



SCHOOL of
GRADUATE STUDIES
EAST TENNESSEE STATE UNIVERSITY

East Tennessee State University
Digital Commons @ East
Tennessee State University

Electronic Theses and Dissertations


Student Works

8-2016

The Combined Effects of Leptin and Coenzyme Q10 in Ameliorating Obesity- Induced Infertility in Female Rats

Adekunle Adedeji
East Tennessee State University

Follow this and additional works at: <https://dc.etsu.edu/etd>

 Part of the [Dietetics and Clinical Nutrition Commons](#), and the [Molecular and Cellular Neuroscience Commons](#)

Recommended Citation

Adedeji, Adekunle, "The Combined Effects of Leptin and Coenzyme Q10 in Ameliorating Obesity- Induced Infertility in Female Rats" (2016). *Electronic Theses and Dissertations*. Paper 3131. <https://dc.etsu.edu/etd/3131>

This Thesis - Open Access is brought to you for free and open access by the Student Works at Digital Commons @ East Tennessee State University. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons @ East Tennessee State University. For more information, please contact digilib@etsu.edu.

The Combined Effects of Leptin and Coenzyme Q₁₀ in Ameliorating Obesity- Induced
Infertility in Female Rats

A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Biology

by

Adekunle Oluseye Adedeji

August 2016

Effiong Otukonyong, Ph.D., Chair

W. Andrew Clark, PhD

Jonathan Peterson, PhD

Edward M. Onyango, PhD

Keywords: Leptin, CoQ₁₀, Leptin receptor, FSH, LH, EH, E-cadherin, β -catenin

ABSTRACT

The Combined Effects of Leptin and Coenzyme Q₁₀ in Ameliorating Obesity- Induced Infertility in Female Rats

by

Adekunle O. Adedeji

Infertility is one of the major problems of obesity. Studies have shown that administration of leptin reversed obesity-induced infertility in rats and mice. Coenzyme Q₁₀ (CoQ₁₀) is an antioxidant and also supplies the energy needed for ovulation and embryo development. We hypothesized that leptin when combined with CoQ₁₀ could greatly improve obesity-induced infertility. The results showed a significant decrease in food intake, body weight, and the regular estrous cycle was restored after treatment with leptin+CoQ₁₀. There was a significant increase ($p < 0.05$) in follicle stimulating hormones after treatment. It was observed that CoQ₁₀ significantly ($p < 0.05$) down-regulates the expression of β -catenin in the ovary while E-cadherin failed to be expressed. In conclusion, administration of leptin with CoQ₁₀ can improve fertility in obese infertile female rats. This study could provide a novel therapeutic strategy for the treatment of infertility and formulation of new drugs for the treatment of obesity-induced infertility in females.

ACKNOWLEDGEMENTS

I would like to express my profound gratitude and appreciation to my committee chair and research advisor, Dr. Effiong Otukonyong for his scholarly advice and encouragement throughout my course of study. I would also like to acknowledge the support and offer my heartfelt gratitude to my committee members, Dr. Jonathan Peterson, Dr. Edward Onyango and Dr. W. Andrew Clark for the knowledge, technical and in-depth contribution towards the success of this study. I thank the entire staff, and faculty of the Department of Health Sciences for the graduate assistantship given to me, the RCD fund provided for this study and for offering me this great opportunity and making the environment conducive to learning and research work. Dr. Clark (Research Lab Valleybrook) and Dr. Peterson (Research Lab) thank you for the materials and equipment provided. I cannot forget to recognize the technical assistance and support given by Dr. Sean Fox, Robin Grindstaff, and Eric Jones. To my fellow old and new graduate students, I acknowledge and appreciate your support and encouragement throughout my course of study.

I would be an ingrate if I did not acknowledge my parents, Mr. and Mrs. Adedeji who gave me the greatest gift in life, education. Thank you. To my family and friends for the continued support and encouragement throughout the Master's program. If it were not for you, this research could not have been completed. To those who have contributed meaningfully to my life and are too numerous to be mentioned, I say thank you all.

DEDICATION

This work is dedicated to God Almighty and to everyone that has contributed to knowledge.

TABLE OF CONTENTS

	Page
ABSTRACT	2
ACKNOWLEDGEMENTS	3
DEDICATION	4
LIST OF TABLES	8
LIST OF FIGURES.....	9
Chapter	
1. INTRODUCTION.....	11
Leptin	11
The Role of Leptin in Food Intake and Energy Expenditure	11
Leptin Receptors.....	13
Role of Leptin in Obesity, Infertility and Reproduction	15
Epithelium Cadherin and Beta Catenin	17
Epithelium Cadherin (E-Cadherin)	17
Beta Catenin	18
E-Cadherin/B-Catenin Complex	18
E-Cadherin/B-Catenin Complex Expression in Rat Ovary.....	19
Coenzyme Q ₁₀	19
Hypothesis	23
Objectives	23
2. MATERIALS AND METHODS.....	24
Animals Model	24
Determination of Oestrus Cycle	24

Irregular Oestrus Cycle	26
Blood and Tissue Collection.....	28
FRAP Assay.....	28
Hormonal Assays using ELISA kits.....	29
Tissues Harvesting and Analysis	29
Western Blotting for Expressions of E-cadherin, β - catenin and Leptin Receptor.....	30
Total Body Assay	31
Sample Preparation for Proximal Analysis	31
Kjehldhal Analysis	32
Soxhlet Anaysis	33
Ashing Analysis.....	34
Bomb Calorimetry	34
Statistical Analysis	35
3. RESULTS.....	36
Leptin Regulates Body Weight.....	36
Leptin Regulates Food Intake	37
CoQ ₁₀ Increases Concentration of Plasma Leptin.....	39
Reproductive Hormone Concentration in the blood.....	39
Combination of Leptin with CoQ ₁₀ Regulates Follicle Stimulating Hormone (FSH)....	39
Regulation of Luteinizing Hormone (LH) by Leptin+CoQ ₁₀	40
Regulation of Plasma Estrogen Hormone (EH) by Leptin+CoQ ₁₀	41
Total Body Composition of Rats using Proximal Analysis	42
Effect of Leptin and CoQ ₁₀ on Crude Fats.....	42
Effect of Leptin and CoQ ₁₀ on Crude Protein	43
Effect of Leptin and CoQ ₁₀ on Total Body Inorganic Content	44

Leptin Combined with CoQ ₁₀ Helps in the Regulation of Total Body Calories.....	45
CoQ ₁₀ is Involved in the Immunological Reaction of the Body	47
Measurement of Total antioxidants	48
Expression of Beta Catenin and E-Cadherin in the Rats' ovary	49
CoQ ₁₀ Down-Regulate the Expression of Beta Catenin in the Ovary.....	49
Expression of E- Cadherin in the Ovary	51
Leptin Receptor (LEPR) Expression in the Hypothalamus.....	53
4. DISCUSSION AND CONCLUSION.....	56
Conclusion	63
Future Direction	64
REFERENCES.....	65
VITA	73

LIST OF TABLES

Table	Page
1. Groups and treatments administered	27
2. Analysis of average body weight	37
3. Analysis of average food intake	38
4. Comparison of average calories in high-fat diet and regular chow diet	47
5. Analysis of average spleen weight	48

LIST OF FIGURES

Figure	Page
1. Biological response of leptin in low (starvation/fasting) and high levels (obesity).....	14
2. Response of leptin in the reproductive system.....	16
3. The role of CoQ ₁₀ in mitochondria electron transport chain.....	20
4. Microscopy view of oestrus cycle stages.....	25
5. Representation of an irregular oestrus cycle	26
6. Samples on freeze dryer	32
7. 6200 automatic isoperibol oxygen bomb calorimeter	35
8. Effects of leptin and CoQ ₁₀ on the body weights.....	36
9. Effects of leptin and CoQ ₁₀ on the food intake	38
10. Leptin concentration in the blood by ELISA.....	39
11. Effects of leptin and CoQ ₁₀ on plasma follicle stimulating hormone (FSH).....	40
12. Plasma luteinizing hormone (LH) concentration by ELISA.....	41
13. Plasma estrogen concentration by ELISA.....	42
14. Total body crude fat measurement by Soxhlet method	43
15. Total body crude protein by Kjeldhal method	44
16. Estimation of body inorganic content by “ashing” method.....	45

17a. Total body calories estimation using “bomb” calorimetry method.....	46
17b. Comparison of average total calories in rats' diets.....	6
18. Graph showing spleen weight measured by digital weighing balance.....	47
19. Plasma antioxidant concentration measured by FRAP assay	49
20. Expression of β - catenin in the ovary by western blot	50
21. β - catenin quantification in the ovary.....	50
22. Expression of an unknown protein at 50kDa that bind with E-cadherin antibody ...	51
23. Western blot showing expression of E- cadherin with positive a control	52
24. The unidentified protein quantification in the ovary	53
25. Western blot showing expression of small proteins that binds with LEPTR antibody in the hypothalamus	54
26. Leptin receptor quantification in the hypothalamus	55

CHAPTER 1 INTRODUCTION

Leptin

Leptin is a 16kDa protein product produced by obese (*ob*) gene. It was first identified and characterized in 1994 by Zhang *et al*⁸². Leptin is secreted primarily by adipose tissue (fat cells) mainly white adipose tissue⁸² and produced in a small amount by the skeletal muscle, mammary gland, placenta and stomach¹³. It plays a significant role in the physiologic functions of the body by regulating food intake, body weight and energy expenditure¹⁷. Leptin regulates the neuroendocrine, metabolic, reproductive as well as immunological processes¹³. Leptin exerts its main effects on the nuclei of the basomedial hypothalamic area of the brain where leptin receptors are located¹⁹.

The Role of Leptin in Food Intake and Energy Expenditure

The major function of the leptin is to maintain homeostatic control of fats by regulating the food intake, body weight and energy expenditure¹⁷. Leptin is secreted primarily by the adipose tissue (fat cells), released into the blood stream and then transported to the hypothalamus of the brain². One of the major functions of the hypothalamus is to regulate food intake and energy expenditure by recognition of peripheral signals such as leptin⁴³. Once leptin is released into the blood stream by the adipose tissue, it is transported to the brain where it binds with the neurons of the leptin receptors (LEPR) located within the nuclei of the basomedial hypothalamus; arcuate (ARC), dorsomedial hypothalamic and ventromedial hypothalamic nuclei of the brain. The neurons relay information about the energy status of the body to the brain¹⁹. The neurons exhibit two forms; orexigenic (increases appetite) and anorexigenic (decreases

appetite) neuropeptides. orexigenic neuropeptides expression modulated by leptin include Neuropeptide Y (NPY), agouti-related protein (AGRP), melanin-concentrating hormone (MCH), orexin, galanin and galanin-like peptide (GALP) ¹⁹. Anorexigenic peptides expression modulated by leptin include pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript, neurotensin, corticotropin-releasing hormone (CRH) and brain-derived neurotrophic factor ⁷. Studies have shown that central administration of orexigenic neuropeptides such as NPY increase food intake and decrease the energy expenditure⁵³. Central administration of leptin causes leptin to bind with the LEPRs located within the nuclei of the basomedial hypothalamus (particularly ARC), thereby inhibiting secretion of orexigenic neuropeptides (which stimulate appetite) but stimulate the release of anorexigenic neuropeptides to decrease appetite. This results in decrease in food intake and increase in energy expenditure with respect to the total body fats and body mass index ⁵³. NPY is associated with the regulation of gonadotropin-releasing hormone (GnRH) by binding directly with GnRH via the brain and / or pituitary and thus regulates gonadotropin secretion (Fig. 2). The GnRH stimulates anterior pituitary to release follicle stimulating hormone (FSH) for folliculization and luteinizing hormone (LH) stimulates the gonads in preparedness for ovulation (Fig. 2). In 1996, Schwartz et al. demonstrated that the level of circulating leptin in peripheral blood is dependent on total body fat and body mass; The higher the total body fat and the body mass index the higher the circulating leptin ⁶⁴. When fat mass falls, the circulating leptin level falls, stimulating appetite and suppressing energy expenditure until the fat mass is restored. However, when fat mass increases, circulating leptin level also increases, suppressing appetite until the weight is lost. This

mechanism by leptin helps the body to maintain and regulate homeostatic control and energy balance of the body⁴⁷. Other factors that causes an increase in circulating leptin include food intake^{41,40}, glucose uptake⁷³ and gender- higher in females than males⁵⁶. However, exercises³⁷ and an increase in age⁵⁶ can also decrease the level of circulating leptin.

Leptin Receptors

Leptin receptors (LEPRs) are classified as class 1 cytokine receptors because it has a common downstream signal pathway and homologous structure with interleukin 6³⁶. LEPRs are located mainly in the hypothalamus, site for control of appetite, reproduction, growth and metabolism⁴³. LEPR are also expressed in small amounts in other parts of the brain such as the cerebellum, anterior pituitary and other parts of tissues such as placenta and stomach⁴³. Leptin receptor family arises from alternative splicing, consists of six isoforms called obese receptors (*ob-R*) isoforms and are grouped into two forms; short and long forms. The short forms include *ob-Ra*, *ob-Rc*, *ob-Rd*, *ob-Rf*, and soluble form, *ob-Re* which circulates in the plasma. The only long isoform, *ob-Rb* is mainly expressed in the hypothalamus¹⁷. The *ob-Rb* contains a long intracellular domain which has two protein motifs that signal via Janus-activated kinases (JAK)-signal transducers and activators of transcription (JAK-STAT) pathway⁷⁰. The short intracellular isoforms signal via phosphatidyl inositol-3 (PI-3) kinase or mitogen-activated protein kinase (MAPK) pathway. The short forms do not activate JAK-START pathway because they contain only one of the two JAK – binding domains⁷⁰.

Leptin binds with LEPR majorly in the hypothalamus and activates different signals that relay information regarding the physiological status of the body to the brain ².

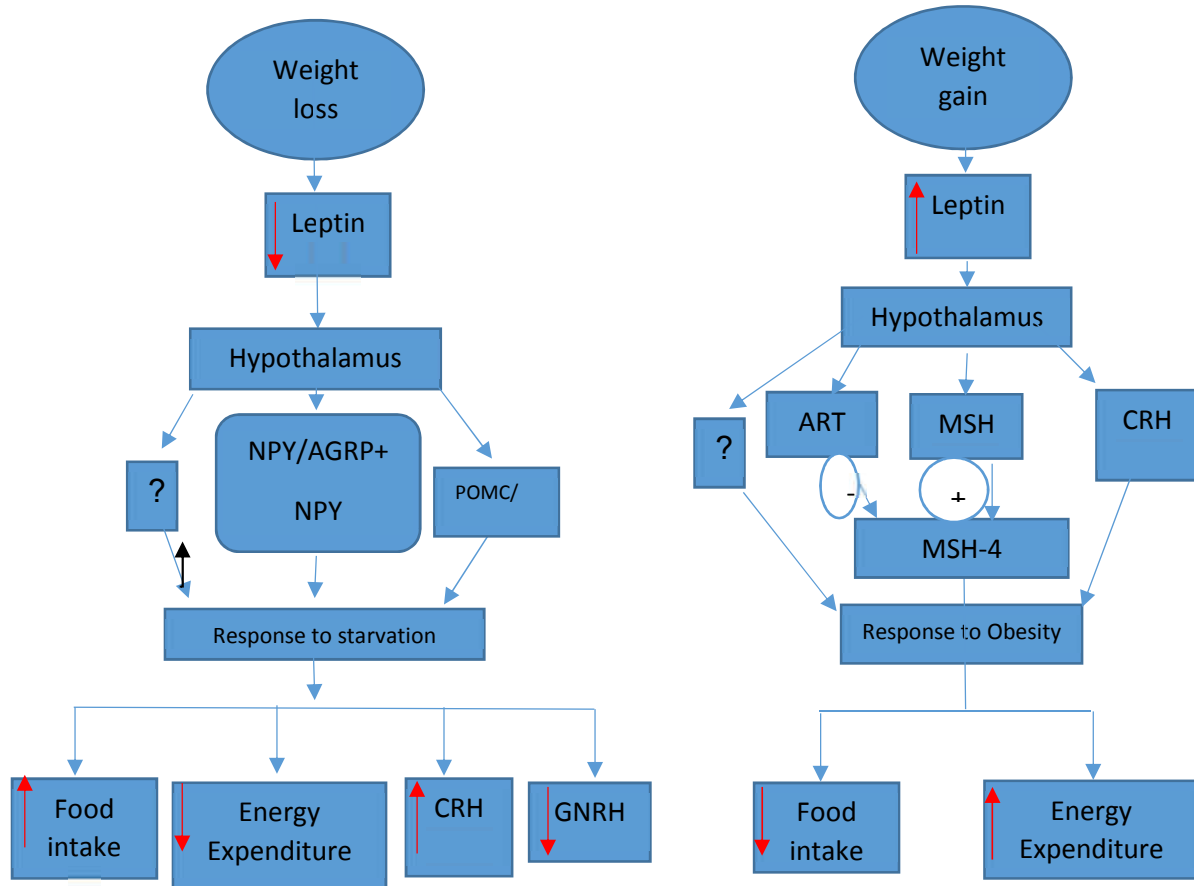


Fig. 1. Biological response of leptin in low (starvation/fasting) and high levels (obesity).

During starvation/fasting/hunger, there is a decrease in adiposity which leads to a decrease in concentration of leptin in the blood. Leptin binds to leptin receptors in the hypothalamus of the brain and conversely activates several pathways; increase NPY/AgRP, neuropeptide Y/Agouti-related peptide; decrease POMC, proopiomelanocortin; CART, cocaine- and amphetamine-regulated transcript. This pathway relays the information about the energy status of the body to the brain and

ultimately leads to increase food intake and a decrease in energy expenditure as well as metabolism. However, in conditions such as obesity, the reverse is the case. Several hormones are involved in leptin response to energy status. These include; gonadotropin releasing hormone (GnRH), corticotrophin release hormone (CRH), melanin-concentrating hormone (MCH), thyroid-stimulating hormone (TSH), thyroid-releasing hormone (TRH), adrenocorticotrophic hormone (ACTH), growth hormone (GH); IGF-I, insulin-like growth factor I as well as reproductive hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estrogen^{32, 64}.

Role of Leptin in Obesity, Infertility and Reproduction

One of the neglected underlying causes of infertility is obesity. Infertility is associated with obesity and overweight, especially when it occurs at the early stage of life or during puberty⁵⁷. Obesity reduces the chances of ovulation and pregnancy in women that ovulate regularly²⁶. Previous studies suggested that the impairments in fertility caused by obesity has its reproductive targets on hypothalamus, ovary, ovarian follicle, oocyte, embryo as well as uterine endothelium^{79,8, 77, 61, 71}. The role of leptin in infertility was first demonstrated in 1997 by Chehab¹⁸. It was observed that defective *ob/ob* female mice whose failure to make leptin were attributed to a gene mutation that prevented the mice from ovulating and thus resulted to infertility due to hormonal abnormalities (gonadotropin secretion impairment)¹⁸. Fertility was restored after administration of exogenous leptin¹⁸. The success of reproduction is dependent on food intake and energy reserves of the body⁵⁷. Pasquali and Gambineri study showed that excessive fat accumulation in adipose tissue (fat cells) and the associated release of high concentrations of free fatty acids into various organs can impair fertility⁵⁷.

Evidently, there is a linkage between nutritional status, obesity, and reproduction⁵⁵. Previous researchers have also demonstrated that leptin is involved in reproduction; neuroendocrine regulation of prolactin, luteinizing hormone, estrogen, follicle stimulating hormone, progesterone and GnRH^{1,24}. Leptin has its effects on the hypothalamus–pituitary–gonadal (HPG) axis, viz-a-vis, gonadal, adrenal, thyroid, pancreatic islet, and growth hormone¹⁷. Study by Yu *et al.* in rats showed that leptin can directly stimulate the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and luteinizing hormone (LH) from the pituitary⁸⁰. This finding was also corroborated in a study by Zieba, Amstalden, & Williams in cattle⁸³. The hypothalamic control of gonadotropin secretion is regulated by GnRH⁸⁰ which has effects on reproduction¹⁷.

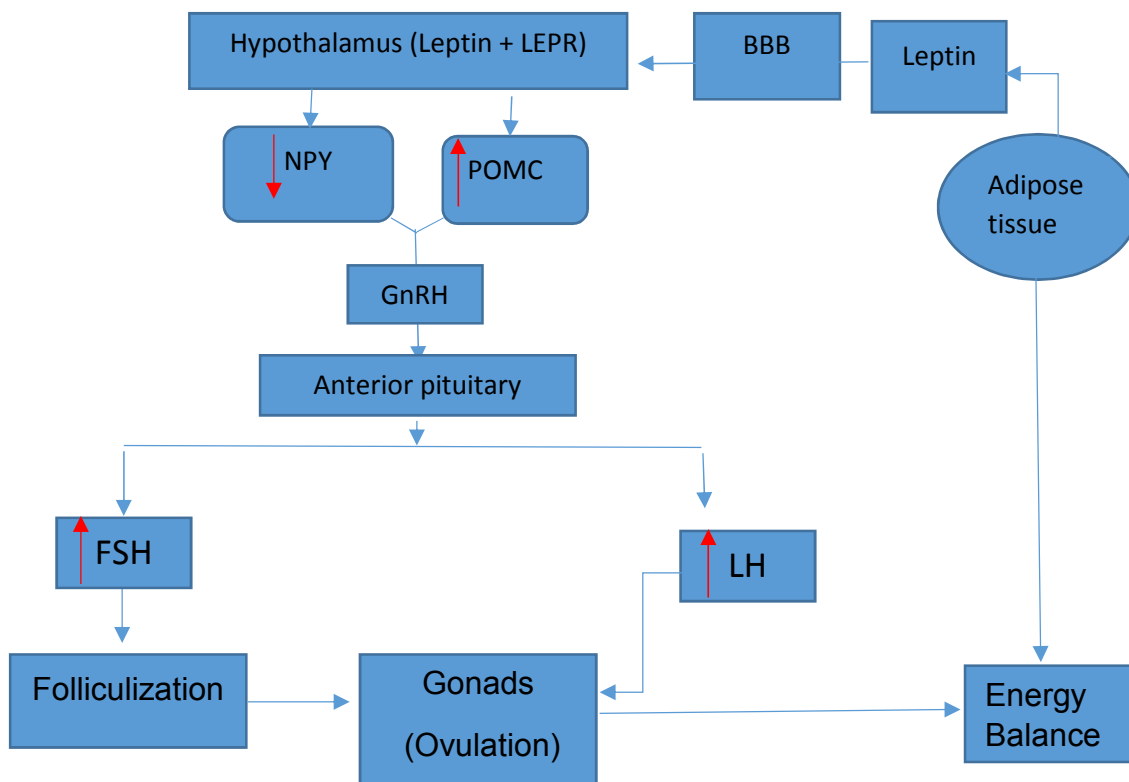


Fig. 2. Response of leptin in the reproductive system.

The adipose tissue stimulates the synthesis of leptin which is then transported to the brain (hypothalamus) via the blood-brain barrier (BBB) where different neuropeptide signals are being activated. Leptin binds with leptin receptors in the preoptic area of the hypothalamus and causes an increase in gonadotropin-releasing hormone (GnRH) secretion. The GnRH stimulates anterior pituitary to release follicle stimulating hormone (FSH) for folliculization and luteinizing hormone (LH) for stimulation of the gonads in preparedness for ovulation (Fig. 2).

Epithelium Cadherin and Beta Catenin

Epithelium Cadherin (E-cadherin)

Cadherin belongs to the superfamily of transmembrane glycoproteins that contain repeated units of approximately 100 residues in their extracellular domain. The primary function of cadherin is to mediate calcium dependent cell-cell interaction and also play key roles in normal tissue development and proliferation of many organs⁶⁸. Disruption of cadherin function has been linked to different kind of developmental and pathological conditions such as cancer⁷⁶. The classical cadherin subfamily includes E, P, R, B and N and other different subtypes. The subtypes are given the name depending on the site they are found. For example, E- cadherin, the most studied member of the family is found in the epithelial tissue⁴⁵. N-cadherin is found in the neuron; P-Cadherin is found in the placental; R-cadherin in the retina; VE- Cadherin in vascular endothelial⁴. E-cadherin also known as cadherin 1 is a protein that is encoded by CDH1, tumor suppressor gene in human⁷⁶. It consists of five repeated cadherin residue, EC1 to EC5 in the extracellular domain, one transmembrane domain, and an

intracellular domain (cytoplasmic tail) that interact with p120-catenin and beta catenin. The intracellular domain contains a highly-phosphorylated region and facilitates the binding to β -catenin, and thus promotes cell to cell interaction with E-cadherin function. E-cadherin down-regulation decreases the strength of cellular adhesion within a tissue, resulting in an increase in cellular motility ⁷⁶.

Beta Catenin

Beta (β -) catenin also known as catenin beta-1 is a CTNNB1 protein encoded by CTNNB1 gene in human ⁴⁶. It was discovered that mammalian β - catenin is homologous both in structure and function with drosophila protein called *armadillo*. *Armadillo* (ARM) is a repeated unit of amino acids (approximately 40) folded into single, elongated, rigid protein domain. The *armadillo* is involved in mediating the morphogenic effects of *Wingless/Wnt* ³⁸. β -catenin is a subtype of catenin protein family and homologous to γ -catenin (plakoglobin). It is expressed in tissue such as cardiac muscle and ovary ⁵⁹. The β -catenin is a key downstream effector protein in the *Wnt* signaling pathway that has been linked to different kind of developmental and pathological conditions such as cancer ¹⁶. β -catenin is involved in two major biological processes: early embryonic development ⁷⁵ and tumorigenesis ⁶⁰. β -catenin is involved in cell to cell adhesion as well as gene transcription ⁷⁴.

E-cadherin/ β -Catenin Complex

E-cadherin cytoplasmic domain is linked to the actin cytoskeleton through the β catenin, γ catenin and p120 catenin to form cell to cell adhesion junction. The β and γ -catenin is linked to α - catenin to form E-cadherin/ β -catenin complex which in turn linked

to the actin cytoskeleton. This complex formation enables the E-cadherin to maintain the structural integrity and polarity of the epithelial cells and tissues. β and γ -catenin play structural roles in the junctional complex while p120 regulates the adhesive activity and trafficking ⁷⁴.

E-cadherin/ β -Catenin Complex Expression in Rat Ovary

Expression of E-cadherin/ β -catenin complex is involved in the development of tissues and proliferation of organs ⁶⁸. E-cadherin/ β -catenin complex expression is increased in the theca and interstitial cells of the rat ovaries during follicular development. However, granulosa cell has more expression of β -catenin ⁶⁷. The cytoplasmic tail of E-cadherin (plasma membrane) is linked to β -catenin (nucleus) to form a complex that helps in the regulation of cytoskeletal structures and intracellular signaling. Studies have shown that modulation of E-cadherin/ β -catenin complex expression forms an integral component of remodeling processes which include folliculogenesis and corpus luteum formation in the ovary ⁶⁷. During these processes, E-cadherin/ β -catenin complex is under the influence of hormones such as estrogen³⁰.

Coenzyme Q₁₀

Coenzyme Q₁₀ (CoQ₁₀) is a fat-soluble vitamin-like compound ³ produced naturally in most cells of the body with higher concentration found in tissues with high energy stores (mitochondria) such as the brain, heart, liver, kidney ²⁷. The oocyst (ovary) has the highest number of mitochondria and mitochondrial DNA more than any cells with high energy requirements such as muscle cells and neurons ¹⁰. CoQ₁₀ can also be found in small amounts in diets such as meats (especially organ meats),

sesame oil, soybeans, nuts, dark leafy green vegetables like spinach, kale and broccoli, seafood, and avocado ¹⁴. CoQ₁₀ is made up of benzoquinone and a hydrophobic tail that comprises of isoprenoids units which can be reduced to ubisemiquinone and finally reduced to ubiquinol which is the biological active ⁶⁹. There are two forms of CoQ₁₀; ubiquinone and ubiquinol. CoQ₁₀ starts off as ubiquinone and converted to the more potent active antioxidant, ubiquinol in the cell. Most CoQ₁₀ supplements are available in the form of ubiquinone. ubiquinol does not need to be converted by the body. The main function of CoQ₁₀ is the transfer of electron in the mitochondrial electron transport chain, used for adenosine triphosphate (ATP) synthesis through ATP synthetase. The ATP synthesis is responsible for creating cellular energy. CoQ₁₀ is also a powerful lipid-soluble antioxidant, its tissue concentration is about five to ten folds higher than the main fat- soluble antioxidants such as vitamin E ⁹. It inhibits lipid peroxidation by providing protection and scavenging free radical or oxidant damage caused by reactive oxygen species (ROS) or reactive nitrogen species (RNS) in the mitochondria ⁴²

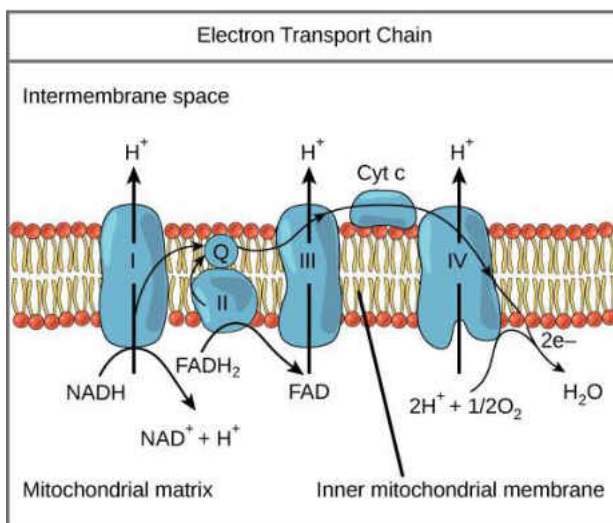


Fig. 3. The role of CoQ₁₀ in mitochondria Electron transport chain. Molnar et al. (2013) ⁸⁵

CoQ₁₀ has shown to be a promising compound in the management and treatment of a wide range of diseases partly or directly caused by mitochondrial disorder such as ischemic heart disease, muscular degeneration, Parkinson, Alzheimer, epilepsy, Diabetes, congestive heart failure, hypertension, cancer, as well as infertility^{66,10,14}. Increase in age, as well as administration of drugs such as Statins decrease the concentration of CoQ₁₀ in tissues⁴⁹. CoQ₁₀ increases ATP synthesis in the mitochondria thus enhancing mitochondrial function and decreases the oxidative stress that causes DNA damage in both animals and human¹⁰. Studies have shown that the presence of ubiquinol in the cell membrane help to reduce cell and DNA damage caused by free radicals, which has a negative impact on health¹⁰. Bentov, Esfandiari, Burstein, & Casper (2010) study showed that mitochondrial DNA damage can be reversed by the supplementation with antioxidants particularly CoQ₁₀¹⁰. In reproduction, CoQ₁₀ increases the availability of energy for egg quality, sperm motility, maturation of oocyte, embryo development as well as implantation in infertile females¹⁰. The survival and quality of oocyte in the ovary rely extensively on the production of ATP, therefore, an increase in the concentration of CoQ₁₀ in the plasma could produce a desirable and positive outcome in fertility. The study conducted by Burstein et al., (2009) in mice concluded that supplementation of CoQ₁₀ improves mitochondrial function as well as an ovarian response in old mice. It has also been shown that combination of CoQ₁₀ with other supplements such as Zinc, lipoic acids, omega-3 fatty acid, vitamins C and E improves mitochondrial disorders than when used alone^{1,68}. Also, findings from

Rodriguez *et al.* study in 2007 showed that combination of CoQ₁₀ with other supplements such as lipoic acid and creatine was more effective in the treatment of mitochondrial disorders than the lone pair in health related diseases ⁶². Abdali, Samson, and Grover (2015) study also described that antioxidants supplementations are beneficial in the management of type 2 diabetes and obesity, especially when supplemented with a combination of antioxidants such as Vit C, E, Lipoic acid, Zinc than CoQ₁₀ alone. The study also recommended testing this hypothesis in high energy health related disease such as infertility ¹. Tarnopolsky (2008) also corroborated other researches that supplementation of CoQ₁₀ with another compound could greatly improve and produce positive impacts in fertility than lone administration ⁶⁹. It is important to conduct this research among females because females are the first target during diagnosis of infertility among couples. With significant evidence showing a positive effect of leptin in reversing obesity-induced infertility and CoQ₁₀ showing a promising outcome in the treatment of some disease conditions such as male infertility, it is important to test if the combination of leptin with CoQ₁₀ could actually yield a more positive outcome in obesity-induced infertility in female rats. This study is novel research as there is no available data on effects of the combination of leptin and CoQ₁₀ in obesity-induced female infertility. This study could provide a therapeutic strategy for the treatment of infertility as well as formulation of new drugs for the treatment of obesity-induced infertility in females.

Hypothesis

The combination of leptin and CoQ₁₀ greatly improve obesity-induced infertility in female rats.

Objectives

To measure the spleen weight, body weight and food intake.

To measure the total antioxidants level in the blood.

Evaluation of expression of β -catenin and E-cadherin proteins in the ovary.

Evaluation of expression of leptin receptors in the hypothalamus.

Determination of plasma level of luteinizing, estrogen and follicle stimulating hormones.

Evaluation of total body composition of each group of the rats.

CHAPTER 2

MATERIALS AND METHODS

Animals Model

Twenty-one female Sprague-Dawley rats of weight range 150-250g were acquired from Harland and used in this study (Protocol #100501). The animal use protocol was approved by the University Committee on Animal Care (UCAC) of East Tennessee State University. Animals were housed individually and kept in a well-controlled, specific pathogen-free room, located in the Brown Hall DLAR facility at ETSU. Food and water were made available ad libitum. Four of the rats were fed regular chow diet (RCD), and the remaining seventeen were fed with high-fat diet (HFD) to induce infertility.

Determination of Oestrus Cycle

A normal regular oestrus cycle of rats is between 4-5 days and consists of proestrus, estrus, metestrus, and diestrus stages (Fig. 4). Vaginal cytology is used to determine different stages of the estrous cycle. A vaginal cytology was conducted using a wet mount process to visualize the vaginal cells. A small amount (10 μ l) of saline solution was introduced into the vaginal orifice of each rat and then aspirated and placed on a clean dry grease free slide. The cells were then checked using a compound microscope at 10X and 40X for analysis.

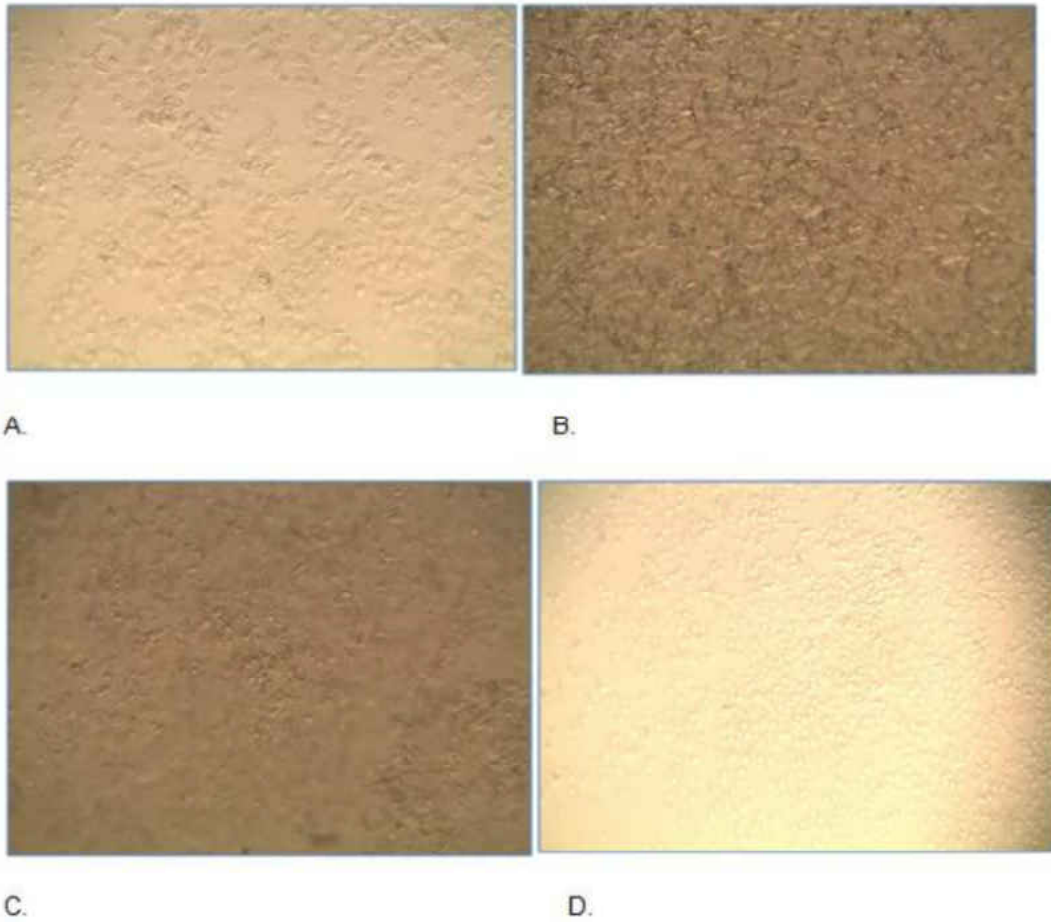


Fig 4. Microscopy view of oestrus cycle stages

Vaginal smears pictures that were taken at 10X using digital camera microscope.

Each of the pictures represents stages of the oestrus cycle.

(A). Proestrus: Predominance of nucleated epithelial cells.

(B). Estrus: Predominance of anucleated cornified cells.

(C). Metestrus: Nucleated epithelial cells, anucleated cornified cells, and leukocytes.

(D). Diestrus: Predominance of leukocytes.

Irregular Oestrus Cycle

The irregularity of the oestrus cycle of the rats fed with HFD was first noticed toward the end of the 12th week of the study (Fig. 5), which signified the onset of infertility. Infertility occurs when any of the stages; proestrus, estrus, metestrus, or diestrus of the 4-day oestrus cycle is skipped particularly estrus phase or maintained for more than two days. The ability of each rat to go through a 4-day cycle without skipping any stage signifies a regular oestrus cycle. The rats' body weight and food intake were measured twice weekly while the estrus cycle checked daily until the oestrus cycle became disrupted.

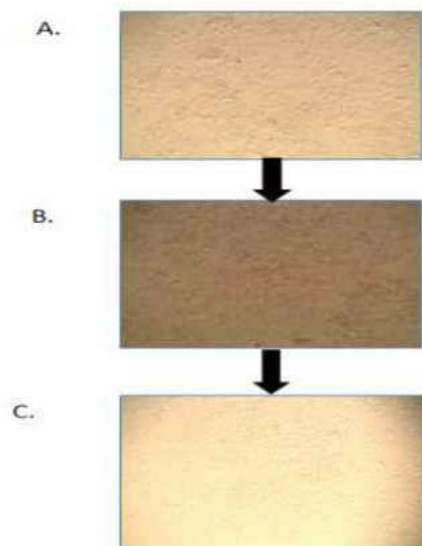


Fig 5. Representation of an irregular oestrus cycle.

The irregular oestrus cycle moved from (A) Proestrus: predominance of nucleated epithelial (skipping estrus stage) to (B) Metestrus: nucleated epithelial cells, anucleated cornified cells, and few leukocytes, and then (C) Diestrus: predominance of leukocytes.

Animals were divided into five groups and treated as follows:

Group I: Fed regular chow diet (RCD), received d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) (10mg/ml/kg/b.w/day) intraperitoneally (i.p).

Group II: Fed high fat diet (HFD), received TPGS (10mg/ml/kg/b.w/day) for two weeks intraperitoneally (i.p)

Group III: Fed HFD, received leptin (100ug/ml/kg/b.w/twice/day) for two days i.p.

Group IV: Fed HFD, received CoQ₁₀ (100mg/ml/kg/b.w/day) for two weeks i.p

Group V: Fed HFD, received leptin (100ug/ml/kg/b.w/twice/day) for two days and CoQ₁₀ (100mg/ml/kg/b.w/day) i.p .

Table 1: Groups and treatments administered

Group	Name	Diet	Leptin	CoenzymeQ10	TPGS
I	RCD	CHOW	-	-	+
II	HFD	HFD	-	-	+
III	HFDleptin	HFD	+	-	+
IV	HFDcoQ10	HFD	-	+	+
V	HFDleptin+CoQ10	HFD	+	+	+

High-fat diet (HFD), regular chow diet (RCD), Tocopheryl polyethylene glycol 1000 succinate (TPGS). TPGS (10mg/ml/kg/b.w/day), leptin (100ug/ml/kg/b.w/twice/day) CoQ₁₀ (100mg/ml/kg/b.w/day).

Group I was fed a regular chow diet (RCD) while groups II to V were fed a high-fat diet (HFD) for 14 weeks to induce infertility. Estrous cycle was checked daily. Food intake and body weight measured twice weekly before and after treatments. All the animals were sacrificed fourteen days after the last day of treatments (18 weeks). Blood, tissues and carcasses were collected for further analysis.

Blood and Tissue Collection

Blood samples were collected from each rat into a 5 ml EDTA tube and placed on ice. It was later centrifuged at room temperature for 10 minutes at 2000Xg using Sorvall Legend XTR centrifuge (Thermo scientific, USA). Once centrifuged, the supernatant (plasma) was pipetted into 3ml glass amber vials and stored at -30 °C for further analysis of leptin, antioxidant status, ferric reducing ability of plasma (FRAP) and reproductive hormones concentrations. The red blood cell (RBC) pellet at the bottom of the EDTA tube was diluted with equal parts of normal saline (0.9% NaCl), vortexed briefly to disrupt the RBC pellet, and centrifuged for 5 minutes at 2000Xg. The clear supernatant was removed and discarded. This process was repeated two more times with the clear supernatant removed and discarded each time. Red blood cell pellet of 100 µl was transferred to clear 150mm screw-top test tube with Teflon lined caps and 2ml of n-hexane, 95% (Fisher Scientific, cat. # 130939) was. This process was done in duplicate and stored at 30°C prior to fatty acid methylation of the RBC.

FRAP Assay

The plasma was evaluated for antioxidant capacity using a spectrophotometric technique, ferric reducing ability of plasma (FRAP) assay, as described and modified by Benzie & Strain (1996). It works on the principle that the intensity of the color produced is directly proportional to the concentration of antioxidant present in the sample read at an absorbance of 593nm. FRAP assay is used to determine total antioxidant capacity in serum/plasma samples. The FRAP reagent was prepared fresh before each series of measurements to ensure validity. The reagent contained the following materials: 200 ml of acetate buffer (300mM, pH 3.6), 20 ml TPTZ solution (0.062 g 2,4,6-tripyridyl-s-

triazine, or TPTZ, in 20ml 40mM HCl), 20 ml FeCl₃ solution (0.2748 g FeCl₃-6H₂O in 50 ml DDW), and 24ml DDW. Frozen plasma samples were thawed at room temperature. The absorbance was determined against a blank containing 1000µl of FRAP reagent and 80µl of DDW. The sample preparation contained 20µl of the sample, 60 ul DDW, and 1ml of FRAP reagent. The standard curve was calibrated using a series of standards from diluting 1mM ferrous sulfate solution. The concentration for the series of dilutions included: 0.1, 0.2, 0.4 at increasing increments until the concentration reached 1.0mM. FRAP values were determined by UV-visible spectrophotometer (Thermo Scientific, BioMate 3S cat. # 840-208400) read at 593nm. An average FRAP value of the triplicate samples was determined for each individual measured. Each study group average FRAP values were determined and overall mean for each case group was calculated.

Hormonal Assays using ELISA kits

Leptin (Life Technologies, cat. # KRC2281), FSH (Biotang, cat. # R6403), LH (Shibayagi, cat. # AKRLH-010S) and estrogen (Calbiotech, cat. #ES180S-100) were measured using rats' ELISA kit according to manufacturers' protocols with microplate reader.

Tissues Harvesting and Analysis

Tissues removed from the rats such as ovary, hypothalamus were immediately snap frozen in liquid nitrogen and stored at -80 °C for homogenization and Western Blotting. The remaining body carcasses were stored at -80 °C for later proximal analysis

to determine the percentage of crude protein, fats, minerals, total calories and fatty acids profiles of isolated fat.

Western Blotting for Expressions of E-cadherin, β -catenin and Leptin Receptor

E-cadherin, β -catenin (from the ovary) and leptin receptors (from the hypothalamus) expressions were examined using Western blots. Each sample was homogenized using RIPA lysis buffer (Triton X-100 10%, Sodium Deoxycholate 1%, SDS 0.10%, NaCl 0.15M, NaH₂P₀₄ 0.01M, NaF 50mM, EDTA 2mM) containing protease inhibitor cocktail (Thermo scientific, cat. #78442) and the cell lysates extracted upon refrigerator centrifugation. The protein concentration of the cell lysates was determined by bicinchoninic acid (BCA) assay protein (Thermo scientific kit, 23236). Protein samples were run with ladder (Fisher scientific, cat # BP 3603-500) and separated by electrophoresis on 8-12% SDS-polyacrylamide gel and electro-transferred onto hybond-ECL nitrocellulose membranes. Blotted membranes were blocked for 1hr with 2% non-fat milk at room temperature. The membranes were washed in 10% TBST contains 1xTris buffered saline (Mediatech, Inc. cat. #46-012-CM) with 10%Tween[®] 20 (Acros, cat #9005-64-5) three times at 5mins each. They were incubated with primary antibodies diluted 1:1000 (5ug of primary antibody to 5ml TBST) for E-cadherin and β -catenin while leptin receptor at 1:2000 overnight at 4°C. The primary antibodies used were mouse antibody (Cell Signaling Technology Cat#14472s) for E- cadherin, rabbit antibodies (Cell Signaling Technology Cat# 8480S RRID:AB_11127855) for β -catenin and leptin receptor (rabbit IG) (Thermo Fisher Scientific Cat# PA1-053 RRID:AB_2296991). β -actin (Sigma-Aldrich Cat# F3022 RRID:AB_476970). After primary antibody incubation, the blots were washed three

times in TBST for 5minutes each before been probed with secondary antibody conjugated with horseradish peroxidase at a dilution of 1:10000 (5ug to 50ml of TBST plus with 2% non-fat milk) at 4⁰c overnight. The secondary antibodies used are goat anti-mouse (Cell Signaling Technology Cat# 7076S, RRID:AB_10695470) for E-cadherin and goat anti-rabbit (Thermo Fisher Scientific Cat# 31460 RRID:AB_228341) for β -catenin and leptin receptor. After the incubation with secondary antibody, it was washed three times in TBST at 5minutes each. The HRP-conjugated antibody–chemoluminescence reagent, Lumigen TMA-6 solution A and B (Catalog no TMA-100) from Lumigen® ECL Ultra was used for visual development by mixing 1 parts of solution A to 1 part of solution B. The substrate was incubated on the membranes for 5minutes under dark. Excess solution was removed and blot images were obtained using chemoluminescence digital imaging, Fluorchem M. Protein simple (Santa Clara, CA, U.S.A).

Total Body Assay

The total body and feeds compositions were conducted using proximal analysis techniques; Soxhlet method was used to evaluate percentage crude fat, Kjeldahl method for percentage of crude protein, bomb calorimetry for total calories and “ashing” for total inorganic content.

Sample Preparation for Proximal Analysis

Whole animal carcass and feeds were weighed and freeze dried using a freeze dryer (Labconco Freezone 2.5 Cat.# 7670520) (Fig. 6) to remove the water content. Whole animals' carcasses and feeds were reweighed after drying. The samples were then grounded, sieved and then stored at -30 ⁰C for later analysis.



Fig. 6. Samples on freeze dryer (Labconco Freezone 2.5)

Kjeldhal Analysis

This method is used to evaluate the percentage of crude protein present in the sample. 1000-1200mg of the sample was measured into Kjeldahl flask (place flask in 250ml beaker to hold upright). 1.9 g of K_2SO_4 , 80 mg of mercuric oxide, 2 ml of concentrated H_2SO_4 and 2 boiling chips were added into the flask with gentle mixing for 1-2 minutes. The Kjeldahl flask was placed on the burner with the neck of the flask inserted into the glass manifold. The heater was switched to 3 with the air on under the hood. It was refluxed for 8 to 12 hours (sample will look clear with black specks). It was allowed to cool, 10ml of deionized distilled water was added and the mixture was filtered. It was distilled by placing beaker with 5 ml of boric acid with several drops of Kjeldahl indicator at the bottom of the apparatus. The sample was added slowly to the top of the distilled apparatus. 10ml of $NaOH/Na_2O_3S_2$ was added slowly (solution

turned black). The total volume of boric acid was allowed to measure 20-30ml, blue in color. The solution was titrated back with 0.1N dilute HCl until changed to steady pink. The samples were run in duplicates. Percentage of crude protein was calculated using the nitrogen content of the sample as follows;

Nitrogen (g)/kg = (ml-HCl – ml blank) x Normality x 14.01/ weight (g) x aliquot volume.

Soxhlet Analysis

This method is used to determine the percentage of fatty acids present in a sample. 2.5g of the sample was mixed thoroughly with 2.5g of anhydrous NaSO₄ in mortar and pestle. 2g of the mixture was placed into cellulose thimble, top with glass wool, and labeled the thimble with a pencil. 175ml of petroleum ether (PET) was measured into the round bottom flasks with 2 boiling chips and the thimble was put into Soxhlet sample extraction chamber. The assembled apparatus includes; round bottom flask (seated at the base), Soxhlet extraction chamber (middle), and condenser on top. Each condenser was attached firmly to each other by tubing. The water was turned on and a steady stream of water going through it with the light on. The mantle heater was turned to 3 and allowed to reflux for 8-12 hours. After reflux, it was allowed to cool off (10-20 minutes) by turning off the heat, drained the fluid from thimble into Soxhlet extraction chamber and finally into round bottom flasks. The drained thimbles were placed in an oven at 60 °c for 24 hours, allow to cool in a desiccator for about 1-2hours and then reweighed. The samples were run in duplicates. The percentage of crude fatty acids was calculated as;

Soxhlet = ((weight. before extraction (g) –Weight. after extraction (g))/ Weight before extraction) x 100

Ashing Analysis

This method is used to evaluate the percentage of crude organic minerals present in a sample. Crucible was first weighed. 5g of the sample was transferred into the crucible and then placed in an oven (Heat) at 700⁰C for 5 – 24 hours. The crucible plus sample was placed in a desiccator (1-2 hours) after “ashing” to cool and then reweighed. The percentage organic minerals (Ash) is calculated as:

Percentage Ash = ((Weight before- Weight after ashing)/ weight before)) x100.

Bomb Calorimetry

This method is used to measure the total calories present in a sample. About 1 – 1.2 g of sample was measured into combustion capsule. The combustion capsule was then placed into ringer holder. The ignition thread was twisted around the wire and draped into combustion capsule, making sure it touched the sample. The valve was closed and charged with oxygen. 2000ml of deionized distilled water was added into the chamber (water) bucket. “bomb” was placed into water bucket and ignition wires were attached. The sample weight was input into the bomb calorimeter computer and start sequence (Fig. 7). The “bomb” explodes the sample and print off the results. The bomb was removed from the bucket and slowly release the combustion capsule assembly. The “bomb housing” and combustion capsule assembly was rinsed with deionized distilled water into a 250ml beaker. 2-4drops of methyl red indicator was added into the

beaker and then titrated against 0.1N of NaOH until a yellow uniform color change is observed. The samples were run in duplicates.

Total calories of dry matter = Calories on receipt – ml of titration (nitrogen)



Fig. 7. Paar 6200 Automatic Isoperibol Oxygen Bomb calorimeter.

Statistical Analysis

The Mean value and standard error of the mean were calculated for leptin, body weight, food intake, spleen weight, FRAP, organic minerals, FSH, LH, estrogen, total calories, crude protein, fatty acids, and CoQ₁₀. Data was analyzed using general descriptive models program in SPSS version 23 (IBM Corp. Armonk, NY, USA) and Graph Pad statistical software (Prism 6 for window version 6.07 Graph Pad, CA) using multiple comparisons (uncorrected Fisher's LSD). The level of significance was determined at $p < 0.05$ for all analysis.

CHAPTER 3

RESULTS

Leptin Regulates Body Weight

The average body weight of all the groups were monitored weekly before and after the treatments with digital weighing scale. There was a decrease in the body weights of the groups treated with HFD leptin and HFD leptin+CoQ₁₀ when compared with the control (HFD) at the 16th week when the treatments started (Fig. 8). This correspond to previous published work showing that leptin decreases the body weight of animals^{25,31,58}. Therefore, leptin plays a significant role in the regulation of body weight. However, CoQ₁₀ did not have an effect on body weight when used alone (Fig. 8).

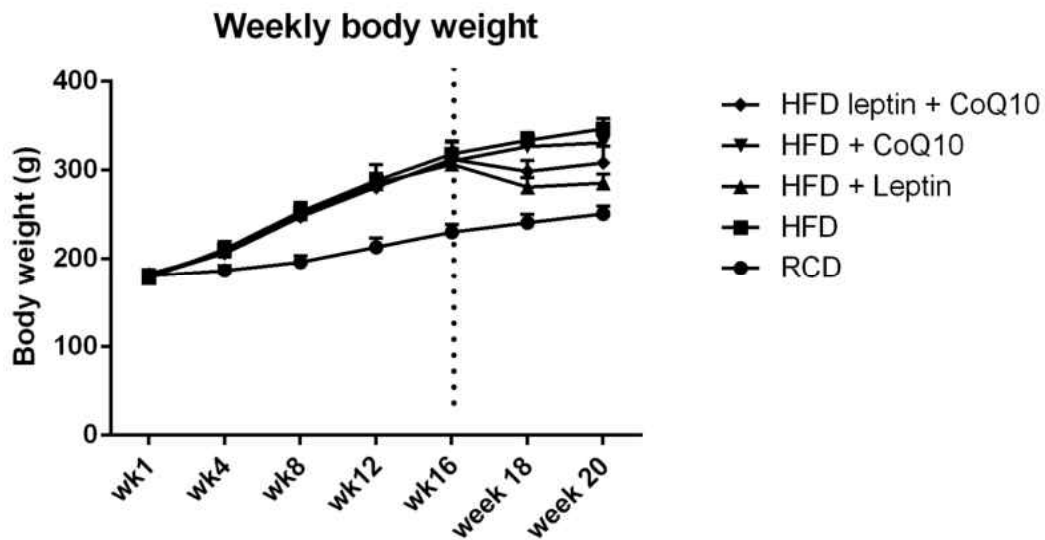


Fig. 8. Effects of leptin and CoQ₁₀ on the body weight.

Table 2: Analysis of average weekly body weight of rats in grams and reported as the mean \pm standard error of the mean.

GROUP	WEEK 1	WEEK 4	WEEK 8	WEEK 12	WEEK 16	WEEK 18	WEEK 20
RCD	180.03 \pm 3.55	186.04 \pm 2.97	195.98 \pm 3.74	212.70 \pm 5.44	229.73 \pm 4.46	240.52 \pm 4.86	250.34 \pm 4.61
HFD	177.91 \pm 1.61	210.38 \pm 3.07	252.71 \pm 3.69	287.94 \pm 6.33	317.82 \pm 5.16	333.17 \pm 3.17	346.18 \pm 4.14
HFD+LEP	181.16 \pm 0.94	208.03 \pm 5.25	250.6 \pm 4.13	284.33 \pm 4.18	306.01 \pm 5.99	280.40 \pm 5.47	285.26 \pm 4.97
HFDCoQ ₁₀	179.93 \pm 1.61	206.24 \pm 5.59	248.11 \pm 6.45	281.71 \pm 7.00	309.93 \pm 6.00	326.16 \pm 7.78	330.53 \pm 7.51
HFDLEP+CoQ ₁₀	182.35 \pm 2.41	205.8 \pm 5.03	247.33 \pm 6.95	280.41 \pm 8.97	311.99 \pm 11.27	298.26 \pm 7.08	307.88 \pm 10.80

Leptin Regulates Food Intake

In this study, the average weekly food intake was measured using a digital weighing scale. It was observed that there was a decrease in the food intake among the treatments groups of HFD leptin and HFDleptin+CoQ₁₀ when compared with the control, HFD at the 16th week when the treatment started. This correspond to previous published work²⁸ showing that leptin is involved in the decrease in the appetite of the rats which ultimately decreases their food intake, while CoQ₁₀ did not show effects on food intake in all the groups when used alone (Fig. 9). It was also observed that the RCD control group consumed more food that the HFD groups, but this did not transform to increase in their body weight due to lower fats and calorie contents in their feed unlike the HFD (Fig. 9).

Table 3: Analysis of average weekly food intake of rats in grams and reported as the mean \pm standard error of the mean.

Group	WK 1	WK 4	WK 8	WK 12	WK 16	WK 18	WK 20
RCD	17.52 \pm 1.69	15.65 \pm 0.68	15.95 \pm 0.63	16.37 \pm 1.28	16.57 \pm 0.68	16.15 \pm 0.58	15.87 \pm 0.73
HFD	17.76 \pm 1.16	10.46 \pm 0.34	10.68 \pm 0.25	10.47 \pm 0.33	10.41 \pm 0.40	9.85 \pm 0.36	10.43 \pm 0.52
HFD+LEPT	16.85 \pm 1.35	11.7 \pm 1.2	11.6 \pm 1.80	11.65 \pm 0.05	11.2 \pm 0.4	7.5 \pm 0.3	8.9 \pm 0.3
HFD+CoQ ₁₀	16.67 \pm 0.83	10.32 \pm 0.20	10.3 \pm 0.11	10.85 \pm 0.69	10.025 \pm 0.46	9.5 \pm 0.28	9.76 \pm 0.16
HFDLEP+CoQ ₁₀	15.4 \pm 1.57	10.76 \pm 0.17	11.53 \pm 0.27	11.33 \pm 0.63	11.40 \pm 0.15	9.23 \pm 0.21	9.46 \pm 0.46

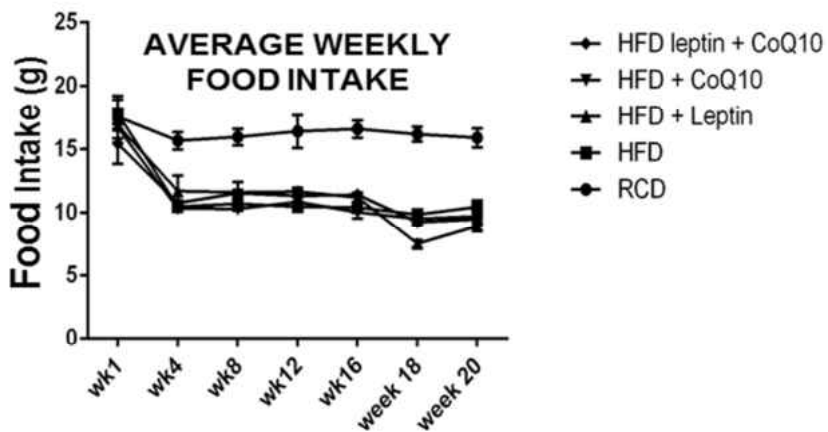


Fig. 9 Effects of leptin and CoQ₁₀ on food intake.

There was a decrease in the food intake observed in the HFD leptin and HFD leptin+CoQ₁₀ groups at week 16 of the treatment. This suggests that leptin plays a significant role in the regulation of food intake.

CoQ₁₀ Increases Concentration of Plasma Leptin

The plasma leptin concentration was measured using ELISA. There was statistical significance increase ($p < 0.05$) in the level of plasma leptin in the HFDCoQ₁₀+leptin treatment group ($p = 0.03$) when compared with control HFD (Fig. 10). This suggests that CoQ₁₀ enhances the concentration of leptin in the blood. Therefore, combination of leptin and CoQ₁₀ increases the concentration of leptin in the blood.

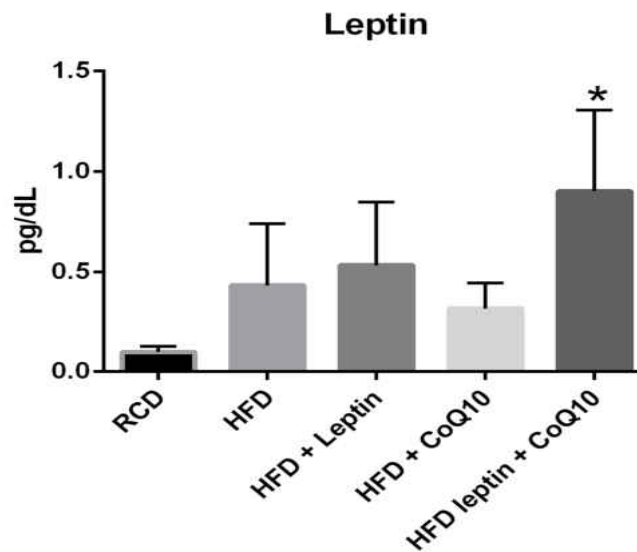


Fig. 10. Leptin concentration in the blood by ELISA.

The result showed an increase in the level of plasma leptin in HFDleptin+ CoQ₁₀ treated groups when compared with HFD control group.

Reproductive Hormones Concentration in the Blood

Combination of Leptin with CoQ₁₀ Regulates Follicle Stimulating Hormone (FSH)

Plasma FSH concentration was measured using ELISA. There was a significant increase ($P = 0.0004$) in the level of plasma concentration in the HFD leptin+CoQ₁₀ group

when compared with the control HFD (Fig.11). This suggests that combination of leptin and CoQ₁₀ could enhance the concentration of FSH in the blood.

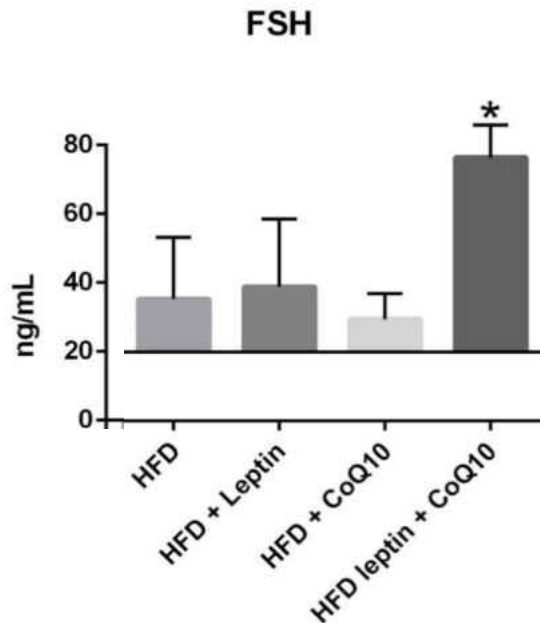


Fig. 11. Follicle stimulating hormone (FSH) concentration by ELISA.

The results showed an increase in the level of FSH in HFD leptin+ CoQ₁₀ treated group ($P=0.0004$) when compared with HFD control group.

Regulation of Luteinizing Hormone (LH) by Leptin with CoQ₁₀

Plasma LH was measured using ELISA. Leptin plus CoQ₁₀ treated group showed an insignificant increase ($p > 0.05$) in the level of plasma LH when compared with the control HFD (Fig. 12). This suggests that leptin+CoQ₁₀ may not have effect in the regulation of LH in the blood.

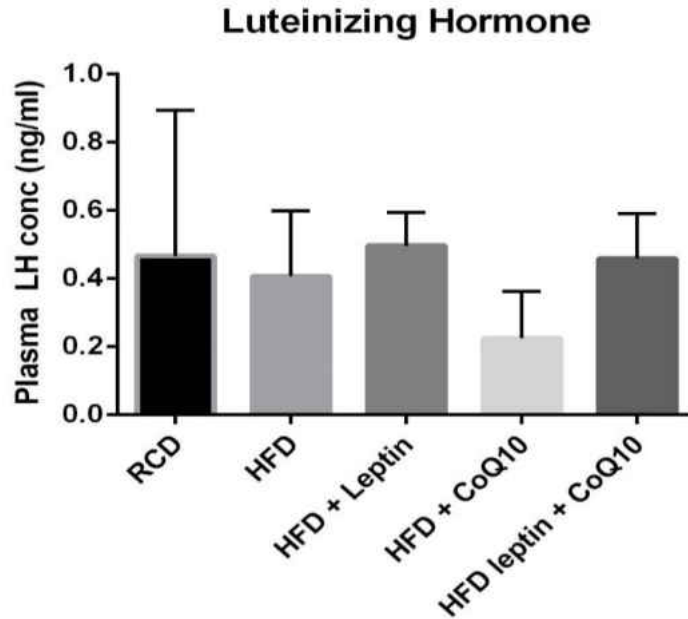


Fig. 12. Plasma luteinizing hormone (LH) concentration by ELISA.

The results showed statistically insignificant ($p > 0.05$) increase in the level of LH in HFD+ Leptin-treated group followed by HFD leptin+ CoQ₁₀ when compared with HFD control group.

Regulation of Plasma Estrogen Hormone (EH) By Leptin with CoQ₁₀

Plasma EH was measured using ELISA. There was a slight insignificant increase ($p > 0.05$) in the level of EH in HFD leptin+CoQ₁₀, HFDCoQ₁₀, and HFD leptin treated group when compared with the control HFD. This suggests that Leptin plus CoQ₁₀ may not have an effect on the regulation of plasma EH (Fig. 13).

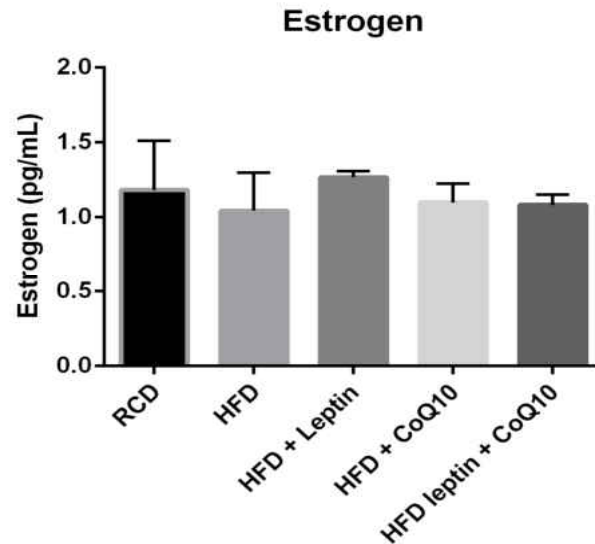


Fig. 13. Plasma estrogen concentration by ELISA.

The result showed an insignificant increase ($p>0.05$) in plasma estrogen in the leptin-treated group when compared with HFD. However, no statistical significance was observed ($p>0.05$) among the treated groups when compared with the HFD control group.

Total Body Composition of Rats using Proximal Analysis

This is a system for approximating the value or materials of the body (animals, foods) based on the body components. For this study, the rats' total body compositions were analyzed into different fractions according to their feeding values. The fractions analyzed include; crude protein, crude fats (ether extract), inorganic content (ash) and total calories.

Effect of Leptin with CoQ₁₀ on Crude Fat

The rat's total body crude fat was estimated using the Soxhlet method. There was significance increase, $p=0.01$ in the percentage of crude fats in the HFDleptin+CoQ₁₀ treated group when compared with HFD control group (Fig. 14). The result suggests that leptin+CoQ₁₀ could increase the percentage of total body crude fat.

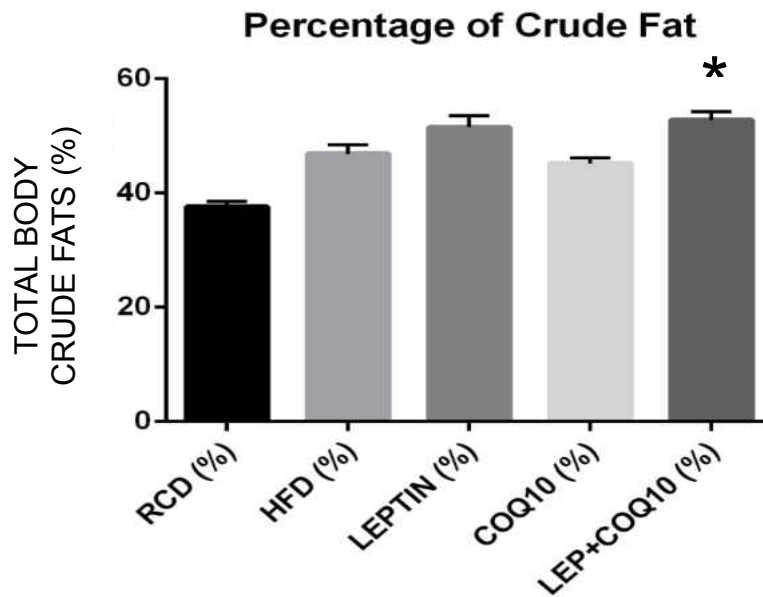


Fig. 14. Total body crude fat measurement by Soxhlet method.

The result showed statistical significance increase ($p<0.05$) in HFD leptin+ CoQ₁₀ ($p=0.01$) treated group when compared with HFD control group.

Effect of Leptin with CoQ₁₀ on Crude Protein

The percentage crude protein was assayed using Kjeldahl method. The result showed no significance difference in the treated HFD leptin+CoQ₁₀ when compared with the control HFD (Fig. 15) ($p>0.005$). Hence, leptin combined with CoQ₁₀ does not

regulate crude protein in the body because CoQ₁₀ functions as an antioxidant and electron transport chain enhancer.

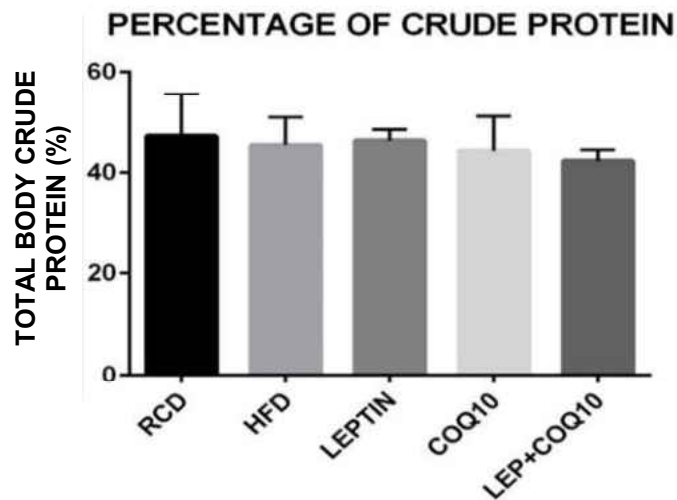


Fig. 15. Total body crude protein by Kjeldahl method

The result showed no statistical significance, $p > 0.05$ in all the treated groups when compared with HFD control group.

Effect of Leptin and CoQ₁₀ on Total Body Inorganic Content.

The percentage of total body minerals was estimated using “ashing” method. The result showed no statistical significance, $p > 0.05$ in the treated group HFDleptin+CoQ₁₀ when compared with HFD control group (Fig. 16). This shows that combination of leptin and CoQ₁₀ does not have an effect on the inorganic content of the body. However, the higher amount seen in the RCD when compared with HFD may be connected with HFD tying up minerals (fat) in the small intestine and excreting them.

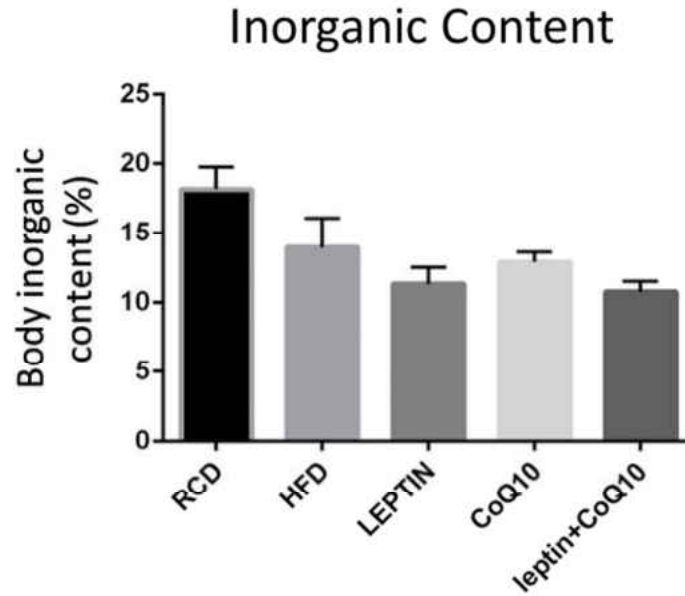


Fig. 16. Estimation of body inorganic content using “ashing” method.

The result showed no statistical significance, $p > 0.05$ in all the treated group when compared with HFD control group.

Leptin Combined with CoQ₁₀ Regulates Total Body Calories

The total body calories were estimated using the “bomb” calorimetry method. The result showed a significance increase in the total calories of the HFDLeptin+CoQ₁₀ ($p=0.0006$) treated group when compared with the HFD control group (Fig. 17). This indicates that combined leptin and CoQ₁₀ could increase in total body calories.

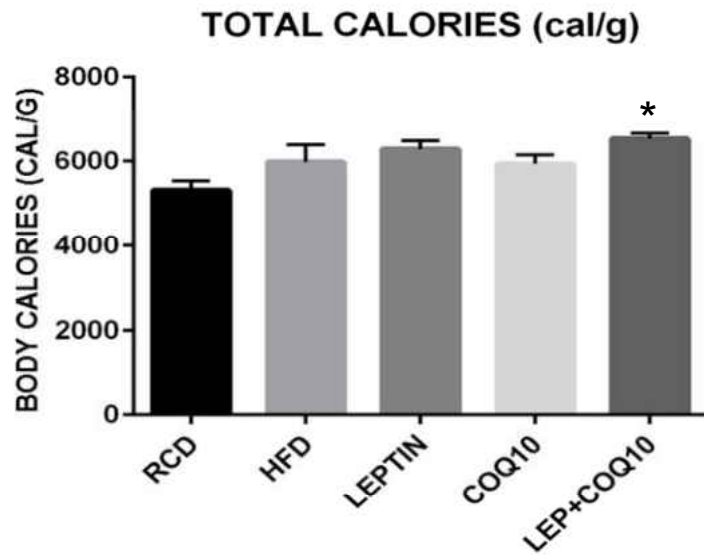


Fig.17A. Total body calories estimation using the “bomb” calorimetry method.

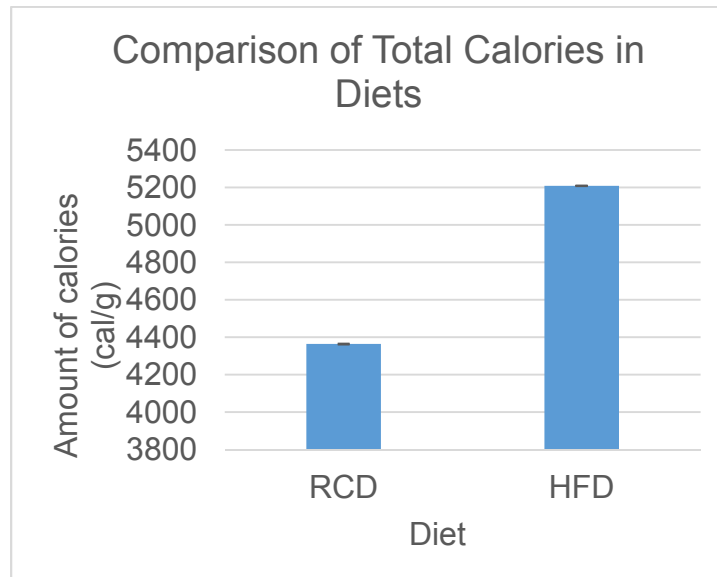


Fig.17b. Comparison of average total calories in diets (RCD and HFD) consumed by the rats. The HFD has more calories than RCD.

Table 4: Comparison of average total calories in diets (cal/g) between regular chow diet (RCD) and high-fat diet (HFD), reported as the mean± standard error of the mean.

RCD	HFD
4363.75±2.83	5209.335±2.21

CoQ₁₀ is involved in immunological reaction of the body

The weight of the spleen was measured using a digital weighing scale. The result showed that there was a significant weight increase ($p < 0.05$) in the spleen weight of HFDCoQ₁₀ ($p = 0.0016$) and HFDleptin+CoQ₁₀ ($p = 0.002$) treated groups when compared with the HFD control group (Fig 18). This suggests that CoQ₁₀ has an effect on immunological response in the body and thus improved immune function in the rats. The effect may be attributed to splenomegaly observed in HFDCoQ₁₀ and HFDleptin+CoQ₁₀ treated groups directly or partially caused by CoQ₁₀ action in the body.

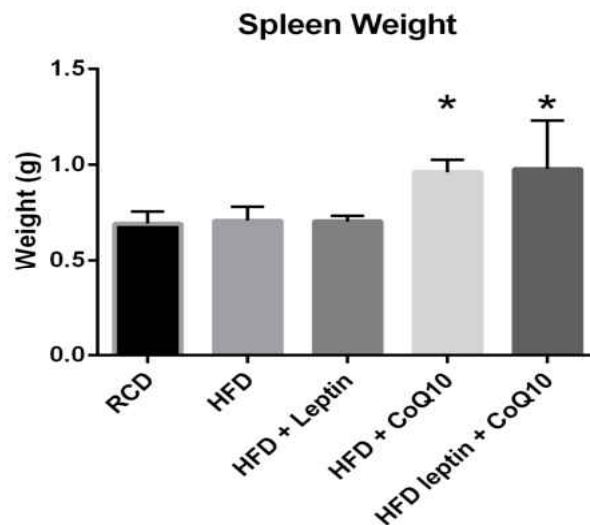


Fig. 18. Graph showing spleen weight measured by digital weighing balance

The results showed statistical significance increase in the spleen weight in HFDCoQ₁₀ ($p=0.0016$) and HFDleptin+CoQ₁₀ ($p= 0.002$) groups when compared with HFD control group.

Table 5: Analysis of average spleen weight in grams and reported as the mean± standard error of the mean.

RCD	HFD	HFD+LEP	HFD+COQ10	LEP+COQ10
0.69225±0.031	0.706375±0.026	0.7035±0.020	0.961±.032	0.9767±0.145

Measurement of Total Antioxidants

Ferric reducing ability of plasma (FRAP) assay was used to estimate the total antioxidant present in the plasma. The ferric reducing ability of plasma in the HFDleptin+CoQ₁₀ treatment group showed a statistical significance increase ($p<0.05$) when compared with the HFD control group (Fig. 19). This result suggests that combination of leptin with CoQ₁₀ could help to enhance the protective effect of CoQ₁₀ against free radicals that causes DNA damage in tissues.

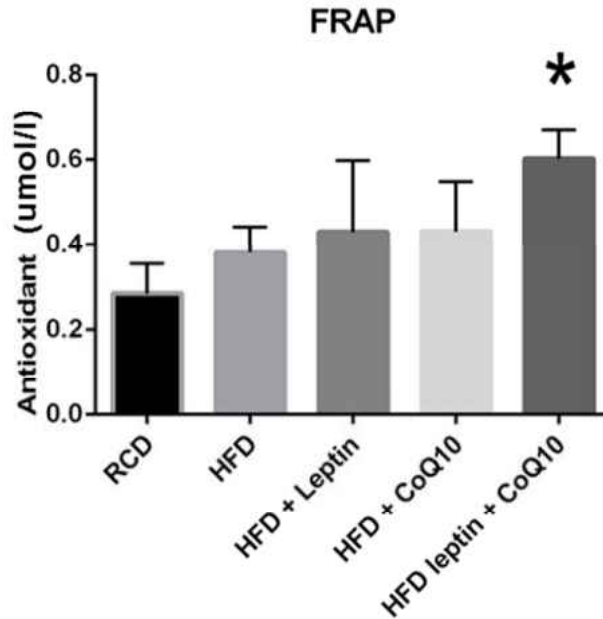


Fig.19. Plasma antioxidant concentration measured by FRAP assay.

The result showed statistical significance increase ($p=0.0016$) in the level of antioxidants in HFD leptin+ CoQ₁₀ treatment group when compared with HFD control group.

Expression of Beta Catenin and Epithelium Cadherin in the Rats' Ovary

In the study, the expression of β -catenin and E-cadherin were analyzed by Western blot using the rats' ovary lysate.

CoQ₁₀ Down-Regulate the Expression of Beta Catenin in the Ovary

The result showed that CoQ₁₀ down-regulated the expression of β -catenin in the ovary (Fig. 20). β -catenin expression was higher in rats fed with HFD. However, there was a decrease in expression in the HFDleptin+CoQ₁₀ treatment group, followed by

HFD CoQ_{10} and then HFD leptin treatment group when compared with HFD group (Fig. 20). This shows that CoQ_{10} down-regulates the expression of β -catenin in the ovary.

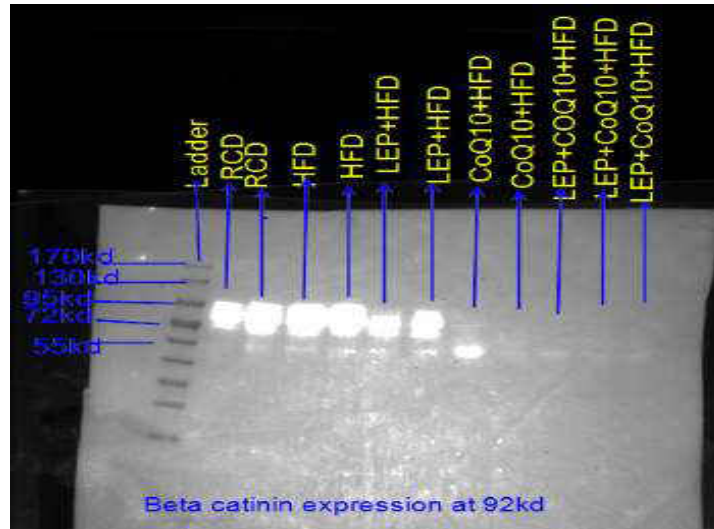


Fig. 20. Expression of β -catenin in the ovary by Western blot

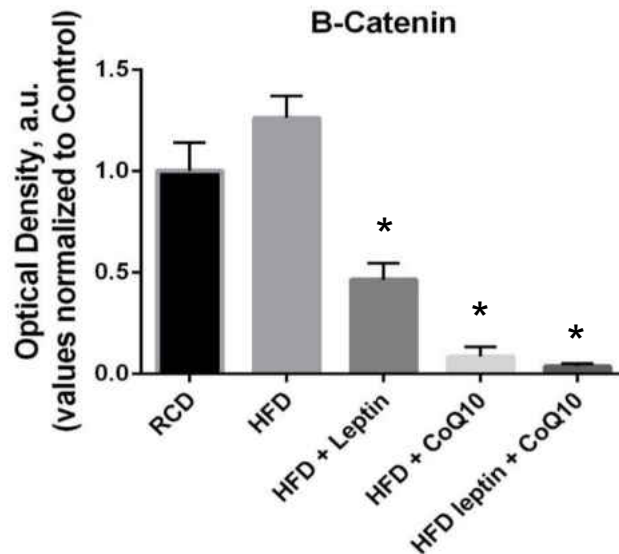


Fig. 21. β -catenin quantification in the ovary

Quantification of the β -catenin showed significance decrease ($p < 0.05$) in the expression of β -catenin in leptin HFD, CoQ_{10} HFD and leptin+ CoQ_{10} +HFD treated

groups when compared with HFD control. This shows that CoQ₁₀ downregulates the expression of β -catenin in the ovary.

Expression of E-cadherin in the ovary

In this study, expression of E-cadherin in the ovary was examined using western blot. The result showed no detection of E-cadherin expression in all the groups at 135kd (which is the size at which E-cadherin was to be expressed according to the protocol used). However, multiple repeated analysis of the lysate for E-cadherin showed consistent detection of an unidentified protein band expressed with E-cadherin antibody at 50kd in all the groups (Fig. 22).

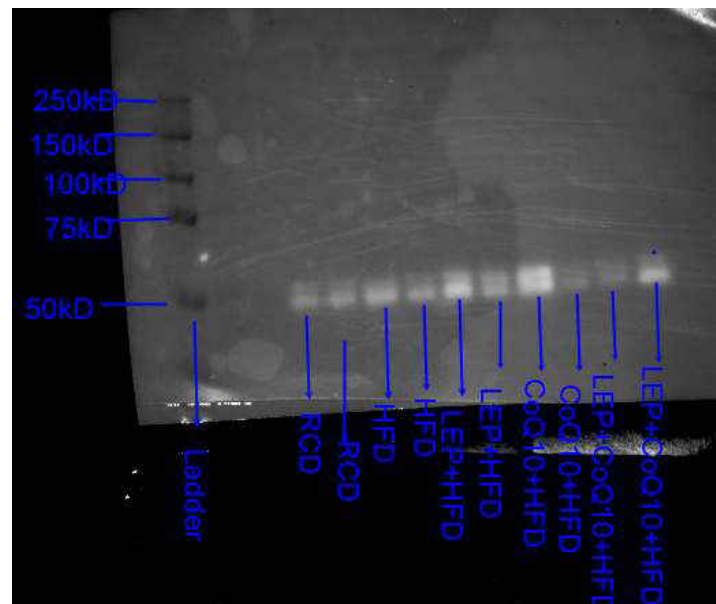


Fig. 22: Western blot showing band of unknown protein at 50kDa that was expressed with E-cadherin antibody.

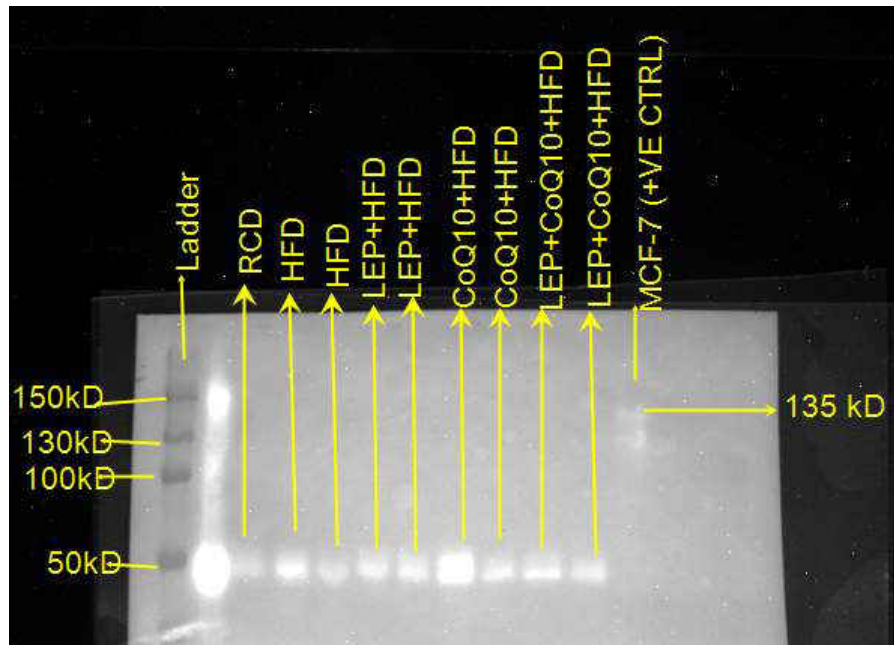


Fig. 23. Western blot showing expression of E-cadherin with positive control (MCF-7)

A positive control, MCF-7 was run along with the samples. The positive control showed expression of E-cadherin at 135kDa while samples showed expression of an unknown protein that binds with E-cadherin antibody at 50kDa.

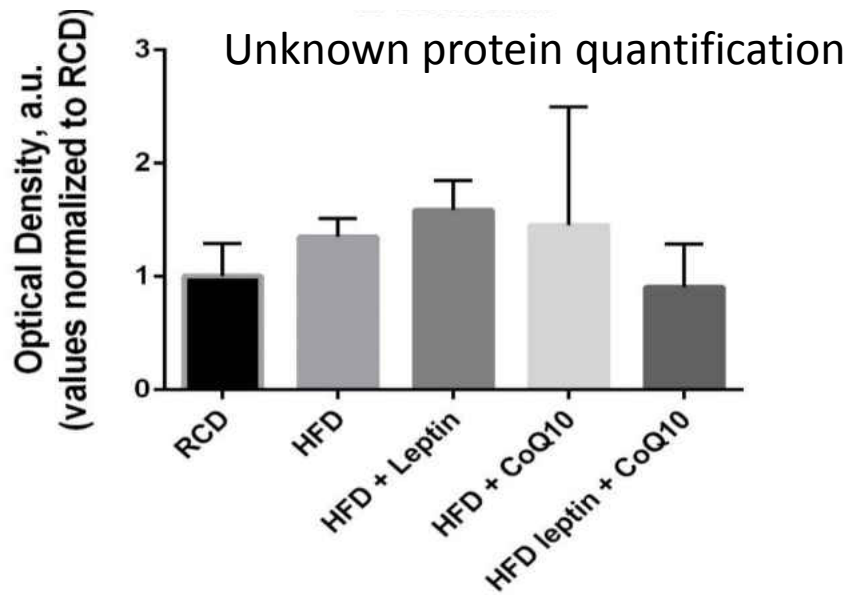


Fig. 24: The unknown protein quantification in the ovary that shows expression with E-cadherin antibody.

Quantification of the unknown band showed no significance ($p > 0.05$) among the groups. However, there was a decrease in the expression of the band in HFDleptin+CoQ₁₀ and slight insignificant increase ($p > 0.05$) in the expression between HFD Leptin and HFD+CoQ₁₀ treated groups when compared with HFD control group (Fig. 24). This suggests that CoQ₁₀ might decrease the expression of the band when combined with leptin.

Leptin Receptor (LEPR) Expression in the Hypothalamus

Hypothalamus lysate was analyzed for expression of leptin receptor (LEPTR). In this study, expression of long isoform (125kDa) and a short isoform (100kDa) of LEPTR was checked. The LEPTRs, long and short forms were not expressed at the sizes; 125kDa and 100kDa respectively. However, there were small unknown proteins that showed expression with leptin receptor antibody detected at sizes smaller than 70kDa.

(Fig. 25). The protocol for the assay indicated that two smaller unrelated proteins could be detected at 31kDa and 35kDa. Quantification of protein detected at 50kDa was done and analyzed (because of higher expression). The result showed downward regulation of the unknown LEPTR in HFDLeptin+CoQ₁₀ and HFD leptin groups when compared with the HFD (Fig. 25).

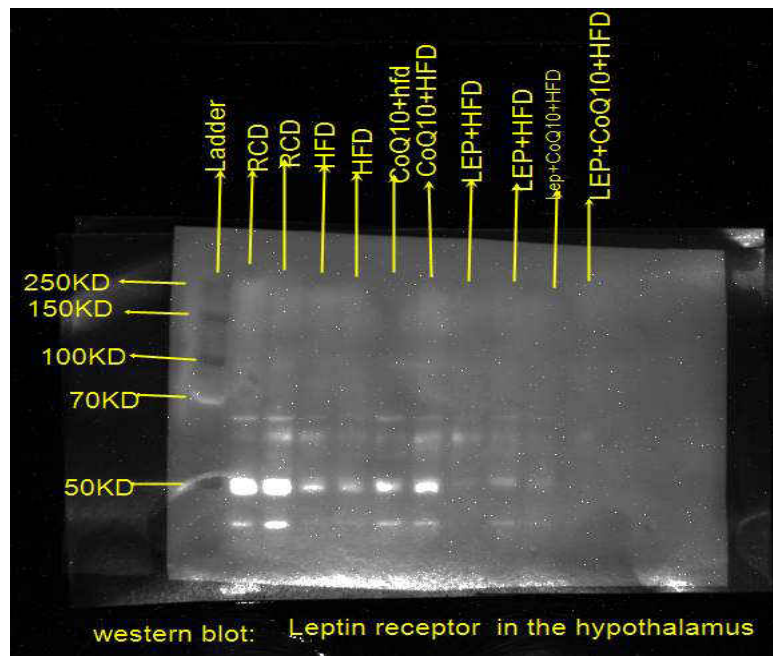


Fig. 25: Western blot showing expression of small proteins that binds with LEPTR antibody in the hypothalamus.

There was statistical significance ($p < 0.05$) in the upward regulation of the small LEPTR in RCD when compared with HFD group (Fig. 26). This shows that leptin tends to decrease the expression of this small protein detected at the 50kDa. There was a slight insignificant increase ($p > 0.05$) in the expression of HFDCoQ₁₀ treatment group, when compared with the HFD group (Fig. 26).

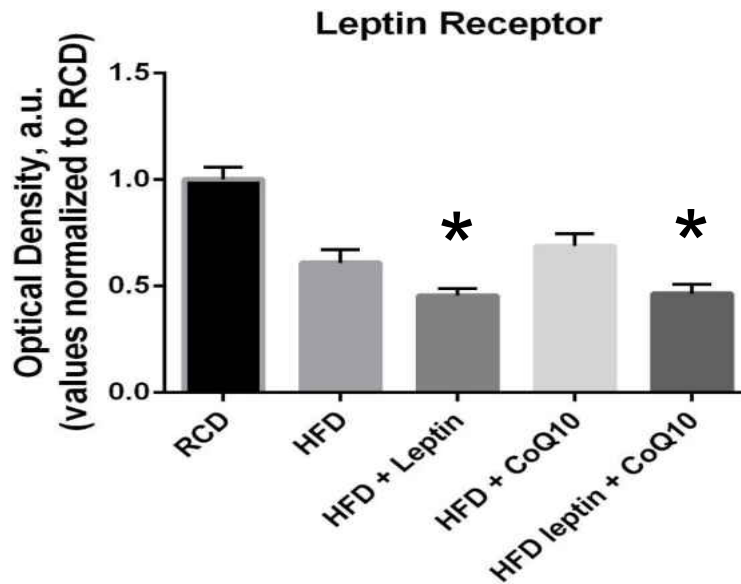


Fig. 26. *Leptin receptor quantification in the hypothalamus*

Quantification of the LEPTR showed significance decrease ($P < 0.05$) in the expression of the small unknown protein in HFD leptin and HFD leptin+CoQ₁₀ treated groups when compared with HFD control. From the graph above, it can be suggested that there may be less small isoform of LEPTRs (if identified as LEPTRs protein) that bind with the LEPTR antibody in the HFD leptin and HFD leptin+CoQ₁₀ groups unlike the HFD group. The decrease in expression can cause delay in relay of information of energy status of the body to the brain, and this may have negative effect on hypothalamic-pituitary-gonadal axis of the body.

CHAPTER 4

DISCUSSION AND CONCLUSION

Leptin is a hormone synthesized primarily by adipose tissues and secreted into the blood stream to drive physiological functions of the body. Leptin helps to regulate food intake, body weight, immunological responses as well as infertility^{6,36}. Leptin has been shown to reverse infertility as well as improve reproduction⁸². Coenzyme Q₁₀ (CoQ₁₀) is an important component of electron transport chain in the mitochondria of human and animal body cell. CoQ₁₀ helps in the synthesis of ATP and acts as a powerful antioxidant and quenches free radicals in the mitochondrial and lipid membranes⁶². Research has shown that CoQ₁₀ helps in reproduction as well as improved fertility⁶². It has been suggested that antioxidant such as CoQ₁₀ supplementation could alternate infertility and improve reproduction^{62,69}. Therefore, this study focused on the combination effects of CoQ₁₀ and leptin on infertile female rats. Various results from this study indicate that combination of leptin with CoQ₁₀ can play a significant role in biological functions that could results in an increase in reproductive functions as well as fertility.

The first observation from the study was the role of leptin in the regulation of food intake, body weights, and energy expenditure. The food intake and body weight of the rats were monitored before and after treatment. This was measured to determine if leptin, CoQ₁₀ and leptin plus CoQ₁₀ reduce food intake and body weight. Previous researches have shown that leptin plays a key role in regulatory centers to reduce feed intake, regulate the body weight and energy expenditure of animals^{25,31,58}. Results (Fig.

VIII) showed that reduction in the body weight started at week 16 when the treatment started among the treated groups, HFD leptin (280.41 ± 5.47), and HFD leptin+CoQ₁₀ (298.26 ± 7.08) but not in the HFD+CoQ₁₀ (326.16 ± 7.78) treated group and the control group, HFD (333.17 ± 3.17) (Table 2). It was also observed that decrease in feed intake (Fig. IX) started at week 16 when the treatment started among the treatment groups; HFD + leptin (7.5 ± 0.3) and HFD leptin+CoQ₁₀ (9.23 ± 0.21) but not in the HFD+CoQ₁₀ group (9.5 ± 0.28) and the control group, HFD (9.85 ± 0.36) (Table 3). This result is in consistent with the study conducted by Pellemounter et al. (1995)⁵⁸ and Halaas et al. (1995)³¹ that leptin regulates body weight and food intake animals. Coll, Farooqi, and O'Rahilly (2007)¹⁹ findings also showed that leptin plays a major role in the downstream anorexigenic pathways in the reduction of food intake in animals. From this study, CoQ₁₀ does not have an effect on the body weight and food intake when administered alone. This result was in line with review by Abdali, Samson, and Grover (2015)¹ that CoQ₁₀ has no effect on the body weight and fat mass. In recent research conducted by Gopi, Purushotha, and Chandrasek (2015)⁸ in broiler chicks, it was observed that CoQ₁₀ does not have an effect on the feed intake or body weight when administered alone.

The role of leptin in fertility was also observed in this study. Previous researches have shown that leptin administration reverses obesity-induced infertility^{25,35,82}. The irregularity of the oestrus cycle was first noticed at the end of the 12th week in the HFD groups. At the 16th week, it was observed that the rats in the HFD groups had disrupted estrous cycle indicating infertility. Normal regular oestrus cycle in female rats is between 4-5 days. Oestrus cycle consists of proestrus, estrus, metestrus and diestrus phases. The continuation of a particular phase of the cycle for more than 2 days or skipping of

estrus phase signified infertility in the rats. Leptin administration restored fertility and oestrus cycle became regular in the HFD leptin and HFDLeptin+CoQ₁₀ treatment groups. It was observed that the HFD control group still maintained the irregular oestrus cycle 2 weeks after treatment. This result supported the study conducted by Chehab (1997)¹⁸ in obese mice and Hoggard, Hunter, Trayhurn, Williams, and Mercer (1998)³⁵ that administration of exogenous leptin reverses obese-induced infertility in animals.

Previous researches have demonstrated that circulating leptin level in the blood is lower in males and post-menopausal women when compared with females and premenopausal women respectively^{52,54}. In addition, when leptin signaling is disrupted in rats they do not experience puberty. This is an indication that leptin regulates gonadal function and reproduction, its deficiency or resistance can result to reproduction abnormalities^{52,54}. Studies have shown that leptin has an effect on gonadotropin-releasing hormone (GnRH) at the anterior pituitary gland, thus stimulating the secretion of serum FSH, LH⁵⁴ and estrogen⁶⁵. CoQ₁₀ can also help in reproduction⁶⁹. CoQ₁₀ helps in synthesis of ATP needed by tissues (oocyte) for the process of ovulation, implantation and embryo development¹⁰. In this study, it was observed that there was an insignificant increase ($p>0.05$) in the level of plasma LH in the HFD leptin and HFD leptin+CoQ₁₀ treatment groups when compared with the control HFD. This result showed that leptin may induce plasma LH concentration. The insignificant increase observed might be connected to the follicular stage when the sample was collected because LH and FSH tend to increase from early follicular stage – mid follicular-ovulation and the peak observe at the luteal stage of follicular developement before declining back to the early follicular stage²⁹. Results from Moschos, Chan, and

Mantzoros, (2002)⁵⁴; Yu, Kimura, Walczewska, Karanth, and McCann, (1997)⁸⁰; Donato et al. (2009)²² studies indicated that leptin stimulates the production of LH and FSH in the blood. The result (Fig.11) from plasma FSH concentration showed significance increase ($p < 0.05$) in plasma FSH in HFDleptin+CoQ₁₀ treated group when compared with the HFD control group. This indicates that combination of leptin and CoQ₁₀ could stimulate an increase in the concentration of FSH in the blood. This is a novel finding as previous studies have suggested that CoQ₁₀ if combined with another compound could help improve fertility^{62,69}.

While there are conflicting reports on the role of leptin on plasma estrogen, some researchers asserted that leptin does not have effects on plasma estrogen while other believe that leptin plays a role in the regulation of plasma estrogen²⁹. The result from Shimizu et al. (1997) shows that leptin increases the level of estrogen in female rats⁶⁵. The result of our study showed that leptin has a slight insignificance increase ($p > 0.05$) in the level of plasma estrogen in the HFD leptin treatment group when compared with the control group, HFD. There was no significance difference ($p > 0.05$) in the level of HFDleptin+CoQ₁₀ and CoQ₁₀ treatment groups when compared with the control, HFD. This suggests that leptin and CoQ₁₀ may not have direct effects on plasma estrogen. However, this insignificant difference maybe dependent on the estrous stage which the sample was collected. Estrogen (like leptin) decreases feed intake, reduce body weight as well as energy expenditure⁸⁴. Estrogen tends to induce leptin. The effects of estrogen can be mimicked by leptin action which can be the result of insignificant difference observed in estrogen level in HFD leptin and HFDleptin+CoQ₁₀ when compared with HFD.

The observational changes noticed in the reproductive hormones from this study may be connected to the phase of the estrous cycle when the blood sample was collected. Leptin which is also a regulator of gonadal function and reproduction is dependent on the functional stage of the estrous cycle. Several studies have shown that leptin concentration increases from early follicular stage to mid-follicular to ovulatory and reach the peak level in early luteal phase and start declining in late luteal phase to early follicular stage, and this can be attributed with adipocyte leptin gene expression^{44,52}.

CoQ₁₀ significantly increases ($p < 0.05$) the concentration of plasma leptin (Fig 10). This shows that combination of CoQ₁₀ with leptin could enhance the effect of leptin in decreasing body weight, food intake, increasing energy expenditure as well as improving reproduction⁶⁹.

It was observed that combination of leptin with CoQ₁₀ significantly increased ($p < 0.05$) the level of antioxidants present in the blood when compared HFDleptin+CoQ₁₀ group with the HFD control group (Fig.19). This indicates that the combined effect of CoQ₁₀ and leptin could help to reduce oxidative stress, the amount of oxidants and thus quench the free radicals that cause DNA damage especially in the reproductive organs and conversely promoting fertility. This is suggestive that higher amount of plasma antioxidant level could produce a positive effect in fertility and improve reproductive functions especially against oxidative stress and DNA damage in the reproductive system. This result is consistent with Tarnopolsky (2008)⁶⁹ submission that supplementation of antioxidants with another compound produces a more positive effect than lone administration.

Another observation from this study is that combination of leptin and CoQ₁₀ significantly increases ($p < 0.05$) the total body calories of the rats when compared with the control group. This may be due in parts to the higher level of body crude fat measured by Soxhlet (Fig. 14) as amount of calories tends to increase with an increase in fat.

From the study, it was observed that CoQ₁₀ significantly increased ($p < 0.05$) the spleen weight in HFDCoQ₁₀ and HFDleptin+CoQ₁₀ treatments group when compared with the HFD group (Fig. 18). This shows that CoQ₁₀ increases spleen weight and therefore is involved in the immunological response of the body. This supports the previous observation by Gopi, Purushotha, and Chandrasek, (2015)²⁸ in broiler chick that CoQ₁₀ supplementation at 20 mg kg⁻¹ increases the spleen weight.

As expected, combination of leptin with CoQ₁₀ does not have an effect on the total crude protein of the rats. There was no significance difference among the groups ($p > 0.05$) (Fig. 15).

β -catenin and E-cadherin are among the major structural proteins found in the ovary to maintain epithelial integrity. They assemble to form cell to cell adherence junction for E-cadherin/ β -catenin complex in the ovary. E-cadherin/ β -catenin complex has been found to be involved in follicular development, ovulation and corpus luteum formation in immature rat ovary⁶⁷. These proteins are also highly expressed in benign and malignant tumor epithelial cells⁶⁷, acting as a tumor suppressor²⁰.

One of the novel findings of the study is that CoQ₁₀ down-regulates the expression of β -catenin in the rat ovary. It was observed that CoQ₁₀ significantly down-

regulates ($p < 0.05$) the expression of β -catenin in the treatment groups, HFDCoQ₁₀ and HFD leptin +CoQ₁₀ when compared with the HFD control group. Leptin effects also significantly down-regulates ($p < 0.05$) the expression of β -catenin in HFD leptin group when compared with the HFD control group. The observation from this study suggests that CoQ₁₀ may have a negative effect on β -catenin in providing cell-to cell adhesion, ovulation as well as follicular development of the ovary. On the other hand, β -catenin (as well as E-cadherin) is mostly expressed (biomarkers) in metastatic tissues and cancer cells⁶⁷, the down-regulation of this protein maybe an indication for protective role of CoQ₁₀ in the ovary.

The expression of E-cadherin was not detected at 135kDa, however, there was a consistent expression of an unknown protein at 50kDa which binds with E-cadherin antibody. The observation from this study suggests that E-cadherin may have a particular stage at which it is adequately expressed in the ovary since the follicular stage of development for the study was not established when the sample was collected. Research conducted by Davies (1998) compared the expression of E-cadherin in normal ovarian epithelium, benign and malignant ovarian tumor⁶⁷. The result showed no expression of E-cadherin detection in four out of six normal ovaries examined and the other two ovaries showed very superficial expression. Although, E-cadherin expression was detected in normal fallopian tube and mammary gland (but not the ovary). However, expression of E-cadherin was detected in twenty-eight out of the thirty differentiated ovarian carcinomas examined²⁰. In another study conducted by Sundfeldt, Piontkewitz, Billig, and Hedin (2000)⁶⁷ using rat ovary, E-cadherin expression was not detected in antral follicular but in the theca, interstitial cells, surface epithelium

of immature ovary and higher expression in benign and cancer cells⁶⁷. E-cadherin and β -catenin serve as biomarkers for cancer and metastatic cells. Observation from this study suggests that E-cadherin may not be detected in normal rat ovary and also dependent on the follicular stage of development as well as the age of the ovary.

The expression of long isoform (125kDa) and short isoform (100kDa) leptin receptor (LEPTR) from the hypothalamus lysate was not detected in this study. However, small proteins (lesser than 70kDa) that bind with the polyclonal leptin receptor antibody were detected. These proteins may be small short isoforms of leptin receptors (*ob-Ra*, *ob-Rc*, *ob-Rd*, *ob-Re* or *ob-Rf*). The small protein which showed highest expression was quantified. The result showed statistical significance ($p < 0.05$) decrease in HFD leptin and HFD leptin+CoQ₁₀ when compared with HFD control group. However, comparing the two control groups, the normal diet (RCD) showed statistical significance increase ($p < 0.05$) when compared with high-fat diet group (HFD). This result indicates that leptin may downregulate the small protein that binds with LEPTR antibody at 50kDa.

Conclusion

Overall, this research supports that combination of leptin with CoQ₁₀ can play a significant role in biological functions that could results in an increase in reproductive functions as well as fertility in obese infertile female rats. The regular estrous cycle was restored after administration with leptin+CoQ₁₀. The administration of combined leptin and CoQ₁₀ increases the level of reproductive hormones, with significance increase ($p < 0.05$) in the level of follicle stimulating hormone while luteinizing hormone and estrogen

showed insignificant increase ($P > 0.05$) in the blood. These reproductive hormones help the ovary in folliculization as well as preparedness for ovulation. Leptin and CoQ₁₀ increased the concentration of antioxidant and improved immune function in the rats.

Future Direction

This experiment should be carried out in a larger sample size with male /pregnant female models, evaluating the reproductive hormones and number of pups. Emphasis should be made on estrous cycle stages during collection of samples. The unidentified protein expression detected at 50kDa with E-cadherin antibody and the small proteins expressed below 70kDa with leptin receptors antibody should be analyzed for further studies.

REFERENCES

1. Abdali D, Samson SE, Grover AK. How effective are Antioxidant supplements in obesity and diabetes? *Medical Principles and Practice*. 2015 March 14;24(3):201–215.
2. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. *Endocrinology and Metabolism Clinics of North America*. 2008 Dec;37(4):811–823.
3. Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS. Leptin accelerates the onset of puberty in normal female mice. *Journal of Clinical Investigation*. 1997 Feb 1;99(3):391–395.
4. Angst BD, Marcozzi C, Magee A. The cadherin superfamily: diversity in form and function. *J. Cell Sci*. 2001;114, 629-641
5. Arroyo A, Navarro F, Navas P, Villalba JM. Ubiquinol regeneration by plasma membrane ubiquinone reductase. *Protoplasma*. 1998 March;205(1-4):107–113.
6. Barb CR, Kraeling RR. Role of leptin in the regulation of gonadotropin secretion in farm animals. *Animal Reproduction Science*. 2004 Jul;82-83:155–167.
7. Bariohay B, Lebrun B, Moyse E, Jean A. Brain-derived Neurotrophic factor plays a role as an Anorexigenic factor in the Dorsal Vagal complex. *Endocrinology*. 2005 Dec;146(12):5612–5620.
8. Bellver J, Ayllón Y, Ferrando M, Melo M, Goyri E, Pellicer A, Remohí J, Meseguer M. Female obesity impairs in vitro fertilization outcome without affecting embryo quality. *Fertility and Sterility*. 2010 Jan 15;93(2):447–454.
9. Bentinger M, Brismar K, Dallner G. The antioxidant role of coenzyme Q. *Mitochondrion*. 2007 Jun;7:S41–S50.
10. Bentov Y, Esfandiari N, Burstein E, Casper RF. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. *Fertility and Sterility*. 2010 Jan;93(1):272–275.
11. Benzie IFF, Strain JJ. The Ferric reducing ability of plasma (FRAP) as a measure of 'Antioxidant Power': The FRAP assay. *Analytical Biochemistry*. 1996 Jul;239(1):70–76.

12. Bhattacharya S, Pandey S, Maheshwari A. The impact of female obesity on the outcome of fertility treatment. *Journal of Human Reproductive Sciences*. 2010;3(2):62.
13. Bluher S, Mantzoros CS. Leptin in humans: Lessons from translational research. *American Journal of Clinical Nutrition*. 2009 Jan 28;89(3):991S–997S.
14. Bonakdar R, Guarneri E. Coenzyme Q10. *Am Fam Physician*. 2005 Sep 15;72(6):1065-1070.
15. Burstein E, Perumalsamy A, Bentov Y, Esfandiari N, Jurisicova A, Casper RF. Co-enzyme Q10 supplementation improves ovarian response and mitochondrial function in aged mice. *Fertility and Sterility*. 2009 Sep;92(3): S31.
16. Cadigan KM, Nusse R. Wnt signaling: A common theme in animal development. *Genes & Development*. 1997 Dec 15;11(24):3286–3305.
17. Caprio M, Fabbrini E, Isidori AM, Aversa A, Fabbri A. Leptin in reproduction. *Trends in Endocrinology & Metabolism*. 2001 March;12(2):65–72.
18. Chehab FF. Early onset of reproductive function in normal female mice treated with Leptin. *Science*. 1997 Jan 3;275(5296):88–90.
19. Coll AP, Farooqi IS, O’Rahilly S. The hormonal control of food intake. *Cell*. 2007 Apr;129(2):251–262.
20. Davies. Expression of E-cadherin, alpha-catenin and beta-catenin in normal ovarian surface epithelium and epithelial ovarian cancers. *Histopathology*. 1998 Jan;32(1):69–80.
21. Denver RJ, Bonett RM, Boorse GC. Evolution of Leptin structure and function. *Neuroendocrinology*. 2011;94(1):21–38.
22. Donato J, Silva RJ, Sita LV, Lee S, Lee C, Lacchini S, Bittencourt JC, Franci CR, Canteras NS, Elias CF. The Ventral Premammillary nucleus links Fasting-Induced changes in Leptin levels and coordinated Luteinizing hormone secretion. *Journal of Neuroscience*. 2009 Apr 22;29(16):5240–5250.
23. Faggioni R, Jones-Carson J, Reed DA, Dinarello CA, Feingold KR, Grunfeld C, Fantuzzi G. Leptin-deficient (ob/ob) mice are protected from T cell-mediated hepatotoxicity: Role of tumor necrosis factor alpha and IL-18. *Proceedings of the National Academy of Sciences*. 2000 Feb 11;97(5):2367–2372.

24. Feuermann Y, Mabjeesh SJ, Shamay A. Mammary fat can adjust Prolactin effect on mammary Epithelial cells via Leptin and estrogen. *International Journal of Endocrinology*. 2009; 2009:1–8.
25. Friedman JM. Leptin, Leptin receptors, and the control of body weight. *Nutrition Reviews*. 2009 Apr 27;56: S38–S46.
26. Gesink Law DC, Maclehose RF, Longnecker MP. Obesity and time to pregnancy. *Human Reproduction*. 2006 Sep 27;22(2):414–420.
27. Ghule AE, Kulkarni CP, Bodhankar SL, Pandit VA. Effect of pretreatment with coenzyme Q10 on isoproterenol-induced cardiotoxicity and cardiac hypertrophy in rats. *Current Therapeutic Research*. 2009 Dec;70(6):460–471.
28. Gopi M, Purushotha MR, Chandrasek D. Influence of Coenzyme Q10 Supplementation in high energy broiler diets on production performance, Hematological and slaughter parameters under higher environmental temperature. *Asian Journal of Animal and Veterinary Advances*. 2015 Jul 1;10(7):311–322.
29. Goumenou AG, Matalliotakis IM, Koumantakis GE, Panidis DK. The role of leptin in fertility. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2003 Feb;106(2):118–124.
30. Günther K, Brabletz T, Kraus C, Dworak O, Reymond MA, Jung A, Hohenberger W, Kirchner T, Köckerling F, Ballhausen WG. Predictive value of nuclear betacatenin expression for the occurrence of distant metastases in rectal cancer. *Diseases of the Colon & Rectum*. 1998 Oct;41(10):1256–1261.
31. Halaas J, Gajiwala K, Maffei M, Cohen S, Chait B, Rabinowitz D, Lallone R, Burley S, Friedman J. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*. 1995 Jul 28;269(5223):543–546.
32. Harris RBS, Zhou J, Redmann SM, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ. A Leptin dose-response study in obese (*ob/ob*) and lean (+/?) mice. *Endocrinology*. 1998 Jan;139(1):8–19.
33. Himms-Hagen J. Physiological roles of the Leptin endocrine system: Differences between mice and humans. *Critical Reviews in Clinical Laboratory Sciences*. 1999 Jan;36(6):575–655.

34. Hirano S. The cadherin superfamily in neural development diversity function and interaction with other molecules. *Frontiers in Bioscience*. 2003;8(4):d306–356.
35. Hoggard N, Hunter L, Trayhurn P, Williams LM, Mercer JG. Leptin and reproduction. *Proceedings of the Nutrition Society*. 1998 Aug;57(03):421–427.
36. Houseknecht KL, Portocarrero CP. Leptin and its receptors: Regulators of whole-body energy homeostasis. *Domestic Animal Endocrinology*. 1998 Nov;15(6):457–475.
37. Keller P. Leptin gene expression and systemic levels in healthy men: Effect of exercise, carbohydrate, interleukin-6, and epinephrine. *Journal of Applied Physiology*. 2005 Jan 20;98(5):1805–1812.
38. Kemler R. From cadherins to catenins: Cytoplasmic protein interactions and regulation of cell adhesion. *Trends in Genetics*. 1993 Sep;9(9):317–321.
39. Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF. Responses of leptin to short-term fasting and refeeding in humans: A link with ketogenesis but not ketones themselves. *Diabetes*. 1996 Nov 1;45(11):1511–1515.
40. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. *The Journal of Clinical Endocrinology & Metabolism*. 1996 Nov;81(11):4162–4165.
41. Korbonits M, Trainer PJ, Little JA, Edwards R, Kopelman PG, Besser GM, Svec F, Grossman AB. Leptin levels do not change acutely with food administration in normal or obese subjects, but are negatively correlated with pituitary-adrenal activity. *Clinical Endocrinology*. 1997 Jun;46(6):751–757.
42. Kwong LK, Kamzalov S, Rebrin I, Bayne A-CV, Jana CK, Morris P, Forster MJ, Sohal RS. Effects of coenzyme Q10 administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. *Free Radical Biology and Medicine*. 2002 Sep;33(5):627–638.
43. Lin J, Barb CR, Kraeling RR, Rampacek GB. Growth hormone releasing factor decreases long form leptin receptor expression in porcine anterior pituitary cells. *Domestic Animal Endocrinology*. 2003 March;24(2):95–101.

44. Ludwig M, Klein HH, Diedrich K, Ortmann O, Medicine I, Medical. Reference tools. Archives of Gynecology and Obstetrics. 2000 Feb 1 [accessed 2016 May 24];263(3):101–99.
45. MacCalman CD, Farookhi R, Blaschuk OW. Estradiol regulates E-cadherin mRNA levels in the surface epithelium of the mouse ovary. Clinical & Experimental Metastasis. 1994 Jul;12(4):276–282.
46. MacDonald BT, Tamai K, He X. Wnt/ β -catenin signaling: Components, mechanisms, and diseases. Developmental Cell. 2009 Jul;17(1):9–26
47. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, et al. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature Medicine. 1995 Nov;1(11):1155–1161.
48. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising Leptin levels may signal the onset of puberty 1. The Journal of Clinical Endocrinology & Metabolism. 1997 Apr;82(4):1066–1070.
49. Marcoff L, Thompson PD. The role of Coenzyme Q10 in Statin-Associated Myopathy. Journal of the American College of Cardiology. 2007 Jun;49(23):2231–2237.
50. May-Panloup P¹, Chretien MF, Malthiery Y, Reynier P. Mitochondrial DNA in the oocyte and the developing embryo. Curr Top Dev Biol. 2007; 77:51-83.
51. Mayela PH, Jose AT, Pedro RC, Carmen Elisa PD, Fanny OR, Jose SQ. Role of leptin in Cattle Production: Review. Journal of Animal and veterinary Advances, 14(4): 81-90.
52. Messinis I. Leptin in human reproduction. Human Reproduction Update. 1999 Jan 1;5(1):52–63.
53. Morrison CD. Leptin signaling in brain: A link between nutrition and cognition? Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2009 May;1792(5):401–408.
54. Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: A review. Fertility and Sterility. 2002 March;77(3):433–444.

55. Nutrition and reproduction in women. *Human Reproduction Update*. 2006 May 1;12(3):193–207.
56. Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *The Journal of Clinical Endocrinology & Metabolism*. 1996 Nov;81(11):3909–3913.
57. Pasquali R, Gambineri A. Metabolic effects of obesity on reproduction. *Reproductive BioMedicine Online*. 2006 Jan;12(5):542–551.
58. Pelleymounter M, Cullen M, Baker M, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*. 1995 Jul 28;269(5223):540–543.
59. Perriard J. Dilated Cardiomyopathy A disease of the Intercalated disc? *Trends in Cardiovascular Medicine*. 2003 Jan;13(1):30–38.
60. Polakis P. The oncogenic activation of β -catenin. *Current Opinion in Genetics & Development*. 1999 Feb;9(1):15–21.
61. Robker RL. Evidence that obesity alters the quality of oocytes and embryos. *Pathophysiology*. 2008 Aug;15(2):115–121.
62. Rodriguez MC, MacDonald JR, Mahoney DJ, Parise G, Beal MF, Tarnopolsky MA. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. *Muscle & Nerve*. 2007;35(2):235–242.
63. Rosenfeldt F, Hilton D, Pepe S, Krum H. Systematic review of effect of coenzyme Q 10 in physical exercise, hypertension and heart failure. *BioFactors*. 2003;18(1-4):91–100.
64. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D. Cerebrospinal fluid leptin levels: Relationship to plasma levels and to adiposity in humans. *Nature Medicine*. 1996 May;2(5):589–593.
65. Shimizu H, Shimomura Y, Nakanishi Y, Futawatari T, Ohtani K, Sato N, Mori M. Estrogen increases in vivo leptin production in rats and human subjects. *Journal of Endocrinology*. 1997 Aug 1;154(2):285–292.
66. Shin E-J, Jeong JH, Chung YH, Kim W-K, Ko K-H, Bach J-H, Hong J-S, Yoneda Y, Kim H-C. Role of oxidative stress in epileptic seizures. *Neurochemistry International*. 2011 Aug;59(2):122–137.

67. Sundfeldt K, Piontkewitz Y, Billig H, Hedin L. E-cadherin-catenin complex in the rat ovary: Cell-specific expression during folliculogenesis and luteal formation. *Reproduction*. 2000 March 1;118(2):375–385.
68. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science*. 1991 March 22;251(5000):1451–1455.
69. Tarnopolsky MA. The mitochondrial cocktail: Rationale for combined nutraceutical therapy in mitochondrial cytopathies☆. *Advanced Drug Delivery Reviews*. 2008 Oct;60(13-14):1561–1567.
70. Tartaglia LA. The Leptin receptor. *Journal of Biological Chemistry*. 1997 March 7;272(10):6093–6096.
71. Tortoriello DV, McMinn J, Chua SC. Dietary-induced obesity and hypothalamic infertility in female DBA/2J mice. *Endocrinology*. 2004 March;145(3):1238–1247.
72. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of Fasting, Refeeding, and dietary fat restriction on plasma Leptin levels 1. *The Journal of Clinical Endocrinology & Metabolism*. 1997 Feb;82(2):561–565.
73. Wellhoener P. Glucose metabolism rather than insulin is a main determinant of Leptin secretion in humans. *Journal of Clinical Endocrinology & Metabolism*. 2000 March 1;85(3):1267–1271.
74. Wheelock MJ, Johnson KR. cadherins as modulators of cellular phenotype. *Annual Review of Cell and Developmental Biology*. 2003 Nov;19(1):207–235.
75. Wodarz A, Nusse R. Mechanisms of wnt signaling in development. *Annual Review of Cell and Developmental Biology*. 1998 Nov;14(1):59–88.
76. Wong AST, Gumbiner BM. Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *The Journal of Cell Biology*. 2003 Jun 16;161(6):1191–1203.
77. Woodruff TK, Shea LD. A new hypothesis regarding ovarian follicle development: Ovarian rigidity as a regulator of selection and health. *Journal of Assisted Reproduction and Genetics*. 2010 Sep 25;28(1):3–6.
78. Wu R, Hendrix-Lucas N, Kuick R, Zhai Y, Schwartz DR, Akyol A, Hanash S, Misek DE, Katabuchi H, Williams BO, et al. Mouse model of human ovarian

Endometrioid Adenocarcinoma based on somatic defects in the Wnt/ β -catenin and PI3K/Pten signaling pathways. *Cancer Cell*. 2007 Apr;11(4):321–333.

79. Yang X, Wu LL, Chura LR, Liang X, Lane M, Norman RJ, Robker RL. Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertility and Sterility*. 2012 Jun;97(6):1438–1443.
80. Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. *Proceedings of the National Academy of Sciences*. 1997 Feb 4;94(3):1023–1028.
81. Zamorano PL, Mahesh VB, De Sevilla LM, Chorich LP, Bhat GK, Brann DW. Expression and localization of the Leptin receptor in endocrine and Neuroendocrine tissues of the rat. *Neuroendocrinology*. 2008 Apr 9;65(3):223–228. 10.1159/000127276.
82. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994 Dec 1;372(6505):425–432.
83. Zieba DA, Amstalden M, Williams GL. Regulatory roles of leptin in reproduction and metabolism: A comparative review. *Domestic Animal Endocrinology*. 2005 Jul;29(1):166–185.
84. Gao Q, Horvath TL. Cross-talk between estrogen and leptin signaling in the hypothalamus. *AJP: Endocrinology and Metabolism*. 2008 Feb 19;294(5):E817–E826.
85. Molnar C, Gair J, Fowler S, Roush, R, Wise, J, Avissar Y, Choi J, DeSaix J, Jurukovski V, Wise R, et al. 2013. *Concepts of Biology*, 1st Canadian edition. Oxidative phosphorylation. © 1999–2016, Rice University. Retrieved 8/3/16 from: http://cnx.org/contents/GFy_h8cu@9.85:7oTVAgrZ@7/Oxidative-Phosphorylation

VITA

ADEKUNLE O. ADEDEJI

- Personal Data: Date of Birth: June 26, 1982
 Place of birth: Osun, Nigeria
 Marital Status: Single
- Education: Akinorun Grammar School, Osun, Nigeria
 West African Examination Council (1999)
 Ambrose Alli University, Edo, Nigeria
 Bachelor of Medical Lab Science. (BMLS) (2010).
 East Tennessee State University, Johnson City, TN
 M. S Biology, concentration in Microbiology (2016).
- Professional East Tennessee State University, TN.
Experience: Department of Health Sciences. Research and
 Graduate Assistant, Aug., 2014 – May, 2016.
 Pathcare Medical Diagnostic Lab, Lagos, Nigeria.
 Medical Laboratory Scientist. Sept., 2013 – July,
 2014.
 Clina-Lancet Laboratories Ltd, Lagos, Nigeria.
 Medical Laboratory Scientist. March, 2013 - July,
 2013.
 General Hospital, Osun, Nigeria. Medical Laboratory
 Scientist Feb., 2012 - Feb, 2013.
- Publications: Imeh-Nathaniel A, **Adedeji A**, Huber R, Nathaniel TI.
 The rewarding properties of methamphetamine in an

invertebrate model of drug addiction. *Physiology & Behavior*. 2016 Jan;153:40–46.

Nathaniel, Soyinka J, **Adedeji A**, Imeh-Nathaniel A.
Molecular and physiological factors of
Neuroprotection in Hypoxia-tolerant models:
Pharmacological clues for the treatment of stroke.
Journal of Experimental Neuroscience. 2015 Feb:1.

Adedeji A, and Obiazi A. The Occurrence of Extended
Spectrum Beta-Lactamase (ESBL) Producing
Escherichia coli among Health Care Centers in the
South Western Nigeria. B.Sc. *thesis* submitted to the
Department of Medical Laboratory Science, Ambrose
Alli University, Nigeria. *Archive of Biomedical Science
and Health*. Vol. 1(2). *in press*.2011.

Training:

Federal Medical center, Ogun, Nigeria

Medical Laboratory Intern. (2011).

Irrua Specialist Hospital, Edo, Nigeria Medical Laboratory
Scientist. (2009).

Obafemi Awolowo University Teaching Hospital, Osun,
Nigeria. Medical Laboratory Scientist trainee (2004-
2007).

Presentation:

Adedeji, A., Otukonyong, E., Peterson J., and Clark W. A.
The Combined Effects of Leptin and Coenzyme Q₁₀ in
Ameliorating Obesity- Induced Infertility in Female
Rats. (2016). ETSU Appalachian Student Research
Forum in Johnson City, TN.