QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

ROLE OF CHRONIC CADMIUM EXPOSURE ON ADIPOSE TISSUE FUNCTION

BY

Sarra Mohammed Attia

A Thesis Submitted to

The College of Arts and Sciences

in Partial Fulfillment of the Requirements for the Degree of

Masters of Science in Environmental Science

June 2022

© 2022 Sarra Attia. All Rights Reserved.

COMMITTEE PAGE

The members of the Committee approve the Thesis of
Sarra Mohammed Attia defended on 22/05/2022.

Dr. Hamda A. Al-Naemi
Thesis/Dissertation Supervisor

Dr. Nabil Zouari
Committee Member

Dr. Kavitha Varadharajan
Committee Member

Approved:

Ahmed Elzatahry, Dean, College of Arts and Sciences

ABSTRACT

ATTIA,SARRA,M., Masters : June : 2022, Environmental Science

Title:Role of Chronic Cadmium Exposure on Adipose Tissue Function

Supervisor of Thesis: Dr.Hamda A. Al-Naemi.

Cadmium (Cd) is a toxic environmental pollutant with a bioaccumulation feature that exceeds 35 years without a known biological role in the living systems. Recently, Cd was found to be accumulated in adipose tissue (AT) which is known for its vital role in energy homeostasis and endocrine functions. The main goal of this study is to investigate the effect of low-dose chronic exposure of Cd on the function of AT in different locations. Sprague-Dawley male rats were exposed to low Cd dose (15 ppm) for ten weeks, then subcutaneous AT, abdominal AT, and retroperitoneal AT were extracted for molecular analysis. Adiponectin and leptin expression levels were evaluated to assess AT function, and Monocyte Chemoattractant Protein-1(MCP-1) was evaluated to assess the adipose tissue macrophages function. Our results showed significant downregulation of adiponectin and leptin mRNA expression in SUB-AT compared to other depots. Also, MCP-1 mRNA and the protein expression levels were downregulated in SUB-AT. These results suggest that chronic exposure to low-dose Cd disrupts the function of WAT in a depot-specific manner by altering the expression profile of the adipocytokines.

DEDICATION

To my resilient self

To my beloved father

To the memory of my mother

To those who inspired me not to give up.

ACKNOWLEDGMENTS

After all the praises to Allah, I would like to express my deepest gratitude to my supervisor Dr. Hamda Al-Naemi for her unlimited support, patience, motivation, and immense knowledge that she provided during this journey. Her guidance helped me overcome many difficult situations and encouraged me to complete my thesis. Besides my advisor, I would like to thank Dr. Kavitha Varadharajan for her training, guidance, and continuous support during this project. Without her consistent guidance, this research could not have been possible. I am also indebted to the members of LARC, including Dr. Muralitharan Kona and Dr. Hamda aboujassoum, for their help in the lab and their willingness to share their knowledge and experiences during this project.

I want to acknowledge the support of Qatar University for providing Qu-grant: QUST-2-LARC-2020-1 to fund this research. My sincere thanks to the Biological and Environmental Sciences department including Dr.Nabil Zouari and Ms.Dhabia Al-Thani for providing all the needed help for my project with dedication and sincerity.

Last but not least, I would like to thank my family and friends (Mariam Yasser, Sandra Das, Hadil El-Sayyed, Reem Al-Asmar) who supported me mentally and encouraged all my steps through this journey.

Table of Contents

DEDICATION .... **IV**

ACKNOWLEDGMENTS .... **V**

LIST OF TABLES .... **VIII**

LIST OF FIGURES .... **IX**

CHAPTER 1: INTRODUCTION **1**

CHAPTER 2: LITERATURE REVIEW **7**

Adipose Tissue Structure *8*

Cadmium and WAT Structure *9*

Adipose Tissue Function *11*

Leptin *12*

Adiponectin *14*

Cadmium-correlated metabolic disease *16*

Research Objectives *19*

CHAPTER 3: MATERIALS AND METHODS **20**

Adipose Tissue Samples *20*

Gene Expression Assay *20*

Protein Expression Study *21*

Western Blotting *22*

Statistical Analysis *23*

CHAPTER 4: RESULTS **24**

Effect of Cadmium on the Expression level of Adipokines in Adipose Tissue *24*

Adipokines Gene Expression Level in AT of Cd-treated Rats *25*

MCP-1 and Adipokines Gene Expression Levels in each AT Type *26*

Gene Expression of MCP-1 and the Inflammatory Mediators in AT *27*

Comparison Between Gene Expression Levels of Adipokines and MCP-1 in all AT Types *28*

Total protein Quantification 30

Total protein separation of AT depots from control and Cd-treated Rats 31

.. 32

.. 33

.. 33

Protein expression of Adiponectin in AT depots.. ***Error! Bookmark not defined.***

.. 34

Protein expression of Leptin in AT depots 34

Protein expression of MCP-1 in AT depots 35

.. 35

The protein expression of Adipokines and MCP-1 in all AT depots 35

CHAPTER 5: DISCUSSION 37

CONCLUSION.. 45

REFERENCES 46

LIST OF TABLES

Table 1. TaqMan Gene Expression Assays 21

Table 2. RNA Quantification of Adipose Tissue from Control and Cd-treated rats ... 24

Table 3. Protein Quantification of Adipose Tissue from Control and Cd-treated rats. 31

Table 4. The Protein Expression of Adipokines and MCP-1 in AT depots................ 36

LIST OF FIGURES

Figure 1. Effect of Cd-treatment on the expression level of adiponectin and leptin in adipose tissue of male Sprague-Dawley rats. 26

Figure 2. Relationship between the expression level of MCP-1 and adipokines of three different adipose tissue depots of male Sprague-Dawley rats after Cd-treatment....... 27

Figure 3. Effect of Cd-treatment on the expression level of MCP-1 in three different adipose tissue depots of male Sprague-Dawley rats and its relation with selected inflammatory markers. 28

Figure 4.Comparison between the expression level of adiponectin, leptin and MCP-1 in AT depots of male Sprague-Dawley rats. 29

Figure 5. Standard Calibration Curve for BCA assay.Standard used in this assay is bovine serum albumin. Assay was performed in duplicates. 30

Figure 6. Representative image of stain-free gel of subcutanous AT after SDS-PAGE. 32

Figure 7. Representative image of stain-free gel of Abdominal AT after SDS-PAGE. 32

Figure 8. Representative image of stain-free gel of retroperitoneal AT after SDS-PAGE. 33

Figure 9. Protein expression of adiponectin in AT depots of subcutaneous, abdominal, and retroperitoneal. 34

Figure 10. Protein expression of leptin in AT depots of subcutaneous, abdominal, and retroperitoneal. 34

Figure 11. Protein expression of MCP-1 in AT depots of subcutaneous and abdominal. 35

CHAPTER 1: INTRODUCTION

In a world of advanced industries and expanded urbanization, the health of all living organisms is under risk due to the high rate of environmental pollution. Patterns of environmental exposure vary from one place to another and depends on many factors such as source point, duration, and doses at the contact points. Countries with high industrial and agricultural production are more susceptible for higher rates of environmental pollutions. According to the US Environmental Protection Agency (EPA), endocrine disrupting chemicals (EDCs) are defined as exogenous substances that affect the endocrine system through altering its function by eliminating endogenous hormone or interfering with synthesis, secretion, metabolism, transport and receptor binding. As a result, endocrine system will be altered causing disrupting of homeostatic system (Lauretta et al., 2019).

The main concern about some EDCs is their resistance to degradation due to their lipophilic nature. Thus, they can accumulate and remain for many years in AT (Regnier & Sargis, 2014; Sabir et al., 2019). A recent report stated that, EDCs may have the ability of disrupting the homeostasis of the energy metabolism by altering the AT, attenuating the production of adipocytokine and endocrine regulation (Street et al., 2018). One of the main EDCs is Cadmium (Cd), which is a widely spreading heavy metal that is naturally occurring in the Earth's crust with average concentration of 0.1 mg/kg (Cimboláková et al., 2020).

Cadmium is classified as a toxic heavy metal and listed seventh on the priority list of Agency for Toxic Substances and Disease Registry (ATSDR, 2019). The recent global production of Cd reaches 23,000 metric tons which highlights the current challenge of Cd exposure worldwide (USGS, 2020). According to World health organization (WHO), the contentious atmospheric deposition, the elevated Cd level in

the marine environment, and the significant Cd stockpiled in the landfills are the main factors that facilitate Cd exposure (COWI A/S, 2003). These factors allow the increasing of Cd content in the agricultural top soil, harming the marine environment, and posing a serious risk to the health and the environment components. In addition to that, anthropogenic activities such as smelting, mining, metal industries, pigments, fossil fuels combustion and fertilizers are great contributors to the Cd environmental contamination (Godt et al., 2006; Dyer, 2007).

The main routes of Cd exposure are inhalation, ingestion of contaminated food and water, and cigarette smoke (Andjelkovic et al., 2019; Fatima et al., 2019). Potential sources of Cd exposure that are highly consumed are seafood, cereals, bread, leafy vegetables, nuts, wild mushrooms, rice, and cocoa powder (COWI A/S, 2003; Tchounwou et al., 2012; Kijoon Kim et al., 2018). The Cd intake from planted food is the highest (Fristachi & Choudhury, 2017; Chen et al., 2018; Huang et al., 2021; Rusin et al., 2021). Thus, populations who depend on certain eating habits that includes cereals, vegetables and seafood are more susceptible for high Cd intake. Additionally, tobacco plant is an important source of Cd intake in smokers since it belongs to the Cd hyper-accumulators plants (Thévenod & Lee, 2013; Sabir et al., 2019). Smoking is the second main cause on Cd exposure and the estimated amount of Cd in one cigarette is around 1-2 µg (Fristachi & Choudhury, 2017; Sabir et al., 2019). The concentration of Cd in the blood of smokers is found to be 4-5 times higher compared to non-smokers (Thévenod & Lee, 2013). Moreover, Cd tends to bioaccumulate in the soil which makes its impact not limited to the food plants but also reaches to the groundwater (Rahimzadeh et al., 2017; Xie et al., 2019; Luo et al., 2020).

Food intake is a key source of the body's nutrition and energy; thus,

consumption of Cd-contaminated food may have an adverse effect on the systemic biological processes. The ingested Cd targets major organs such as the liver and kidneys which predominantly exhibit high rates of Cd accumulation (Jacobo-Estrada et al., 2017; Genchi et al., 2020). After ingestion, Cd binds to albumin and blood erythrocytes which facilitate its migration into body's organs and tissues where it can forms Cd-MT complexes by binding to metallothionein (MT) (Fatima et al., 2019). MT is a small protein that is rich in cysteine and characterized with high affinity to metal ions like Zinc (Zn), Copper (Cu) and is involved in metal detoxification by binding to toxic metals (Jacobo-Estrada et al., 2017). The high affinity of MT toward metals is due to the presence of the highly reactive thiol groups in MT (Sabolić et al., 2013). In physiological conditions, Zn-MT complex is the usual binding, however in the presence of Cd it becomes Cd-MT complex. The affinity toward Cd is higher and the stability of Cd-MT complex is 1000 fold higher than Zn-MT complex (Sabolić et al., 2013). Reports declared that Cd induces the gene expression of MT, therefore it is used as biomarker to indicate Cd exposure and its distribution through the tissues of the body (Tchounwou et al., 2012; Andjelkovic et al., 2019).

Vital biological processes include glucose and lipid metabolism, and energy homeostasis are regulated by adipose tissue. Also, adipose tissue (AT) is responsible for releasing factors known as adipokines that regulate appetite, energy expenditure, fat distribution and systemic hemostasis. Thus, the potential effects of environmental pollutants on AT function are associated with disrupted metabolic homeostasis and increased risk for metabolic diseases such as type 2 diabetes. Traditionally the known primary function of adipose tissue is to serve as a body cushion to help protect inner organs, also it works as an insulator to prevent heat loss of the body (Hui & Feng, 2018). Additionally it has a main role in energy storage that provides the required systemic

energy (Kwon & Pessin, 2013). In the case of cold or low food intake, AT provides the desired energy to the body's tissues through releasing glycerol and free fatty acids from the stored triglycerides. Also, AT is now recognized as an endocrine organ that produces a number of metabolic and hormonal active substances known as adipocytokines or adipokines (Frühbeck & Gómez-Ambrosi, 2013). There are two main types of AT, white adipose tissue (WAT) and brown adipose tissue. Both types of adipose tissue have distinct features and functions, and both are involved in systemic homeostasis maintenance. Nevertheless, WAT is considered the main site of metabolic dysregulation in several metabolic diseases (Henriques et al., 2019). Thus, WAT is the main focus in this study.

The interaction between the bio-elements and Cd affects several biological processes that involve transporter proteins, ion channels, metabolism, absorption of nutrients and cellular hemostasis (Matović et al., 2011; Jacobo-Estrada et al., 2017). Many studies were done on the interaction between Ca, Zn and Cd and reported that Cd has a toxic effect in their cellular hemostasis due to their competition on the same binding site and ligands since both elements belong to the same group and are able to form tetrahedral complex (Matović et al., 2011). According to Andjelkovic et al. (2019), chronic exposure to Cd reduces the level of Zn in blood and some organs, affects the essential absorption of magnesium (Mg) and disrupts Cu, Zn and Mg tissue level. Furthermore, one of the main mechanisms of Cd toxicity is inducing oxidative stress which leads to lipid peroxidation and depletion of glutathione (GSH) (Bernhoft, 2013; Atieh et al., 2017). Cadmium can cause an enhancement in the production of reactive oxygen species (ROS) through binding to the sulfhydryl groups of GSH protein and inhibiting the activity of anti-oxidant enzymes (Matović et al., 2011; Rahimzadeh et al., 2017). Moreover, Cd main features that poses its toxic risk are the non-

biodegradability and prolonged biological half-life (10-30 years) with no known beneficial physiological function (Jacobo-Estrada et al., 2017; Rahimzadeh et al., 2017).The toxicity of Cd comes from the ionized form (Cd^{2+}) when released from their binding protein (Sabolić et al., 2013; Jacobo-Estrada et al., 2017; Mezynska & Brzóska, 2018). Cadmium can bind to the mitochondria and inhibit both oxidative phosphorylation and cellular respiration even at low concentration (Patrick, 2003; Rahimzadeh et al., 2017). It has been reported that, Cd affects the cellular activities and induces cell apoptosis indirectly through modulating the level of Ca and consequently the caspases activities and the nitrogen-activated protein kinases (MRPKs) in the cell (Godt et al., 2006; Jacobo-Estrada et al., 2017; Rahimzadeh et al., 2017).

The main targets for Cd are the liver and kidneys due to their ability to synthesis MT. However, when the amount of Cd passes their ability of synthesis MT, the detriment effect is induced. There are several mechanism of Cd- induced renal and hepatic toxicity such as dysregulation of autophagy, oxidative stress, endoplasmic reticulum stress, inflammatory cell infiltration and Cd-induced apoptosis (J.-Y. Lee et al., 2019; Zou et al., 2020). An example of Cd toxic mechanism is by triggering the accumulation of the apoptosis-inducing protein (P53) through inhibiting its degradation (J.-Y. Lee et al., 2019). Toxicity of Cd is not limited to the liver and kidney, it has been reported that, Cd toxic effect impacts also muscles, bones, pancreas, glands, brain and adipose tissue (Kawakami et al., 2010; Fristachi & Choudhury, 2017). The toxic effect on pancreas is disrupting the function of insulin in the body. As an endocrine disruption effect, many explanations could be provided such as Cd induces receptor degradation or alteration of the gene encoding receptor-protein at the DNA level, thus less insulin receptors may be synthesized (Ficková et al., 2003). Also, since the pancreatic β-cells are among the highest Zn concentration cells, Cd competes with Zn ions for several

binding sites including zinc transporters, thus Cd can utilize these transporters (Sabir et al., 2019). Therefore, Cd is deposited abundantly in pancreas causing disruption in insulin hemostasis. Additionally, Cd was found to be adversely impacting the function of several glands by disrupting the relative hormones homeostasis. For example, Cd was found to disrupt the function of pituitary gland which affects its patterns of releasing essential hormones (Caride et al., 2010; Fiordelisio et al., 2020). According to Calderoni et al. (2005) high Cd exposure induces apoptosis in pituitary cells and inhibit the production of prolactin. Moreover, for the thyroid gland, an association was reported between high Cd exposure and high thyroid hormone level and thyroglobulin level in adults (Buha et al., 2018). In addition to that, several reports suggest that Cd acts as a metalloestrogen since it can mimic estrogen activity in mammary gland (Johnson et al., 2003; Ali et al., 2010; Bimonte et al., 2021) .This could be associated with nuclear ERα and its hormone-binding domain and the membrane-bound estrogen receptors activation (Pup et al., 2016). Also, Androgen hormone found to be a target for Cd toxic effects. Cd has the ability to mimic androgen by binding to its receptors and disrupt cell growth stimulation and gene expression modulation (Ye et al., 2000; Martin et al., 2002; Neslund-Dudas et al., 2018). Collectively, these data indicate that Cd has an endocrine effect that adversely impacts the endocrine system and consequently the pattern of systemic hormones. Thus, the leading aim of this study is to investigate the Cd effect on the endocrine function of AT by targeting specific adipokines namely leptin and adiponectin.

CHAPTER 2: LITERATURE REVIEW

Adipose tissue is a potential target for heavy metals accumulation in addition to the liver and kidney. Recent studies reported Cd accumulation in AT of the human body (Echeverría et al., 2019; Egger et al., 2019). Egger et al. (2019) stated that the median concentration of Cd in AT was about 12.6 µg/kg. Moreover, the results of Echeverría et al. (2019) showed that the mean Cd concentrations in AT of breast and waist regions were 32 and 42 µg/kg respectively. They correlated this accumulation of Cd in AT with several parameters such as age, smoking, the types of food consumed and body mass index. In agreement with these studies, Salcedo-Bellido et al. (2021) reported that persons exposed to Cd exposure from sources such as smoking had more accumulated Cd in their AT. Data collected from studies using rodent models showed similar results. Kawakami et al. (2010) reported a correlation between Cd doses increment and Cd concentration elevation in AT of male SIc: ICR mice. This was represented by their data where Cd concentration in AT recorded 0.114 µg/kg at the lowest dose (5 µmol/kg), and 0.404 µg/kg at the highest (20 µmol/kg). Altogether, these data suggest that AT is a potential site for Cd accumulation. The risk of Cd accumulation in AT includes disrupting its capability to accommodate the surplus energy and produce the required adipokines for its endocrine function. Consequently, this may affect the systemic homeostasis since AT occupies a large part of the whole body.

Adipose Tissue Structure

Adipose tissue has a dynamic role, reflected by its heterogeneity, wide distribution, and unique structure. White adipose tissue (WAT) comprises a special loose connective tissue that is composed of unilocular adipocytes, parenchymal cells and the stromal vascular fraction (Frühbeck & Gómez-Ambrosi, 2013; Henriques et al., 2019). The stromal vascular fraction includes preadipocytes, endothelial cells, fibroblasts and immune cells such as macrophages (Henriques et al., 2019). The unilocular adipocyte is a single large droplet that occupies most of the cell besides the cytoplasm and the peripheral nucleus. Both the unilocular adipocyte and the low mitochondrial density characterize WAT morphology (Walker et al., 2014; Henriques et al., 2019). The lipid droplet size determines the size of adipocyte, which can range from 20 µM to 200 µM(Walker et al., 2014). Accordingly, the elasticity feature of the adipocytes is maintained since their size can vary in response to different physiological conditions (Walker et al., 2014). Due to the wide abundance of WAT, it can be classified based on the regional distribution throughout the body. The two main subtypes of WAT are the subcutaneous (SUB) and the visceral (VAT). The latter also can be further subdivided into omental, mesenteric, perirenal, and peritoneal fat depots (Choe et al., 2016). Subcutaneous AT is located in the innermost layers of the skin and has a primary function of energy storage (Badimon & Cubedo, 2017; Henriques et al., 2019; Kahn et al., 2019). Moreover, SUB-AT is responsible for thermal insulation and providing a protective cushion against mechanical damage (Choe et al., 2016; Chait & den Hartigh, 2020). On the other hand, the VAT is located in the internal organs and is known for its high metabolic response (Badimon & Cubedo, 2017; Kahn et al., 2019).

Both SUB-AT and VAT have different metabolic functions and different adipokine expression profiles.

According to *in vivo* studies, VAT shows a high inflammatory profile indicating its association with pro-inflammatory conditions (Badimon & Cubedo, 2017). Another study compared SUB-AT and VAT in the case of type 2 diabetes and reported that VAT had a higher expression level of adipokines involved in inflammation (Samaras et al., 2010). In contrast, SUB-AT was found to have a higher expression level of adipokines that are involved in energy expenditure, such as leptin and adiponectin (Samaras et al., 2010; Item & Konrad, 2012). Under physiological conditions, SUB-AT accounts for 80% of the total fat mass, while VAT represents around 10-20% (Henriques et al., 2019; Chait & den Hartigh, 2020). The ratio of SUB-AT to VAT varies depending on several factors such as nutrition, age, sex, and the homeostasis of each specific depot (Schoettl et al., 2018). As reported by Frühbeck & Gómez-Ambrosi (2012), the regional distribution of fats is associated with several inner organs like the kidney, liver, and heart which could be a key indicator for several metabolic alterations. Subsequently, AT responds to these alterations by remodeling adipocytes through changes either in the number, size, or both (Choe et al., 2016).

Cadmium and WAT Structure

Adipose tissue structure is associated with adipogenesis which is responsible for the maturation of preadipocytes to adipocytes. The main differentiation markers of AT are CCAAT/enhancer-binding protein (C/EBP) and Peroxisome proliferator-activated receptor-gamma (PPAR- γ). The disruption of adipogenesis leads to the impairment of the differentiation capacity of adipocytes, which then induces dysfunction of AT structure. *In vitro*, Cd was found to inhibit the differentiation of

3T3-L preadipocytes in a dose-dependent manner (E. Lee et al., 2012). The researchers, Lee et al. (2012), suggested that the inhibition occurred through the pathways of C/EBP and PPAR- γ since their expression level was found to be drastically decreased. *In vivo* study done by Kawakami et al. (2010), reported a reduction pattern of both C/EBP and PPAR- γ expression levels after Cd-exposure in a dose-dependent manner. This was accompanied by a significant decrease in mice AT weight and adipocytes size (Kawakami et al., 2010). Moreover, under acute Cd exposure, the size of the adipocytes of MT- null mice was found to be significantly reduced (Kawakami et al., 2013). In addition to that, the expression level of both C/EBP and PPAR- γ were significantly decreased (Kawakami et al., 2013). According to Kawakami et al. (2013), these results suggested that Cd has direct effects on accelerating the lipolysis process since it suppresses the expression level of adipogenic markers such as PPAE-γ and C/EBP-α. Furthermore, a recent study reported a significant reduction in body weight and adipocyte size after eight weeks of Cd exposure in the mice model (Prabhu et al., 2020).

These results are in agreement with the results of other heavy metals studies. For example, Rizzetti et al. (2019), found that mercury (Hg) induces WAT disruption, which negatively reflectes the signaling events and metabolic activities. Their results suggested that the reduced size of adipocytes that occurred due to Hg exposure led to endoplasmic reticulum (ER) stress, attenuated antioxidant defenses, and disrupted the mRNA expression level of GRP78, PPARα, PPARγ, leptin, and adiponectin in WAT. However, two studies that explored the Cd effect on AT in rat models reported no change in body weight and adipocyte size (Ficková et al., 2003; Treviño et al., 2015). Collectively, Cd exposure has the potential to induce alterations into the structure of AT. It is worth mentioning that the structural changes resulted from Cd exposure lake consistency due to differences in experimental design that includes doses, duration, and

species. Considering the wide distribution of AT throughout the body, any changes related to the maturation of AT, or its structure may impact AT's primary functions.

Adipose Tissue Function

The main function of AT is to regulate the systemic energy storage and release through lipogenesis and lipolysis (Matafome & Seiça, 2017). Under the condition of excess energy, AT stores it in the form of triglycerides via lipogenesis. While under the systemic demand of energy, triglycerides are released through lipolysis into free fatty acids and glycerol. The balance between lipogenesis and lipolysis indicates healthy functional AT which can be reflected in the circulating lipid profile. The relationship between the circulating lipid profile and the main functions of adipose tissue, such as adipogenesis, lipogenesis, and lipolysis under Cd exposure, was extensively reviewed by Attia et al. (2021). A recent study investigating the effect of chronic low-dose Cd exposure on the circulating lipid profile reported a significant increase in serum levels of triglycerides (TGs), low-density lipoprotein (LDL), and total cholesterol. In contrast, high-density lipoprotein (HDL) and glutathione serum levels were reduced significantly in SD rats (Samarghandian et al., 2015). This study is consistent with those of Afolabi et al. (2012) and Treviño et al.(2015), who conducted it using Wister rats with acute high dose exposure to Cd and chronic exposure to Cd with multiple doses, respectively. The increment of TGs, LDL, and total cholesterol accompanied by a decrease in HDL in the circulation could be attributed to adipocyte dysfunction.

Furthermore, the endocrine function of AT is vital, where adipokines are released to contribute to a complex network of signals (Frühbeck & Gómez-Ambrosi, 2013). Adipokines that AT produces are involved in the metabolic regulation and play an essential role in maintaining systemic functions such as inflammatory and

immunological responses, vascular events, reproductive functions, appetite regulation, and insulin sensitivityy (Henriques et al., 2019). Additionally, some of these secreted adipokines exert both autocrine and paracrine actions, which mainly affect the processes of AT remodeling, angiogenesis, and adipogenesis (Ordovas & Corella, 2008; Henriques et al., 2019). The AT secretory status depends on the changes of cellular tissue composition, including alterations in the phenotypes, numbers, and site of adipose tissue depots (Ouchi et al., 2011). There are two types of adipokines, pro-inflammatory such as leptin, monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) and anti-inflammatories such as adiponectin and interleukin 10 (IL-10) (Mancuso, 2016; Hui & Feng, 2018; Henriques et al., 2019). Studies showed that adipocyte secretion of adiponectin, leptin, resistin and TNF-α is related to increased risk of type 2 diabetes and arteriosclerosis (Kawakami et al., 2010; Samaras et al., 2010). The initial adipokines to be discovered were leptin and adiponectin and accordingly, AT was identified as an endocrine organ.

Leptin

Leptin is an adipokine secreted by AT and was the main reason for adipose tissue recognition as an endocrine organ when first discovered in 1994 (Zhang et al., 1994). Studies found that WAT secrets leptin into the bloodstream and act on LEPR/LepR receptors in the hypothalamus to regulate food intake and energy expenditure in both animals and humans (M. Li, 2011; Münzberg & Morrison, 2015). The circulating level of leptin was found to be directly proportional to body fat mass, and its receptor is expressed abundantly in many tissues (Stern et al., 2016). Accordingly, leptin signals have an essential contribution to regulating AT metabolism, appetite, satiety, puberty, fertility, and reproductive function (Fasshauer & Blüher, 2015; Stern et al., 2016). The long-form receptor LepRb initiates intracellular signaling

by activating the tyrosine kinase (JAK-2), including multiple downstream factors such as STAT-3 and SHP-2. These factors regulate activation of ERK and suppression of cytokine signaling 3 (SOCS3) and PI3K pathways which contribute to the innate immune response (Mancuso, 2016; Kahn et al., 2019). Moreover, leptin can directly increase pro-inflammatory cytokines such as TNF-α and IL-6 in monocytes. Also, it enhances the production of chemokines like MCP-1 and IL-8 in macrophages and the lipid mediators PGE2 cysteinyl leukotrienes (Ouchi et al., 2011; Mancuso, 2016). Also, leptin in monocytes can stimulate ROS production, promoting cell proliferation and migratory response (Ouchi et al., 2011). Since leptin's primary known function is appetite regulation, in the case of starvation/fasting, low leptin enhances the stimulation of high food intake and low energy expenditure and prevents starvation-induced changes (Kahn et al., 2019). In addition, studies showed that leptin plays a role in glucose metabolism regulation, exerts insulin-sensitizing effects, and is considered a key regulator of β-cells (Denroche et al., 2012; Fasshauer & Blüher, 2015; Stern et al., 2016). Furthermore, leptin has been found to have a protective role for β-cells from lipotoxicity in multiple rodent models and culture cell lines (Stern et al., 2016). According to Denroche et al. (2012), leptin has an essential effect on glucose homeostasis since it can normalize glucose levels in the blood when insulin deficiency (type 1 diabetes) occurs in non-obese rodents. Also, leptin deficiency causes multiple metabolic disorders such as hyperphagia, diabetes, morbid obesity and immunologic dysfunction (Henriques et al., 2019).

In terms of Cd exposure and its effect on leptin, a study done by Levy et al. (2000) reported a reduction of leptin expression level under the Cd exposure in a dose-dependent manner (Levy et al., 2000). Similar results reported that Cd exposure decreases the expression level of leptin (Kawakami et al., 2010, 2012, 2013). *In vitro*,

treated adipocytes with high Cd concentration showed decreased in leptin secretion and phosphor-diesterase activity stimulation (Levy et al., 2000; Kawakami et al., 2010). These results are consistent with studies of other heavy metals on AT and adipokines. A study was done in China on children exposed to lead pollution and tested the adipokines level in their blood (Yang et al., 2014). Both leptin and IL-8 adipocytokine were detected. However, children with high blood lead levels had higher IL-8 than leptin. On the other hand, children with low blood lead levels, had higher leptin than IL-8. Thus, heavy metals exposure even at low doses has a potential impact on the pattern of leptin expression, suggesting induction of function disruption.

Adiponectin

Adiponectin is an adipokin produced exclusively by adipocytes with a high level in the blood that ranges between 3 to 30 µg/ml, and it targets different cell types (Fasshauer & Blüher, 2015; Mancuso, 2016). As a complex molecule, adiponectin has three forms which are low, intermediate, and high molecular weight complexes in the circulation. Also, it has two receptors, AdipoR1 and AdipoR2, which mediate the actions of adiponectin and activate the AMP-activated protein kinase (AMPK) pathway (Fasshauer & Blüher, 2015; Mancuso, 2016). The metabolic properties of adiponectin are favorable since it is an anti-inflammatory adipokine that can inhibit the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). As a result, it inhibits inflammation, reduces the expression of TNFα and IL-6, and regulates glucose metabolism and energy homeostasis (Chandrasekar et al., 2008; Novotny et al., 2012; Vasiliauskaité-brooks et al., 2017). The promotor of the adiponectin gene was found to have a various number of transcription factor binding sites such as PPARs (α & γ), C/EBPs, and response element-binding protein (SREBP) (Astapova & Leff, 2012). These transcriptional factors positively regulate the transcription and expression

of the adiponectin gene (Astapova & Leff, 2012). Thus, any alterations on these factors could adversely affect adiponectin expression. Furthermore, adiponectin is found to have a substantial role in improving insulin secretion through stimulating the insulin gene expression and the exocytosis of insulin granules (Fasshauer & Blüher, 2015). Adiponectin overexpression leads to the improvement of the insulin sensitivity system, while the opposite is related to lowering insulin sensitivity (Holland et al., 2011). According to Awazawa et al. (2009), the signals of adipoR1 mediate the liver kinase B (LKB-AMPK) pathway, which reduces the genes expression involved in both hepatic lipogenesis and cholesterol synthesis. This was attained by suppressing the expression of sterol response element binding protein-1C (SREBP1c). Accordingly, adiponectin contributes to reducing hepatic lipogenesis and the elevation of β-oxidation via both receptors that mediate the activation of AMPK and PPAR-α in skeletal muscle and liver (Ouchi et al., 2011; Stern et al., 2016). Activation of AMPK increases fatty acid oxidation and glucose uptake in the muscles tissue and inhibits gluconeogenesis in the liver (Ouchi et al., 2011). As a result, the deficiency of AdipoR1 reduces the AMPK activation and increases the production of glucose. While in the case of AdipoR2, the signaling activity of the PPARα pathway is decreased, and insulin resistance is enhanced (Ouchi et al., 2011; Iwabu et al., 2015). Thus, the disruption of both AdipoR1 and AdipoR2 receptors has a negative effect on adiponectin's binding ability and, consequently, its actions (Ouchi et al., 2011). This was confirmed by Iwabu et al. (2015) when using AdipoR1- and AdipoR2-knockout mice and found that the binding ability of adiponectin has been abolished in this animal model. That indicates the critical role of both receptors in mediating the action of adiponectin in the body. Correspondingly, when the secretion of adiponectin is decreased due to obesity or obesity-metabolic

disease, it causes insulin resistance and glucose intolerance (Capeau, 2007; Iwabu et al., 2015).

Few studies explored the effect of Cd on adiponectin expression. Under Cd acute exposure condition, the mRNA expression level of adiponectin in AT showed a significant reduction (Kawakami et al., 2010). Similarly, in MT-null mice, the expression level of adiponectin was significantly decreased in a dose-dependent manner (Kawakami et al., 2013). Moreover, the expression levels of the essential transcriptional factors peroxisome proliferator-activator receptor gamma (PPARγ) and CCAAT/enhancer-binding protein alpha (C/EBPα) were found to be reduced (Kawakami et al., 2013). These results match the collected data from *in vivo* studies after treatment with arsenic and mercury exposure (Farkhondeh et al., 2019; Rizzetti et al., 2019). These changes in adiponectin under unhealthy conditions could be used as a potential marker for AT dysfunction.

Cadmium-correlated metabolic disease

Adipose tissue dysfuncation has been suggested to have a significant role in enhancing the risk of metabolic disease development (Ordovas & Corella, 2008). As mentioned earlier, Cd has the ability to disrupt the structure and function of AT. This can lead to the development of metabolic, cardiovascular, and inflammatory diseases (Tellez-Plaza et al., 2013; Planchart et al., 2018; Das et al., 2021). Risk evaluation of metabolic diseases that is correlated with Cd can be assessed by following parameters: circulating lipid profile, GLUT4, insulin, and pro-inflammatory cytokines. According to several studies, under Cd exposure the circulating lipid profile was shown to change by increasing the levels of free fatty acids, triglycerides, low density lipo-protein, very-low-density lipoprotein, and decreasing of high-density lipoprotein (Alvarez et al.,

2007; Afolabi et al., 2012; Kisok Kim, 2012; Olisekodiaka et al., 2012; Treviño et al., 2015; Zhou et al., 2016). These changes in the circulating lipid profile are reflecting the disruption of AT metabolism and function. Another disrupted process is the insulin homeostasis. Cadmium was found to reduce GLUT 4 (insulin-responsive glucose transporter) which impairs glucose uptake. Han et al. (2003) reported a significant reduction in GLUT4 mRNA and GLUT4 protein levels in adipocytes of Cd-treated rats. In addition to that, Cd was found to reduce the insulin receptors in AT which undesirably impact the insulin signaling (Ficková et al., 2003). This is related to the adverse impact of Cd on leptin and adiponectin since both are involved in the insulin signaling regulation. Adiponectin is known for GLUT4 induction and glucose uptake enhancement (Ceddia et al., 2005; Nicholson et al., 2018). As for leptin, is related to the adipocyte size and food intake flux that either stimulates or inhibits insulin. By using insulin resistance adipocytes dysfunction index (IDA-IR), Cd-treated AT exhibited a significant increment of insulin resistance (Treviño et al., 2015). Similarly, a recent study found a positive correlation between the high Cd level of smoker's AT and the increased level of both HOMA-IR and insulin (Salcedo-Bellido et al., 2021). These data illustrate the negative effects of Cd on insulin signaling and its promotion for insulin resistance which play a crucial role in developing type-2-diabetes. In addition to that, several studies linked Cd with the induction of inflammatory events. The most produced pro-inflammatory cytokines (TNF-α and IL-6) in the condition of AT macrophage infiltration were found to be upregulated by Cd (Guzik et al., 2017; Salama et al., 2019). In contrast, an anti-inflammatory cytokine (IL-10) was found to be significantly downregulated (Salama et al., 2019; Choudhury et al., 2021). Cytokines such as TNF-α and IL-6 are known to activate nuclear factor kappa B (NF-κB) signaling pathway. Consequently, NF-κB signaling pathway activates the transcription of

proteins and inflammatory factors that are involved in inflammatory pathways (Matafome & Seiça, 2017; Hossein-Khannazer et al., 2020). A study by Freitas & Fernandes (2011) reported that Cd highly activates NF-κB signaling pathway and induces TNF-α, and IL-6 release in monocytes. As a result, upregulation of pro-inflammatory inhibit the anti-inflammatory adipocytokines such as adiponectin and IL-10 (Fasshauer et al., 2003; Astapova & Leff, 2012; Upadhyaya et al., 2014; Mancuso, 2016). Moreover, TNF-α and Il-6 were found to downregulate the gene expression of adiponectin (Astapova & Leff, 2012).

Most of the above-reported studies investigated the effect of acute Cd exposure on organs such as liver and related biological function. However, few studies investigated chronic exposure of Cd on AT function. Thus, this study is carried out to further investigate the effect of chronic exposure to low dose of Cd on AT function through evaluating the pattern of adipocyte secretions and testing. It is hypothesized that chronic low dose cadmium exposure affects the functionality of white adipose tissue.

Research Objectives

1. Evaluation of expression level of adipokines (adiponectin and leptin) in rat adipose tissues after Cd exposure.
2. Effect of Cd on the level of MCP-1expression in rat adipose tissues after Cd exposure.
3. Comparison between three adipose depots (subcutaneous, abdominal and retroperitoneal).

CHAPTER 3: MATERIALS AND METHODS

Adipose Tissue Samples

Preserved adipose tissue samples (Retroperitoneal, subcutaneous and abdominal) were obtained from Dr. Hamda Al-Naemi tissue repository at Laboratory Animal Research Center (LARC), Qatar University. Tissue samples were collected under study approved by Institutional Animal Care and Use Committee IACUC # QU-IACUC 038/2017 and conducted by Al-Naemi and Das (2020) at Laboratory Animal Research Center (LARC); Qatar University.

Study Design and Treatment Procedures are reported in details by (Al-Naemi & Das, 2020). Briefly, adult male Sprague Dawley (SD) rats were divided into two groups, control and cadmium-treated. The control group received normal drinking water while Cd treated group received Cd in drinking water with dose of 15 mg Cd/kg body weight (B.W.) as $CdCl_2$ (BDH Chemicals, England) for 10 weeks. Then, the animals were sacrificed using anesthesia with sodium thiopentone (40 mg/kg B.W., i.p.) and adipose tissue (Retroperitoneal, subcutaneous, and abdominal) were collected, frozen in liquid nitrogen and stored in the repository at − 80 °C.

Gene Expression Assay

Adipose tissue previously stored at -80°C were homogenized using both liquid nitrogen and probe sonicator, then total RNA was extracted from adipose tissue using TRIzol™ LS Reagent (ThermoFisher Scientific, USA; 10296010). Slight modifications were followed at the washing step, where the RNA pellet was resuspended in ice-cold 75% ethanol and kept at-20°C for overnight. Then, washed three times to enhance the RNA purity. Total RNA was quantified using nanophotometer (Implen; P330). A known amount of RNA samples (150 ng) was reverse transcribed into cDNA using the high-capacity cDNA transcription kit individually (Applied Biosystems, Lithuania)

following the manufacturer's instructions. Final volume of the obtained cDNA was 20 µl and stored at -20°C until the performance of RT-PCR gene expression assays. RT-PCR was performed using diluted cDNA (1:3) and TaqMan® Fast Advanced Master Mix (Applied Biosystems, USA) for six targets as summarized in Table 1. GAPDH (Rn01775763_g1) was assigned as the endogenous gene. The amplification was carried out by QuantStudio 6 flex (Applied biosystem™). The relative quantity of gene expression was calculated using 2-ΔΔCt method. Results are presented as fold change (log2) versus the mean values of the control samples normalized against the endogenous gene.

Table 1. TaqMan Gene Expression Assays

Targeted Gene	Taqman Assay ID
Adiponectin	Rn00595250_m1
Leptin	Rn00565158_m1
MCP-1	Rn00580555_m1
IL-6	Rn01410330_m1
IL-10	Rn01483988_g1
TNF-α	Rn01525859_g1

Protein Expression Study

The followed protocol of protein extraction was adopted from Marin et al. (2019) with few modifications. Briefly, adipose tissue samples were homogenized using liquid nitrogen and with probe sonicator. Then, further homogenized sample was subjected to cell lysis using cell lysis buffer (CLB) which is a mixture of RIPA buffer (Thermo-Fisher Scientific, USA) and cocktail protease & phosphatase inhibitor (Thermo-Fisher Scientific, USA). A fixed amount of CLB was added to each tissue sample with a ratio

of 600 **μ**l (CLB)/ 100 mg (tissue weight) individually. Then, samples were incubated in ice for 1h, then centrifuged three times; each round was 15 minutes on a speed of 20,000 rcf at 4°C. Upper lipid layer was removed after each centrifugation and the supernatant was transferred to a new tube. Finally, total protein was quantified using Bicinchoninic acid (BCA) kit (Thermo-Fisher Scientific, USA) with bovine serum albumin (BSA) as reference by following the manufacture instructions, and the absorbance was measured at 562 nm using Versamax Multiplate Reader (Molecular Devices).

Western Blotting

Protein samples (containing 30 μg/well) were loaded onto 10% SDS-PAGE gel and separated by electrophoresis using Tricine buffer. Electrophoresis conditions and protein visualization steps were adopted with few modifications from Hermann Schägger (2006) and Haider et al. (2012). For gel imaging, stain-free gel (TGX stain free FastCast Acrylamide starter kit, 10% - BioRad) was used to capture the separation pattern of each tissue type. Then, the separated proteins were transferred to PVDF membrane (0.2 μm) at 90 V for 35-51 minutes in 1x TGS transfer buffer. PVDF membrane was selected to facilitate the probing and stripping process as citied in multiple studies (Alvarez et al., 2007; Buettner et al., 2008; Diaz Marin et al., 2019). Next, PVDF membranes were washed with 1X TBS buffer containing 0.1% Tween 20 (washing buffer) and blocked in 5% skimmed milk buffer for 1 h. After three washes, PVDF membranes were incubated with specific primary antibodies against adiponectin (Abcam, ab62551) at 1:500 dilution, leptin (Abcam, ab3583) at 1:500 and MCP-1 (Abcam, ab9669) at 1:4000, all for overnight at 4°C. This was followed by three washes with washing buffer, then the PVDF membranes were incubated with the secondary anti-rabbit (Abcam, ab205718) at 1:15,000 for 1h at room temperature. The

immunoreactive bands were detected using ECL method using Syngene G box Gel documentation system.

Statistical Analysis

Data is presented as means ± SEM. Statistical significance of differences in means is determined by a one-way or two-way ANOVA followed by comparison test. P-value <0.05 is considered a significant value. All statistical analysis is performed using GraphPad Prism version 9.

CHAPTER 4: RESULTS

Effect of Cadmium on the Expression level of Adipokines in Adipose Tissue

The RNA extraction process from AT is challenging due to the nature of AT structure and composition, such as high-triglycerides content, low cell count, and the presence of other cells (i.e., endothelial cells and fibroblasts). However, RNA of adipose tissue was successfully extracted using the optimized TRIzol™ LS method. Total RNA was quantified using the nano-photometer to determine the concentration and quality of the extracted RNA. Table 2 shows the concentrations of control (C) and Cd-treated (T) samples. The purity of the extracted RNA is represented by the ratio of (260/280) and ranges between 1.9-2.0, indicating a high purity level. Accordingly, 150 ng of RNA/sample were reverse transcribed into cDNA using the high-capacity cDNA transcription kit for RT-PCR gene expression.

Table 2. RNA Quantification of Adipose Tissue from Control and Cd-treated rats

Tissue Type	Sample	RNA Concentration (ng/µl)	A260/A280	A260/A230
Retroperitoneal Adipose Tissue	Control- 1	452	1.965	1.725
	Control- 2	476	1.951	1.889
	Control- 3	468	1.983	1.934
	Treated-1	420	1.927	2.333
	Treated-2	590	1.916	1.161
	Treated-3	476	1.915	2.164
	Treated-4	494	1.960	1.350
	Treated-5	510	1.962	2.179
	Treated-6	422	1.901	1.486

Tissue Type	Sample	RNA Concentration (ng/µl)	A260/ A280	A260/A230
Abdominal Adipose Tissue	Control- 1	492	1.937	2.216
	Control- 2	648	1.976	2.09
	Control- 3	348	1.933	1.891
	Treated-1	464	1.983	1.95
	Treated-2	596	1.961	1.560
	Treated-3	470	1.942	1.880
	Treated-4	686	1.983	1.874
	Treated-5	534	1.963	1.628
	Treated-6	436	1.982	1.874
Subcutaneous Adipose Tissue	Control- 1	680	1.977	1.628
	Control- 2	582	1.966	1.802
	Control- 3	1302	1.997	1.429
	Treated-1	1340	1.994	2.094
	Treated-2	2488	2.016	2.170
	Treated-3	914	1.978	2.175
	Treated-4	796	1.990	2.221
	Treated-5	776	1.970	1.896
	Treated-6	1028	1.977	1.941

Adipokines Gene Expression Level in AT of Cd-treated Rats

The level of adipokines (adiponectin and leptin) expression levels were investigated. Figure.1 shows a common trend of downregulation for both adiponectin and leptin within the three AT types. The gene expression levels of adiponectin and leptin in AB-AT (B) and REtrop-AT (C) showed a decrease without a significant difference in the gene expression levels of targeted adipokines based on $2^{-\Delta\Delta Ct}$ calculations. However, between the adipokines, a significant difference was found between leptin and adiponectin in AB-AT. Unlike REtrop-AT and AB-AT, there is a significant downregulation of both adiponectin and leptin gene expression levels in SUB-AT (A).

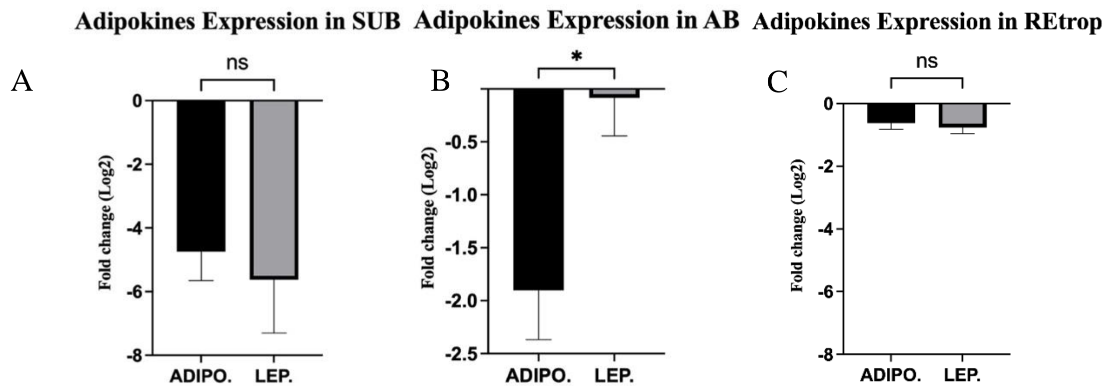

Figure 1. Effect of Cd-treatment on the expression level of adiponectin and leptin in adipose tissue of male Sprague-Dawley rats.
(A) subcutaneous adipose tissue, (B) abdominal adipose tissue, (C) retroperitoneal adipose tissue. Gene expression results were generated using 2-ΔΔCt method. An unpaired t-test comparison was performed using GraphPad Prism version 9, the significant different is represented by (* P-value < 0.05), n=6.

MCP-1 and Adipokines Gene Expression Levels in each AT Type

The levels of MCP-1 and adipokines (adiponectin and leptin) expression levels within each AT type were investigated. No significant differences were observed between the expression level of MCP-1 and adipokines in SUB-AT and REtrop-AT. However, in AB-AT, a statistically significant difference was found between the expression levels of MCP-1 and adiponectin. Another observation was noted regarding the higher expression level of examined targets in SUB- AT compared with REtrop-AT and AB-AT.

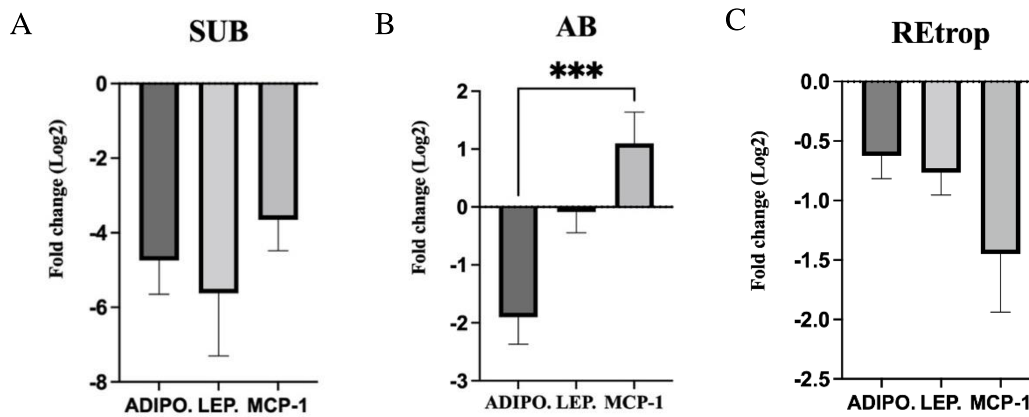

Figure 2. Relationship between the expression level of MCP-1 and adipokines of three different adipose tissue depots of male Sprague-Dawley rats after Cd-treatment. ((A) subcutaneous adipose tissue, (B) abdominal adipose tissue, (C) retroperitoneal adipose tissue. Gene expression results were generated using $2^{-\Delta\Delta Ct}$ method. One-way ANOVA was performed using GraphPad Prism version 9, the significant different is represented by (*** P-value < 0.001), n=6.

Gene Expression of MCP-1 and the Inflammatory Mediators in AT

A trend of downregulation is shown in REtrop-AT and SUB-AT for the expression level of MCP-1 as shown in Figure 3. Whereas in AB-AT, a non-significant upregulation in the expression level of MCP-1is observed. The gene expression of IL-6 tended to decrease in both REtrop-AT and AB-AT, and it was under the detection limit in SUB-AT. Similarly, IL-10 gene expression showed a trend of downregulation in all AT tissues. TNF-α gene expression was found to be downregulated in SUB-AT. While in REtrop-AT ad AB-AT, an upregulation of TNF-α was observed but is not significant based on $2^{-\Delta\Delta Ct}$ calculations. Notably, SUB-AT showed a higher expression level in terms of the MCP-1 and IL-10 fold change that recoded 4.5 and 3.5 respectively. Also, a statistically significant difference was found between the TNF-α and MCP-1 gene expression levels in SUB-AT.

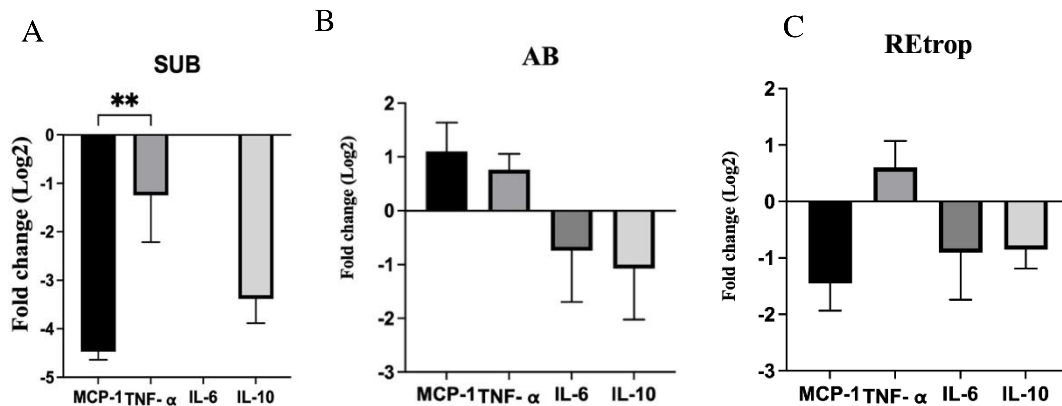

Figure 3. Effect of Cd-treatment on the expression level of MCP-1 in three different adipose tissue depots of male Sprague-Dawley rats and its relation with selected inflammatory markers.
(A) subcutaneous adipose tissue, (B) abdominal adipose tissue, (C) retroperitoneal adipose tissue. Gene expression results were generated using $2^{-\Delta\Delta Ct}$ method. One-way ANOVA was performed using GraphPad Prism version 9, the significant different is represented by (** P-value < 0.01), n=6.

Comparison Between Gene Expression Levels of Adipokines and MCP-1 in all AT Types

The expression pattern of adipokines varies from one AT depot to another. Accordingly, further investigation was done to compare the gene expression level of adiponectin, leptin, and MCP-1 in REtrop-AT, AB-AT, and SUB-AT of Cd-treated rats. Using Two-way ANOVA, SUB-AT showed high significant difference relative to REtrop-AT and AB-AT ($P < 0.001$). As shown in Figure 4, adiponectin and leptin expression levels were found to be significantly expressed in SUB-AT, whereas both REtrop-AT and AB-AT showed less significant expression. Also, a significant difference ($P<0.001$) was found between the gene expression of MCP-1 in SUB-AT and AB-AT.

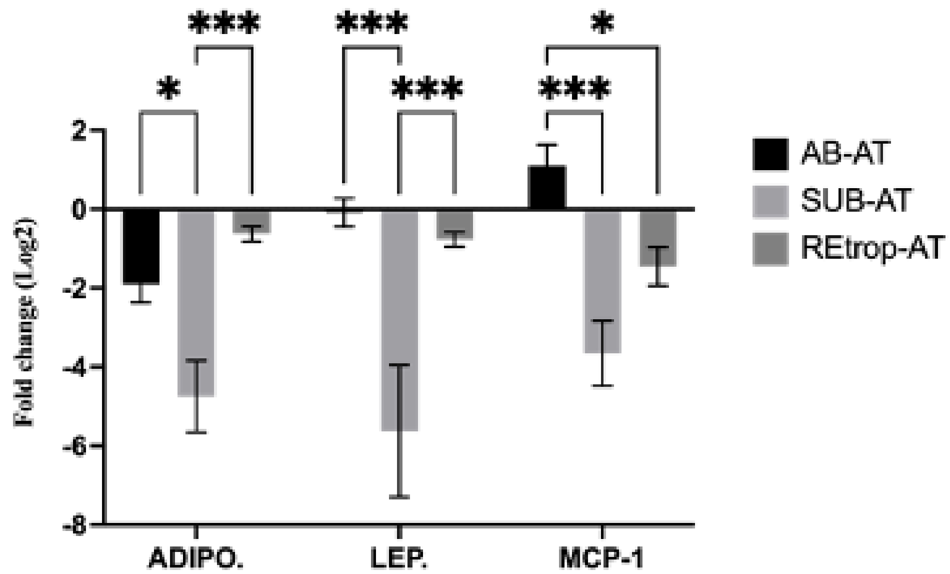

Figure 4.Comparison between the expression level of adiponectin, leptin and MCP-1 in AT depots of male Sprague-Dawley rats.
abdominal adipose tissue(AB-AT), subcutaneous adipose tissue (SUB-AT), retroperitoneal adipose tissue (Retrop-AT). Gene expression results were generated using $2^{-\Delta\Delta Ct}$ method. 2way ANOVA was performed using GraphPad Prism version 9, the significant different is represented by (* P-value < 0.5, **P<0.1, ***P<0.001), n=6

Total protein Quantification

Total protein quantification was determined using BCA protein assay against the standard protein BSA. The concentration of total protein extracted from each depot is shown in Table 3 where BCA protein assay was performed in duplicate. And the mean of protein concentration is presented as mean ± SEM. Protein concentrations were calculated based on the standard curve of standard protein BSA as shown in Figure 5. The calculated concentrations of total protein extracted from SUB-AT were the highest whereas the concentrations of protein extracted from REtrop-AT were lowest (Table 3).

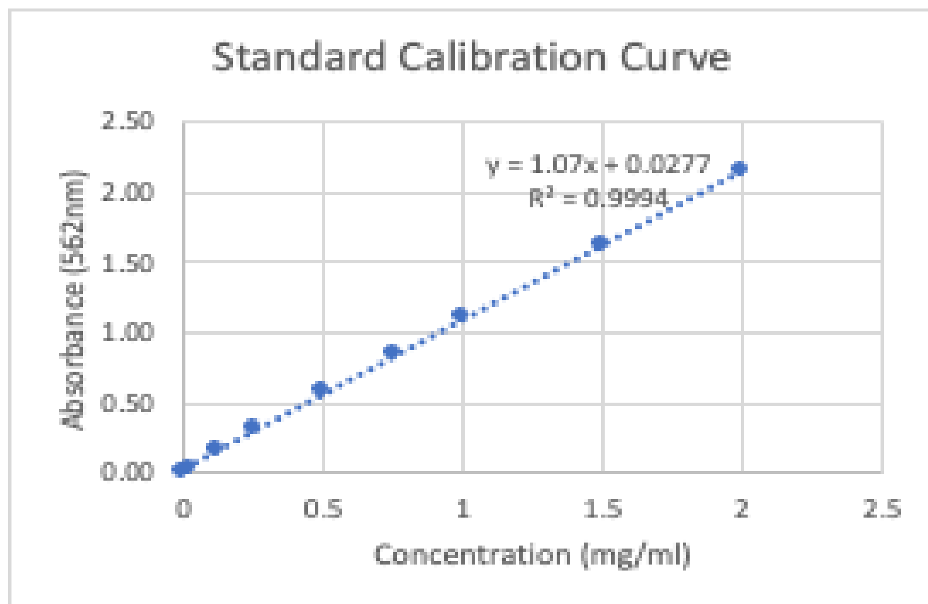

Figure 5. Standard Calibration Curve for BCA assay.Standard used in this assay is bovine serum albumin. Assay was performed in duplicates.

Table 3. Protein Quantification of Adipose Tissue from Control and Cd-treated rats.

Tissue Type	Sample	Mean of Protein Concentration (mg/ml)
Subcutaneous Adipose Tissue	Control- 1	1.3 ± 0.153
	Control- 2	
	Control- 3	
	Treated-1	1.9 ± 0.251
	Treated-2	
	Treated-3	
	Treated-4	
	Treated-5	
Abdominal Adipose Tissue	Control- 1	0.9 ± 0.140
	Control- 2	
	Control- 3	
	Treated-1	1.6 ± 0.06
	Treated-2	
	Treated-3	
	Treated-4	
	Treated-5	
Retroperitoneal Adipose Tissue	Control- 1	0.9 ± 0.07
	Control- 2	
	Control- 3	
	Treated-1	1.0 ± 0.05
	Treated-2	
	Treated-3	
	Treated-4	
	Treated-5	

Total protein separation of AT depots from control and Cd-treated Rats

To evaluate the expression level of adipokines, 30 μg of extracted protein from each AT type was separated in 10% SDS-PAGE. The area of focus for the targeted proteins starts form 50 kDa and below. As shown in Figure 6 bands are shown in the aimed molecular weights. However, at REtrop-AT the lower the molecular weight the less band are detected as shown in Figure 7&8 especially below 20 kDa. This accord with the concentration of extracted protein from each depot.

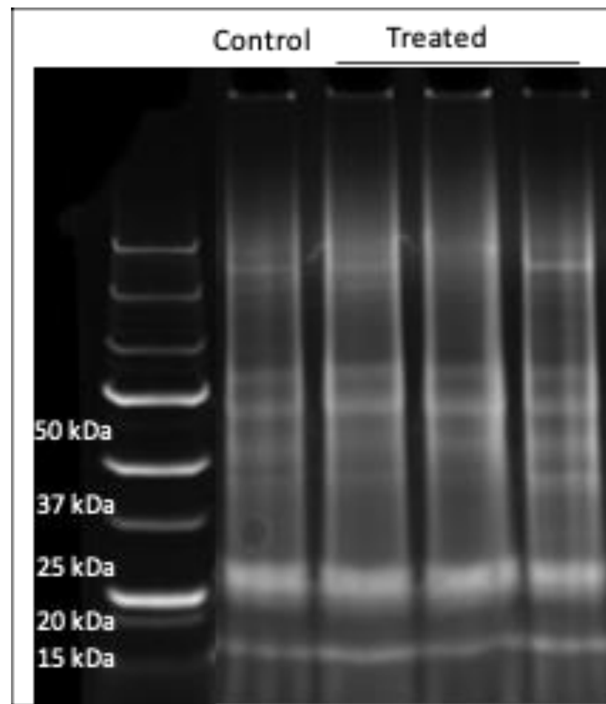

Figure 6. Representative image of stain-free gel of subcutanous AT after SDS-PAGE.

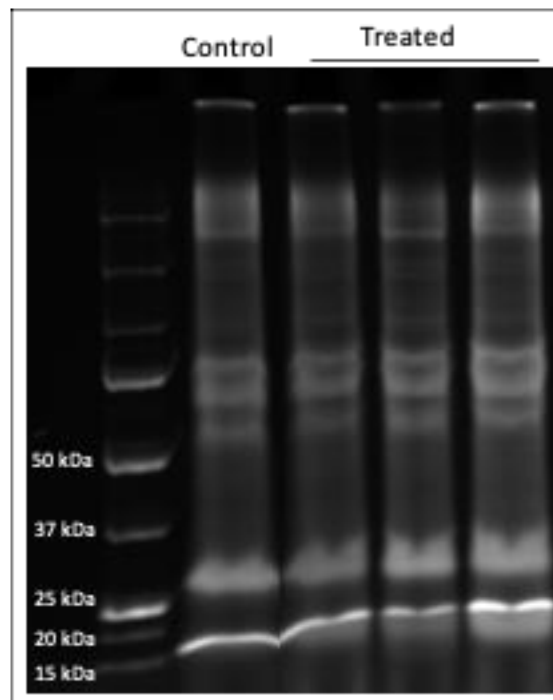

Figure 7. Representative image of stain-free gel of Abdominal AT after SDS-PAGE.

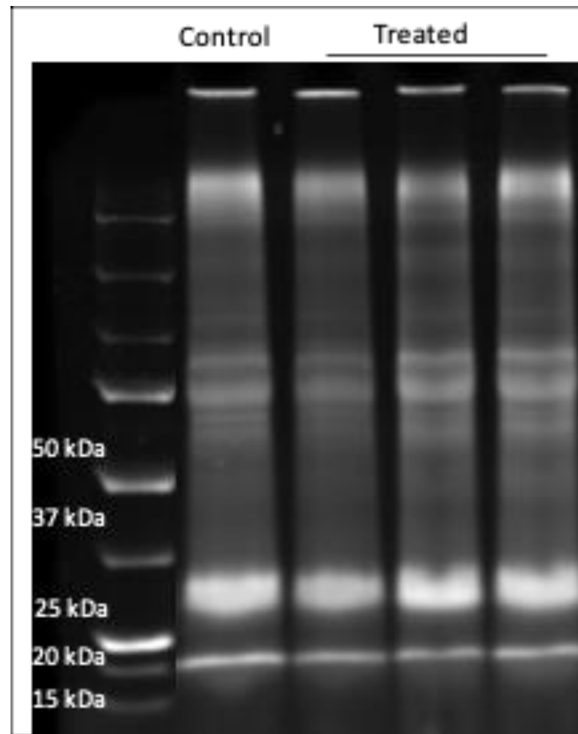

Figure 8. Representative image of stain-free gel of retroperitoneal AT after SDS-PAGE.

Protein expression of Adiponectin in AT depots

The expression of adiponectin was evaluated using western blot qualitative analysis. As shown in Figure 9, expression of adiponectin was detected in all AT depots. It was observed that adiponectin at all AT depots detected at 50 kDa which is higher than the theoretical value due to the fats that hinder the migration of protein through the gel. Adiponectin expression in SUB-AT was found to be higher than AB-AT and REtrop-AT.

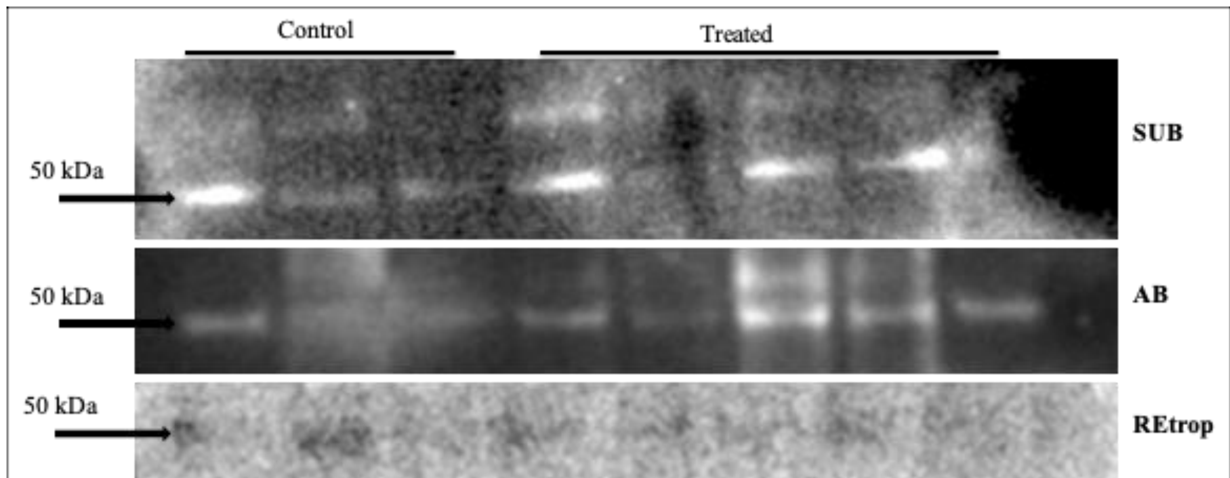

Figure 9. Protein expression of adiponectin in AT depots of subcutaneous, abdominal, and retroperitoneal.

Protein expression of Leptin in AT depots

The expression of leptin was evaluated using western blot qualitative analysis. As shown in Figure 10, expression of leptin was detected in all AT depots at 25 kDa. It was observed that leptin expression was the lowest at Retrop-AT compared to SUB-AT and AB-AT. However, SUB-AT expressed more leptin than AB-AT and REtrop with noticeable decrease in the treated samples.

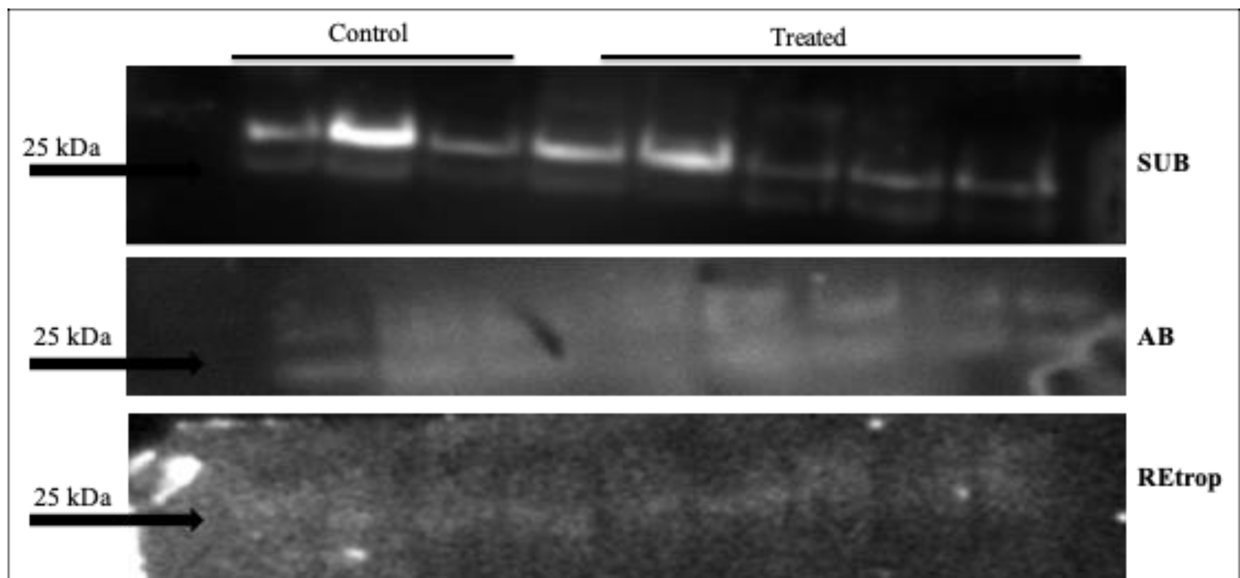

Figure 10. Protein expression of leptin in AT depots of subcutaneous, abdominal, and retroperitoneal.

Protein expression of MCP-1 in AT depots

The expression of MCP-1 was evaluated using western blot qualitative analysis. As shown in Figure 11, expression of MCP-1 was detected in all SUB-AT and AB-AT at 20 kDa. It was observed that MCP-1 was not detected in most of the treated samples compared to control. It is worth mentioning that the least protein concentration is the one extracted from REtrop-AT, though the initial weight of all AT depots was the same (Table 3). Moreover, the stained gel of REtrop-AT samples showed almost no bands at the molecular weights below 20 kDa (figure 8) where MCP-1 is detected. Therefore, MCP-1 protein expression was not detected in all the samples of REtrop-AT.

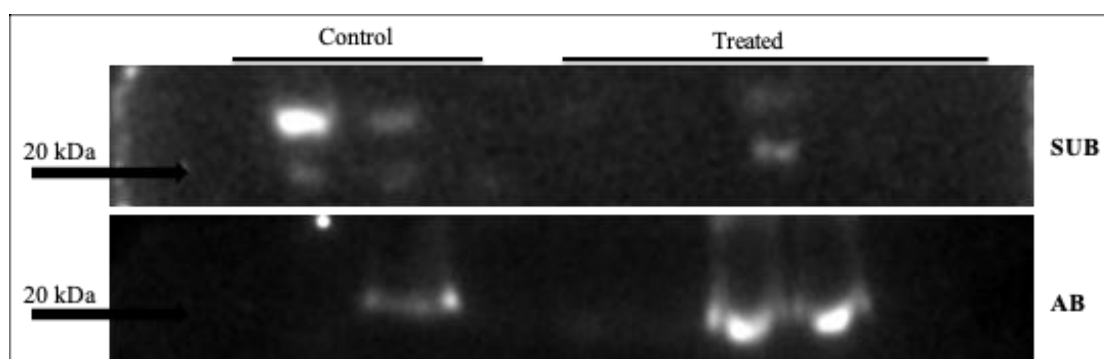

Figure 11. Protein expression of MCP-1 in AT depots of subcutaneous and abdominal.

The protein expression of Adipokines and MCP-1 in all AT depots

The protein expression of each target was evaluated in AT depots (Table 4). Adiponectin and leptin were found to have a higher expression in SUB-AT compared to AB-AT and REtrop-AT. On the other hand, the lowest MCP-1 expression was found at SUB-AT compared to AB-AT.

Table 4. The Protein Expression of Adipokines and MCP-1 in AT depots.

Tissue Type	Target	Control			Treated		
Subcutaneous Adipose Tissue	Adiponectin	++	+++	+	+++	+++	+
	Leptin	+++	++	++	+	+	+
	MCP-1	+	-	-	+	-	-
Abdominal Adipose Tissue	Adiponectin	++	++	+	+++	+++	+
	Leptin	++	+	++	++	+	+
	MCP-1	+	-	-	+	+	-
Retroperitoneal Adipose Tissue	Adiponectin	+++	++	++	+	++	+
	Leptin	+	+	+	+	+	+
	MCP-1	UN	UN	UN	UN	UN	UN

Expressed (+), Not expressed (-), Un-detected (UN). Increasing the number of (+) means higher intensity.

CHAPTER 5: DISCUSSION

Cadmium is a toxic environmental pollutant that has high accumulation properties in live tissues such as liver, kidney and adipose tissue. According to the literature, Cd accumulation imposes adverse effects on the function of targeted tissues (Genchi et al., 2020). To our knowledge, there are limited investigations on the link between chronic Cd exposure and WAT function. Therefore, this study attempted to study the impact of chronic Cd exposure on WAT function using three different AT depots of adult male SD rats. The expression levels of adiponectin and leptin in subcutaneous (SUB-AT) and visceral depots (AB-AT and REtrop-AT) were evaluated. Our results showed that adiponectin and leptin mRNA expression levels were significantly downregulated in SUB-AT. Moreover, a significant difference was found between subcutaneous AT and visceral AT (Retroperitoneal and Abdominal); indicating a depot-location properties when exposed to Cd. Conventionally, the expression patterns of adipokines differ between adipose depots (M. Lee et al., 2013). Subcutaneous adipose tissue expresses leptin and adiponectin more than visceral adipose tissue (Samaras et al., 2010; M. Lee et al., 2013; Mazaki-Tovi et al., 2016). This aligns with our results where the expression level of adiponectin and leptin is greater in SUB-AT than AB-AT and REtrop-AT.

Adipokines such as adiponectin and leptin are produced by mature adipocytes, which help adipocytes maintain the body energy homeostasis. Therefore, disrupting their production could be an indication of adipocytes dysfunction that might be linked to adipocytes maturation. Adipocyte's maturation is regulated by critical factors such as PPARγ and C/EBPα. A previous *in vitro* study reported that Cd adversely affects the differentiation of preadipocytes by downregulating the expression level of PPARγ and C/EBPα in 3T3-L1 adipocytes (E. Lee et al., 2012). This also accords with results

reported by Kawakami et al. (2010, 2013) that showed Cd exposure altered the expression level of the critical regulators of adipogenic differentiation PPARγ and C/EBPα in mice models. Linking these results with the downregulation of adiponectin and leptin supports the hypothesis that Cd adversely affects the differentiation capacity of the adipocytes. Under acute Cd exposure, similar significant downregulation of adiponectin mRNA expression level was detected (Kawakami et al., 2010). Another study conducted by Kawakami et al.(2013) using MT-null mice showed that acute exposure reduced the mRNA expression level of leptin and adiponectin in a dose-dependent manner.

In the present study, the protein production for adiponectin and leptin by adipocytes was investigated in the three adipose depots. Since adiponectin and leptin are secretory proteins, most studies assess their levels in blood samples. Although both adipokines have paracrine and endocrine effects and act on other tissues through their specific receptors, the AT remains to be their main production site. However, to our knowledge there is no available literature investigating the Cd impact on the protein production of adipokines at adipose depots. The results generated from the current study showed that chronic low dose Cd exposure does not inhibit both adipokines protein expression in all AT depots. According to the literature, Cd adversely affects the adiponectin circulating level (Valcke et al., 2019; X. Wang et al., 2021). A similar effect was observed with other heavy metals such as lead, mercury, and arsenic, where their exposure negatively affected the circulating level of adiponectin (Song et al., 2017; Valcke et al., 2019; Tinkov et al., 2021; X. Wang et al., 2021). Nevertheless, when the protein level of adiponectin in visceral AT and subcutaneous AT was compared with its level in circulation, no correlation was found (Jonas et al., 2017). Moreover, no correlation was found between the adiponectin mRNA-expression level and

adiponectin protein concentration in normal and abnormal adipose tissue (Jonas et al., 2017). Unlike adiponectin circulating level, adiponectin gene expression level did not correlate with protein content in AT and its secretion in both SUB and VAT (Kovacova et al., 2012; Korac et al., 2021). Similarly, an *in vitro* study reported that the reduction of adiponectin in the media did not mirror a reduction in adiponectin tissue content (Phillips et al., 2008). This suggests a posttranscriptional mechanism that might regulate the protein amount or posttranslational changes that affect the protein stability and secretion rates.

In addition, adiponectin has three isoforms in circulation, which are trimer (low molecular weight), hexamer (medium molecular weight), and multimeric (high molecular weight). The distribution of the circulating adiponectin oligomers is thought to be primarily regulated at the stage of secretion from adipocytes (Coelho et al., 2013). The adiponectin multimerization has a vital role in its biological function (Kovacova et al., 2012). As mentioned earlier in Chapter 2, it is evident that adiponectin exerts its action through two essential receptors, which are AdipoR1 and AdipoR2. The studies of Cd exposure effect on adiponectin receptors are scarce. However, a study reported that lead exposure caused downregulation of adiponectin receptor (Meyer et al., 2020). Several studies found that adiponectin receptors expression at gene and protein levels are positively associated with adiponectin circulating level (Kern et al., 2003; Blüher et al., 2007; Kollias et al., 2011; Jonas et al., 2017). This indicates the vital role of AdipoR1 and AdipoR2 in adiponectin functionality. Thus, the expressed adiponectin in the current study could be reflecting the non-secretory form of the protein that could be a result of deficiency in corresponding receptors. Further investigation is required to assess the expression level of both receptors at the gene level and protein level in AT which could provide further knowledge about adiponectin functionality under chronic

Cd exposure.

Unlike adiponectin, leptin production and secretion into the blood is proportional to the adipocytes size and numbers (Park & Ahima, 2014). As discussed above, leptin is produced by differentiated adipocytes, and its low expression could indicate a deficiency of the adipocytes maturation process. The data collected from the present study showed a low protein expression of leptin in SUB-AT when compared to adiponectin (Table 4). Also, the most downregulated adipokine in SUB-AT was leptin (Figure 2A). Together, this data supports the hypothesis that chronic Cd exposure dysregulates adipocytes maturation process which affects their secretion capacity. Leptin secretion rate increased or decreased independently of its mRNA expression (Barr et al., 1997) due to the presence of small vesicular stores in adipocytes (Harris, 2014). Thus, chronic low-dose Cd exposure could affect the secretion and production of leptin but was not enough to exhaust the leptin stores and entirely suppress leptin expression. In addition, leptin expression varies between fat depots and the favored depot to produce leptin is subcutaneous. Accordingly, the expression level of leptin in visceral AT (AB-AT & REtrop-AT) seems unaffected and not fluctuated compared to leptin expression in SUB-AT (Figure 9). Considering that SUB-AT is the depot to most accumulate Cd (unpublished data); this explains the dysregulated expression of adipokines at both protein and mRNA levels in SUB-AT compared to other depots.

There is an established link between the abnormality of adipocytes and the induction of inflammation, especially with obesity (Rull et al., 2010; Greevenbroek et al., 2016). In the case of obesity, well-known inflammatory markers in ATs are TNF-α, IL-6, and the elevated number of macrophages. In the present study, we investigated the effect of chronic Cd exposure on the adipose tissue macrophages (ATM) by assessing the expression level of MCP-1 as a functional protein for

monocytes/macrophages recruitment. MCP-1 is mainly produced under the state of AT abnormality. Our results showed a trend of downregulation in the mRNA expression level of MCP-1 in SUB-AT and REtrop-AT whereas it showed a non-significant up-regulation in AB-AT (Figure 3). The significant downregulation was found in SUB-AT with fold change of 4. Additionally, the protein expression of MCP-1 in most treated samples was suppressed in SUB-AT and AB-AT (Figure 10). Unlike adiponectin, leptin can enhance the production of MCP-1 and stimulates the macrophages infiltration in response to an excess food intake as part of its pro-inflammatory properties (Coelho et al., 2013; Mancuso, 2016). However, in the current work, leptin is downregulated which could explain the downregulation trend of MCP-1. Moreover, experimental results in the literature demonstrate a correlation between the AT leptin expression and markers of inflammation (Harris, 2014). The present study demonstrated that both mRNA and protein expressions of MCP-1 matched in SUB-AT (Figure 2A & Figure 10). The downregulation of MCP-1 protein expression might be a result of the low protein expression of leptin. To the best of our knowledge there are very limited studies that explore the effect of Cd on adipose tissue macrophages activity, especially at the protein level. Therefore, most of the interpretation of our data is based on the available literature of mRNA expression levels of MCP-1, the cytokines levels from macrophages cell lines as well as macrophages in AT under obesity condition. However, few studies reported that low dose Cd decreases essential proteins in macrophages which inhibit the motility of the macrophages and alter their interactions and migration ability. Of relevance, there is a noteworthy difference between the impact of high-dose Cd and low-dose Cd, especially on the macrophages activities and inflammatory mediators levels. The high-dose encourages their pro-inflammatory events while low-dose inhibits those events (Kiremidjian-Schumacher et al., 1981;

Olszowski et al., 2015; Mirkov et al., 2021). These pro-inflammatory events include: activating NF-κB pathway, inducing oxidative stress and recruiting more immune cells (Thévenod & Lee, 2013; Andjelkovic et al., 2019).

Macrophages quantity, activation state, and metabolic phenotypes are the main factors that determine macrophages function (Y. Li et al., 2020). Under pathological conditions, ATM can exhibit mixed phenotypes in response to local regulation (Z. Wang et al., 2021). However, the maintained cellular communication and crosstalk between adipocytes and macrophages is demonstrated by the ability of preadipocytes to differentiate into macrophages (Thomas & Apovian, 2017). Published data indicated that Cd adversely affects the differentiation capacity of preadipocytes into macrophages and interferes with the immune cells development (Z. Wang et al., 2021). Taken together, these research findings suggest that Cd might impair the differentiation of preadipocytes through diminishing the transcription factor PPARγ that could influence the differentiation of macrophages (Thomas & Apovian, 2017). The AT-derived MCP-1 expression in normal-weight and overweight women is associated with resident macrophages content, stromal vascular cells, and AT location as reported by Bruun et al.(2005). Accordingly, impaired adipogenesis leads to a decrease in macrophages differentiation capacity, which translates into the downregulation of MCP-1.

On the other hand, Kawakami et al.(2013) reported an increment in the number of macrophages and elevation of the mRNA expression level of MCP-1 after Cd treatment in the MT-null mice model in a dose-dependent manner. Another study reported acute Cd exposure caused an upregulation of MCP-1 mRNA expression levels in glioblastoma cell lines (Kasemsuk et al., 2020). Moreover, chronic low dose exposure to Cd was found to induce inflammatory cells infiltration in liver tissue associated with upregulation of MCP-1 mRNA expression level in the hepatocytes of

pubertal mice model (X. Li et al., 2021). These results are opposing to the results of our study that might be due to differences in the experimental design including the different animal models, Cd dose, and mode of administration (Kuester et al., 2002). Despite the dose, acute exposure of Cd caused an upregulation of MCP-1 mRNA expression level within the first 24h, while the chronic Cd exposure caused downregulation of MCP-1 expression level which is consistent with our results (Harstad, 2002). Moreover, a recent *in vitro* study compared mouse and rat macrophages cell lines and reported that mouse macrophages cell lines were more sensitive to Cd exposure than rat macrophages cell lines (García-Mendoza et al., 2019). To the best of our knowledge, this study is the first to investigate the effect of chronic Cd exposure on MCP-1 mRNA and protein expression levels in adult male SD rats

Macrophages produces certain cytokines which mediate the inflammatory response and can be used as markers for macrophages activity. Therefore, we further investigated the expression patterns of the inflammatory cytokines, namely TNF-α, IL-6, and IL-10. Our result showed a trend of downregulation of both IL-6 and IL-10 in all AT depots. However, TNF-α showed a slight upregulation in AB-AT and REtrop-AT but not in SUB-AT. The expression pattern of the inflammatory cytokines agrees with the MCP-1 expression pattern. Early evidence demonstrated that Cd significantly decreased the phagocytic activity of murine macrophages in a dose-dependent manner (Loose et al., 1978). *In vitro* experiments conducted by Jin et al.(2016) showed that Cd exposure decreased the inflammatory responses of murine macrophages in a dose and time-dependent manner. Moreover, a subtoxic dose of Cd (10μM) was found to inhibit the expression level of both IL-10 and IL-6 in murine macrophages (Riemschneider et al., 2015). Cox et al. (2016) proposed that Cd induces immune dysfunction in macrophages. This was confirmed it with lipopolysaccharide treatment after Cd

exposure wherein the ability of macrophages to transcribe and release cytokines was disrupted. A single-cell transcriptomic study reported that chronic Cd exposure induces phenotypic alterations in the immune system and reduces the number of monocytes when comparing the circulating immune system with the plasma Cd level (Lu et al., 2021). Together, these data suggest that chronic Cd exposure disturb the function of immune cells, especially the macrophages.

Former studies that investigated the macrophages infiltration patterns in different AT depots reported that in normal-weight and obese conditions, VAT was found to have more macrophages than SUB-AT (Jonas et al., 2015). Subcutaneous AT acts as a metabolic sink that stores excess free fatty acids and glycerol in the form of TGs. Visceral AT accumulates when the capacity of SUB-AT is exceeded due to chronic stress (Ibrahim, 2010). Also, VAT is associated with inflammatory events. This could explain the upregulation trend of MCP-1 and TNF-α in AB-AT of our study. Although the MCP-1 expression level was found to be disrupted, the inflammatory markers were found to be downregulated which suggests that chronic exposure to low-dose Cd is not an inflammatory promotor. It is possible that Cd exposure negatively affected SUB-AT but not to the level that causes lipid accumulation in VAT. Hence, REtrop-AT was found to be the least affected AT, which is reflected in the amount of extracted protein (low fat cells) and non-significant adipokines expressions.

To summarize the results of the present study, the author suggests that chronic low-dose Cd exposure affects WAT function, that results in significant downregulation of mRNA expression levels of adiponectin and leptin mainly at SUB-AT. This elucidates the protective role of SUB-AT microenvironment against Cd toxicity among other tested WAT depots.

CONCLUSION

The main goal of the current study was to examine the effect of chronic exposure to chronic low-dose Cd on the WAT function. To achieve this goal, the main adipokines produced and secreted by WAT were evaluated in different WAT depots, namely, subcutaneous, abdominal, and retroperitoneal adipose tissues. The most significant finding that emerged from this study is that chronic exposure to low-dose Cd adversely affects the secretion pattern of fundamental adipokines in SUB-AT compared to other depots. Moreover, chronic Cd exposure disrupts the function of adipose tissue macrophages in SUB-AT compared to visceral depots. However, Cd exposure to low-dose dose did not induce inflammation in SUB-AT. This study is one of the first attempts to thoroughly examine the effect of chronic exposure to low-dose Cd in different WAT depots at both gene and protein levels. It contributes to the existing knowledge by providing insights into the impact of chronic exposure to low-dose Cd on the function of different adipose depots that occupy different locations throughout the body. In terms of directions for future research, further work investigating the effect of chronic Cd on the protein expression of adiponectin and leptin and their receptors is required using other techniques other than western blot analysis. This could enhance our understanding of the potential alterations in the protein sequencing and the mechanisms regulating their production and secretion. Henceforth, combining the protein expression in WAT with the adipokines circulating level will further explain the association between the production and the secretion of adipokines by the adipocytes.

REFERENCES

Afolabi, O. K., Oyewo, E. B., Adekunle, A. S., Adedosu, O. T., & Adedeji, A. L. (2012). *Original article : IMPAIRED LIPID LEVELS AND INFLAMMATORY RESPONSE IN RATS EXPOSED TO CADMIUM*. 677–687.

Al-Naemi, H. A., & Das, S. C. (2020). Cadmium-induced endothelial dysfunction mediated by asymmetric dimethylarginine. *Environmental Science and Pollution Research*, *27*(14), 16246–16253. https://doi.org/10.1007/s11356-020-08116-5

Ali, I., Penttinen-Damdimopoulou, P. E., Mäkelä, S. I., Berglund, M., Stenius, U., Åkesson, A., Håkansson, H., & Halldin, K. (2010). Estrogen-Like Effects of Cadmium in Vivo Do Not Appear to be Mediated via the Classical Estrogen Receptor Transcriptional Pathway. *Environmental Health Perspectives*, *118*(10), 1389–1394. https://doi.org/10.1289/ehp.1001967

Alvarez, S. M., Gómez, N. N., Scardapane, L., Fornés, M. W., & Giménez, M. S. (2007). Effects of chronic exposure to cadmium on prostate lipids and morphology. *BioMetals*, *20*(5), 727–741. https://doi.org/10.1007/s10534-006-9036-9

Andjelkovic, M., Djordjevic, A. B., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D., & Bulat, Z. (2019). Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. *International Journal of Environmental Research and Public Health*, *16*(2). https://doi.org/10.3390/ijerph16020274

Astapova, O., & Leff, T. (2012). Adiponectin and PPARγ: Cooperative and Interdependent Actions of Two Key Regulators of Metabolism. In *Vitamins and Hormones* (Vol. 90, pp. 143–162). https://doi.org/10.1016/B978-0-12-398313-

8.00006-3

Atieh, M. A., Ji, Y., & Kochkodan, V. (2017). Metals in the Environment: Toxic Metals Removal. In *Bioinorganic Chemistry and Applications* (Vol. 2017). https://doi.org/10.1155/2017/4309198

ATSDR. (2019). *ATSDR's Substance Priority List*. https://www.atsdr.cdc.gov/spl/#

Attia, S. M., Varadharajan, K., Shanmugakonar, M., Das, S. C., & Al-Naemi, H. A. (2021). Cadmium: An Emerging Role in Adipose Tissue Dysfunction. *Exposure and Health*. https://doi.org/10.1007/s12403-021-00427-3

Awazawa, M., Ueki, K., Inabe, K., Yamauchi, T., Kaneko, K., Okazaki, Y., Bardeesy, N., Ohnishi, S., Nagai, R., & Kadowaki, T. (2009). Adiponectin suppresses hepatic SREBP1c expression in an AdipoR1/LKB1/AMPK dependent pathway. *Biochemical and Biophysical Research Communications*, *382*(1). https://doi.org/10.1016/j.bbrc.2009.02.131

Badimon, L., & Cubedo, J. (2017). Adipose Tissue Depots and Inflammation: Effects on Plasticity and Resident Mesenchymal Stem Cell Function. *Cardiovascular Research*, *113*(9), 1064–1073. https://doi.org/10.1093/cvr/cvx096

Barr, V. A., Malide, D., Zarnowski, M. J., Taylor, S. I., & Cushman, S. W. (1997). Insulin Stimulates Both Leptin Secretion and Production by Rat White Adipose Tissue. *Endocrinology*, *138*(10), 4463–4472. https://doi.org/10.1210/endo.138.10.5451

Bernhoft, R. A. (2013). Cadmium toxicity and treatment. *The Scientific World Journal*, *2013*. https://doi.org/10.1155/2013/394652

Bimonte, V. M., Besharat, Z. M., Antonioni, A., Cella, V., Lenzi, A., Ferretti, E., & Migliaccio, S. (2021). The Endocrine Disruptor Cadmium: A New Player in the Pathophysiology of Metabolic Diseases. *Journal of Endocrinological*

Investigation, *44*(7), 1363–1377. https://doi.org/10.1007/s40618-021-01502-x

Blüher, M., Williams, C. J., Klöting, N., Hsi, A., Ruschke, K., Oberbach, A., Fasshauer, M., Berndt, J., Schön, M. R., Wolk, A., Stumvoll, M., & Mantzoros, C. S. (2007). Gene Expression of Adiponectin Receptors in Human Visceral and Subcutaneous Adipose Tissue Is Related to Insulin Resistance and Metabolic Parameters and Is Altered in Response to Physical Training. *Diabetes Care*, *30*(12), 3110–3115. https://doi.org/10.2337/dc07-1257

Bruun, J. M., Lihn, A. S., Pedersen, S. B., & Richelsen, B. (2005). Monocyte Chemoattractant Protein-1 Release Is Higher in Visceral than Subcutaneous Human Adipose Tissue (AT): Implication of Macrophages Resident in the AT. *The Journal of Clinical Endocrinology & Metabolism*, *90*(4), 2282–2289. https://doi.org/10.1210/jc.2004-1696

Buettner, C., Muse, E. D., Cheng, A., Chen, L., Scherer, T., Pocai, A., Su, K., Cheng, B., Li, X., Harvey-White, J., Schwartz, G. J., Kunos, G., & Rossetti, L. (2008). Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nature Medicine*, *14*(6), 667–675. https://doi.org/10.1038/nm1775

Buha, A., Matovic, V., Antonijevic, B., Bulat, Z., Curcic, M., Renieri, E. A., Tsatsakis, A. M., Schweitzer, A., & Wallace, D. (2018). Overview of cadmium thyroid disrupting effects and mechanisms. *International Journal of Molecular Sciences*, *19*(5). https://doi.org/10.3390/ijms19051501

Calderoni, A. M., Oliveros, L., Jahn, G., Anton, R., Luco, J., & Giménez, M. S. (2005). Alterations in the lipid content of pituitary gland and serum prolactin and growth hormone in cadmium treated rats. *BioMetals*, *18*(3), 213–220. https://doi.org/10.1007/s10534-005-0581-4

Capeau, J. (2007). The story of adiponectin and its receptors AdipoR1 and R2: To

follow. *Journal of Hepatology*, *47*(5). https://doi.org/10.1016/j.jhep.2007.06.002

Caride, A., Fernández-Pérez, B., Cabaleiro, T., Tarasco, M., Esquifino, A. I., & Lafuente, A. (2010). Cadmium chronotoxicity at pituitary level: effects on plasma ACTH, GH, and TSH daily pattern. *Journal of Physiology and Biochemistry*, *66*(3), 213–220. https://doi.org/10.1007/s13105-010-0027-5

Ceddia, R. B., Somwar, R., Maida, A., Fang, X., Bikopoulos, G., & Sweeney, G. (2005). Globular Adiponectin Increases GLUT4 Translocation and Glucose Uptake but Reduces Glycogen Synthesis in Rat Skeletal Muscle Cells. *Diabetologia*, *48*(1), 132–139. https://doi.org/10.1007/s00125-004-1609-y

Chait, A., & den Hartigh, L. J. (2020). Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. *Frontiers in Cardiovascular Medicine*, *7*. https://doi.org/10.3389/fcvm.2020.00022

Chandrasekar, B., Boylston, W. H., Venkatachalam, K., Webster, N. J. G., Prabhu, S. D., & Valente, A. J. (2008). Adiponectin blocks interleukin-18-mediated endothelial cell death via APPL1-dependent AMP-activated protein kinase (AMPK) activation and IKK/NF-κB/PTEN suppression. *Journal of Biological Chemistry*, *283*(36), 24889–24898. https://doi.org/10.1074/jbc.M804236200

Chen, H., Yang, X., Wang, P., Wang, Z., Li, M., & Zhao, F.-J. (2018). Dietary cadmium intake from rice and vegetables and potential health risk: A case study in Xiangtan, southern China. *Science of The Total Environment*, *639*, 271–277. https://doi.org/10.1016/j.scitotenv.2018.05.050

Choe, S. S., Huh, J. Y., Hwang, I. J., Kim, J. I., & Kim, J. B. (2016). Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Frontiers in Endocrinology*, *7*(APR), 1–16. https://doi.org/10.3389/fendo.2016.00030

Choudhury, C., Mazumder, R., Biswas, R., & Sengupta, M. (2021). Cadmium Exposure Induces Inflammation Through the Canonical NF-κB Pathway in Monocytes/Macrophages of Channa punctatus Bloch. *Fish & Shellfish Immunology*, *110*, 116–126. https://doi.org/10.1016/j.fsi.2021.01.002

Cimboláková, I., Uher, I., Veszelits Laktičová, K., Vargová, M., Kimáková, T., & Papajová, I. (2020). Heavy Metals and the Environment. In *Environmental Factors Affecting Human Health: Vol. i* (Issue tourism, p. 13). IntechOpen. https://doi.org/10.5772/intechopen.86876

Coelho, M., Oliveira, T., & Fernandes, R. (2013). Biochemistry of Adipose Tissue: an Endocrine Organ. *Archives of Medical Science*, *2*(2), 191–200. https://doi.org/10.5114/aoms.2013.33181

COWI A/S. (2003). *Nordic Council of Ministers Cadmium Review*. *January*, 26. http://www.who.int/ifcs/documents/forums/forum5/nmr_cadmium.pdf

Cox, J. N., Rahman, M. A., Bao, S., Liu, M., Wheeler, S. E., & Knoell, D. L. (2016). Cadmium attenuates the macrophage response to LPS through inhibition of the NF-κB pathway. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, *311*(4), L754–L765. https://doi.org/10.1152/ajplung.00022.2016

Das, S. C., Varadharajan, K., Shanmugakonar, M., & Al-Naemi, H. A. (2021). Chronic Cadmium Exposure Alters Cardiac Matrix Metalloproteinases in the Heart of Sprague-Dawley Rat. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.663048

Denroche, H. C., Huynh, F. K., & Kieffer, T. J. (2012). The role of leptin in glucose homeostasis. *Journal of Diabetes Investigation*, *3*(2), 115–129. https://doi.org/10.1111/j.2040-1124.2012.00203.x

Diaz Marin, R., Crespo-Garcia, S., Wilson, A. M., & Sapieha, P. (2019). RELi

protocol: Optimization for protein extraction from white, brown and beige adipose tissues. *MethodsX*, *6*(December 2018), 918–928. https://doi.org/10.1016/j.mex.2019.04.010

Dyer, C. A. (2007). Heavy Metals as Endocrine-Disrupting Chemicals. In *Endocrine-Disrupting Chemicals*. https://doi.org/10.1007/1-59745-107-x_5

Echeverría, R., Vrhovnik, P., Salcedo-Bellido, I., Iribarne-Durán, L. M., Fiket, Ž., Dolenec, M., Martin-Olmedo, P., Olea, N., & Arrebola, J. P. (2019). Levels and Determinants of Adipose Tissue Cadmium Concentrations in an Adult Cohort from Southern Spain. *Science of The Total Environment*, *670*, 1028–1036. https://doi.org/10.1016/j.scitotenv.2019.03.114

Egger, A. E., Grabmann, G., Gollmann-Tepeköylü, C., Pechriggl, E. J., Artner, C., Türkcan, A., Hartinger, C. G., Fritsch, H., Keppler, B. K., Brenner, E., Grimm, M., Messner, B., & Bernhard, D. (2019). Chemical Imaging and Assessment of Cadmium Distribution in the Human Body. *Metallomics*, *11*(12), 2010–2019. https://doi.org/10.1039/C9MT00178F

Farkhondeh, T., Samarghandian, S., & Azimi-Nezhad, M. (2019). The role of Arsenic in Obesity and Diabetes. *Journal of Cellular Physiology*, *234*(8), 12516–12529. https://doi.org/10.1002/jcp.28112

Fasshauer, M., & Blüher, M. (2015). Adipokines in Health and Disease. *Trends in Pharmacological Sciences*, *36*(7), 461–470. https://doi.org/10.1016/j.tips.2015.04.014

Fasshauer, M., Kralisch, S., Klier, M., Lossner, U., Bluher, M., Klein, J., & Paschke, R. (2003). Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications*, *301*(4). https://doi.org/10.1016/S0006-291X(03)00090-1

Fatima, G., Raza, A. M., Hadi, N., Nigam, N., & Mahdi, A. A. (2019). Cadmium in Human Diseases: It's More than Just a Mere Metal. *Indian Journal of Clinical Biochemistry*, *34*(4), 371–378. https://doi.org/10.1007/s12291-019-00839-8

Ficková, M., Eybl, V., Kotyzová, D., Mičková, V., Möstbök, S., & Brtko, J. (2003). Long lasting cadmium intake is associated with reduction of insulin receptors in rat adipocytes. *BioMetals*, *16*(4), 561–566. https://doi.org/10.1023/a:1023485130767

Fiordelisio, T., Santiago, Y., & Romero, I. (2020). SAT-714 Cadmium, an Endocrine Disruptor of the Reproductive Axis in Mice. *Journal of the Endocrine Society*, *4*(Supplement_1). https://doi.org/10.1210/jendso/bvaa046.1958

Freitas, M., & Fernandes, E. (2011). Zinc, Cadmium and Nickel Increase the Activation of NF-κB and the Release of Cytokines from THP-1 Monocytic Cells. *Metallomics*, *3*(11), 1238. https://doi.org/10.1039/c1mt00050k

Fristachi, A., & Choudhury, H. (2017). Cadmium. In *International Encyclopedia of Public Health* (pp. 316–319). Elsevier. https://doi.org/10.1016/B978-0-12-803678-5.00043-6

Frühbeck, G., & Gómez-Ambrosi, J. (2013). Adipose Tissue: Structure, Function and Metabolism. In *Encyclopedia of Human Nutrition* (Vols. 1–4, pp. 1–13). Elsevier. https://doi.org/10.1016/B978-0-12-375083-9.00001-5

García-Mendoza, D., Han, B., Berg, H. J. H. J., & Brink, N. W. (2019). Cell-specific Immune-modulation of Cadmium on Murine Macrophages and Mast Cell Lines in Vitro. *Journal of Applied Toxicology*, *39*(7), 992–1001. https://doi.org/10.1002/jat.3788

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity. *International Journal of Environmental Research*

and Public Health, *17*(11), 3782. https://doi.org/10.3390/ijerph17113782

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., & Groneberg, D. A. (2006). The toxicity of cadmium and resulting hazards for human health. *Journal of Occupational Medicine and Toxicology*, *1*(1), 1–6. https://doi.org/10.1186/1745-6673-1-22

Greevenbroek, M. M. J., Schalkwijk, C. G., & Stehouwer, C. D. A. (2016). Dysfunctional Adipose Tissue and Low-grade Inflammation in the Management of the Metabolic Syndrome: Current Practices and Future Advances. *F1000Research*, *5*(0), 2515. https://doi.org/10.12688/f1000research.8971.1

Guzik, T. J., Skiba, D. S., Touyz, R. M., & Harrison, D. G. (2017). The Role of Infiltrating Immune Cells in Dysfunctional Adipose Tissue. *Cardiovascular Research*, *113*(9), 1009–1023. https://doi.org/10.1093/cvr/cvx108

Haider, S. R., Reid, H. J., & Sharp, B. L. (2012). *Tricine-SDS-PAGE* (pp. 81–91). https://doi.org/10.1007/978-1-61779-821-4_8

Han, J. C., Park, S. Y., Hah, B. G., Choi, G. H., Kim, Y. K., Kwon, T. H., Kim, E. K., Lachaal, M., Jung, C. Y., & Lee, W. (2003). Cadmium Induces Impaired Glucose Tolerance in Rat by Down-regulating GLUT4 Expression in Adipocytes. *Archives of Biochemistry and Biophysics*, *413*(2), 213–220. https://doi.org/10.1016/S0003-9861(03)00120-6

Harris, R. B. S. (2014). Direct and Indirect Effects of Leptin on Adipocyte Metabolism. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1842*(3), 414–423. https://doi.org/10.1016/j.bbadis.2013.05.009

Harstad, E. B. (2002). Analysis of Strain Difference in Sensitivity to Cadmium-Induced Hepatotoxicity in Fischer 344 and Sprague-Dawley Rats. *Toxicological Sciences*, *67*(2), 329–340. https://doi.org/10.1093/toxsci/67.2.329

Henriques, F., H. Bedard, A., & Luiz Batista Júnior, M. (2019). Adipose Tissue Inflammation and Metabolic Disorders. In *Adipose Tissue - An Update: Vol. i* (Issue tourism, p. 13). IntechOpen. https://doi.org/10.5772/intechopen.88631

Holland, W. L., Miller, R. A., Zhao V. Wang, Kai Sun, Barth, B. M., Hai H. Bui, Davis, K. E., Bikman, B. T., Halberg, N., Rutkowski, J. M., Wade, M. R., Tenorio, V. M., Kuo, M.-S., Brozinick, J. T., Zhang, B. B., Birnbaum, M. J., Summers, S. A., & Philipp E. Scherer. (2011). Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nature Medicine*, *47*(3), 549–562. https://doi.org/10.1097/CCM.0b013e31823da96d.Hydrogen

Hossein-Khannazer, N., Azizi, G., Eslami, S., Alhassan Mohammed, H., Fayyaz, F., Hosseinzadeh, R., Usman, A. B., Kamali, A. N., Mohammadi, H., Jadidi-Niaragh, F., Dehghanifard, E., & Noorisepehr, M. (2020). The Effects of Cadmium Exposure in the Induction of Inflammation. *Immunopharmacology and Immunotoxicology*, *42*(1), 1–8. https://doi.org/10.1080/08923973.2019.1697284

Huang, W.-L., Chang, W.-H., Cheng, S.-F., Li, H.-Y., & Chen, H.-L. (2021). Potential Risk of Consuming Vegetables Planted in Soil with Copper and Cadmium and the Influence on Vegetable Antioxidant Activity. *Applied Sciences*, *11*(9), 3761. https://doi.org/10.3390/app11093761

Hui, H. X., & Feng, T. (2018). Adipose Tissue as an Endocrine Organ. In *Intech: Vol. i* (Issue tourism, p. 13). https://doi.org/http://dx.doi.org/10.5772/57353

Ibrahim, M. M. (2010). Subcutaneous and visceral adipose tissue: structural and functional differences. *Obesity Reviews*, *11*(1), 11–18. https://doi.org/10.1111/j.1467-789X.2009.00623.x

Item, F., & Konrad, D. (2012). Visceral fat and metabolic inflammation: The portal theory revisited. *Obesity Reviews*, *13*(SUPPL.2). https://doi.org/10.1111/j.1467-789X.2012.01035.x

Iwabu, M., Okada-Iwabu, M., Yamauchi, T., & Kadowaki, T. (2015). Adiponectin/adiponectin receptor in disease and aging. *Npj Aging and Mechanisms of Disease*, *1*(1), 1–6. https://doi.org/10.1038/npjamd.2015.13

Jacobo-Estrada, T., Santoyo-Sánchez, M., Thévenod, F., & Barbier, O. (2017). Cadmium Handling, Toxicity and Molecular Targets Involved during Pregnancy: Lessons from Experimental Models. *International Journal of Molecular Sciences*, *18*(7), 1590. https://doi.org/10.3390/ijms18071590

Jin, Y., Liu, L., Zhang, S., He, R., Wu, Y., Chen, G., & Fu, Z. (2016). Cadmium exposure to murine macrophages decreases their inflammatory responses and increases their oxidative stress. *Chemosphere*, *144*, 168–175. https://doi.org/10.1016/j.chemosphere.2015.08.084

Johnson, M. D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., Clarke, R., Sholler, P. F., Lirio, A. A., Foss, C., Reiter, R., Trock, B., Paik, S., & Martin, M. B. (2003). Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nature Medicine*, *9*(8), 1081–1084. https://doi.org/10.1038/nm902

Jonas, M. I., Kurylowicz, A., Bartoszewicz, Z., Lisik, W., Jonas, M., Domienik-Karlowicz, J., & Puzianowska-Kuznicka, M. (2017). Adiponectin/resistin interplay in serum and in adipose tissue of obese and normal-weight individuals. *Diabetology and Metabolic Syndrome*, *9*(1), 1–9. https://doi.org/10.1186/s13098-017-0293-2

Jonas, M. I., Kurylowicz, A., Bartoszewicz, Z., Lisik, W., Jonas, M., Wierzbicki, Z.,

Chmura, A., Pruszczyk, P., & Puzianowska-Kuznicka, M. (2015). Interleukins 6 and 15 levels are higher in subcutaneous adipose tissue, but obesity is associated with their increased content in visceral fat depots. *International Journal of Molecular Sciences*, *16*(10), 25817–25830. https://doi.org/10.3390/ijms161025817

Kahn, C. R., Wang, G., & Lee, K. Y. (2019). Altered Adipose Tissue and Adipocyte Function in the Pathogenesis of Metabolic Syndrome. *Journal of Clinical Investigation*, *129*(10), 3990–4000. https://doi.org/10.1172/JCI129187

Kasemsuk, T., Phuagkhaopong, S., Yubolphan, R., Rungreangplangkool, N., & Vivithanaporn, P. (2020). Cadmium induces CCL2 production in glioblastoma cells via activation of MAPK, PI3K, and PKC pathways. *Journal of Immunotoxicology*, *17*(1), 186–193. https://doi.org/10.1080/1547691X.2020.1829211

Kawakami, T., Nishiyama, K., Kadota, Y., Sato, M., Inoue, M., & Suzuki, S. (2013). Cadmium Modulates Adipocyte Functions in Metallothionein-null Mice. *Toxicology and Applied Pharmacology*, *272*(3), 625–636. https://doi.org/10.1016/j.taap.2013.07.015

Kawakami, T., Nishiyama, K., Tanaka, J., Kadota, Y., Sato, M., & Suzuki, S. (2012). Changes in macrophage migration and adipokine gene expression induced by cadmium in white adipose tissue of metallothionein-null mice. *Annual Meeting of the Japanese Society of Toxicology*, *39.2*, AP-46. https://doi.org/10.14869/toxpt.39.2.0.AP-46.0

Kawakami, T., Sugimoto, H., Furuichi, R., Kadota, Y., Inoue, M., Setsu, K., Suzuki, S., & Sato, M. (2010). Cadmium reduces adipocyte size and expression levels of adiponectin and Peg1/Mest in adipose tissue. *Toxicology*, *267*(1–3), 20–26.

https://doi.org/10.1016/j.tox.2009.07.022

Kern, P. A., Di Gregorio, G. B., Lu, T., Rassouli, N., & Ranganathan, G. (2003). Adiponectin Expression From Human Adipose Tissue. *Diabetes*, *52*(7), 1779–1785. https://doi.org/10.2337/diabetes.52.7.1779

Kim, Kijoon, Melough, M., Vance, T., Noh, H., Koo, S., & Chun, O. (2018). Dietary Cadmium Intake and Sources in the US. *Nutrients*, *11*(1), 2. https://doi.org/10.3390/nu11010002

Kim, Kisok. (2012). Blood cadmium concentration and lipid profile in Korean adults. *Environmental Research*, *112*, 225–229. https://doi.org/10.1016/j.envres.2011.12.008

Kiremidjian-Schumacher, L., Stotzky, G., Likhite, V., Schwartz, J., & Dickstein, R. A. (1981). Influence of cadmium, lead, and zinc on the ability of sensitized guinea pig lymphocytes to interact with specific antigen and to produce lymphokine. *Environmental Research*, *24*(1), 96–105. https://doi.org/10.1016/0013-9351(81)90136-5

Kollias, A., Tsiotra, P. C., Ikonomidis, I., Maratou, E., Mitrou, P., Kyriazi, E., Boutati, E., Lekakis, J., Economopoulos, T., Kremastinos, D. T., Dimitriadis, G., & Raptis, S. A. (2011). Adiponectin levels and expression of adiponectin receptors in isolated monocytes from overweight patients with coronary artery disease. *Cardiovascular Diabetology*, *10*(1), 14. https://doi.org/10.1186/1475-2840-10-14

Korac, A., Srdic-Galic, B., Stancic, A., Otasevic, V., Korac, B., & Jankovic, A. (2021). Adipokine signatures of subcutaneous and visceral abdominal fat in normal-weight and obese women with different metabolic profiles. *Archives of Medical Science*, *17*(2), 323–336. https://doi.org/10.5114/aoms/92118

Kovacova, Z., Tencerova, M., Roussel, B., Wedellova, Z., Rossmeislova, L., Langin, D., Polak, J., & Stich, V. (2012). The impact of obesity on secretion of adiponectin multimeric isoforms differs in visceral and subcutaneous adipose tissue. *International Journal of Obesity*, *36*(10), 1360–1365. https://doi.org/10.1038/ijo.2011.223

Kuester, R. K., Waalkes, M. P., Goering, P. L., Fisher, B. L., McCuskey, R. S., & Sipes, I. G. (2002). Differential Hepatotoxicity Induced by Cadmium in Fischer 344 and Sprague-Dawley Rats. *Toxicological Sciences*, *65*(1), 151–159. https://doi.org/10.1093/toxsci/65.1.151

Kwon, H., & Pessin, J. E. (2013). Adipokines Mediate Inflammation and Insulin Resistance. *Frontiers in Endocrinology*, *4*, 71. https://doi.org/10.3389/fendo.2013.00071

Lauretta, R., Sansone, A., Sansone, M., Romanelli, F., & Appetecchia, M. (2019). Endocrine Disrupting Chemicals: Effects on Endocrine Glands. *Frontiers in Endocrinology*, *10*(March), 1–7. https://doi.org/10.3389/fendo.2019.00178

Lee, E., Moon, J. Y., & Yoo, B. S. (2012). Cadmium inhibits the differentiation of 3T3-L1 preadipocyte through the C/EBPα and PPARγ pathways. *Drug and Chemical Toxicology*, *35*(2), 225–231. https://doi.org/10.3109/01480545.2011.591401

Lee, J.-Y., Tokumoto, M., & Satoh, M. (2019). *Novel Mechanisms of Cadmium-Induced Toxicity in Renal Cells* (pp. 153–162). https://doi.org/10.1007/978-981-13-3630-0_12

Lee, M., Wu, Y., & Fried, S. K. (2013). Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. *Molecular Aspects of Medicine*, *34*(1), 1–11. https://doi.org/10.1016/j.mam.2012.10.001

Levy, J. R., Gyarmati, J., Lesko, J. M., Adler, R. A., & Stevens, W. (2000). Dual Regulation of Leptin Secretion: Intracellular Energy and Calcium Dependence of Regulated Pathway. *American Journal of Physiology-Endocrinology and Metabolism*, *278*(5), E892–E901. https://doi.org/10.1152/ajpendo.2000.278.5.E892

Li, M. (2011). Leptin and beyond: An odyssey to the central control of body weight. In *Yale Journal of Biology and Medicine* (Vol. 84, Issue 1).

Li, X., Li, H., Cai, D., Li, P., Jin, J., Jiang, X., Li, Z., Tian, L., Chen, G., Sun, J., & Bai, W. (2021). Chronic oral exposure to cadmium causes liver inflammation by NLRP3 inflammasome activation in pubertal mice. *Food and Chemical Toxicology*, *148*, 111944. https://doi.org/https://doi.org/10.1016/j.fct.2020.111944

Li, Y., Yun, K., & Mu, R. (2020). A Review on the Biology and Properties of Adipose Tissue Macrophages Involved in Adipose Tissue Physiological and Pathophysiological Processes. *Lipids in Health and Disease*, *19*(1), 164. https://doi.org/10.1186/s12944-020-01342-3

Loose, L. D., Silkworth, J. B., & Simpson, D. W. (1978). Influence of cadmium on the phagocytic and microbicidal activity of murine peritoneal macrophages, pulmonary alveolar macrophages, and polymorphonuclear neutrophils. *Infection and Immunity*, *22*(2), 378–381. https://doi.org/10.1128/iai.22.2.378-381.1978

Lu, Y., Wu, J., Gu, W., Huang, Z., Shu, Z., Huang, M., Chen, J., Zhou, M., Bai, Y., Chen, X., Xiao, Y., Shen, M., Luo, D., Deng, Q., Chai, L., He, M., Gong, J., Yuan, H., Xu, Q., & Cai, J. (2021). Single-cell transcriptomics uncovers phenotypic alterations in the monocytes in a Chinese population with chronic cadmium exposure. *Ecotoxicology and Environmental Safety*, *211*, 111881.

https://doi.org/10.1016/j.ecoenv.2020.111881

Luo, C., Routh, J., Dario, M., Sarkar, S., Wei, L., Luo, D., & Liu, Y. (2020). Distribution and Mobilization of Heavy Metals at an Acid Mine Drainage Affected Region in South China, a Post-remediation Study. *Science of The Total Environment*, *724*, 138122. https://doi.org/10.1016/j.scitotenv.2020.138122

Mancuso, P. (2016). The Role of Adipokines in Chronic Inflammation. *ImmunoTargets and Therapy*, *5*, 47. https://doi.org/10.2147/ITT.S73223

Martin, M. B., Voeller, H. J., Gelmann, E. P., Lu, J., Stoica, E.-G., Hebert, E. J., Reiter, R., Singh, B., Danielsen, M., Pentecost, E., & Stoica, A. (2002). Role of Cadmium in the Regulation of AR Gene Expression and Activity. *Endocrinology*, *143*(1), 263–275. https://doi.org/10.1210/endo.143.1.8581

Matafome, P., & Seiça, R. (2017). Function and Dysfunction of Adipose Tissue. In L. Letra & R. Seiça (Eds.), *Obesity and Brain Function* (pp. 3–31). Springer International Publishing. https://doi.org/10.1007/978-3-319-63260-5_1

Matović, V., Buha, A., Bulat, Z., & Đukić-Ćosić, D. (2011). Cadmium Toxicity Revisited: Focus on Oxidative Stress Induction and Interactions with Zinc and Magnesium. *Archives of Industrial Hygiene and Toxicology*, *62*(1), 65–76. https://doi.org/10.2478/10004-1254-62-2011-2075

Mazaki-Tovi, M., Bolin, S. R., & Schenck, P. A. (2016). Differential secretion of adipokines from subcutaneous and visceral adipose tissue in healthy dogs: Association with body condition and response to troglitazone. *Veterinary Journal*, *216*, 136–141. https://doi.org/10.1016/j.tvjl.2016.08.002

Meyer, D. N., Crofts, E. J., Akemann, C., Gurdziel, K., Farr, R., Baker, B. B., Weber, D., & Baker, T. R. (2020). Developmental exposure to Pb2+ induces transgenerational changes to zebrafish brain transcriptome. *Chemosphere*, *244*,

125527. https://doi.org/10.1016/j.chemosphere.2019.125527

Mezynska, M., & Brzóska, M. M. (2018). Environmental Exposure to Cadmium— A Risk for Health of the General Population in Industrialized Countries and Preventive Strategies. *Environmental Science and Pollution Research*, *25*(4), 3211–3232. https://doi.org/10.1007/s11356-017-0827-z

Mirkov, I., Popov Aleksandrov, A., Ninkov, M., Tucovic, D., Kulas, J., Zeljkovic, M., Popovic, D., & Kataranovski, M. (2021). Immunotoxicology of cadmium: Cells of the immune system as targets and effectors of cadmium toxicity. *Food and Chemical Toxicology*, *149*, 112026. https://doi.org/10.1016/j.fct.2021.112026

Münzberg, H., & Morrison, C. D. (2015). Structure, production and signaling of leptin. In *Metabolism: Clinical and Experimental* (Vol. 64, Issue 1). https://doi.org/10.1016/j.metabol.2014.09.010

Neslund-Dudas, C. M., McBride, R. B., Kandegedara, A., Rybicki, B. A., Kryvenko, O. N., Chitale, D., Gupta, N., Williamson, S. R., Rogers, C. G., Cordon-Cardo, C., Rundle, A. G., Levin, A. M., Dou, Q. P., & Mitra, B. (2018). Association between cadmium and androgen receptor protein expression differs in prostate tumors of African American and European American men. *Journal of Trace Elements in Medicine and Biology*, *48*, 233–238. https://doi.org/10.1016/j.jtemb.2018.04.006

Nicholson, T., Church, C., Baker, D. J., & Jones, S. W. (2018). The role of adipokines in skeletal muscle inflammation and insulin sensitivity. *Journal of Inflammation (United Kingdom)*, *15*(1), 1–11. https://doi.org/10.1186/s12950-018-0185-8

Novotny, D., Vaverkova, H., & Karasek, D. (2012). Adiponectin: A Perspective Adipose Tissue Marker with Antiinflammatory and Antiaterogenic Potencial. In *Lipoproteins - Role in Health and Diseases* (p. 13). InTech.

https://doi.org/10.5772/47754

Olisekodiaka, M. J., Igbeneghu, C. A., Onuegbu, A. J., Oduru, R., & Lawal, A. O. (2012). Lipid, Lipoproteins, Total Antioxidant Status and Organ Changes in Rats Administered High Doses of Cadmium Chloride. *Medical Principles and Practice*, *21*(2), 156–159. https://doi.org/10.1159/000333385

Olszowski, T., Gutowska, I., Baranowska-Bosiacka, I., Piotrowska, K., Korbecki, J., Kurzawski, M., & Chlubek, D. (2015). The Effect of Cadmium on COX-1 and COX-2 Gene, Protein Expression, and Enzymatic Activity in THP-1 Macrophages. *Biological Trace Element Research*, *165*(2), 135–144. https://doi.org/10.1007/s12011-015-0234-6

Ordovas, J. M., & Corella, D. (2008). Metabolic syndrome pathophysiology: The role of adipose tissue. *Kidney International*, *74*(SUPPL. 111). https://doi.org/10.1038/ki.2008.517

Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in Inflammation and Metabolic Disease. *Nature Reviews Immunology*, *11*(2), 85–97. https://doi.org/10.1038/nri2921

Park, H.-K., & Ahima, R. S. (2014). Leptin signaling. *F1000Prime Reports*, *6*. https://doi.org/10.12703/P6-73

Patrick, L. (2003). Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. In *Alternative Medicine Review* (Vol. 8, Issue 2, pp. 106–128).

Phillips, S. A., Ciaraldi, T. P., Oh, D. K., Savu, M. K., & Henry, R. R. (2008). Adiponectin secretion and response to pioglitazone is depot dependent in cultured human adipose tissue. *American Journal of Physiology-Endocrinology and Metabolism*, *295*(4), E842–E850.

https://doi.org/10.1152/ajpendo.90359.2008

Planchart, A., Green, A., Hoyo, C., & Mattingly, C. J. (2018). Heavy Metal Exposure and Metabolic Syndrome: Evidence from Human and Model System Studies. *Current Environmental Health Reports*, *5*(1), 110–124. https://doi.org/10.1007/s40572-018-0182-3

Prabhu, R., Ribeiro, M., & Kajdacsy-Balla, A. (2020). The Toxic Effect of Environmental Cadmium on Visceral Adipose Tissue. *The FASEB Journal*, *34*(S1), 1–1. https://doi.org/10.1096/fasebj.2020.34.s1.06577

Pup, L. Del, Mantovani, A., Cavaliere, C., Facchini, G., Luce, A., Sperlongano, P., Caraglia, M., & Berretta, M. (2016). Carcinogenetic mechanisms of endocrine disruptors in female cancers (Review). *Oncology Reports*, *36*(2), 603–612. https://doi.org/10.3892/or.2016.4886

Rahimzadeh, M. R., Rahimzadeh, M. R., Kazemi, S., & Moghadamnia, A. A. (2017). Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*, *8*(3), 135–145. https://doi.org/10.22088/cjim.8.3.135

Regnier, S. M., & Sargis, R. M. (2014). Adipocytes under assault: Environmental disruption of adipose physiology. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1842*(3), 520–533. https://doi.org/10.1016/j.bbadis.2013.05.028

Riemschneider, S., Herzberg, M., & Lehmann, J. (2015). Subtoxic Doses of Cadmium Modulate Inflammatory Properties of Murine RAW 264.7 Macrophages. *BioMed Research International*, *2015*, 295303. https://doi.org/10.1155/2015/295303

Rizzetti, D. A., Corrales, P., Piagette, J. T., Uranga-Ocio, J. A., Medina-Gomez, G., Peçanha, F. M., Vassallo, D. V., Miguel, M., & Wiggers, G. A. (2019). Chronic Mercury at Low Doses Impairs White Adipose Tissue Plasticity. *Toxicology*,

418(December 2018), 41–50. https://doi.org/10.1016/j.tox.2019.02.013

Rull, A., Camps, J., Alonso-Villaverde, C., & Joven, J. (2010). Insulin Resistance, Inflammation, and Obesity: Role of Monocyte Chemoattractant Protein-1 (orCCL2) in the Regulation of Metabolism. *Mediators of Inflammation*, *2010*, 1–11. https://doi.org/10.1155/2010/326580

Rusin, M., Domagalska, J., Rogala, D., Razzaghi, M., & Szymala, I. (2021). Concentration of cadmium and lead in vegetables and fruits. *Scientific Reports*, *11*(1), 11913. https://doi.org/10.1038/s41598-021-91554-z

Sabir, S., Akash, M. S. H., Fiayyaz, F., Saleem, U., Mehmood, M. H., & Rehman, K. (2019). Role of Cadmium and Arsenic as Endocrine Disruptors in the Metabolism of Carbohydrates: Inserting the Association into Perspectives. *Biomedicine & Pharmacotherapy*, *114*(December 2018), 108802. https://doi.org/10.1016/j.biopha.2019.108802

Sabolić, I., Breljak, D., Herak-Kramberger, C. M., & Ljubojević, M. (2013). Cadmium and Metallothionein. In *Encyclopedia of Metalloproteins* (pp. 342–352). Springer New York. https://doi.org/10.1007/978-1-4614-1533-6_27

Salama, S. A., Arab, H. H., Hassan, M. H., Al robaian, M. M., & Maghrabi, I. A. (2019). Cadmium-induced Hepatocellular Injury: Modulatory Effects of γ-glutamyl Cysteine on The Biomarkers of Inflammation, DNA damage, and Apoptotic Cell Death. *Journal of Trace Elements in Medicine and Biology*, *52*, 74–82. https://doi.org/10.1016/j.jtemb.2018.12.003

Salcedo-Bellido, I., Gómez-Peña, C., Pérez-Carrascosa, F. M., Vrhovnik, P., Mustieles, V., Echeverría, R., Fiket, Ž., Pérez-Díaz, C., Barrios-Rodríguez, R., Jiménez-Moleón, J. J., & Arrebola, J. P. (2021). Adipose Tissue Cadmium Concentrations as a Potential Risk Factor for Insulin Resistance and Future Type

2 Diabetes Mellitus in GraMo Adult Cohort. *Science of The Total Environment*, *780*, 146359. https://doi.org/10.1016/j.scitotenv.2021.146359

Samaras, K., Botelho, N. K., Chisholm, D. J., & Lord, R. V. (2010). Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. *Obesity*, *18*(5), 884–889. https://doi.org/10.1038/oby.2009.443

Samarghandian, S., Azimi-Nezhad, M., Shabestari, M. M., Azad, F. J., Farkhondeh, T., & Bafandeh, F. (2015). Effect of chronic exposure to cadmium on serum lipid, lipoprotein and oxidative stress indices in male rats. *Interdisciplinary Toxicology*, *8*(3), 151–154. https://doi.org/10.1515/intox-2015-0023

Schägger, H. (2006). Tricine–SDS-PAGE. *Nature Protocols*, *1*(1), 16–22. https://doi.org/10.1038/nprot.2006.4

Schoettl, T., Fischer, I. P., & Ussar, S. (2018). Heterogeneity of Adipose Tissue in Development and Metabolic Function. *Journal of Experimental Biology*, *221*(Suppl_1). https://doi.org/10.1242/jeb.162958

Song, X., Li, Y., Liu, J., Ji, X., Zhao, L., & Wei, Y. (2017). Changes in Serum Adiponectin in Mice Chronically Exposed to Inorganic Arsenic in Drinking Water. *Biological Trace Element Research*, *179*(1), 140–147. https://doi.org/10.1007/s12011-017-0950-1

Stern, J. H., Rutkowski, J. M., & Scherer, P. E. (2016). Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. *Cell Metabolism*, *23*(5), 770–784. https://doi.org/10.1016/j.cmet.2016.04.011

Street, M. E., Angelini, S., Bernasconi, S., Burgio, E., Cassio, A., Catellani, C., Cirillo, F., Deodati, A., Fabbrizi, E., Fanos, V., Gargano, G., Grossi, E., Iughetti, L., Lazzeroni, P., Mantovani, A., Migliore, L., Palanza, P., Panzica, G., Papini,

A. M., … Amarri, S. (2018). Current knowledge on endocrine disrupting chemicals (EDCs) from animal biology to humans, from pregnancy to adulthood: Highlights from a national italian meeting. *International Journal of Molecular Sciences*, *19*(6). https://doi.org/10.3390/ijms19061647

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). *Heavy Metal Toxicity and the Environment* (pp. 133–164). https://doi.org/10.1007/978-3-7643-8340-4_6

Tellez-Plaza, M., Guallar, E., Howard, B. V., Umans, J. G., Francesconi, K. A., Goessler, W., Silbergeld, E. K., Devereux, R. B., & Navas-Acien, A. (2013). Cadmium exposure and incident cardiovascular disease. *Epidemiology*, *24*(3). https://doi.org/10.1097/EDE.0b013e31828b0631

Thévenod, F., & Lee, W.-K. (2013). Toxicology of Cadmium and Its Damage to Mammalian Organs. In *Metal Ions in Life Sciences* (Vol. 11, pp. 415–490). https://doi.org/10.1007/978-94-007-5179-8_14

Thomas, D., & Apovian, C. (2017). Macrophage functions in lean and obese adipose tissue. In *Metabolism: Clinical and Experimental* (Vol. 72). https://doi.org/10.1016/j.metabol.2017.04.005

Tinkov, A. A., Aschner, M., Ke, T., Ferrer, B., Zhou, J.-C., Chang, J.-S., Santamaría, A., Chao, J. C.-J., Aaseth, J., & Skalny, A. V. (2021). Adipotropic Effects of Heavy Metals and their Potential Role in Obesity. *Faculty Reviews*, *10*. https://doi.org/10.12703/r/10-32

Treviño, S., Waalkes, M. P., Flores Hernández, J. A., León-Chavez, B. A., Aguilar-Alonso, P., & Brambila, E. (2015). Chronic Cadmium Exposure in Rats Produces Pancreatic Impairment and Insulin Resistance in Multiple Peripheral Tissues. *Archives of Biochemistry and Biophysics*, *583*, 27–35.

https://doi.org/10.1016/j.abb.2015.07.010

Upadhyaya, S., Kadamkode, V., Mahammed, R., Doraiswami, C., & Banerjee, G. (2014). Adiponectin and IL-6. *Adipocyte*, *3*(1), 39–45. https://doi.org/10.4161/adip.26553

USGS. (2020). *Cadmium*. https://pubs.usgs.gov/periodicals/mcs2020/mcs2020-cadmium.pdf

Valcke, M., Ouellet, N., Dubé, M., Laouan Sidi, E. A., LeBlanc, A., Normandin, L., Balion, C., & Ayotte, P. (2019). Biomarkers of cadmium, lead and mercury exposure in relation with early biomarkers of renal dysfunction and diabetes: Results from a pilot study among aging Canadians. *Toxicology Letters*, *312*, 148–156. https://doi.org/10.1016/j.toxlet.2019.05.014

Vasiliauskaité-brooks, I., Sounier, R., Rochaix, P., & Bellot, G. (2017). *Europe PMC Funders Group Structural insights into adiponectin receptors suggest ceramidase activity*. *544*(7648), 120–123. https://doi.org/10.1038/nature21714.Structural

Walker, G. E., Marzullo, P., Ricotti, R., Bona, G., & Prodam, F. (2014). The pathophysiology of abdominal adipose tissue depots in health and disease. In *Hormone Molecular Biology and Clinical Investigation* (Vol. 19, Issue 1). https://doi.org/10.1515/hmbci-2014-0023

Wang, X., Karvonen-Gutierrez, C. A., Mukherjee, B., Herman, W. H., & Park, S. K. (2021). Urinary metals and adipokines in midlife women: The Study of Women's Health Across the nation (SWAN). *Environmental Research*, *196*, 110426. https://doi.org/10.1016/j.envres.2020.110426

Wang, Z., Sun, Y., Yao, W., Ba, Q., & Wang, H. (2021). Effects of Cadmium Exposure on the Immune System and Immunoregulation. In *Frontiers in*

Immunology (Vol. 12). https://doi.org/10.3389/fimmu.2021.695484

Xie, T., Wang, M., Chen, W., & Uwizeyimana, H. (2019). Impacts of Urbanization and Landscape Patterns on the Accumulation of Heavy Metals in Soils in Residential Areas in Beijing. *Journal of Soils and Sediments*, *19*(1), 148–158. https://doi.org/10.1007/s11368-018-2011-6

Yang, Y., Zhang, X., Fu, Y., & Yang, H. (2014). Leptin and IL-8: Two novel cytokines screened out in childhood lead exposure. *Toxicology Letters*, *227*(3), 172–178. https://doi.org/10.1016/j.toxlet.2014.03.009

Ye, J., Wang, S., Barger, M., Castranova, V., & Shi, X. (2000). Activation of androgen response element by cadmium: A potential mechanism for a carcinogenic effect of cadmium in the prostate. *Journal of Environmental Pathology, Toxicology and Oncology*, *19*(3).

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, *372*(6505). https://doi.org/10.1038/372425a0

Zhou, Z., Lu, Y., Pi, H., Gao, P., Li, M., Zhang, L., Pei, L., Mei, X., Liu, L., Zhao, Q., Qin, Q.-Z., Chen, Y., Jiang, Y., Zhang, Z., & Yu, Z. (2016). Cadmium Exposure is Associated with the Prevalence of Dyslipidemia. *Cellular Physiology and Biochemistry*, *40*(3–4), 633–643. https://doi.org/10.1159/000452576

Zou, H., Sun, J., Wu, B., Yuan, Y., Gu, J., Bian, J., Liu, X., & Liu, Z. (2020). Effects of Cadmium and/or Lead on Autophagy and Liver Injury in Rats. *Biological Trace Element Research*, *198*(1), 206–215. https://doi.org/10.1007/s12011-020-02045-7