UNIVERSITI TEKNOLOGI MARA

LEPTIN-INDUCED CHANGES IN THE MALE REPRODUCTIVE SYSTEM: ROLE OF AMPK PATHWAY AND EFFECTS OF PROFORTIL®

IFRAH ALAM MALIK

Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Medicine)

Faculty of Medicine

October 2023

ABSTRACT

Excessive levels of leptin have a detrimental effect on the male reproductive system eventually leading to infertility. However, is still unclear how these effects are brought about. Several different signaling pathways are involved in mediating leptin's effects. One of these pathways is the AMPK pathway. Therefore, to understand if the AMPK pathway is involved in mediating leptin's adverse effects, Dorsomorphin was used to inhibit the AMPK pathway and observe if that would lead to any decrease or inhibition of the effects of leptin on sperm and testicular tissue in Sprague-Dawley rats. Furthermore, leptin causes these adverse effects by inducing oxidative stress. Therefore, Profortil®, a concoction of several different antioxidants, was administered to leptin-treated rats to observe if it could prevent the adverse effects of leptin in the male reproductive system of Sprague-Dawley rats. In study I, adult male Sprague-Dawley rats were divided into three groups: Control, which was given 0.1ml normal saline. Leptin, which was treated with 60μg/kg/day of leptin, and leptin+Dorsomorphin which was concurrently treated with leptin and Dorsomorphin at 5mg/kg/day. Treatments were given for 2 weeks via the intraperitoneal route. Food intake and body weight of the rats was measured weekly. At the end of the treatment, the rats were euthanized, and testes and epididymides were collected to measure the organ weight, total sperm count, percentage of sperm with abnormal morphology, ST diameter and epithelial height, expression of connexin-43 and occludin, concentration of 8-OHdG, testosterone, inhibin B, Acetyl-CoA carboxylase and phosphorylated AMPK. In study II, adult Sprague-Dawley rats were divided into 4 groups: Control, which was given 0.1ml normal saline. Leptin, which was administered 60μg/kg/day leptin for 2 weeks. Leptin+Profortil, which was given leptin and Profortil at 50 mg/kg/day. Profortil was given initially for 1 week, followed by another two weeks concurrently with leptin. The last group received only Profortil at 50mg/kg/day for 3 weeks. Normal saline and leptin were given via the intraperitoneal route. Profortil was given by oral gavage. Food intake and body weight of the rats was measured weekly. At the end of the treatment, sperm and testes were collected to measure the organ weight, total sperm count, percentage of sperm with abnormal morphology, concentration of testosterone, CYP17a1, CYP19a1, 17β-HSD, total antioxidant capacity, 8-OHdG, SOD and catalase activity, gene expression of catalase, concentration of phosphorylated AKT and PIP3, testicular cell apoptosis and sperm DNA fragmentation. Results from study I showed that leptin administration caused a decrease in total sperm count, increase in percentage of sperm with abnormal morphology, decrease in ST epithelial height, increase in concentration of 8-OHdG and decrease in gene expression of occludin and connexin-43 in the testicular tissue. Dorsomorphin administration in the leptin-induced rats did not have any significant effect on any of the parameters that were affected by leptin administration. In study II, leptin administration caused a decrease in total sperm count, increase in fraction of sperm with abnormal morphology, increased 8-OHdG concentration, decrease in catalase activity and gene expression, decreased gene expression of connexin-43 and occludin, increased testicular cell apoptosis and sperm DNA fragmentation. Treatment with Profortil prevented the effect of leptin on the total sperm count. However, no other effects of Profortil were observed on the rest of the leptin-induced parameters measured. The results from study I indicate that perhaps the AMPK pathway may not be involved in mediating the adverse effects of leptin on the sperm and testes in the Sprague-Dawley rats. The results from study II indicate that Profortil administration was able to alleviate some effects of leptin on the male reproductive system but not all.

ACKNOWLEDGEMENT

First and foremost, praise be to Allah for His blessings. Without Him I would not have had this opportunity or the perseverance to go through this long PhD journey.

I would like to acknowledge our grant 600-RMI/FRGS 5/3 (0011/2016) for funding this research.

To Assoc. Prof. Dr. Damayanthi Durairajanayagam, thank you not just for being my supervisor, but also my friend. Your constant encouragement and words of motivation are the reason I was able to continue this journey. You were incredibly kind, caring and understanding. I hope to become a supervisor like you someday. To Prof. Dr. Harbindar Jeet Singh, thank you for being the best supervisor and for choosing me to be your student. I am very lucky and honoured to have had this opportunity to learn from you and I hope to continue learning from you in the future. You have always pushed me to improve myself and I cannot thank you enough for that.

To my Adipokine Interest Group (AIG) – Dr. Fayez, Faizatul, Amir, Saleh, Maryam and Zurain; thank you for being a part of my journey. Thank you for the support, motivation and help in the lab. I have learned a lot from all of you, and I hope I was able to have the same impact in your lives that you had on mine. To Norasikin, endless thank yous for having my back from the first day. You have never backed away from helping me and have always been there whenever I needed a friend. Thank you for listening to my rants and being patient with me always.

To my parents, Prof Alam Sher Malik and Prof Rukhsana, thank you for always being there for me and believing in me. You are my role models. I hope to be just as kind, compassionate, loving and understanding as you both someday. I hope I make you proud. To my brothers – Azveed and Azlan – thank you for supporting me through my journey and your words of encouragement.

To my husband Jibran – thank you for being my rock. Without your constant support I would never have been able to complete this. Thank you for taking care of our beautiful kids while I immersed myself in my research. To my wonderful kids – Inaaya, Amayra and Ashar, I cannot express how lucky I feel to be your mother. I hope that one day I can be good enough to be your role model, just as my parents are mine. This PhD is for you all. I hope this inspires you to be the best you can be and reach for the stars!

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

Research Background

A journey of more than 40 years led to the discovery of the cytokine hormone leptin (Ingalls et. al., 1950; Zhang et. al., 1994). Primarily produced by the white adipose tissue (Trayhurn & Woods, 2005), it was initially thought to be involved only in the regulation of food intake, body weight, and energy expenditure. However, research over the years has shown it to be a pleiotropic factor effecting different organ systems of the body. Leptin has roles in the immune system, neuroendocrine system, inflammation, skeletal system, and reproductive system (reviewed by Almabhouh et. al., 2019).

Leptin performs its actions through its receptors. Six isoforms of the leptin receptor have been identified and classified according to their structure. The long form is responsible for leptin's actions, the short isoforms are involved in transporting leptin across the cell membrane, and the secretory form aids in transporting leptin in circulation (reviewed by Singh, 2009). The receptors are widespread across many organs indicating the numerous roles of leptin in the different body systems. These receptors are present in the hypothalamus, pancreas, kidneys, lungs, skeletal muscles, ovaries, testes, and spermatozoa (Houseknecht & Portocarrero, 1998; Keiffer et. al., 1996; Sharma & Considine, 1998; Jope et. al., 2003; Abir et. al., 2005; Guerra et. al., 2007).

Leptin performs its functions by binding to the receptors and activating a number of signaling pathways, including the JAK-STAT, AMPK, PI3K, mTOR, and MAPK pathways respectively (Frühbeck, 2006; Kwon et. al., 2016; Wauman et. al., 2017).

In the reproductive system, leptin's role is well-established. It has a role as a permissive factor in initiating or triggering puberty, particularly in females. As it is produced by the white adipose tissue, when adequate amounts of adipose tissue are present in the body, and leptin levels reach a certain level, puberty is triggered (Frisch, 1980; Farooqi et. al., 1999). Furthermore, it has been observed that subjects with leptin deficiency or with impaired leptin signaling, have hypothalamic hypogonadism (Batt et. al., 1982; Bivens & Olster, 1997; Farooqi et. al., 2007). In leptin-deficient mice,