, T-lymphocyte cells, B-lymphocyte cells, fat cells, keratinocytes, hepatocytes, fibroblasts, and dendritic cells ." IL-6 exerts effects on a similarly broad array of cellular targets expressing the functional IL-6 receptor , including (T-lymphocyte cells, B-lymphocyte cells, vascular endothelial cells, monocytes, and hepatocytes) (Copaescu *et al.,* 2020; Uciechowski and Dempke, 2020).

Interlukin- 6 contributes to the regulation of the coordination of innate and acquired immune systems , additionally, interleukin-6 plays an important role in regulating metabolism , in neurodevelopment and survival , and in developing and maintaining various types of cancer (Rose-John , 2020) .

Interleukin- 6 has several functions, in addition to its role in hematopoiesis, it has many wonderful effects on the maturation and survival of myeloid cells, and it can stimulate the growth of stem cells (Williams *et al*., 2007). Interleukin- 6 has strong effects on coagulation as it stimulates platelet production (Senchenkova *et al.*, 2013), moreover it also affects stimulation of the adrenal axis by stimulating ACTH production (Papanicolaou *et al.*, 1998) and has IL-6 effects on keratinocytes, in the skin that express the IL-6 receptor as well as produce Interleukin-6 (Wang *et al* ., 2004).

Many cytokines, especially IL-6, stimulate the production of acute phase proteins (APPs)in response to various stimuli, the patterns of cytokine production and acute phase response vary with the different inflammatory conditions, acute changes in stage reflect the presence and severity of inflammation (Gabay, 2006).

According to Rose-John (2020),that IL-6 is the major alarm signal in the human body in response to infection , inflammation , and possibly cancer , it has been shown that IL-6 plays a major role in chronic inflammation and that IL-6 levels are elevated in inflammatory diseases in humans , in addition , the expression of IL-6 at the site of inflammation is improved (Gabay, 2006) , overproduction of IL-6 also contributes , through its roles as a growth factor or an anti-apoptotic factor , to the development of malignant diseases such as multiple myeloma and renal cancer (Nishimoto and Kishimoto , 2006) .

Interleukin 6 mediates the acute phase responses , at the beginning of acute inflammation , when its activity as a pro-inflammatory cytokine persists , acute inflammation turns into chronic inflammation that includes immune responses , in chronic inflammation , IL-6 has a detrimental role that favours mononuclear cell accumulation as (lymphocytes and monocytes) at the site of injury , through continuous MCP-1 secretion , angio proliferation and anti-apoptotic functions on T cells (Atreya *et al* ., 2000; Gabay, 2006) .

Obesity increases circulating IL-6 levels, which are positively associated with BMI (Päth *et al.,* 2001) and decreased after weight loss in the adipose tissue and the plasma (Kim and Bajaj, 2014) , plasma IL-6 levels are associated with IR and the risk of T2D (Kim and Bajaj, 2014), IL-6 has major effects on cellular immunity with both pro-inflammatory and anti-inflammatory functions (Copaescu *et al*., 2020).

C-Reactive Protein (CRP) "is a homo pentameric acute-phase inflammatory protein that exhibits elevated expression during inflammatory conditions" (Sproston and Ashworth, 2018), "a member of the pentraxin protein family (molecular weight: 120 kD)" (Kop and Weinstein , 2007) , "the gene for CRP has been localized to chromosome 1 and codes for a mature, 206 amino acid polypeptide" (Ahmed *et al*., 2012) , increased levels of inflammatory cytokines, particularly IL-6 , cause transcriptional induction of the CRP gene in hepatocytes in the liver (Boras *et al*., 2014).

It was first recognized as a material in the serum of people with severe inflammation that interacted with the capsular polysaccharide (C-polysaccharide) of pneumococcus, hence it was known as CRP. Tillet and Francis discovered it in 1930 (Gould and Weiser, 2001;Nehring , *et al*., 2021). CRP is a well-established marker of inflammation , low-grade inflammation (LGI), defined as slightly

increased CRP levels, is associated with increased risk of several diseases (Dinh et al ., 2019).

C-reactive protein is a serum protein that is generated in large amounts as part of the body's innate immune response to infection and tissue damage (Gershov *et al.*, 2000). According to Sproston and Ashworth (2018), CRP is more than just a marker of inflammation or infection, it is also a crucial regulator of inflammatory processes,during trauma, stress, or infection. CRP and other inflammatory proteins in the blood such as "haptoglobin , fibrinogen, and serum amyloid A (SAA) etc..., " serve to restore equilibrium and limit microbial development independently of antibodies (Murata *et al* ., 2004 ; Chen *et al*., 2018).

The acute-phase response is characterized by a change in the plasma proteins released by the liver "including a decrease in albumin and an increase in C-Reactive Protein". CRP, fibrinogen, and serum amyloid A (SAA) are the three acute-phase reactants that have been studied the most extensively, and these APPs are well-documented inflammatory markers and mediators of atherosclerosis (Kop and Weinstein, 2007).

C-reactive protein is mainly generated by hepatocytes in the liver, but it is also produced by " smooth muscle cells, macrophages, endothelial cells, lymphocytes, and fat cells " (Sproston and Ashworth , 2018) , CRP synthesis is primarily stimulated in response to pro-inflammatory cytokines, most notably IL-6 , but also interleukin-1and TNF-α (Zhang *et al* ., 1996 ; Sproston and Ashworth , 2018).

C-reactive protein has been shown to predict cardiovascular mortality independent of serum LDL-C concentrations (Ridker *et al*., 2002; Williams *et al.*, 2006), where there is an increased risk of coronary heart disease in people with elevated baseline levels of CRP in plasma (Danesh *et al* ., 1999 ; Pepys and

Hirschfield , 2003) , many studies have shown the presence of high levels of IL-6 and CRP among individuals with IR and T2D clinically (Festa *et al.*, 2000; Kanmani *et al.*, 2019) , CRP has been correlated with IR and obesity and increased risk for development of T2D and associated cardiovascular disease (Festa *et al.*,2000; Salgado-Bernabé *et al*., 2016).

Chronic bacterial or viral infections, autoimmune disorders , chronic inflammatory conditions, diabetes and metabolic syndrome, high blood pressure, high BMI , and cancer, decrease HDL , high triglycerides, and the use of estrogen and progesterone are all related to elevated CRP levels (Pearson *et al* ., 2003; Knight ,2015) , CRP levels typically rise during pregnancy and fall to baseline shortly after birth (Belo *et al*., 2005;Cicarelli *et al* ., 2005) .

González-Fernández and his colleagues (2017) discovered that infection can increase and decrease CRP levels in pregnant and lactating women, noting that only a deficiency of folic acid during lactation was associated with increased CRP levels. Also, they suggested that the interpretation of CRP levels in pregnant and lactating women who have co-existing nutrient deficiencies and multiple infections requires caution.

On the other hand, IL-6 is secreted by adipose tissue in increased amounts in obesity , and this is the major cytokine regulating the hepatic production of CRP. Thus, WAT may be a major player in the raised circulating levels of CRP in obesity, but through the indirect route of adipocyte-derived IL-6 (Yudkin *et al.*, 2000; Trayhurn and Wood, 2004).

According to Forouhi and his colleagues (2001),obesity and especially visceral adipose tissue, is a major trigger of chronic, low-grade inflammation. This partly explains the association of CRP with markers of metabolic syndrome.

2.8 Lipid profile , related with suckling.

A typical lipid pattern in the blood includes :total cholesterol (TC) (which measures all cholesterol in all lipoprotein particles),High-density lipoprotein cholesterol (HDL-C) (measures cholesterol in HDL particles) is often referred to as "good cholesterol" because HDL-C absorbs excess cholesterol and transfers it to the liver for removal, Low-density lipoprotein cholesterol (LDL-C) is often referred to as bad cholesterol" since it stores excess cholesterol in the walls of blood vessels, contributing to atherosclerosis. The sum of LDL-C is typically measured using the results of total cholesterol, HDL-C, and triglycerides (TG). T G measures all the triglycerides in all the lipoprotein particles, most of which are in the very low-density lipoproteins (VLDL-C).(Heart , 2003; Lee and An, 2020).

Breastfeeding benefits may counteract the negative effects of gestation by raising the mother's basal metabolic rate, mobilizing fat stores, increasing insulin sensitivity, and continuing positive effects on blood lipids, particularly lowering circulating TG in the mother and not lowering HDL-C , which is a strong risk factor for T2D in women. Whereas, the physiological stress of normal gestation is distinguished by an increase in circulating TG and increased IR, reaching its peak in mid-gestation (Gunderson *et al* ., 2007; Ajmera *et al*., 2019).

Furthermore, many maternal adaptations occur during lactation, including a rise in basal metabolic rates, mobilization of fat stores , and the maternal fuel metabolism is altered markedly with a 15%–25% increase in energy expenditure for milk production (Butte *et al* ., 1999 ; Gunderson , 2014) .

Breastfeeding necessitates the mobilization of fat for milk synthesis, which requires fundamental changes in fat metabolism during breastfeeding (Stuebe and Rich-Edwards , 2009).

As lactation becomes established, a mother's body should respond to meet the high nutritional requirements of milk production through a variety of adaptations, including increased food intake, mobilization of lipid and bone stores, and increased glucose production (Sadovnikova *et al.*, 2020), whereas ,the body prepares for the metabolic demands of breastfeeding by increasing maternal lipid deposition during the first and second trimesters of human gestation (Villar *et al.*, 1992 ; Boyle and Le Foll , 2019). The quantity of fat secreted during the course of lactation varies by species, but in general, the processes involved in milk fat production and secretion greatly increase lipid transport requirements. During a six-month period of lactation, the typical woman secretes more than 5 kg of fat, whereas a 30 g mouse dam secretes about 30 g, or the equivalent of her body weight, of lipid into milk over a 21-day period of lactation (Schwertfeger *et al* ., 2003 ; McManaman , 2014) .

Upon approaching childbirth, the induction of lipoprotein lipase activity in the mammary glands increases the use of circulating triglycerides in the preparation for lactogenogenesis (Herrera, 2000 ; Gunderson, 2014).

When measuring regional changes in lipoprotein lipase (LPL) activity as a proxy for lipid deposition in mice , Hamosh and his colleagues (1970) found that fat deposition increases during pregnancy, but fat storage stops in adipose tissue with breastfeeding, and its absorption increases in mammary tissues with the transfer of fat into milk .

In cohort studies , Stuebe and Rich-Edwards (2009) found that lactation has been correlated with a lowered risk of hyperlipidemia and cardiovascular disease.

Ramos-Roman and his colleagues (2020) indicated that lactation effects would be expected to protect against liver lipid accumulation and hyperlipidemia, even in obese subjects, and highlight the need for future research to investigate the influence of prolactin on fatty acid flux outside the mammary gland. Furthermore, many studies have pointed out the role of lactation in lipid profile changes.

According to Knopp and his colleagues (1985), the increase in HDL-C components in lactation is partly due to the increased breakdown of triglyceride-rich lipoproteins by the lactating breast ,while triglyceride, LDL-C and TC levels declined between delivery and six months postpartum and the TC levels remained stable until nine months postpartum, after which, at 2 months postweaning, the levels increased to delivery levels (Kallio *et al.*,1992). Furthermore, triglyceride levels returned to baseline within 13 weeks of the breastfeeding period compared to non-breastfeeding women (Darmady and Postle ,1982; Stuebe , 2015) .

Kjos and his colleagues (1993) reported that breastfeeding, even for a short duration, has a beneficial effect on glucose and lipid metabolism in women with gestational diabetes. They also found higher mean high-density lipoprotein (HDL) cholesterol in the blood in the lactating women after adjusting for maternal age, body mass index, and insulin use during pregnancy compared to the non-lactating women. There were no observed differences in total cholesterol, LDL-C, or triglyceride levels.

In a study, Gunderson and his colleagues (2007) examined changes in metabolic risk factors among lactating women from preconception to post weaning and among non-lactating women from preconception to post-delivery , in comparison with non-pregnancy women, found that average changes in LDL-C and triglycerides are more appropriate with longer breastfeeding duration.

Furthermore, breastfeeding for a longer amount of time may ameliorate the long-range reduction in HDL-C that occur during gestation.

In a study of breastfeeding and non-breastfeeding women, Abdulkareem (2018) demonstrated that HDL-C was higher in the breastfeeding groups than in non-breastfeeding mothers, but the difference was not statistically significant. However, LDL-C was significantly lower in the breastfeeding group than in the non-breastfeeding group, and serum cholesterol, serum triglyceride, and VLDL-C were insignificantly lowers in the breastfeeding group in comparison to the non-breastfeeding group.

On the other hand, Rudolph and his colleagues (2007) indicated that The usage of different resources for triglycerides synthesizing by "mammary epithelial cells" is impacted by both breastfeeding stage and diet, and that three kinds of substrate are used to synthesize milk triglycerides: dietary fat, fatty acids mobilized from adipose tissue stores, and lipids manufactured by denovo synthesis from simple sugar (glucose) and other dietary precursors.

Chapter Three

Materials and Methods

3. Materials And Methods:

3.1. Materials

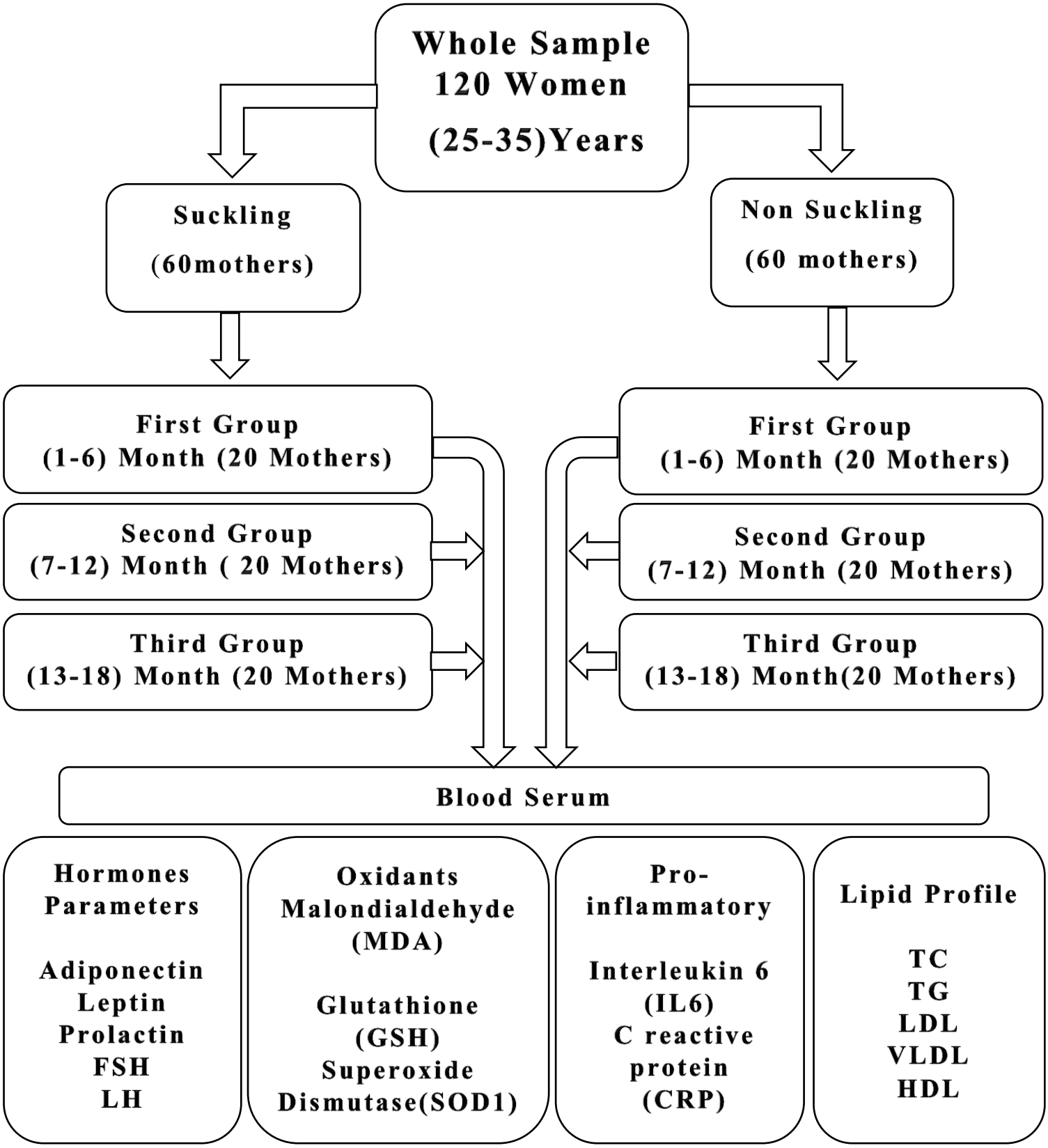
3.1.1. Subjects Of the Study:

The current study was carried out in Maysan province from February - October 2020, the sample of this study included 120 women aged (25–35 years) old, including sixty suckling mothers and sixty non-suckling mothers, who visited Al Sadder Teaching Hospital and some other healthy clinic centers. Each of these suckling and non-suckling mothers was divided into three groups (20 mothers/group) according to the periods of suckling as the following:

- First group (1-6 months) .
- Second group (7-12 months).
- Third group (13-18 months).

Healthy mothers (according to a consultant physician) were taken, and women taking hormonal contraceptives were excluded. The questionnaire is designed to obtain the actual information about the sample.

3.1.2. Experimental design



3.1.3 Instruments and Equipments

The instruments and equipment that used in this study with their companies and countries of origin are listed in table (3.1).

Table (3.1) : The instruments and equipment's used in this study.

NO	INSTRUMENT	COMPANY /ORIGIN
1	Can tube	China
2	Centrifuge	Germany
3	COBAS e411	Roche / Germany
4	Cotton	M.O.H ,Iraq
5	Disposable syringe 10 ml	Trogector3 /Germany
6	Elisa reader	Bio –tek /Germany
7	Elisa washer	Bio – tek /Germany
8	Eppendorf tubes(1.5 ml)	M.O.H/ China
9	Gel tube	Sun /Jordan
10	Gloves	Turkey
11	100 ml and 500 ml graduated cylinders.	China
12	Horizontal microtiter plate shaker	Germany
13	Incubator	Heraeus /Germany
14	Magnetic stirrer	Germany
15	Pipettes for delivery of : 50 µl, 200 µl, 1 ml and 10 ml	Dragon LAP. CO.
16	Refrigerator	Concord /Lebanon
17	Syringe	Zhejiang INI Medical Devices /China
18	Test tubes for dilution	China
19	Tips(10ml,20ml, 100ml and 200 ml)	Star Lab/ UK
20	Vortex mixer	Germany

3.1.4 Laboratory kits.

The laboratory kits used in this study are shown in table (3.2)

Table (3.2) : kits and their suppliers .

No	Kits	Company/Origin
1	Adiponectin	Elabscience / USA
2	C- reactive protein	ROCH / GERMAMY
3	FSH	ROCH / GERMAMY
4	Glutathione	Elabscience / USA
5	Interleukin 6	Elabscience / USA
6	Leptin	Elabscience / USA
7	LH	ROCH / GERMAMY
8	Lipid profile (TC,LDL,HDL, TG)	ROCH / GERMAMY
9	Malondialdehyde	Elabscience / USA
10	Prolactin	ROCH / GERMAMY
11	Malondialdehyde	Elabscience / USA
12	Superoxide dismutase [Cu-Zn]	Elabscience / USA

3.1.5 Diagnostic Kits Found in The Appendix

3.2.Methods

3.2.1 Blood Samples Collection

Five milliliters of venous blood samples were drawn at (9-11 a.m.) using a disposable needle and plastic syringes for each suckling and non-suckling mother. The blood samples were left for 15 minutes to clot at room temperature, to get the serum, which was separated by centrifugation at 3000 (rpm) for 15 (min) , to measure all the parameters for the current study. Serum was transferred into a labeled plain tube and stored at -20 until used for evaluation of hormones and other parameters.

3.2.2 Determination Of Hormonal Parameters

3.2.2.1 Determination of Human Adiponectin (ADP)

Principle of the Assay

Direct sandwich solid-phase immunoassay depends on chromogenic reaction consist of monoclonal antibody one which will be bind to the adiponectin and monoclonal antibody two labeled with biotin to react with HPR-streptavidin enzyme which works on 3,3',5,5'-Tetramethylbenzidine (TMB) substance (Hu *et al.*, 1996) .

3.2.2.2 Determination of Human Leptin (LEP)

Principle of the Assay

Direct sandwich solid-phase immunoassay depends on chromogenic reaction consist of monoclonal antibody one which will be bind to the leptin and monoclonal antibody two labeled with biotin to react with horseradish

peroxidase(HPR)-streptavidin enzyme which works on TMB substance (Considine *et al*., 1996).

3.2.2. 3 Determination of Prolactin

Test principle

The Elecsys Prolactin II assay uses a sandwich two monoclonal antibodies specifically directed against human prolactin (**Fahie-Wilson and Smith, 2013**)

The principle

- ☑ Incubate 10 µL of serum and a biotinylated monoclonal prolactin specific antibody will form the first complex.
- ☑ Adding a second labeled antibody with a ruthenium complex plus streptavidin-coated micro particles, a sandwich complex is formed and the reaction of biotin and streptavidin makes the complex attach to the solid phase.
- ☑ The reaction mixture is drawn into the measurement cell, where the micro particles are magnetically catch on the electrode's surface. Washing to remove and unbound substances . When a voltage is applied to the electrode, chemiluminescent emission occurs, which is measured by a photomultiplier.
- ☑ Using calibration curve and a master curve provided via the reagent barcode or e-barcode to get the results .

Measuring Range

(1.00-10000 µIU/mL)or (0.0470-470 ng/Ml) . In female (4.79- 23.3 ng/Ml).

3.2.2.4 Determination of FSH

Test principle

The principle of the sandwich the assay took 18 minutes in total.

- ☑ Incubate 40 µL of serum , a biotinylated anti-FSH antibody , and an aruthenium compound labeled FSH specific antibody will form the sandwich complex.
- ☑ Adding streptavidin-coated micro particles , the complex becomes attach to the solid phase via interaction of biotin and streptavidin.
- ☑ The mixture is drawn into the measurement cell, where the micro particle are magnetically catch on the electrode's surface. Washing to remove any unbound substances . When a voltage is applied to the electrode, chemiluminescent emission occurs, which is measured by a photomultiplier.
- ☑ Using calibration curve and a master curve provided via the reagent barcode or e-barcode to get the results (Beastall *et al.*, 1987; Scott *et al.*, 1989) .

Measuring Range

(0.100-200 mIU/mL). In female: Follicular Phase: (3.5 -12.5), Luteal Phase: (1.0- 11.4) ,Ovulation Phase : (1.5 - 21.5).

3.2.2.5 Determination of LH

Test principle

The principle of the sandwich the assay took 18 minutes in total.

- ☑ Incubate 20 µL of serum with an antibody that labeled with biotin and an antibody labeled with aruthenium complex will form the sandwich complex.
- ☑ Adding micro particle that are covered with streptavidin , the complex becomes attach to the solid phase via interaction of biotin and streptavidin.
- ☑ The mixture then is drawn into the measurement cell, where the micro particle are magnetically catch on the electrode's surface. Washing to removes any unbound substances. When a voltage is applied to the electrode, chemiluminescent emission occurs, which is measure by a photomultiplier.
- ☑ Using calibration curve and a master curve provided via the reagent barcode or e-barcode to get the results (Bablok *et al.*, 1988).

Measuring Range

(0.100-200 mIU/mL). In femal : FOllicular Phase: (2.4 -12.6), Luteal Phase: (1.0- 11.4) Ovulation Phase : (14.0 - 95.6).

3.2.3 Determination of Oxidant stress

3.2.3.1 Determination of Human Malondialdehyde (MDA)

Principle of the Assay

The Competitive-ELISA technique used, where the wells pre-coated with MDA and will compete with the MDA in the sample or standard for sites on the Ab labeled with biotin and specific for MDA . wash for any excess conjugate and unbound sample or standard then incubation with Avidin conjugated with HRP then add the TMB substrate . The reaction stop later by stop solution to get a color of yellow which its density indicate the MDA concentration (Mène-Saffrané *et al*., 2007).

Reagent Preparation and Storage

Allow 20 minutes for the kit to get to room temperature before using .

1- Wash Buffer: Diluted 30 ml Wash Buffer with D.W. to gets 750 ml Wash Buffer.

2- Standard:

2.1 Add 1 ml Sample/Standard dilution buffer into one Standard tube, left it for 10 m.

2.2 Use a 7 tubes and Fill each with 0.3ml of the Sample/Standard dilution buffer except the seventh one. To get a serial dilution add 0.3 ml of the above standard dilution to first tube and then transfer 0.3 to the next and so on.

3- An antibody labeled with Biotin Solution preparing: add for example depending on the volume you need)1µl of Biotin-labeled antibody into 99µl Antibody Dilution Buffer to get a ratio as 1:100.

4- HRP-Streptavidin conjugate Solution prepare: add for example depending on the volume you need)1µl of HRP-Streptavidin Conjugate into 99µl of its Dilution Buffer to get a ratio as 1:100.

Assay Procedure

Equilibrate the TMB substrate for 30 minutes at 37 °C before adding reagents to the wells. When diluting samples and reagents, they must be well mixed and equally distributed. For each test, it is recommended a standard curve be plotted.

1. Add 50 µL of sample , standard to their position in the wells, for the blank well add only the dilution buffer of standard .

2.Add 50 µL of Antibody Solution that labeled with biotin.

3.Seal and incubate for 3/4 hour at 37°C.

4.wash with 350 uL of washing buffer for three times after decant the well , left one minute between them .

5.Add 100µL HRP Conjugate Solution, seal and incubate for half an hour minutes at 37°C.

6.Aspirate or decant the solution from each well, then repeat step 2 for a total of five washes.

7.Add 90µl TMB substance , seal the plate and incubate 37°C for 15-20 minutes at dark place.

8.Add 50 µL of Stop Solution.

9.Immediately after adding the stop solution, read the O.D. absorbance at 450 nm in a Microplate Reader.

3.2. 4 Determination of Anti-oxidant

3.2.4 .1 Determination of Glutathione (GSH)

Test principle

The Competitive-ELISA technique used, where the wells pre-coated with GSH and will compete with the GSH in the sample or standard for sites on the Ab labeled with biotin and specific for GSH. wash for any excess conjugate and unbound sample or standard then incubation with Avidin conjugated with HRP and then add the TMB substrate. The reaction stop later by stop solution to get a color of yellow which its density indicate the GSH concentration (Kinoshita *et aI.,* 1996).

Reagent preparation

Allow 20 minutes for the kit to get to room temperature before using.

1- Wash Buffer: Dilute 30 ml Wash Buffer with D.W. to get 750 ml Wash Buffer .

2- Standard :

2.1 Add 1 ml Sample/Standard dilution buffer into one Standard tube, left it for 10 m.

2.2 Use a 7 tubes and Fill each with 0.3ml of the Sample/Standard dilution buffer except the seventh one . To get a serial dilution add 0.3 ml of the above standard dilution to first tube and then transfer 0.3 to the next and so on.

3-An antibody labeled with Biotin Solution preparing : add for example depending on the volume you need)1µl of Biotin-labeled antibody into 99µl Antibody Dilution Buffer to get a ratio as 1:100 .

4-HRP-Streptavidin conjugate Solution prepare : add for example (depending on the volume you need)1µl of HRP-Streptavidin Conjugate into 99µl of its Dilution Buffer to get a ratio as 1:100 .

Assay procedure

1. Add 50 µL of sample , standard to their position in the wells , for the blank well add only the dilution buffer of standard .

2. Add 50 µL of Antibody Solution that labeled with biotin .

3. Seal and incubate for 3/4 hour at 37°C.

4. Wash with 350 uL of washing buffer for three times after decant the well , left one minute between them .

5. Add 100µL HRP Conjugate Solution, seal and incubate for half an hour minutes at 37°C.

6. Aspirate or decant the solution from each well, then repeat step 2 for a total of five washes .

7. Add 90µl TMB substance , seal the plate and incubate 37°C for 15-20 minutes at dark place.

8. Add 50 µL of stop solution.

9. Immediately after adding the stop solution, read the O.D. absorbance at 450 nm in a Microplate Reader.

3.2.4.2 Determination of Human SOD1 (Superoxide dismutase [Cu-Zn])

Principle of the Assay

Direct sandwich solid-phase immunoassay depends on chromogenic reaction consist of monoclonal antibody one which will be bind to the SOD1 and monoclonal the second antibody labeled with biotin to react with HPR-streptavidin enzyme which works on TMB substance (Brown and Borchelt, 2014).

3.2.5 Determination of Pro –inflammatory markers

3.2.5.1 Determination of Human Interleukin 6(IL-6)

Principle of the Assay

Direct sandwich solid-phase immunoassay depends on chromogenic reaction consist of monoclonal antibody one which will be bind to the IL6 and monoclonal antibody two labeled with biotin to react with HPR-streptavidin enzyme which works on TMB substance (**Bowcock , 1988**).

3.2.5.1.1 Reagent Preparation (Adiponectin, Leptin, SOD1 and Interleukin- 6)

Allow 20 minutes for the kit to get to room temperature before using .

1. Wash Buffer: Dilute 30 ml Wash Buffer with D.W. to get 750 ml Wash Buffer.
2. Standard:
    - 2.1 Add 1 ml Sample/Standard dilution buffer into one Standard tube, left it for 10 m.
    - 2.2 2.2Use a 7 tubes and Fill each with0.3ml of the Sample/Standard dilution buffer except the seventh one , . to get a serial dilution add 0.3 ml of the above standard dilution to first tube and then transfer 0.3 to the next and so on .
3. An antibody labeled with Biotin Solution preparing : add for example (depending on the volume you need)1µl of Biotin-labeled antibody into 99µl Antibody Dilution Buffer to get a ratio as 1:100 .

4. HRP-Streptavidin conjugate Solution prepare: addfor example (depending on the volume you need)1µl of HRP-Streptavidin Conjugate into 99µl of its Dilution Buffer to get a ratio as 1:100 .

3.2.5.1.2 Assay Procedure (Adiponectin, Leptin , SOD1 and Interleukin- 6)

1. Wash the plate twice. Then add for each well 0.1 ml the standard, test sample, and control (zero) to their position in the wells .
2. Seal the plate with a sealant that found in the kit and incubate for 90 minutes at a temperature of 37°C.
3. Tap the wells to remove its continents and then wash with Wash Buffer for two times .
4. Fill the following wells (standard, test sample & zero wells) with 0.1 ml of Biotin-labeled antibody working solution.
5. Seal and incubation for one hour and at 37°C.
6. Wash with Wash Buffer 3 times with one minute between them .
7. Fill each well with 0.1 ml of HRP-Streptavidin Conjugate Solution that we already diluent , seal the plate and incubate at 37° C for half an hour .8.Wash the plate with
8. Wash Buffer for five times with one to two minute between them .
9. Add for each well a 90µlTMB Substrate, seal it and incubate in the dark at temperature of 37°C for about (15-30) minutes depending on the first three to four wells changing color .
10. Add 50µl of Stop Solution and notice the color changing in to yellow from blue immediately.
11. Immediately after adding the stop solution, read the O.D. absorbance at 450 nm in a Microplate Reader.

3.2.5.2 Determination of C-reactive protein

Test principle

C-Reactive Protein (CRP) was evaluated by particle enhanced immune turbidimetric assay, with human CRP kit (**Price *et al.*, 1987**).

Principle of CRP procedure assay

Human CRP bind tightly with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically .

Measuring Range

(1-200 mg/L) or (0.1-20 mg/dL).

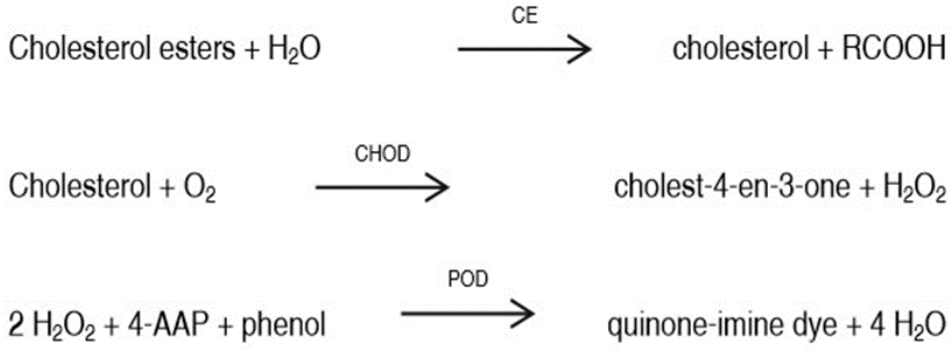
3.2.6 Determination of lipid profile

3.2.6 .1 Determination of Total cholesterol (TC)

TC was evaluated by using Enzymatic colorimetric test , with human cholesterol kit (Roeschlau *et al* ., 1974) .

Principle of assay

Cholesterol esterase cleave the cholesterol esters to get cholesterol and RCOOH . the cholesterol then oxide by Cholesterol oxidase to cholest-4-en-3one and H2O2. H2O2 plus phenol and 4AAP by peroxidase produce a red dye . The dye color density is proportional to the cholesterol concentration. It is determined by photometric .



Measuring Range

(0.25-20.7 mmol/L) or (9.7-800 mg/dL).

3.2.6 .2 Determination of Triglycerides (TG)

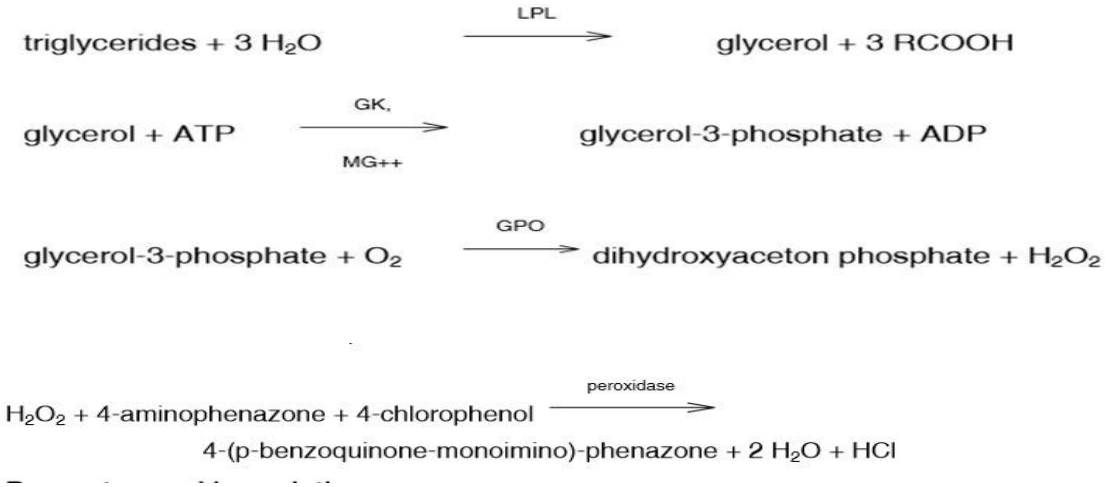
TG was evaluated by using enzymatic colorimetric test , with human TG kit (Siedel *et al* ., 1993) .

Principle of triglycerides procedure assay

Hydrolaysis of TG by LPL to get glycerine plus fatty acids , glycerin phosphated to get glycerol -3- phosphate which oxidiesed to get the red dye . The density of this dye is propotional to concentration of TG .

Measuring Range

(0.1-10 mmol/L) or (8.85-885 mg/dL).

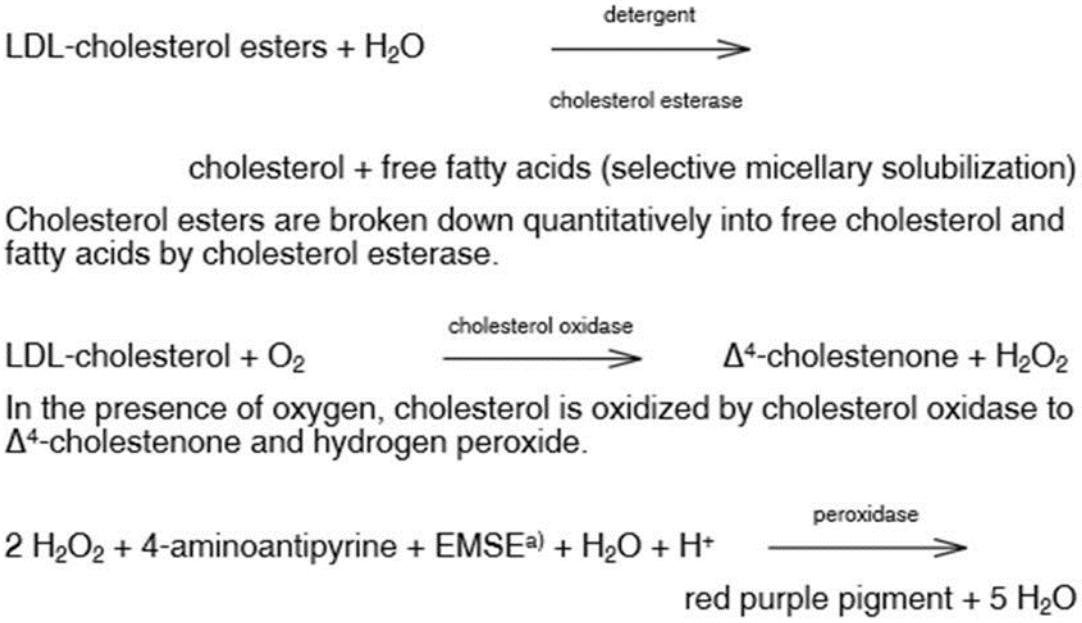


3.2.6 .3 Determination of serum low density lipoprotein cholesterol (LDL-C).

LDL-C was evaluated by using Homogeneous enzymatic colorimetric assay , with human LDL- C kit (Bachorik, 2000).

Principle of assay

Selectively LDL chosen by surfactant compound plus sugar inhibited for other lipoproteins .The Cholesterol esterase work on LDL- cholesterol esters to get Cholesterol which Oxidases to cholestenone and H2O2 . H2O2 by the enzyme peroxidase and EMSE produce a red purple dye . The density of dye are the reflection of the LDL concentration.



Measuring Range

(0.10-14.2 mmol/L) or (3.87-549 mg/dL) .

3.2.6.4 Determination Of Very Low Density Lipoprotein Cholesterol (VLDL-C).

levels of VLDL- cholesterol is determination according to the equation of dividing triglyceride levels by 5 (Friedewald *et al.*, ,1972) **.**

3.2.6 .5 Determination Of High Density Lipoprotein (HDL-C)

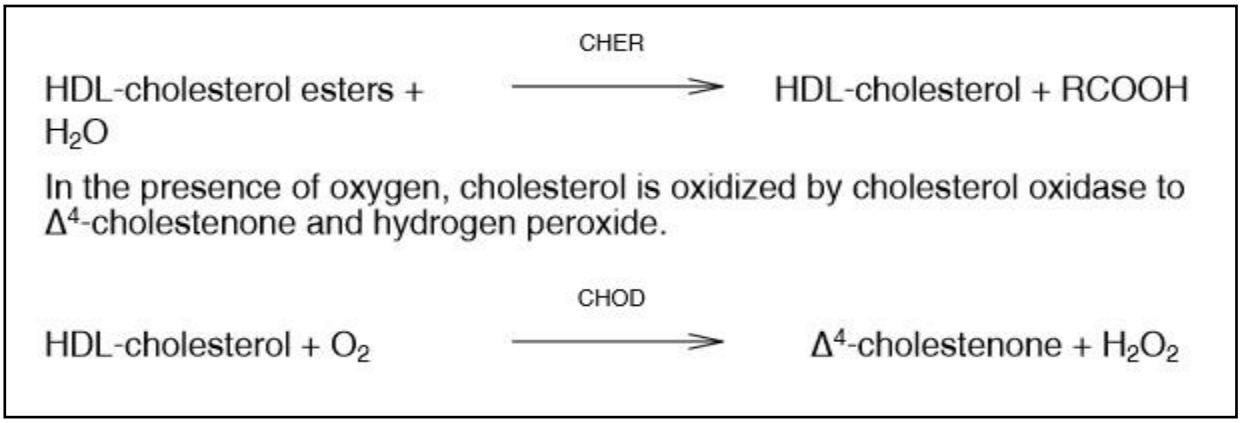
HDL-C was evaluated by using Homogeneous enzymatic colorimetric test , with human HDL- C kit (Katayama, *et al.*, 2009).

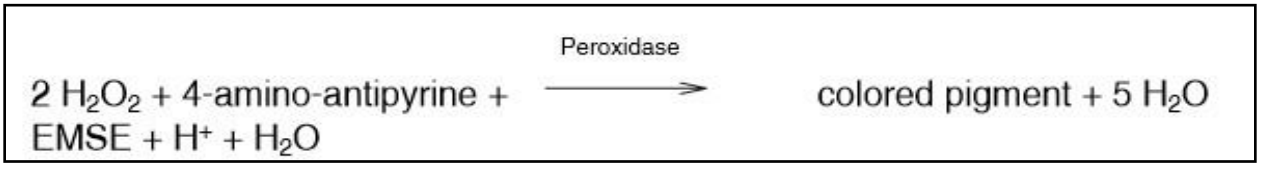
Principle

The prevention of other lipoprotein reaction except HDL after the treat with polyanions and a detergent to form a watersoluble complex. The Cholesterol esterase(CHER) work on HDL- cholesterol esters to get Cholesterol which Oxidases to cholestenone and H2O2 . The H2O2 produced reacts with 4aminoantipyrine and EMSEa to make a dye in the presence of peroxidase .The density of dye are the reflection of the HDL concentration and is measured photometrically.

Measuring Range

(0.08-3.88 mmol/L) or (3.09-150 mg/dL).





3.3 Statistical analysis

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 one-way Analysis Of Variance (ANOVA) , followed by t-test and Duncan's test at ($p \leq 0.05$) significant level.

Chapter Four

Results

4 . Results :

4.1 The levels of hormonal and biochemical parameters in in suckling and non -suckling mothers for a different groups.

4.1.1 Hormonal parameters

4.1.1.1 Adiponectin hormone :

First Group : Results revealed that the adiponectin concentration in the non- suckling mothers (283.470 ±23.467 ng/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (255.070 ±26.886 ng/ml).

Second Group : Results revealed that the adiponectin concentration in the non- suckling mothers (277.110 ± 24.968 ng/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (255.712 ±28.873 ng/ml).

Third Group : Results revealed that the adiponectin concentration in the non-suckling mothers (255.440 ±18.791 ng/ml) raised (not significant) compared with the suckling mothers (249.630 ±15.897 ng/ml). Figure (4.1) ;Table (4.1).

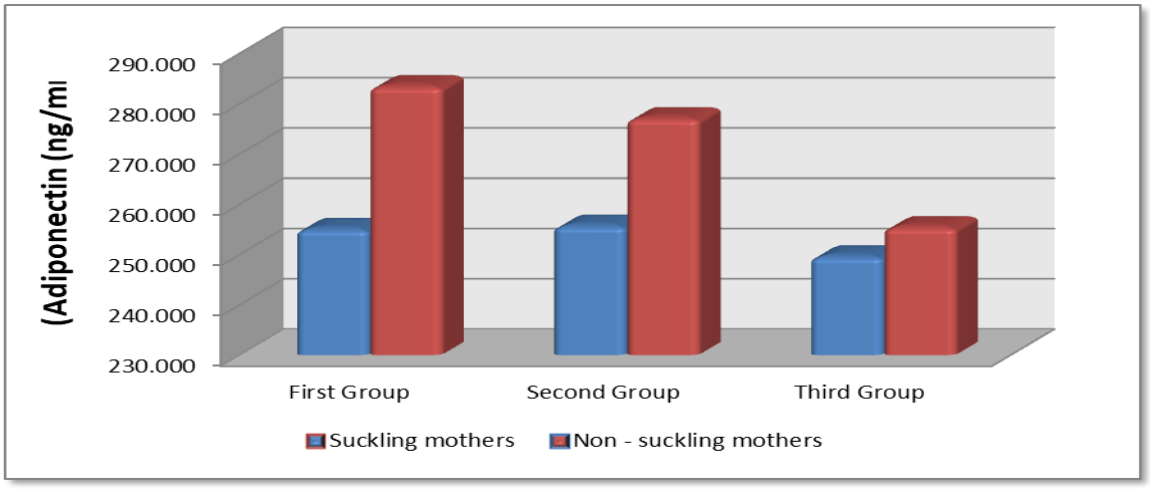


Figure (4.1): Adiponectin levels in suckling and non - suckling mothers for different groups.

***The values represent mean ± SD .**

4.1.1. 2 Leptin hormone:

First Group : Results revealed that the leptin concentration in the non-suckling mothers (6.827 ±0.308 ng/ml) raised (not significant) compared with the suckling (6.705 ±0.374 ng/ml) .

Second Group : Results revealed that the leptin concentration in the non-suckling mothers (6.870 ±0.266ng/ml) raised (not significant) compared with the suckling (6.700±0.390ng/ml) .

Third Group: Results revealed that the leptin concentration in the non-suckling mothers (6.911 ±0.21 8 ng/ml) raised significantly ($P \leq 0.05$) compared with suckling mothers (6.666 ±0.311 ng/ml). Figure (4.2) ;Table (4.1).

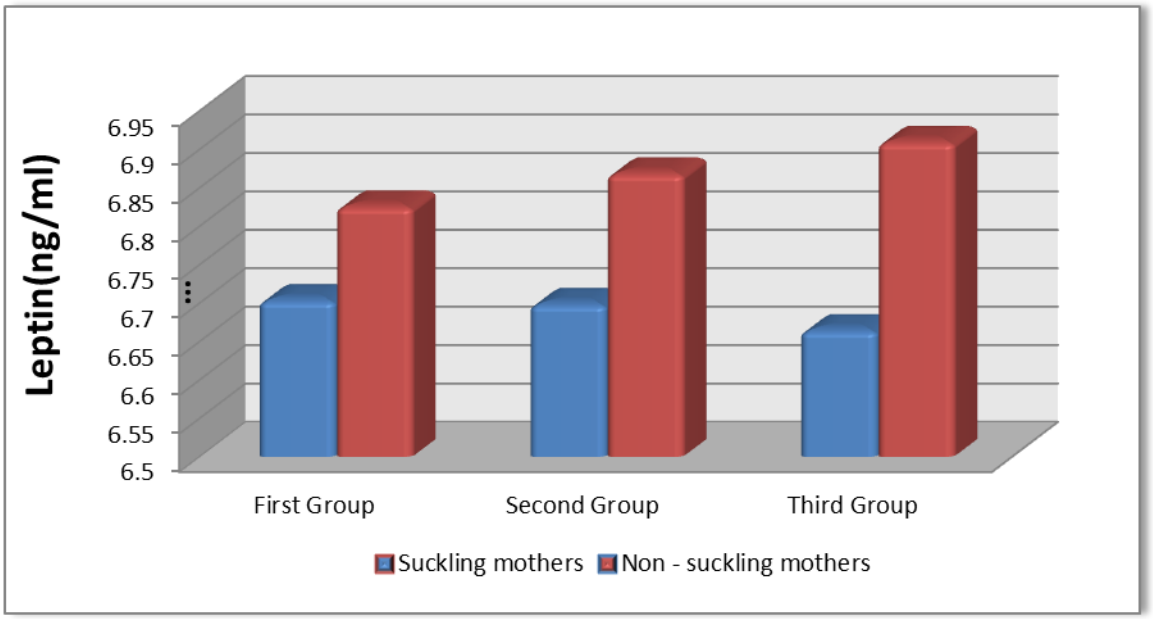


Figure (4.2) : Leptin levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

4.1.1.3 Prolactin hormone(PRL) :

First Group : Results revealed that the prolactin concentration in the non-suckling mothers (25.875 ±4.568 ng/ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (89.958 ±8.844).

Second Group : Results revealed that the prolactin concentration in the non-suckling mothers (24.609 ±5.239 ng/ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (75.282 ±9.802 ng/ml).

Third Group : Results revealed that the prolactin concentration in the non-suckling mothers (18.464 ±3.719 ng/ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (52.679 ±8.646 ng/ml). Figure (4.3),Table (4.1).

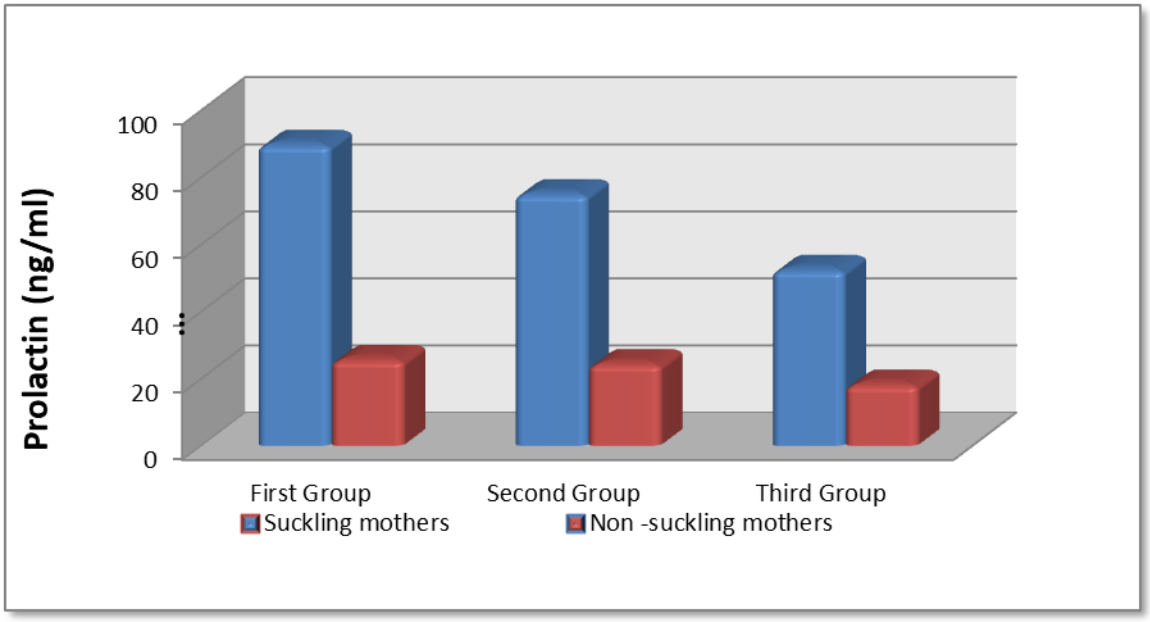


Figure (4.3):Prolactin levels in suckling and non -suckling mothers for different groups .

***The values represent mean ± SD .**

4.1.1.4 Follicle-Stimulating Hormone (FSH) :

First Group: Results revealed that the FSH hormone concentration in the non- suckling mothers (7.811 ±0.560 mlu/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (6.949 ±0.923 mlu/ml).

Second Group: Results revealed that the FSH hormone concentration in the non-suckling mothers (8.083±0.864 mlu/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (7.403 ±0.778 mlu/ml).

Third Group: Results revealed that the FSH concentration in the non-suckling mothers (8.602 ±0.885 mlu/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (7.713 ±0.965 mlu/ml). Figure (4.4) ;Table (4.1).

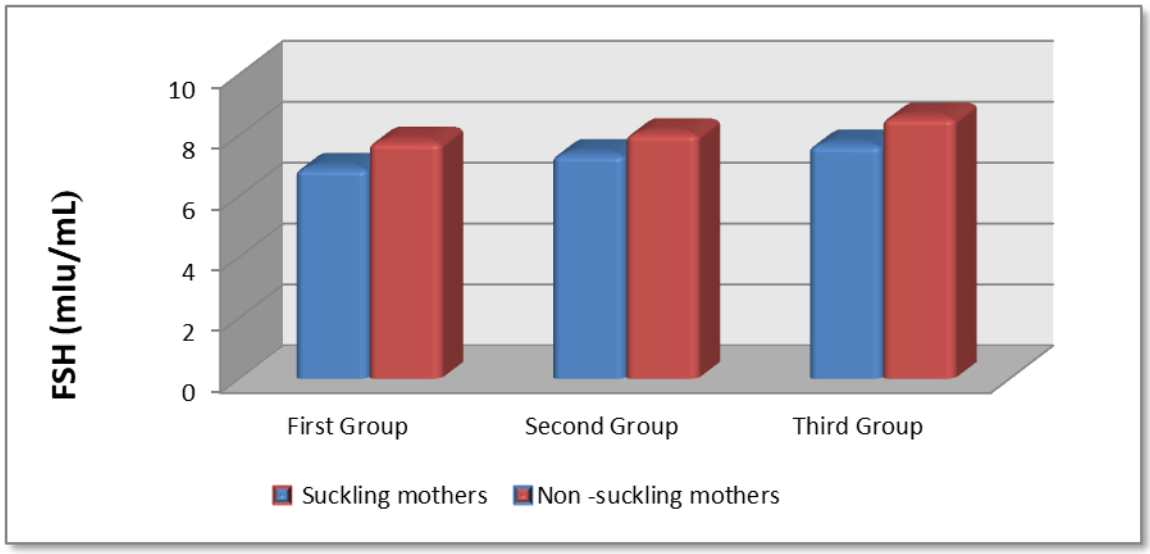


Figure (4.4): FSH hormone levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

4.1.1.5 Luteinizing Hormone (LH):

First Group: Results revealed that the LH hormone concentration in the non-suckling mothers (7.276 ±0.929 mlu/ml) rasied significantly ($P \leq 0.05$) compared with the suckling mothers (5.945 ±0.974 mlu/ml) .

Second Group: Results revealed that the LH hormone concentration in the non -suckling mothers (8.189 ±0.998 mlu/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (6.551 ±0.645 mlu/ml).

Third Group: Results revealed that the LH hormone concentration in the non- suckling mothers (8.643 ±0.949 mlu/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (7.606 ±0.840 mlu/ml). Figure (4.5) ;Table (4.1).

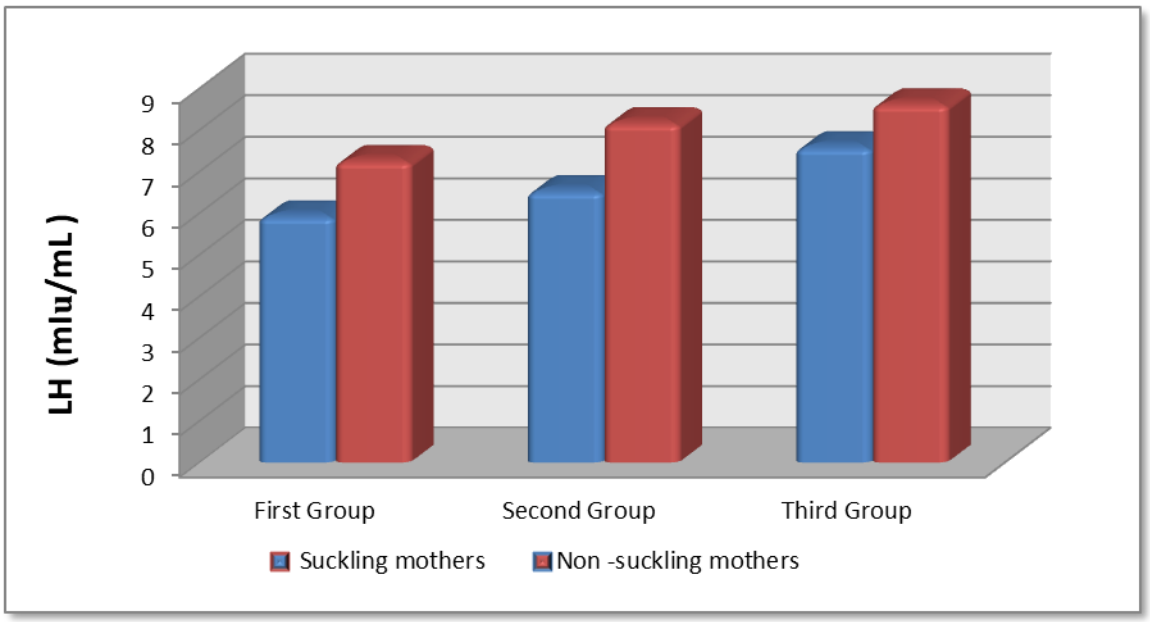


Figure (4.5): LH levels in suckling and non -suckling mothers for different groups.

***The values represent mean ± SD .**

Table (4.1) : Hormonal parameters (Adiponectin , Leptin , Prolactin , FSH and LH) in suckling and non -suckling mothers for a different groups.

Groups / Parameters	First Group		Second Group		Third Group	
	Suckling Mother	Non - Suckling Mothers	Suckling Mothers	Non - Suckling Mothers	Suckling Mothers	Non - Suckling Mothers
Adiponectin(ng/ml)	255.070 a ±26.886	283.470 b ±23.467	255.712 a ±28.873	277.110 b ± 24.968	249.630 a ±15.897	255.440 a ±18.79
Leptin (ng/ml)	6.705 a ±0.374	6.827 a ±0.308	6.700 a ± 0.390	6.870 a ±0.266	6.666 a ±0.311	6.911 b ±0.218
Prolactin (ng/ml)	89.958 a ±8.844	25.875 b ±4.568	75.282 a ±9.802	24.609 b ±5.239	52.679 a ±8.646	18.464 b ±3.719
FSH(mlu/ml)	6.949 a ±0.923	7.811 b ±0.560	7.403 a ±0.778	8.083 b ±0.864	7.713 a ± 0.965	8.602 b ±.885
LH (mlu/ml)	5.945 a ±0.974	7.276 b ±0.929	6.551 a ±0.645	8.189 b ±0.998	7.606 a ±0.840	8.643 b ±0.949

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

*A significantly different ($P \leq 0.05$) within groups is represented by different letters

4.1.2 Oxidant and antioxidant parameters

4.1.2.1 Malondialdehyde (MDA) :

First Group: Results revealed that the Malondialdehyde concentration in the non- suckling mothers (122.672 ±16. 779 ng/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (90.435 ±8.776 ng/ml).

Second Group: Results revealed that the Malondialdehyde concentration in the non- suckling mothers (226.040 ±14.983 ng/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (88.910 ±14.825 ng/ml).

Third Group: Results revealed that the Malondialdehyde concentration in the non- suckling mothers (227.745 ±16.405 ng/ml) raised significantly ($P \leq 0.05$) compared with the suckling mothers (76.030 ±9.802 ng/ml). Figure (4.8); Table (4.2).

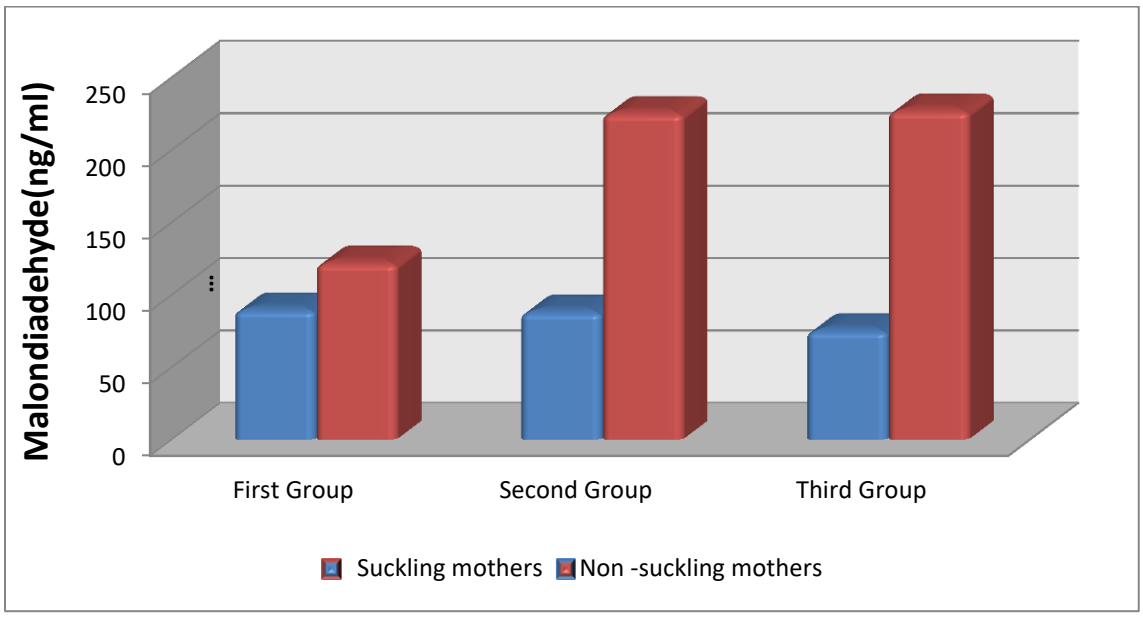


Figure (4.6): Malondialdehyde levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

4.1.2.2 Glutathione (GSH) :

First Group : Results revealed that the glutathione (GSH) concentration in the non- suckling mothers (6.945 ±0.925 µg /ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (8.585 ±0. 950 µg /ml) .

Second Group : Results revealed that the GSH concentration in the non-suckling mothers (5.795 ±0.933 µg /ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (7.050 ±0.623 µg /ml).

Third Group : Results revealed that the GSH concentration in the non-suckling mothers (5.065 ±0.940 µg /ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (6.940 ±0.534 µg /ml). Figure (4.6) ;Table (4.2) .

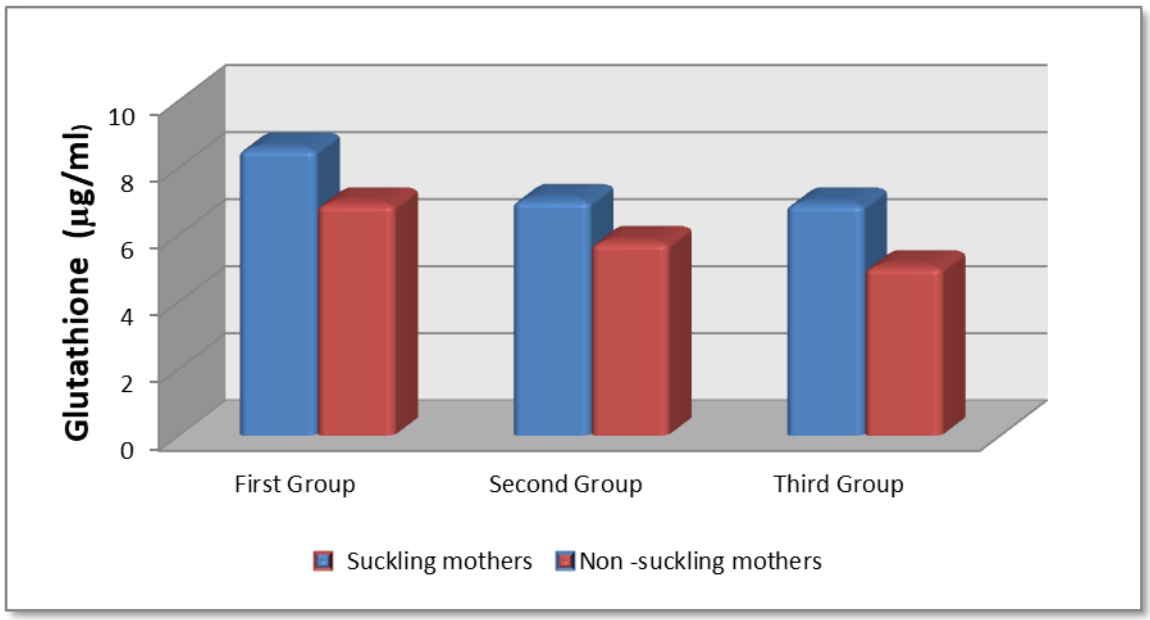


Figure (4.7) : Glutathione levels in suckling and non - suckling mothers for different groups.

***The values represent mean ± SD .**

4.1.2.3 Superoxide Dismutase [Cu-Zn] (SOD1) :

First Group : Results revealed that the superoxide dismutase[Cu-Zn] (SOD1) concentration in the non- suckling mothers (6.165 ±0.914 ng/ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (8.360 ±0.926 ng/ml).

Second Group : Results revealed that the SOD1concentration in the non-suckling mothers (6.906 ±0.899 ng/ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (9.035 ±0.692 ng/ml).

Third Group : Results revealed that the SOD1concentration in the non-suckling mothers (8.720 ±0.905 ng/ml) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (9.948 ±0.888 ng/ml). Figure (4.7); Table (4.2) .

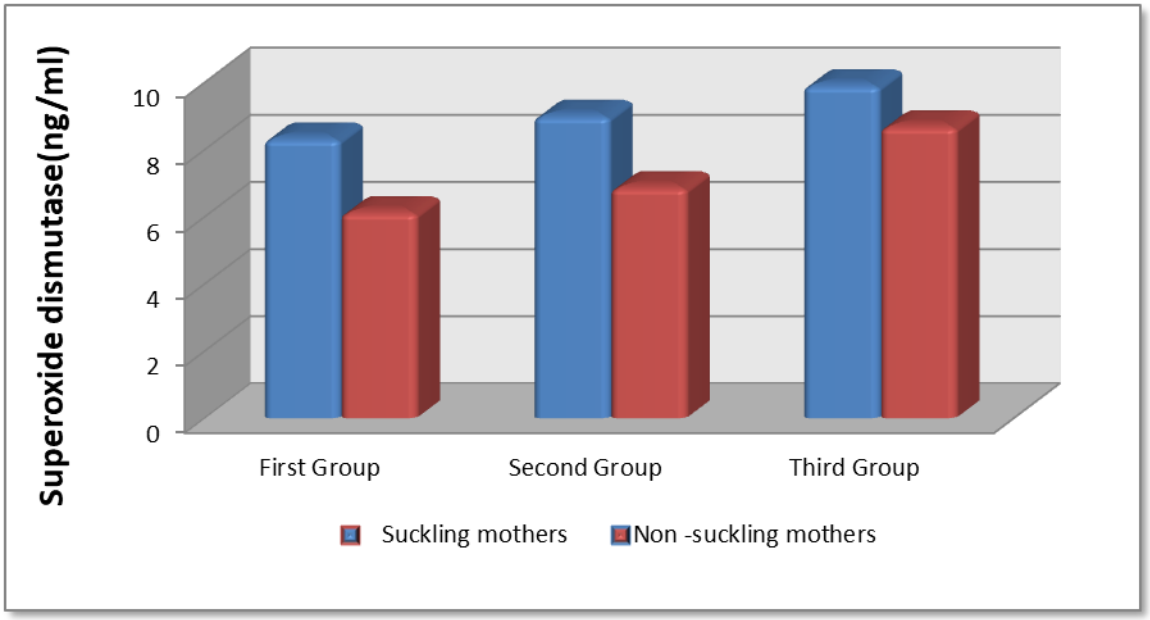


Figure (4.8): Superoxide dismutase[Cu-Zn] levels in suckling and non - suckling mothers for different groups.

***The values represent mean ± SD .**

4.1.3 Pro -inflammatory markers :

4.1.3.1 Interleukin-6 (IL-6):

First Group : Results revealed that the Interleukin -6 concentration in the non- suckling mothers (0.159 ±0.024 pg/ml) raised (not significant) compared with the suckling mothers (0.148 ±0.007 pg /ml).

Second Group : Results revealed that the IL -6 concentration in the non-suckling mothers (0.189 ±0.033 pg /ml) raised (not significant) compared with the suckling mothers (0.184 ±0.019 pg /ml) .

Third Group : Results revealed that the IL -6 concentration in the non-suckling mothers (1.147 ±0.988 pg /ml) raised (not significant) compared with the suckling mothers (1.097 ±0.947 pg /ml) . Figure (4.9) ;Table (4.2).

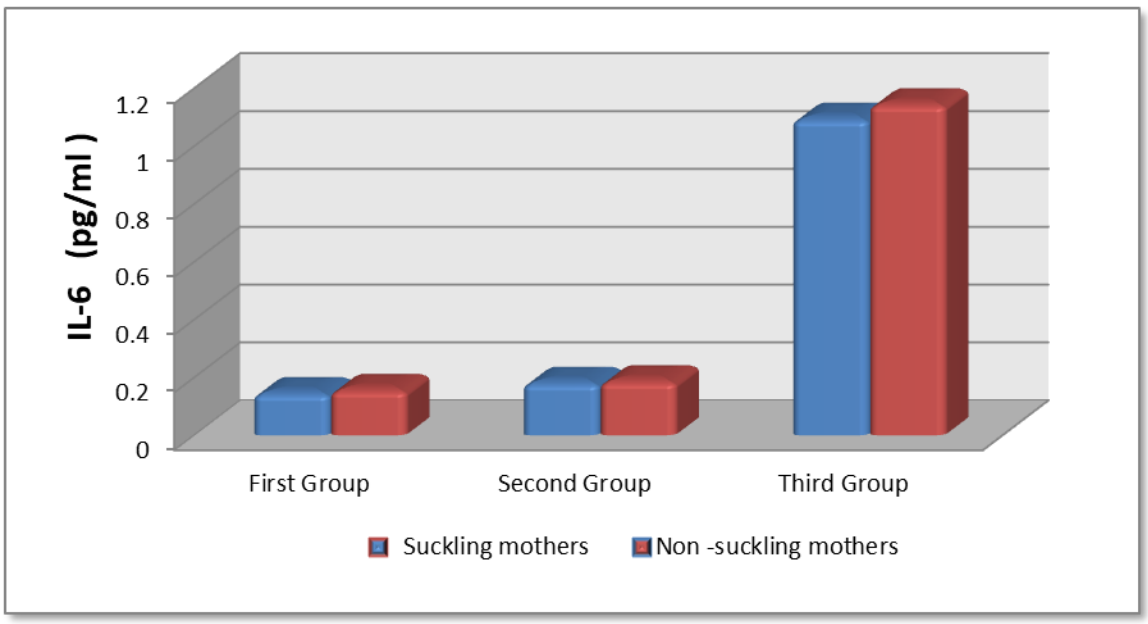


Figure (4.9): Interleukin- 6 levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

4.1.3.2 C-reactive protein (CRP) :

First Group: Results revealed c-reactive protein concentration in the non-suckling mothers (1.690±0.554 mg/dl)) raised significantly ($P \leq 0.05$) compared with suckling mothers (0.716 ±0.202 mg/dl) .

Second Group: Results revealed that the CRP concentration in the non-suckling mothers (1.209 ±0.387mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (0.703±0.170mg/dl) .

Third Group : Results revealed that the CRP concentration in the non-suckling mothers (0.723±0.250mg/dl) raised significantly ($P \leq 0.05$) compared with suckling mothers (0.691±0.186mg/dl) . Figure (4.10); Table (4.2).

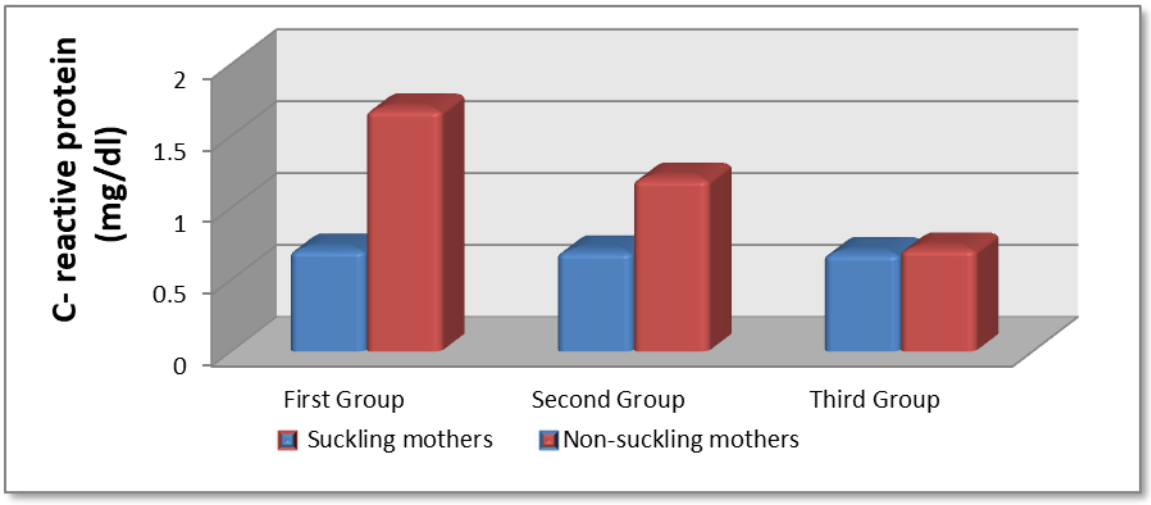


Figure (4.10): C- reactive protein levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

Table (4.2): Oxidant and antioxidant parameters (MDA, GSH and SOD1) and pro -inflammatory markers (IL-6 and CRP) in suckling and non - suckling mothers for different groups.

Groups / Parameters	First Group		Second Group		Third Group	
	Suckling Mothers	Non - Suckling Mothers	Suckling Mothers	Non - Suckling Mothers	Suckling Mothers	Non - Suckling Mothers
MDA(ng/ml)	**90.435 a ±8.776**	**122.672 b ±16.779**	**88.910 a ±14.825**	**226.040b ±14.983**	**76.030 a ±9.802**	**227.745 b ±16. 405**
GSH (µg/ml)	**8.585 a ± 0.950**	**6.945 b ±0.925**	**7.050 a ±0.623**	**5.795 b ±0.933**	**6.940 a ±0.534**	**5.065 b ±0.940**
SOD1(ng/ml)	**8.360 a ±0.926**	**6.165 b ±0.914**	**9.035 a ±0.692**	**6.906 b ±0.899**	**9.948 a ±0.888**	**8.720 b ±0.905**
IL-6(pg /ml)	**0.148 a ±0.007**	**0.159 a ±0.024**	**0.184 a ±0.019**	**0.189 a ±0.033**	**1.097 a ±0.947**	**1.147 a ±0.988**
CRP(mg/dl)	**0.716 a ±0.202**	**1.690 b ±0.554**	**0.703 a ±0.170**	**1.209 b ±0.387**	**0.691 a ±0.186**	**0.723 b ±0.250**

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

4.1.4 The lipid profile

4.1.4.1 Total cholesterol (TC) :

First Group : Results revealed that the cholesterol concentration in the non-suckling mothers (166.673 ±14.725 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (143.545 ±11.541 mg/dl).

Second Group : Results revealed that the cholesterol concentration in the non- suckling mothers (167.285 ± 16.941 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (138.210 ± 13.175 mg/dl).

Third Group : Results revealed that the cholesterol concentration in the non-suckling mothers (169.605 ±18.107 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (135.880 ±14.783 mg/dl). Figure (4.11) ;Table (4.3).

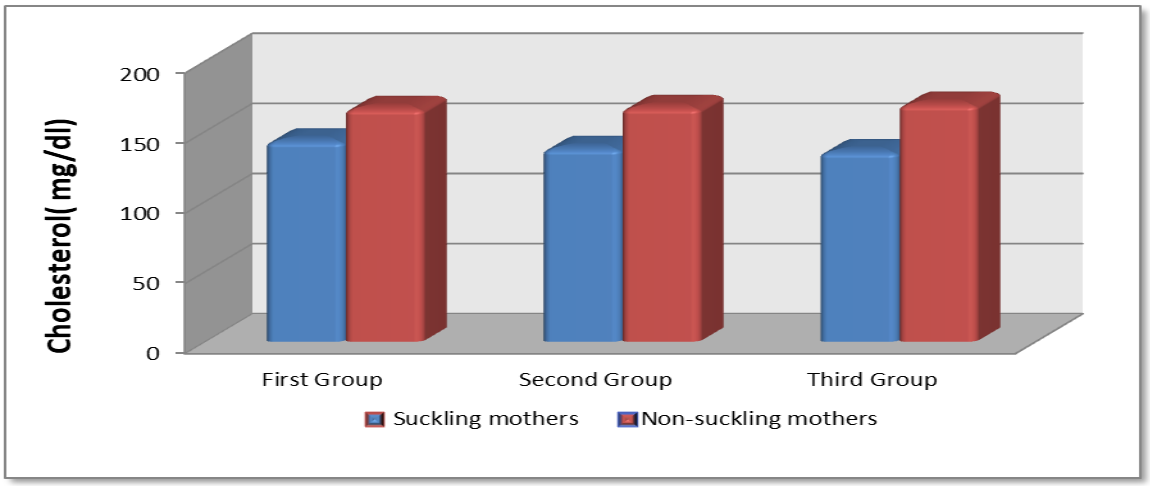


Figure (4.11): cholesterol levels in suckling and non - suckling mothers for different groups.

***The values represent mean ± SD .**

4.1.4.2. Triglycerides (TG):

First Group : Results revealed that the TG concentration in the non- suckling mothers (100.640 ±15.926 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (89.920 ±9.086 mg/dl).

Second Group : Results revealed that the TG concentration in the non-suckling mothers (102.365 ±14.273 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (86.950 ±9.922 mg/dl) .

Third Group : Results revealed that the TG concentration in the non-suckling mothers (108.530 ±9.382 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (85.250 ±9.907 mg/dl). Figure(4.12);Table (4.3).

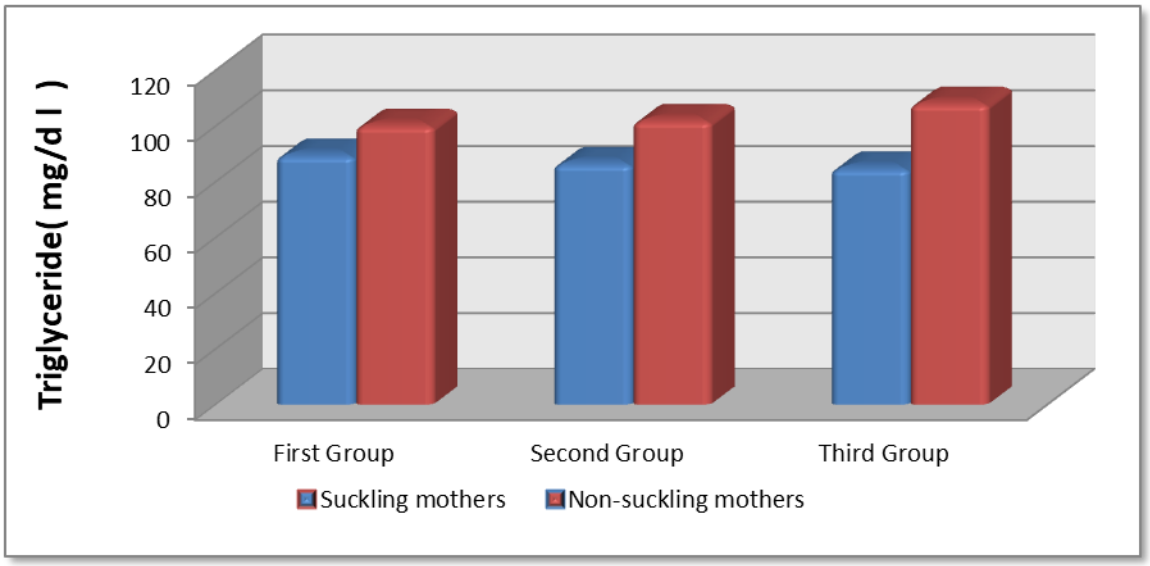


Figure (4.12): Triglyceride levels in suckling and non - suckling mothers with in different groups.

***The values represent mean ± SD .**

4.1.4.3. Low Density Lipoprotein Cholesterol(LDL-C):

First Group : Results revealed that the LDL concentration in the non-suckling mothers (95.256 ±12.646 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (85.130 ±8.241 mg/dl).

Second Group : Results revealed that the LDL concentration in the non -suckling mothers (97.610 ±13.537 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (81.880 ±9.160 mg/dl) .

Third Group : Results revealed that the LDL concentration in the non -suckling mothers (103.020 ±13.497 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (80.075 ± 9.515 mg/dl). Figure (4.13); Table (4.3).

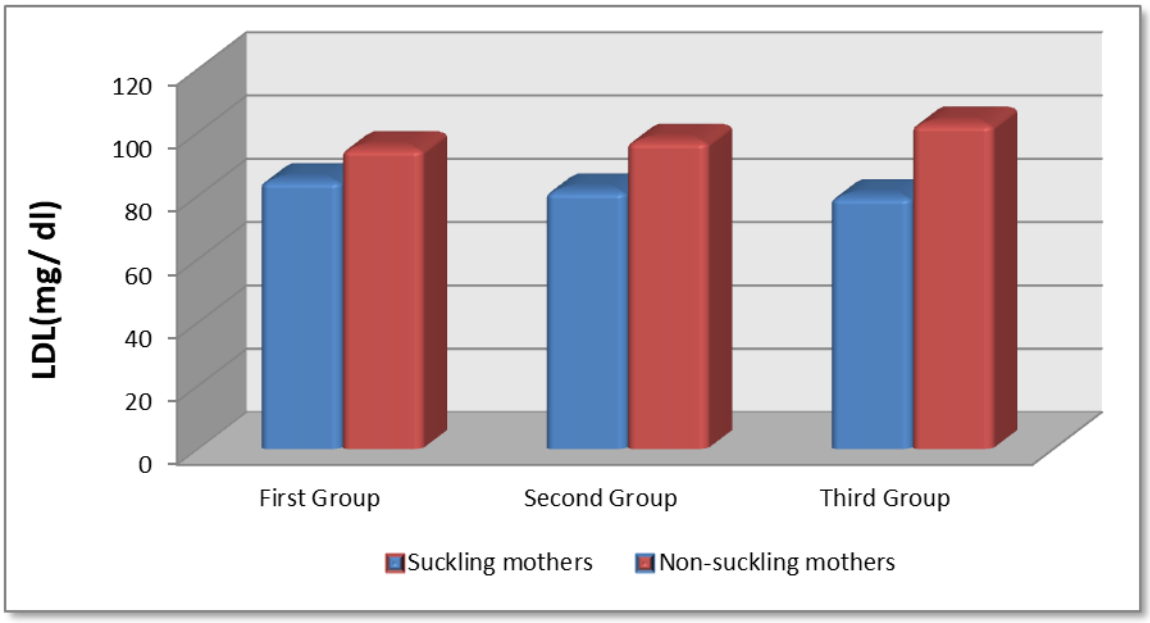


Figure (4.13): LDL levels in suckling and non -suckling mothers for different groups .

***The values represent mean ± SD**

4.1.4.4.Very low density lipoprotein cholesterol(VLDL-C):

First Group : Results revealed that the VLDL concentration in the non - suckling mothers (21.766 ±4.782 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (16.711 ±3.682 mg/dl).

Second Group : Results revealed that the VLDL concentration in the non - suckling mothers (22.215 ±4.887 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (15.931 ± 3.681 mg/dl).

Third Group : Results revealed that the VLDL concentration in the non - suckling mothers (23.363 ±3.999 mg/dl) raised significantly ($P \leq 0.05$) compared with the suckling mothers (14.892 ±3.048 mg/dl). Figure (4.14);Table(4.3).

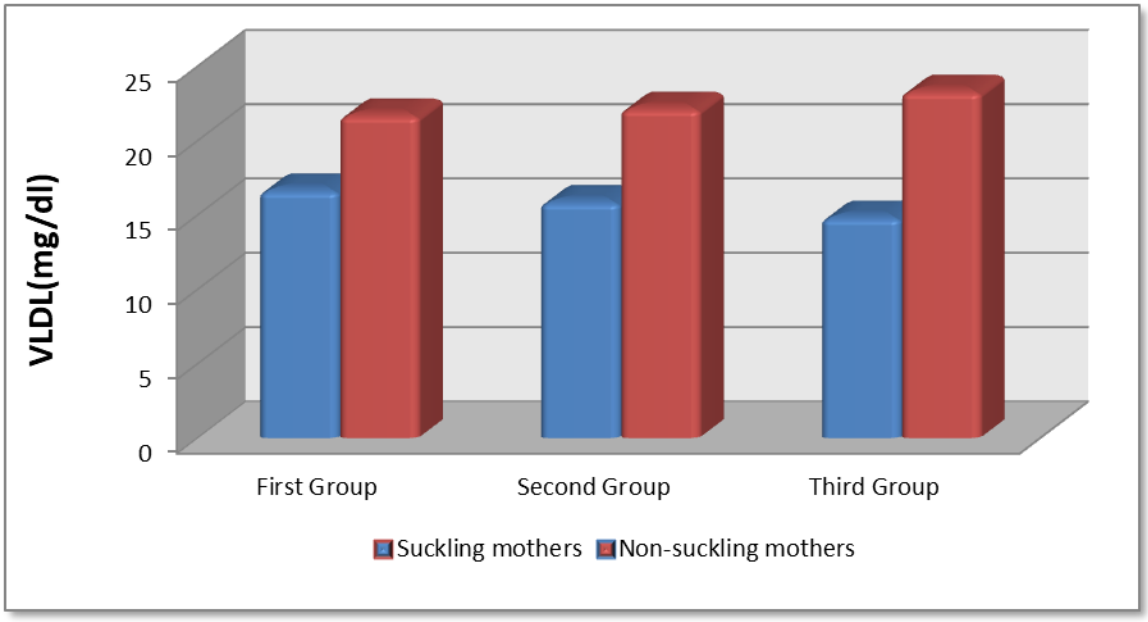


Figure (4.14): VLDL levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

4.1.4.5. High density lipoprotein (HDL-C):

First Group : Results revealed that the HDL concentration in the non - suckling mothers (39.850 ±3.238 mg/dl) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (56.020 ± 3.788 mg/dl).

Second Group : Results revealed that the HDL concentration in the non - suckling mothers (37.245 ± 3.387 mg/dl) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (58.020 ± 4.763 mg/dl).

Third Group : Results revealed that the HDL concentration in the non - suckling mothers (35.265 ±3. 308mg/dl) reduced significantly ($P \leq 0.05$) compared with the suckling mothers (59.380 ±2.590 mg/dl). Figure(4.15); Table (4.3).

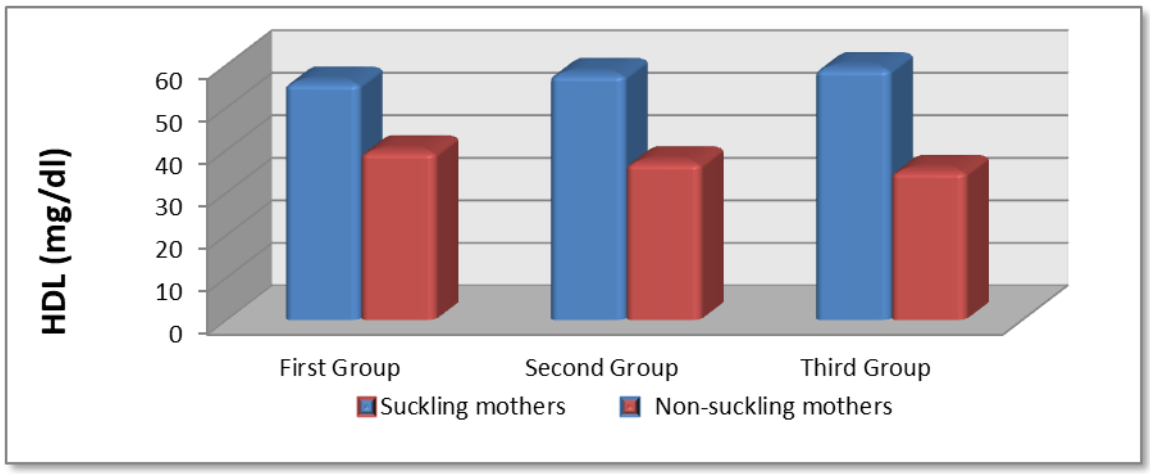


Figure (4.15): HDL levels in suckling and non - suckling mothers for different groups .

***The values represent mean ± SD .**

Table (4.3): Lipid Profile (TC, TG, LDL, VLDL And HDL) In Suckling Mothers And Non- Suckling Mothers In Different Groups.

Groups / Parameters	First Group		Second Group		Third Group	
	Suckling Mothers	Non - Suckling Mothers	Suckling Mothers	Non - Suckling Mothers	Suckling Mothers	Non - Suckling Mothers
TC(mg/dl)	143.545 a ±11.541	166 .673 b ±14.725	138.210a ± 13.175	167.285b ± 16.941	135.880a ±14.783	169.605b ±18.107
TG (mg/dl)	89.920 a ±9.086	100.640 b ±15.926	86.950 a ±9.922	102.365b ±14.273	85.250 a ±9.907	108.530b ±9.382
LDL (mg/dl)	85.130 a ±8.241	95.256 b ±12.646	81.880 a ±9.160	97.610 b ±13.537	80.075 a ± 9.515	103.020b ±13.497
VLDL (mg/dl)	16.711 a ±3.682	21.766 b ±4.782	15.931 a ±3.681	22.215 b ±4.887	14.892 a ±3.048	23.363b ±3.999
HDL(mg/dl)	56.020 a ± 3.788	39.850 b ±3.238	58.020 a ± 4.763	37.245 b ± 3.387	59.380 a ±2.590	35.265b ±3.308

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

4.2 Hormonal Parameters in Suckling Mothers for Different Groups.

4.2.1. Hormonal Parameters

4.2.1.1 Adiponectin Hormone: Results revealed that the adiponectin concentration in the third group (249.630 ±15.897ng/ml) reduced (not significant) compared with the second group (255.712 ±28.873 ng/ml) and with the first group (255.070 ±26.886 ng/ml) . The adiponectin concentration in the second group raised (not significant) compared with the first group . Table (4.4).

4.2.1.2 Leptin Hormone: Results revealed that the leptin concentration in the third group (6.666 ±0.311 ng/ml) reduced (not significant) compared with the second group (6.700±0.390ng/ml) and with the first group (6.705 ±0.374 ng/ml) , the leptin concentration in the second group reduced (not significant) compared with the first group . Table (4.4).

4.2.1.3 Prolactin Hormone (PRL) : Results revealed that the prolactin concentration in the third group (52.679 ± 8.646 ng/ml) reduced significantly ($P \leq 0.05$) compared with the second group (75.282 ±9.802 ng/ml) and with the first group(89.958 ±8.844 ng/ml), the prolactin concentration in the second group reduced significantly ($P \leq 0.05$) compared with the first group . Table (4.4).

4.2.1.4 Follicle-Stimulating Hormone (FSH): Results revealed that FSH concentration in the third group (7.713 ±0.965 mlu/ml) raised significantly ($P \leq 0.05$) compared with the first group (6.949 ±0.923 mlu/ml) and with the second group (7.403 ±0.778 mlu/ml) ,the FSH concentration in the second group raised (not significant) compared with the first group . Table (4.4).

4.2.1.5 Luteinizing Hormone (LH) : Results revealed that the LH concentration in the the third group (7.606 ±0.840 mlu/ml) raised significantly ($P \leq 0.05$) compared with the second group (6.551 ±0.645 mlu/ml) and with the first group (5.945 ± 0.974 mlu/ml) ,the LH concentration in the second group raised significantly ($P \leq 0.05$) compared with the first group . Table (4.4).

Table (4.4) : Hormonal Parameters (Adiponectin , Leptin , Prolactin , FSH And LH Hormones) In Suckling Mothers For Different Groups.

Groups / Parameters	First Group	Second Group	Third Group
Adiponectin (ng/ml)	**255.070 a ±26.886**	**255.712 a ±28.873**	**249.630 a ±15.897**
Leptin(ng/ml)	**6.705 a ±0.374**	**6.700 a ±0.390**	**6.666 a ±0.311**
Prolactin (ng/ml)	**89.958 a ±8.844**	**75.282 b ±9.802**	**52.679 c ±8.646**
FSH (mlu/ml)	**6.949 a ±0.923**	**7.403 a ±0.778**	**7.713 b ±0.965**
LH (mlu/ml)	**5.945 a ±0.974**	**6.551 b ±0.645**	**7.606 c ±0.840**

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

4.2.2 Oxidant And Antioxidant Parameters In Suckling Mothers For Different Groups.

4.2.2.1 Malondialdehyde (MDA): Results revealed that the MDA concentration in the third group (76.030 ±9.802 ng/ml) reduced significantly ($P \leq 0.05$) compared with the second group (88.910 ±14.825 ng/ml) and with the first group (90.435 ±8.776 ng/ml). The MDA concentration in the second group reduced (not significant) compared with first group . Table (4.5).

4.2.2.2 Glutathione (GSH): Results revealed that the GSH concentration in the third group (6.940 ±0.534 µg /ml) reduced significantly ($P \leq 0.05$) compared with the first group (8.585 ±0. 950 µg /ml). The GSH concentration in the second group (7.050 ±0.623 µg /ml) reduced significantly ($P \leq 0.05$) compared with the first group .The GSH concentration in second group raised (not significant)compared with third group . Table (4.5).

4.2.2.3 Superoxide Dismutase [Cu-Zn] (SOD1): Results revealed that the SOD1 concentration in the third group (9.948 ±0.888 ng/ml) raised significantly ($P \leq 0.05$) compared with the second group (9.035 ±0.692ng/ml) and with the first group(8.360 ±0.926 ng/ml) , the SOD1 concentration in the second group raised significantly ($P \leq 0.05$) compared with the first group . Table (4.5).

4.2.3 Pro-Inflammatory Markers In Suckling Mothers For Different Groups.

4.2.3.1 Interleukin 6 (IL-6): Results revealed that the IL-6 concentration in the third group (1.097 ±0.947 pg/ ml) raised significantly ($P \leq 0.05$) compared with the first group (0.148 ±0.007 pg/ ml)and with the second group (0.184 ±0.019 pg/ ml) .The IL-6 concentration in the second group raised (not significant) compared with the first group . Table (4.5).

4.2.3.2 C-reactive protein (CRP): Results revealed that the CRP concentration in the third group (0.691±0.186 mg/dl) reduced (not significant) compared with the first group (0.716 ±0.202 mg/dl) and with the second group (0.703±0.170 mg/dl) .The CRP concentration in the second group reduced (not significant) compared with the first group . Table (4.5).

Table (4.5) : Oxidant and antioxidant parameters (MDA, GSH and SOD) and pro - inflammatory markers (IL6 and CRP) in suckling mothers for different groups.

Groups / Parameters	First Group	Second Group	Third Group
MDA(ng/ml)	**90.435 a ±8.776**	**88.910 a ±14.825**	**76.030 b ±9.802**
GSH(µg /ml)	**8.585 a ±0. 950**	**7.050 b ±0.623**	**6.940 b ±0.534**
SOD1(ng/ml)	**8.360 a ±0.926**	**9.035 b ±0.692**	**9.948 c ±0.888**
IL-6(pg/ ml)	**0.148 a ±0.007**	**0.184 a ±0.019**	**1.097 b ±0.947**
CRP (mg/dl)	**0.716 a ±0.202**	**0.703 a ±0.170**	**0.691 a ±0.186**

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

4.2.4 Lipid Profile In Suckling Mothers For Different Groups .

4.2.4.1 Total Cholesterol (TC): Results revealed that the TC concentration in the third group(135.880 ±14.783 mg/dl) reduced (not significant) compared with the second group (138.210 ± 13.175 mg/dl) and with the first group(143.545 ±11. 541 mg/dl) , the TC concentration in the second group reduced (not significant) compared with the first group . Table (4.6).

4.2.4.2 Triglycerides (TG) : Results revealed that the TG concentration in the third group (85.250 ±9.907 mg/dl) reduced (not significant) compared with the second group (86.950 ±9.922 mg/dl) and with the first group(89.920 ±9.086 mg/dl) , the TG concentration in the second group reduced (not significant) compared with the first group . Table (4.6).

4.2.4.3 Low Density Lipoprotein Cholesterol (LDL-C) : Results revealed that the LDL concentration in the third group (80.075 ± 9.515 mg/dl) reduced (not significant) compared with the second group (81.880 ±9.160 mg/dl) and with the first group(85.130 ±8.241 mg/dl) , the LDL concentration in the second group reduced (not significant) ($P \leq 0.05$) compared with the first group . Table (4.6).

4.2.4.4 Very Low Density Lipoprotein Cholesterol(VLDL-C): Results revealed that the VLDL concentration in the third group (14.892 ±3.048mg/dl) reduced (not significant)compared with the second group (15.931 ± 3.681 mg/dl) and with the first group(16.711 ±3.682 mg/dl) , the VLDL concentration in the second group reduced (not significant) compared with the first group .Table (4.6).

4.2.4.5 High Density Lipoprotein (HDL-C) : Results revealed that the HDL concentration in the third group (59.380 ±2.590 mg/dl) raised significantly ($P \leq 0.05$) compared with the first group (56.020 ± 3.788 mg/dl) , the HDL concentration in the second group (58.020 ± 4.763 mg/dl)raised significantly ($P \leq 0.05$) compared with the first group . Table (4.6).

Table (4.6) : Lipid Profile (TC , TG , LDL , VLDL And HDL) In Suckling Mothers For Different Groups.

Groups / Parameters	First Group	Second Group	Third Group
TC (mg/dl)	**143.545 a ±11.541**	**138.210 a ± 13.175**	**135.880 a ±14.783**
TG (mg/dl)	**89.920 a ±9.086**	**86.950 a ±9.922**	**85.250 a ±9.907**
LDL (mg/dl)	**85.130 a ±8.241**	**81.880 a ±9.160**	**80.075 a ± 9.515**
VLDL(mg/dl)	**16.711 a ±3.682**	**15.931 a ± 3.681**	**14.892 a ±3.048**
HDL(mg/dl)	**56.020 a ±3.788**	**58.020 b ±4.763**	**59.380 b ±2.590**

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

4.3 Hormonal Parameters In Non-Suckling Mothers For Different Groups.

4.3.1 Hormonal Parameters

4.3.1 .1 Adiponectin Hormone: Results revealed that the adiponectin concentration in the third group (255.440 ±18.791 ng/ ml) reduced significantly ($P \leq 0.05$) compared with the first group (283.470 ±23.467 ng/ ml) and with the second group (277.110 ± 24.968 ng/ ml) .The adiponectin concentration in the second group reduced (not significant) compared with the first group . Table (4.7).

4.3.1 .2 Leptin Hormone : Results revealed that the leptin concentration in the third group (6.911 ±0.218 ng/ml) raised (not significant) compared with the second group (6.870 ±0.266ng/ml) and with the first group (6.827 ±0.308 ng/ml) , the leptin concentration in the second group raised (not significant) compared with the first group . Table (4.7).

4.3.1.3 Prolactin Hormone (PRL): Results revealed that the PRL hormone concentration in the third group (18.464 ± 3.719 ng/ml) reduced significantly ($P \leq 0.05$) compared with the second group (24.609 ±5.239 ng/ml) and with the first group(25.875 ±4.568 ng/ml). The PRL concentration in the second group reduced (not significant) compared with the first group .Table (4.7).

4.3.1.4 Follicle Stimulating Hormone (FSH): Results revealed that the FSH concentration in the third group (8.602 ±.885 mlu/ml) raised significantly ($P \leq 0.05$) compared with the second group (8.083±0.864 mlu/ml) and with the first group(7.811 ±0.560 mlu/ml) . The FSH concentration in the second group raised (not significant) compared with the first group . Table (4.7).

4.3.1.5 Luteinizing Hormone(LH) : Results revealed that the LH concentration in the third group (8.643 ±0.949 mlu/ml)raised significantly ($P \leq 0.05$) compared with the first group (7.276 ±0.929 mlu/ml) and raised (not significant) compared with the second group (8.189 ±0.998 mlu/ml).The LH concentration in the second group raised significantly ($P \leq 0.05$) compared with the first group.Table (4.7).

Table (4.7) : Hormonal Parameters (Adiponectin , Leptin , Prolactin , FSH And LH Hormones) In Non- Suckling Mothers For Different Groups.

Groups / Parameter	First Group	Second Group	Third Group
Adiponectin (ng/ml)	283.470 a ±23.467	277.110 a ± 24.968	255.440 b ±18.791
Leptin (ng/ml)	6.827 a ±0.308	6.870 a ±0.266	6.911 a ±0.218
Prolactin(ng/ml)	25.875 a ±4.568	24.609 a ±5.239	18.464 b ±3.719
FSH (mlu/ml)	7.811 a ±0.560	8.083 a ±0.864	8.602 b ±0.885
LH (mlu/ml)	7.276 a ±0.929	8.189 b ±0.998	8.643 b ±0.949

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different small letters.

4.3.2 Oxidant And Antioxidant In Non- Suckling Mothers For Different Groups.

4.3.2.1 Malondialdehyde (MDA) : Results revealed that the MDA concentration in the third group (227.745 ±16.405 ng/ml) raised significantly ($P \leq 0.05$) compared with the first group (122.672 ±16.779 ng/ml) and the MDA concentration in third group raised (not significant) compared with second group (226.040 ±14.983 ng/ml). The MDA concentration in second group raised significantly ($P \leq 0.05$) compared with the first group . Table (4.8).

4.3.2.2 Glutathione (GSH) : Results revealed that the GSH concentration in the third group (5.065 ±0.940 µg /ml) reduced significantly ($P \leq 0.05$) compared with the second group (5.795 ±0.933 µg /ml) and with the first group(6.945 ±0.925 µg /ml). The GSH concentration in the second group reduced significantly ($P \leq 0.05$) compared with the first group . Table (4.8).

4.3.2.3 Superoxide Dismutase [Cu-Zn] (SOD1): Results revealed that the SOD1 concentration in the third group (8.720 ±0.905 ng/ml) raised significantly ($P \leq 0.05$) compared with the second group (6.906 ±0.899 ng/ml) and with the first group(6.165 ±0.914 ng/ml) , the SOD1 concentration in the second group raised significantly ($P \leq 0.05$) compared with the first group . Table (4.8).

4.3.3 Pro-Inflammatory Markers In Non-Suckling Mothers For Different Groups.

4.3.3.1 The Interleukin- 6 (IL-6) : Results revealed that the IL-6 concentration in the third group (1.147 ±0.988 pg / ml) raised significantly ($P \leq 0.05$) compared with the first group (0. 159 ±0.024 pg / ml) and with the second group (0.189 ±0.033 pg/ ml) .The IL-6 concentration in the second group raised (not significant) compared with the first group . Table (4.8).

4.3.3.2 C - Reactive Protein (CRP) : Results revealed that the CRP concentration in the third group (0.723±0.250mg/dl) reduced significantly ($P \leq 0.05$) compared with the first group (1.691 ±0.554mg/dl) and the second group (1.209 ±0.387 mg/dl).The CRP concentration in second group reduced significantly ($P \leq 0.05$) compared with the first group. Table (4.8).

Table (4.8) : Oxidant and antioxidant parameters (MDA , GSH and SOD1) and pro inflammatory markers (IL6 and CRP) in non- suckling mothers for different groups.

Groups / Parameters	First Group	Second Group	Third Group
MDA (ng/ml)	122.672 a ±16.779	226.040 b ±14.983	227.745 b ±16.405
GSH (µg /ml)	6.945 a ±0.925	5.795 b ±0.933	5.065 c ±0.940
SOD1(ng/ml)	6.165 a ±0.914	6.906 b ±0.899	8.720 c ±0.905
IL-6(pg / ml)	0.159 a ±0.024	0.189 a ±0.033	1.147 b ±0.988
CRP(mg/dl)	1.691 a ±0.554	1.209 b ±0.387	0.723 c ±0.250

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

4.3.4 Lipid Profile In Non-Suckling Mothers For Different Groups.

4.3.4.1 Total Cholesterol (TC) : Results revealed that the TC concentration in the third group (169.605 ±18.107 mg/dl) raised (not significant) compared with the second group (167.285 ± 16.941 mg/dl) and with the first group(166.673 ±14.725 mg/dl) , the TC concentration in the second group raised (not significant) compared with the first group. Table (4.9).

4.3.4.2 Triglycerides (TG) : Results revealed that the TG concentration in the third group (108.530 ±9.382 mg/dl)raised (not significant) compared with the second group (102.365 ±14.273 mg/dl) and with the first group (100.640 ±15.926 mg/dl) , the TG concentration in the second group raised (not significant) compared with the first group. Table (4.9).

4.3.4.3 Low Density Lipoprotein Cholesterol (LDL-C): Results revealed that the LDL concentration in the third group (103.020 ±13.497 mg/dl) raised (not significant) compared with the second group (97.610 ±13.537 mg/dl) and with the first group (95.256 ±12.646 mg/dl) , the LDL concentration in the second group raised (not significant) compared with the first group . Table (4.9).

4.3.4.4 Very Low Density Lipoprotein Cholesterol(VLDL-C): Results revealed that the VLDL concentration in the third group (23.363 ±3.999 mg/dl)raised (not significant) compared with the second group (22.215 ±4.887 mg/dl) and with the first group(21.7666 ±4.782 mg/dl) , the VLDL concentration in the second group raised (not significant) compared with the first group . Table (4.9).

4.3.4.5 High Density Lipoprotein (HDL-C): Results revealed that the HDL concentration in the third group (35.265 ±3.308 mg/dl) reduced significantly ($P \leq 0.05$) compared with the first group (39.850 ±3.238 mg/dl), the HDL concentration in the second group (37.245 ± 3.387 mg/dl) decreased significantly ($p \leq 0.05$) compared with the first group . The HDL concentration in the third group reduced (not significant) compared with HDL concentration in the second group . Table (4.9).

Table (4.9) : Lipid Profile (TC , TG , LDL , VLDL And HDL) In Non-Suckling Mothers For Different Groups.

Groups / Parameters	First Group	Second Group	Third Group
TC(mg/dl)	**166.673 a ±14.725**	**167.285 a ±16.941**	**169.605 a ±18.107**
TG (mg/dl)	**100.640 a ±15.926**	**102.365 a ±14.273**	**108.530 a ±9.382**
LDL (mg/dl)	**95.256 a ±12.646**	**97.610 a ±13.537**	**103.020 a ±13.497**
VLDL(mg/dl)	**21.766 a ±4.782**	**22.215 a ±4.887**	**23.363 a ±3.999**
HDL(mg/dl)	**39.850 a ±3.238**	**37.245 b ± 3.387**	**35.265 b ±3.308**

*The values represent mean ± SD .

*Similar letters represent no significantly different within groups .

* A significantly different ($P \leq 0.05$) within groups is represented by different letters.

Chapter Five

Discussion

5. Discussion

5.1 Hormonal Parameters

5.1.1 Adiponectin Hormone

The present results revealed that the adiponectin hormone levels increased significantly (except third group) non-suckling mothers compared with suckling mothers for differing groups. Figure (4.1). Table (4.1) .

The present decreased adiponectin levels in suckling mothers groups 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 mothers groups and a low levels of prolactin in non-suckling mothers groups in current study may be explained the high and low levels of adiponectin in non-suckling and suckling mothers groups , respectively.

Although , the adiponectin hormone levels increased significantly (except third group) in non - suckling mothers in comparison with the suckling mothers for different groups .Table (4.1) , this hormone decreased significantly in third group in comparison with first and second groups for the non - suckling mothers .Table (4.7) , may be point out the absence role of prolactin hormone in those non - suckling mothers .

The present results agreed with many studies concerning the relationship between adiponectin and the prolactin hormone .

Asai-Sato and his colleagues (2006) suggest that prolactin affects the regulation of maternal metabolism through suppression of adiponectin. They also indicate the involvement of adiponectin in the alterations of maternal metabolism during lactation under the influence of prolactin.

Prolactin suppresses lipid storage as well as the release of adipokines including adiponectin , IL-6, and perhaps leptin , according to several lines of evidence.In addition PRL has also been linked to the regulation of adipogenesis (Brandebourg *et al* ., 2007).

Earlier studies by Combs and his colleagues (2003) demonstrated that exogenous prolactin inhibited adiponectin secretion in mice in vivo and the prolactin hormone also suppressed adiponectin secretion by cultured human adipose tissue and decreased the serum adiponectin level in prolactin transgenic mice (Nilsson *et al*., 2005; Asai-Sato *et al.,* 2006).

Combs and his colleagues (2003) showed that adiponectin levels are suppressed from mid-gestation until weaning,of all the endocrine changes that peak around pregnancy, placental lactogens and prolactin best fit the time course for the suppression of adiponectin. Placental prolactin-like molecules appear in the maternal circulation around mid-gestation, whereas pituitary prolactin levels increase gradually during gestation and remain elevated until suckling stops.

Fuglsang and his colleagues (2006) reported serum adiponectin levels peaked in mid- pregnancy and the lowest levels were seen in late gestation. They also observed an inverse association with maternal BMI and hypothesized that the decline in plasma adiponectin levels postpartum might be due to an increase in maternal fat stores during gestation and lactation.

Nevertheless , Asai-Sato and his colleagues (2006) found that plasma concentrations of adiponectin at the third trimester of pregnancy and breastfeeding declined, with no attachment to the degree of gestational weight gain. Thus, the gestational adipose store seems to have a little effect on the regulation of adiponectin secretion at a physiological level.

Maternal adiponectin levels are repressed during the breastfeeding period compared to levels during gestation or those for non-gestation and non-breastfeeding women under normal reproductive cycles (Anderson *et al.,* 2016).

Qi and his colleagues (2004) indicated that adiponectin increased the basal energy expenditure and that a decrease in plasma adiponectin levels suppressed the basal metabolism , thus accelerating the energy storage in mice. Additionally, it is well known that PRL inhibits brown fatty tissue thermogenesis in rodents during suckling (Chan and Swaminathan ,1990) ,this results in a substantial reduction in the energy requirements of the mother, which is an energy sparing advantage for milk production .Therefore, it is probable that the decline in plasma adiponectin influences the energy metabolism of a suckling mother (Asai-Sato *et al*., 2006).

Adiponectin inhibits lipolysis in humans and in mice (Qiao *et al* ., 2011 , Wedellova *et al* ., 2013 :Singh , 2014),therefore , lowered adiponectin concentrations facilitate the rate of lipolysis. Furthermore, the impact of adiponectin on insulin sensitivity and on the metabolism of glucose and fatty acids contributes to nutrient partitioning and thus may affect the nutrient availability in the mammary gland for milk production. In addition, certain pathological conditions such as inflammation and endocrine hormones may affect the expression of adiponectin in adipose tissue (Singh , 2014).

The concentrations of circulated adiponectin in dairy cows decreased from day 21 before delivery, with adiponectin reaching a low at the time of birth and then increasing gradually(Singh *et al*., 2014). The transition period from late pregnancy to lactation is a period of physiological adaptations and overall metabolism to fulfill the mammary gland's nutritional demand for milk production, and since adiponectin is involved in regulating glucose and fatty acid metabolism

(Yamauchi *et al.,* 2002; Singh *et al.*, 2014), therefore, adiponectin concentrations are likely to change during this period of negative energy balance (Singh *et al.*, 2014).

5.1.2 Leptin Hormone

The present results revealed that the leptin hormone levels increased significantly (except first and second group) in the non- suckling mothers compared with suckling mothers of differing groups. Figure (4. 2) . Table (4.2) .

Leptin levels increased slightly in non - suckling mothers between different groups .Table (4.7) . Leptin levels decreased slightly in suckling mothers between different groups .Table (4.4) .

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 mothers groups (especially the third group) ,while the present lipid profile in non-suckling mothers groups indicated a high presence of fatty parameters in these mothers . On the other hand , the present reduction of leptin hormone in suckling mothers 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.

The present results agreed with many studies concerning the role of leptin hormone related with fatty mass and energy balance in suckling and some physiological processes .

Butte and his colleagues (1997) discovered that leptin correlates positively with weight, BMI, fat mass , and fat mass ratio as well , changes in leptin are associated with changes in weight and fat mass ,women who gained more weight

during gestation had higher levels of leptin. Similarly, women who gained weight or failed to lose weight after childbirth had higher levels of leptin .

Circulating leptin concentrations are favorably associated to body fatty quantity (Chan and Mantzoros, 2005 ; Savino *et al.*, 2010) .

Chan and Mantzoros (2005) concluded that the leptin playing amain role in energy availability in cases of energy deficiency.

The fall in leptin plasma levels exceeds the rate at which fat stores are decreased during periods of energy deficit, reduction of the leptin signal induces several neuroendocrine responses that tend to limit weight loss , such as hunger , food-seeking behavior, and inhibition of plasma thyroid hormone levels .In humans, the leptin signaling system appears to be mainly involved in the maintenance of adequate energy stores for survival during periods of energy deficit (Jequier , 2002). Leptin mainly contributes to energy homeostasis and satiation. Its levels in the circulation rise in proportion to fat mass, and circulating leptin conveys information to the hypothalamus regarding the amount of energy stored in adipose tissue, suppressing appetite and affecting energy expenditure (Friedman and Halaas , 1998 ;Gale *et al* ., 2004) .

Leptin might play a critical role in regulating body weight by signalling the size of the fatty tissue mass, where plasma leptin was found to be highly associated with BMI in rodents and in thin and obese people (Maffei *et al*., 1995) .

The proportion of body lipids is linked to serum leptin concentrations, meaning that most obese people are unaffected by endogenous leptin production (Considine *et al*., 1996) .

Leptin circulates in the blood and acts on the brain to regulate food intake and energy expenditure. When fat mass increases, plasma leptin levels rise,

suppressing the appetite until weight is lost. When fat mass falls, leptin levels fall, stimulating appetite and suppressing energy expenditure until fat mass is restored. This system maintains the balanced control of fat tissue mass (Friedman , 2011).

It appears that leptin may act as the critical link between adipose tissue and not only the hypothalamic centers regulating energy homeostasis but also the reproductive system, indicating whether adequate energy reserves are present for normal reproductive function (Moschos *et al*., 2002 ; Gale *et al* ., 2004).

The demands of milk synthesis and release produce a condition of negative energy balance in the suckled mother, and, in laboratory rodents, are accompanied by a dramatic hyperphagia (Crowley, 2011).

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

Pickavance and his colleagues (1998) suggested that hypoleptinaemia may be a significant agent promoting the hyperphagia of lactation .

Brogan and his colleagues (1999) suggested the low concentrations of leptin may be important in allowing the mother to adapt to the suckling condition by removing a signal for satiety that facilitates the increase in food intake .

However, in postpartum women within 3 and 6 months, normalization of leptin was associated with changes not only in weight and fat mass, but also in serum insulin. Furthermore, no major differences were observed in leptin levels between lactating and non-lactating women (Butte *et al.*, 1997).

5.1.3 Prolactin Hormone

The present results revealed that the prolactin hormone levels decreased significantly in non - suckling mothers in comparison with the suckling mothers for different groups . Figure (4.3) . Table (4.1) .

In the same manner , the prolactin hormone decreased significantly in third group in comparison with the first and second groups for non - suckling mothers .Table (4.7).

The high levels of prolactin in suckling mothers be necessary to helps the continuous production of milk via the suckling response , suckling stimulates the synthesis of prolactin hormone , while , the non – suckling mothers (without suckling response) have a less levels of prolactin .

The present results agreed with many studies concerning the role of prolactin during suckling.

Prolactin is the main hormonal signal responsible for stimulation of milk synthesis in the mammary glands (Crowley , 2011) .

Prolactin sends out a comprehensive signal during suckling that promotes the synthesis and secretion of milk components , as well as the survival of the alveolar cell. The quantity of milk produced is determined by the removal of milk from the gland, a function that is dependent on the posterior pituitary gland's release of the hormone oxytocin and the contraction of myoepithelial cells to push the milk out of the alveoli (Neville, 2006) .

While milk secretion is a continuous process, the amount of milk produced is controlled by infant demand, which means it is dependent on the suckling response. Suckling must begin within three to four days postpartum to maintain milk secretion (Moreno- Villares and Germán-Díaz , 2019).

The development of mammary glands , milk synthesis, and milk secretion keeping 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 rising energy requirements of the developing fetus followed by milk output to sustain offspring after childbirth (Lopez-Vicchi *et al.*, 2020) , during gestation, serum prolactin levels increase rapidly due to an increase in the size and quantity of lactotrophs. During breastfeeding, suckling causes rapid PRL production via a neuroendocrine reflex pathway (Saleem *et al* ., 2018).

Prolactin (PRL), one of the most significant breastfeeding-related hormones, is suppressed by the hypothalamus-pituitary dopaminergic system and activated by the hypothalamus-pituitary oxytocinergic system, where this hormone is necessary in all stages of lactation (Ni *et al* . , 2021).

Prolactin levels will not continuously remain at elevated levels even after delivery. Prolactin levels will only spike during periods of nipple stimulation, allowing for control over milk production. As long as suckling is maintained , prolactin levels stay elevated. If the mother does not nurse her infant, PRL concentrations will return to pre-pregnancy concentrations after 1 to 2 weeks (Freeman *et al* ., 2000 ; Al-Chalabi *et al.*, ,2020) .

However , prolactin levels after delivery are similar in both suckling and non-suckling mothers, so that the basic process occurs regardless of whether suckling is begun (Neville and Morton , 2001).

However , the present prolactin finds in decreased significantly between groups for suckling mothers, may be explained to whole body state adaptation in these mothers due to the positive role of suckling .

5.1.4 FSH and LH hormones

The present results revealed that the FSH and LH hormones levels increased significantly in non - suckling mothers compared with the suckling mothers for different groups . Figure (4.4) , Figure (4.5). Table (4.1).

The present FSH and LH increased slightly and significantly between groups in non - suckling mothers .Table (4.7) .

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

The present results agreed with many studies concerning relationship between prolactin hormone and FSH and LH secretion.

Grattan and his colleagues (2007) discovered evidence of PRL-R mRNA expression in a similar miniature subpopulation of GnRH neurons, and their findings support the thought that prolactin limits luteinizing hormone excretion through action that is at lower partially mediated by direct inhibition of the GnRH neuron.

Elevated prolactin may influence reproduction by acting on the hypothalamic GnRH neurons and/or the pituitary gland, influencing gonadotrophin, LH, and FSH secretion (McNeilly , 2001 ; Grattan *et al*., 2007) .

Hyperprolactinemia is a condition in which elevated levels of prolactin suppress gonadotropin secretion during suckling , resulting in amenorrhea (McNeilly,1980).

During seasonal infertility in ungulate males and females, as well as in pathological hyperprolactinemia in both men and women, a common relationship between increased prolactin levels and a decrease in both LH and FSH is observed (McNeilly , 1987) .

The pulsatile release of LH , which reverses hypothalamic gonadotropin-releasing hormone (GnRH) release , is irregular and much slower during breast-feeding-induced amenorrhea than the one pulse per hour needed in the normal follicular phase of the menstrual cycle to drive follicle development , 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).

Since prolactin inhibits the secrete of GnRH from the hypothalamus , a lack of GnRH causes a lack of pulsatile stimulation of gonadotrophic cells, resulting in FSH and LH not being released from the anterior pituitary , as FSH and LH are the primary hormones required to control menstruation , suckling women will experience a phase of transient amenorrhea before breastfeeding is discontinued (Koike *et al*., ,1991;Grattan et al ., 2007;Al-Chalabi *et al*., 2020).

Henderson and his colleagues (2008) in their study showed direct effects of PRL and dopamine at the level of the gonadotroph cell , and interactions between these two hormones in the regulation of gonadotrophin secretion.

On the other hand , the present FSH and LH increased slightly and significantly among groups for suckling mothers table (4.4) , may be attributed to presence the reduction concentrations of prolactin hormone in those suckling mothers .

5.2 Oxidant And Antioxidant Parameters :

5.2.1 Malondialdehyde (MDA)

The present results revealed that malondialdehyde levels increased significantly in non- suckling mothers compared with suckling mothers of differing groups. Figure (4.8). Table (4.2).

The present results, also indicated that MDA levels increased significantly in second and third groups in comparison with the first for non - suckling mothers .Table (4.8), and MAD reduced significantly in third group in comparison with first and second groups for suckling mothers .Table (4.5).

Thepresent results concerning the high levels of MDA in non- suckling mothers attributed to high oxidation and oxidative stress , thus , more production of ROS species that be harmed and damaged to cells and their functions , whereas , the reduction of MDA levels in suckling mothers , reflects high activity of antioxidant system , while , the current study results pointed out high activities of antioxidants in suckling mothers.

The present results agreed with many studies concerning the oxidative stress during suckling .

In case of oxidative stress and the antioxidant defense system of suckling mothers, the oxidative stress level was elevated and the antioxidant power level was lessen in the early puerperium, but the antioxidant power level then showed a clear propensity to recover and the oxidative stress gradually decreased in the first 3 months after delivery (Kuramoto and Kitagawa , 2017).

Garratt and his colleagues (2013) ,reported oxidative damage to proteins was lowered in the livers of mice females with an eight-puppy litter than in mice females with two pups or non-reproductive control mice females. In their

experiment on mice during lactation, they found that although protein oxidation decreased, activity levels of the antioxidant enzyme superoxide dismutase increases in the liver, suggested that this may be one of the mechanisms used to protect against oxidative stress.

According to Hyatt and his colleagues (2017), lactating rats did not show any lasting effects in oxidative damage or antioxidant levels. They also suggested that lactation goes beyond returning females to a nonreproductive baseline and improved metabolic conditions long after reproduction has ended , which is consistent with the Stuebe and RichEdwards' reassignment hypothesis , which indicates "that lactation reverses many of the metabolic changes that occur during pregnancy".

In study by Garratt and his colleagues (2011) in reproductive females mice liver , markers of oxidative damage "malonaldehyde , protein thiols and the proportion of glutathione in the oxidized form" indicates lowers oxidative stress in reproducing mice females when compares with non-reproductive controls , even during at maximum suckling, none of the markers of oxidative damage indicated elevate oxidative stress than among non-reproductive females.

Furthermore , several markers of oxidative stress did not increase in suckling mice compared with that in non-reproductive controls, where MDA levels were significantly decreased in liver, kidneys, skeletal muscle and small intestine in suckling female than in non- reproductive controls , indicating that no evidence of increased oxidative stress was found in reproductive mice (Zheng *et al* . , 2015) .

5.2.2 Glutathione (GSH) And Superoxide Dismutase[Cu-Zn] (SOD1)

The present results revealed that the antioxidants glutathione (GSH) and Superoxide Dismutase[Cu-Zn] (SOD1)levels decreased significantly for in non-suckling mothers compared with suckling mothers of differing groups . Figure (4.6) , Figure (4.7). Table (4.2) .

The present GSH decreased significantly between different groups for non - suckling mothers table (4.8).

The present results SOD1 increased significantly between different groups for suckling mothers table (4.5) .

The present reduction in GSH and SOD1 antioxidants with high MDA level in non - suckling mothers , may be attributed to the overproduction of ROS and decreased production of antioxidants (SOD1 , GSH) . In other words , the high production of ROS overtakes the mother's antioxidant capacity due to the increase of oxidation processes that occurred via the accumulation of storage , unpackaged and un used fats in the lactation process or their metabolites.

The present results agreed with many studies concerning with the role of antioxidants system during in suckling mothers.

Zheng and his colleagues (2015) found that antioxidant activities regulated physiologically in response to elevated ROS output, during the peak of suckling.

Hyatt and his colleagues (2018) found that rats who experienced suckling exhibited select signs of oxidative stress after suckling ceased and mammary tissue regressed. Whereas SOD2 levels were higher in the liver and WAT, no statistical

changes were reported for SOD1, GPX levels in the liver, as well as SOD2, SOD1, CAT, GPX levels in skeletal muscle, as compared to non-suckling rats and non-reproduce rats.

Yang and his colleagues (2013) discovered that nursing gerbils had lower levels of oxidative damage markers such as " MDA and protein carbonyls" , as well as higher levels of superoxide dismutase activity and total antioxidant capacity than non- reproductive gerbils.

Oxidative damage in lipid and proteins in the liver was lower in mice weaning enlarged litters ,than in non-breeding ones , and was intermediate in those with reduced litters , likewise , in the heart , oxidative damage in proteins also tended to be lower in breeding females than in non-breeding ones. However, no correlation was found between the concentration of markers of oxidative damage in the liver and the activity of catalase or the concentration of GSH ,only the catalase was more active , in kidneys ,this indicated a protective action of antioxidants (Ołdakowski *et al* ., 2015) .

NO significant increase in glutathione at peak lactation with the much decrease in MDA , indicates that changes in markers of oxidative damage are not completely related to an increase in GSH and that additional mechanisms are involved (Valko *et al*., 2007; Garratt *et al.,* 2011) .

In breeding , Columbian ground squirrels females showed higher antioxidant defense before to suckling , but with no consequence on offspring development or survival as predicted by the oxidative shielding hypothesis (Viblanc *et al* ., 2018).

Valencak and his colleagues (2016) suggested that metabolism in reproductive females mice is well equipped for the challenges of suckling and

either fights back , bear or even reduces ROS generation , this participate to increasing evidence that there is no linear relationship between oxidative damage and suckling .

However, Garratt and his colleagues (2011) found no substantial increase in GSH correlated with peak lactation during more MDA reduction, which may explain the current findings about low GSH concentrations in suckling mothers.

5.3 Pro- Inflammatory Markers

The present results revealed that the IL-6 and CRP increased significantly (except IL-6) in non-suckling mothers compared with the suckling mothers for different groups . Figure (4.9), Figure (4.10). Table (4.2) .

The present IL-6 results indicated a significant increased between groups (except second group) for non- suckling mothers .Table (4.8).

This significant increase in CRP and slight increase in IL-6 may be indicated a presence of an inflammation state in these non-suckling mothers , in according to the high levels of CRP which used as an indicator of inflammation and its stimulation by several cytokines including IL6 , beside that CRP responds to a variety of maternal factors including the present increase findings i.e leptin hormone , oxidative stress , TG concentrations and a low HDL associated with a high values of CRP in non-suckling mothers .

The present results agreed relatively with many studies concerning the role of cytokines related with inflammation .

C-Reactive Protein levels rise rapidly in response to "trauma, inflammation, and contagion", and fall just as quickly when the condition is resolved. As a result , the measurement of C-Reactive Protein is widely used to monitor various inflammatory condition (Du Clos, 2000).

C-Reactive Protein is the best-known acute phase protein , in humans , almost every type of inflammation is accompanied by an increase in CRP concentration ;until recently, the only known physiological function of CRP was the marking of cells to initiate their phagocytosis (Sheriff *et al.*, 2021).

During an acute-phase response, CRP is produced in large amounts by hepatocytes upon stimulation by the cytokines interleukin 6, TNFα and IL-1β (Vermeire *et al* ., 2005) .

C-Reactive Protein synthesis mainly occurs when stimulated in response to pro-inflammatory cytokines, most notably IL-6 and, to a lesser degree, L1β and TNF- α (Zhang *et al* ., 1996 ; Sproston and Ashworth , 2018) .

According to molecular studies, leptin is able to modulate CRP expression levels both indirectly, through its action on other pro-inflammatory molecules , such as interleukin 6, and directly, by enhancing its production in hepatic and vascular tissues (Chen *et al*., 2006; De Rosa *et al*., 2009 ; Letizia Hribal *et al.*, 2014) .

Leptin increases the release of pro-inflammatory cytokines, such as TNF-α , IL-6 and IL-12, from mouse macrophages and activates human blood neutrophils (Chihara *et al* ., 2020) .

Furthermore, oxidative stress may be a determinate of CRP concentrations and enhance pro-atherosclerotic inflammatory processes at the earliest stages of coronary heart disease development (Abramson *et al*., 2005) .

Dohi and his colleagues (2007) indicated that C-reactive protein levels were correlated with oxidative stress where they found CRP levels were positively connected with 8-isoprostane, (an oxidative stress marker), and TG and negatively connected with HDL-C .

In the case of low HDL and high triglycerides, elevation in human CRP has also been seen (Pearson *et al.*, 2003; Knight, 2015).

Elevated CRP and low HDL- C jointly contribute to the prediction of all-cause, cancer, and cardiovascular mortality. Thus, the interactive relationship between them in mediating inflammatory processes might be explained (Kim *et al* ., 2012).

Ladeia and his colleagues (2006) showed that plasma hs-CRP was correlated with triglycerides and triglyceride-to-HDL ratio, suggesting that strategies to decrease inflammatory activity should focus on the lipid profile.

Meanwhile , pregnancy and lactation involved in adaptations of immune regulation (Kuzawa *et al* ., 2013) Regardless of feeding technique, the postpartum period appears to be oriented toward heightened and activated innate and specific immune defenses, while breastfeeding provides an additional level of potential protection for these mothers and their children (Groer *et al*., 2005) . The immunological effects of pregnancy remains until about 1 year after delivery (Watanabe *et al* ., 1997) .

Interleukin-6 is reported to rise during pregnancy, peak during the first day postpartum and subsequently decrease (Maes *et a l*., 2000 ; Aris *et al.* , 2008 ; Skalkidou *et al*., 2009).

Kuzawa and his colleagues (2013) found that CRP levels were highest in late-pregnancy women, with no evidence of elevated CRP in suckling women. Thus, this pattern is consistent with immune adaptations that occurred during gestation.

Little is known about the role of pro-resolution pathways in normal reproductive function. However, it is anticipated that in physiological reproductive events , their expression may be temporally regulated and important in the

maintenance of proper reproductive function (Jabbour *et al.,* 2009) , therefore , Stuebe and Rich-Edwar s (2009) hypothesized that lactation plays an important role in "resetting" maternal metabolism after pregnancy.

However, the present CRP significantly decreased with progressive periods development (groups) for suckling mothers table (4.8),might be attributed to many factors that interfere with CRP concentrations and its assessment .

5.4 Lipid Profile

The present findings concerning lipid profile revealed that the parameters TC , TG , LDL and VLDL increased significantly and HDL was reduced significantly in non-suckling mothers compared with suckling mothers of differing groups . Figure (4.11) , Figure (4.12) , Figure (4.13) , Figure (4.14) Figure (4.15) . Table (4.3) .

The present findings ,TC , TG , LDL and VLDL increased slightly between different groups for non - suckling mothers and the HDL concentration decreased significantly in second and third groups in comparison with the first group for non - suckling mothers .Table (4.9). The present findings ,TC , TG , LDL and VLDL decreased slightly between different groups for suckling mothers ,and the HDL concentration increases significant in second and third groups in compare with the first group for suckling mothers .Table (4.6).

These present variables revealed the positive role of the breastfeeding process (suckling) in improving these parameters , due to the mobilization of fat stores and their use in the milk production and lactation , while , these present variables have an opposite tendency in the non - suckling mothers.

The present results agreed with many studies about the role of suckling in improving these lipids parameters.

In a comparative analysis, Velarde and his colleagues (2017) found significant differences in lipid profiles between mothers of four-month-old infants based on the type of feeding (completely breastfed, partially breastfed, and formula feeding), and they suggest that exclusively breast feeding may be associated with a better lipid profile, where they found that concentrations of TG and VLDL-C were greater and HDL-C was less among women who fed formula to their infants, in comparison with those who exclusively breastfed. HDL-C was lower among women who partially breastfed their infants in comparison with those who completely breastfed; TG and VLDL-C levels were higher but not significant.

In a study of breastfeeding and non-breastfeeding women, Abdulkareem (2018) demonstrated that HDL-C was higher in the breastfeeding groups than in non-breastfeeding mothers, but the difference was not statistically significant. However, LDL-C was significantly lower in the breastfeeding group than in the non-breastfeeding group, and serum cholesterol, serum triglyceride, and VLDL-C were insignificantly lowers in the breastfeeding group in comparison to the non-breastfeeding group.

In a study, Gunderson and his colleagues (2007) examined changes in metabolic risk factors among lactating women from preconception to post weaning and among non-lactating women from preconception to post-delivery , in comparison with non-pregnancy women, found that average changes in LDL-C and triglycerides are more appropriate with longer breastfeeding duration. Furthermore, breastfeeding for a longer amount of time may ameliorate the long-range reduction in HDL-C that occur during gestation.

Kjos and his colleagues (1993) reported that breastfeeding, even for a short duration, has a beneficial effect on glucose and lipid metabolism in women with gestational diabetes. They also found higher mean high-density lipoprotein (HDL)

cholesterol in the blood in the lactating women after adjusting for maternal age, body mass index, and insulin use during pregnancy compared to the non-lactating women. There were no observed differences in total cholesterol, LDL-C, or triglyceride levels.

According to Knopp and his colleagues (1985), the increase in HDL-C components in lactation is partly due to the increased breakdown of triglyceride-rich lipoproteins by the lactating breast ,while triglyceride, LDL-C and TC levels declined between delivery and six months postpartum and the TC levels remained stable until nine months postpartum, after which, at 2 months postweaning, the levels increased to delivery levels (Kallio *et al.*,1992).Furthermore, triglyceride levels returned to baseline within 13 weeks of the breastfeeding period compared to non-breastfeeding women (Darmady and Postle ,1982; Stuebe , 2015) .

On the other hand, Rudolph and his colleagues (2007) indicated that The usage of different resources for triglycerides synthesizing by "mammary epithelial cells" is impacted by both breastfeeding stage and diet, and that three kinds of substrate are used to synthesize milk triglycerides: dietary fat, fatty acids mobilized from adipose tissue stores, and lipids manufactured by denovo synthesis from simple sugar (glucose) and other dietary precursors .

McClure and his colleagues (2012) discovered that seven years postpartum, visceral fat depots are significantly greater in among mothers who breastfed for less than three months after the birth of each of their infant. Therefore, they suggest these results provide a potential physiologic basis for prior findings that women who do not consistently breastfeed are at an increased risk of diabetes, cardiovascular disease, and the metabolic syndrome.

Gunderson and his colleagues (2014) found that in type 2 diabetic women, increased lactation intensity was connected with more lipid-favorable parameters, such as greater HDL and lower TG.

Chapter Five

Conclusions and Recommendations

1. Conclusions :

The results of the present study included the following conclusions:

1. Adiponectin hormone levels rise significantly (except in the third group) in the non-suckling mothers, which may indicate the absence of the important role of the hormone prolactin coinciding with the absence of the stimulus to suckling.
2. Increasing the levels of leptin hormone significantly (except for the first and second groups) in non-suckling mothers indicates an increase in fat deposition in them.
3. FSH and LH levels increased in non-suckling mothers because prolactin levels were significantly reduced.
4. The decrease in the antioxidants GSH and SOD1 with the high level of MDA significantly in non-suckling mothers indicates the appearance of so-called oxidative damage in non-suckling mothers.
5. Increased inflammatory susceptibility in non-suckling mothers is indicated by increased IL6 and CRP levels.
6. The appearance of some indications about the presence of metabolic disorders due to the results of the lipid profile in non-suckling mothers.
7. The changes in the hormones adiponectin and leptin and the rest of the studied parameters in non-suckling mothers, which caused some metabolic disorders, may indicate the exposure of these mothers in the future to some metabolic syndrome diseases (obesity, high blood pressure, heart disease, and type 2 diabetes) ,if it is not corrected and changed its causes.

2. Recommendations :

Further studies be recommended as the following :

1- More samples , with different ages and different slices of society.

2- More other hormones related to the regulation of metabolism , especially during the period of suckling.

3- More other oxidant and antioxidant parameters.

4- Studying the role of immunological system and their effects on all metabolic parameters in women during suckling.

5- Studying the important role of breastfeeding and its benefits against current life diseases, where breastfeeding reduces the risk of obesity, cardiovascular disease, type 2 diabetes, hypertension, breast cancer, and ovarian cancer.

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APPENDICES

Questionnaire			
Name of mother			
Age			
Address			
Not having any disease			
No pregnancy			
Number of children			
Not taking birth control pills or taking a contraceptive needle			
Breastfeeding Type	Natural	Unnatural	
Breastfeeding Period	(1-6 Month)	(7-12 Months)	(13-18 Months)

ELISA kit : The contents of ELISA kits are shown in the following

Table (3.3) : Adiponectin, Leptin, Malondialdehyde , Superoxide Dismutase[Cu-Zn] Human And Interleukin 6 ELISA Kit Components

NO	Item	Specifications
1	Micro Plate	96 well
2	Standard(dry)	2 phial
3	Diluent Buffer for standard	20ml
4	Antibody labeled with biotin	120ul
5	Antibody Dilution Buffer	10ml
6	HRP-Streptavidin Conjugate(SABC)	120ul
7	Dilution Buffer of SABC	10ml
8	Substrate Reagent (TMB Substrate)	10ml
9	Stop Solution	10ml
10	Concentrated Wash Buffer(25)	30ml
11	Sealer	5pieces
12	Manual	1 copy

Table (3.4) : Glutathione ELISA kit components .

NO	Item	Specifications
1	Micro Plate	96 well
2	Standard	2 phial
3	Biotinylated Detection Ab (100×)	120 uL*1
4	Concentrated HRP Conjugate (100×)	120 μL*1
5	Standard & Sample Diluent	20 mL *1
6	Antibody Diluent	14 mL *1
7	HRP Conjugate Diluent	14 mL *1
8	Wash Buffer (25×)	30 mL *1
9	Substrate	10 mL *1
10	Stop Solution	10 mL *1
11	Plate Sealer	5 pieces
12	Manual	1 copy

الخلاصة

يهدف البحث الى دراسة الملامح الفسيولوجية للأديبونكتين ، واللبتين ، وبعض الهرمونات التناسلية الأخرى ، والإجهاد التأكسدي ، والعلامات المسببة للالتهابات ، والصورة الدهنية للأمهات المرضعات وغير المرضعات في محافظة ميسان خلال المدة من شباط إلى تشرين الاول ٢٠٢٠. بلغت عينة الدراسة الكلية ١٢٠ امرأة (تتراوح أعمارهن بين ٢٥ - ٣٥ سنة) ، تضمنت العينة ٦٠ من الأمهات المرضعات و ٦٠ من الأمهات غير المرضعات ، واللاتي راجعن مستشفى الصدر التعليمي وبعض مراكز العيادات الصحية الأخرى . تم تقسيم كل من هؤلاء الأمهات المرضعات والغير المرضعات إلى ثلاث مجموعات (٢٠ أم لكل مجموعة)

وبحسب فترات الرضاعة وعلى النحو التالي :

- المجموعة الأولى (١-٦شهر) .
- المجموعة الثانية (٧-١٢ شهرًا) .
- المجموعة الثالثة (١٣- ١٨ شهرا) .

أظهرت النتائج :

زيادة مستويات الأديبونكتين معنويا (ما عدا المجموعة الثالثة) (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة بالأمهات المرضعات في المجموعات المختلفة . أنخفاض مستويات الأديبونكتين معنويا (أ≤ ٠,٠٥) (ماعدا المجموعة الثانية بالمقارنة مع المجموعة الأولى) في الأمهات غير المرضعات في المجموعات المختلفة . أنخفاض (عدا المجموعة الثانية) مستويات الأديبونكتين بشكل طفيف في الأمهات المرضعات في المجموعات المختلفة.

زيادة مستويات اللبتين معنوياً (ما عدا المجموعتين الأولى والثانية) (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة بالأمهات المرضعات في المجموعات المختلفة . زادت مستويات اللبتين وانخفضت بشكل طفيف في الأمهات غير المرضعات والأمهات المرضعات على التوالي في المجموعات المختلفة

.أنخفاض مستويات البرولاكتين معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة . أنخفاض مستويات البرولاكتين معنويا (أ≤ ٠,٠٥) (ماعدا المجموعة الثانية مقارنة مع المجموعة

الأولى) في الأمهات غير المرضعات في المجموعات المختلفة . أنخفاض مستويات البرولاكتين معنويا (أ≤ ٠,٠٥) في الأمهات المرضعات في المجموعات المختلفة .

زيادة مستويات الهرمون المنبه للجريب (FSH) معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة. زيادة مستويات FSH معنويا (أ≤ ٠,٠٥) (ماعدا المجموعة الثانية بالمقارنة مع المجموعة الأولى) في الأمهات غير المرضعات و الأمهات المرضعات في المجموعات المختلفة .

زيادة مستويات الهرمون اللوتيني (LH) معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة. زيادة مستويات هرمون LH معنوياً (أ≤ ٠,٠٥) (ماعدا المجموعة الثالثة بالمقارنة مع المجموعة الثانية) في الأمهات غير المرضعات في المجموعات المختلفة . زيادة مستويات هرمون LH معنويا (أ≤ ٠,٠٥) في الأمهات المرضعات في المجموعات المختلفة .

زيادة مستويات المالونديالدهايد (MDA) معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة. زيادة مستويات MDA معنويا (أ≤ ٠,٠٥) (ماعدا المجموعة الثالثة بالمقارنة مع المجموعة الثانية) في الأمهات غير المرضعات في المجموعات المختلفة . انخفاض مستويات MDA معنويا (أ≤ ٠,٠٥) (باستثناء المجموعة الثانية بالمقارنة مع المجموعة الأولى) في الأمهات المرضعات في المجموعات المختلفة .

انخفاض مستويات الجلوتاثيون (GSH) و سوبر أكسيد ديسميوتاز (SOD) معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة . انخفاض مستويات GSH معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات والأمهات المرضعات في المجموعات المختلفة . زيادة مستويات SOD معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات والأمهات المرضعات في المجموعات المختلفة .

زيادة مستويات الانترلوكين - ٦ (IL-6) زيادة طفيفة في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة . زيادة مستويات IL-6 معنويا (أ≤ ٠,٠٥) (باستثناء مستويات IL-6 في المجموعة الثانية مقارنة مع المجموعة الأولى) في الأمهات غير المرضعات و الأمهات المرضعات في المجموعات المختلفة .

زيادة مستويات بروتين سي التفاعلي (CRP) معنويا (أ≤ ٠,٠٥) في الأمهات غير المرضعات مقارنة مع الأمهات المرضعات في المجموعات المختلفة . انخفاض مستويات CRP) معنويا (أ≤ ٠,٠٥) في الأمهات الغير مرضعات في المجموعات المختلفة. انخفاض مستويات CRP بشكل طفيف في الأمهات المرضعات في المجموعات المختلفة.

زيادة الكوليسترول الكلي (TC) والدهون الثلاثية (TG) وكوليسترول البروتين الدهني منخفض الكثافة (- LDL C) وكوليسترول البروتين الدهني منخفض الكثافة جدًا (VLDL- C) معنويا (أ≤ ٠,٠٥) و انخفاض البروتين الدهني عالي الكثافة (HDL- C) معنويا (أ≤ ٠,٠٥) في الأمهات الغير مرضعات مقارنة بالأمهات المرضعات في المجموعات المختلفة.

انخفاض الكوليسترول الكلي (TC) ، والدهون الثلاثية (TG) ، وكوليسترول البروتين الدهني منخفض الكثافة (LDL- C) ، وكوليسترول البروتين الدهني منخفض الكثافة جدا (VLDL- C) بشكل طفيف في الأمهات المرضعات في المجموعات المختلفة. زيادة مستويات HDL- C معنويا (أ≤ ٠,٠٥) (ماعدا المجموعة الثالثة بالمقارنة مع المجموعة الثانية) في الأمهات المرضعات في المجموعات المختلفة .

ان الجوانب الفسيولوجية قد نوقشت أستنادا ألى دور الرضاعة و تأثيرها الايجابي على المعايير الحالية الامر الذي ينعكس ايجابا على تطور صحة هذه الامهات .

وزارة التعليم العالي والبحث العلمي
جامعة ميسا ن
كلية العلوم

قسم علوم الحيا ة

تقيـــيم مســـتويات الأديبـــونكتين,اللبتـــين ,هرمونـــات التكـــاثر وبعـــض المعـــايير الكيموحيويـــة ومضـــادات الأكســـدة لـــدى النســـاء المرضـــعات وغيـــر المرضـــعات في محافظة ميسان.

رسالة مقدمة
الى مجلس كلية العلوم / جامعة ميسان
وهي جزء من متطلبات نيل درجة ماجستير علوم في علوم الحياة
من قبل

ميسون محمد خنجر
بكالوريوس علوم / جامعة البصرة (٢٠٠٣)
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