THE INVESTIGATION OF INTESTINAL ZINC AND GLUCOSE METABOLISM DUE TO INCREASED GLUCOSE AMOUNT IN OBESITY

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ABSTRACT

THE INVESTIGATION OF INTESTINAL ZINC AND GLUCOSE METABOLISM DUE TO INCREASED GLUCOSE AMOUNT IN OBESITY

Obesity is defined as an excessive accumulation of adipose tissue within the human body. The global prevalence of obesity has seen a marked increase, and our country is no exception to this trend. The pathogenesis of obesity is intricately linked to the consumption of glucose, a phenomenon in which glucose absorption plays a pivotal role via the enterocytes of the intestinal lining. In individuals with obesity, both dietary and blood glucose levels are elevated, thereby intensifying the exposure of enterocyte cells to glucose.

This thesis results delved into the interactions between high-glucose-associated zinc metabolism and zinc-dependent glucose metabolism within enterocyte cells. Results revealed a correlation between elevated glucose concentrations and reduced zinc levels within the cells, coupled with the regulation of mRNA levels on zinc transporters. Additionally, observed a reduction in cellular 2-deoxy-glucose uptake upon zinc treatment. Also, indicated that zinc treatments, whether administered to the basolateral or both apical and basolateral sides of the cells, led to the regulation of mRNA levels for glucose transporters, compared to control groups. Remarkably, also observed that specific treatments involving zinc on the polarized sides of Caco-2 cells increased the efflux of glucose from these cells.

In conclusion, results suggest that zinc may play a pivotal role in the development of obesity concerning glucose metabolism.

ÖZET

OBEZİTEDE ARTAN GLİKOZ MİKTARINA BAĞLI İNCE BAĞIRSAK ÇİNKO VE GLUKOZ METABOLİZMASININ İNCELENMESİ

Günümüzde besin tüketiminin dengesizliği ve artan paketli gıda tüketimlerinin yanında karbonhidrat odaklı tüketimin de yaygın olması obezite ve tip 2 diyabet risklerini her yaş grubunda arttırmaktadır. Ayrıca obezite birçok diğer metabolik hastalıklar içinde risk faktörü olarak değerlendirilebilir. Beslenme şeklimiz ne olursa olsun genel olarak besin öğeleri vücudumuza bağırsak yolu ile girmektedir. Bağırsak dokusu bulunduğu konum açısından sindirilen besinler için vücut dışı-içi arasındaki geçiş noktasıdır. Bu toplam hücre popülasyonu içersinde en yüksek oran enterosit hücrelerine aittir ve besin emiliminden sorumlu olması canlılığın sürdürülebilmesi adına önemlidir. Besin-entersosit hücre ilişkisi dikkate alındığında son yıllarda bu hücrelerin besin öğlerinin çeşidini ve miktarını nasıl algıladıkları ve obezite gibi metabolik hastalıklarla ilişkileri üzerine çalışmalar gittikçe artmaktadır. Bu alanda yaptığımız çalışmalarda, enterosit hücrelerinin yüksek oranda glikoz ile maruziyeti sonrası çinko metabolizmasına etki edebileceğine dair bazı deneysel sonuçlar elde edilmişti. Sonuçları daha ileri taşımak ve sonrasında yapılabilecek çalışmalar adına bu tez planlanmıştır. Özellikle farklı dokularda hücre için çinkonun glikoz ve yağ metabolizması için önemli olduğu gösterilmiştir. Bunun yanında obezite durumunda serum çinko seviyesinin düşük olduğu gözlemlenmiştir. Bu bilgiler glikoz ve çinko arasında fizyolojik ilişkinin olduğunu işaret etmektedir. Yapılan çalışmalarda ince bağırsak enterosit hücreleri için glikoz ve çinko metabolizmasının olası fizyolojik ilişkisi hakkında yeterli bilgi olmaması ve özellikle bu iki besin öğesi için enterosit hücrelerinin geçiş noktası olması yapılan çalışmayı öneli hale getirmiştir.

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CHAPTER 1

INTRODUCTION

The first chapter of the thesis covers a general overview of comprehensive insight into the interplay among glucose metabolism, obesity, and zinc metabolism. This part explains the intricate connections between glucose metabolism and obesity, as well as the consequential impact of glucose, zinc, and obesity on metabolic processes at the small intestine level.

1.1. Small Intestine Glucose Metabolism and Its Correlation with Obesity

Glucose, a fundamental carbohydrate monomer abundantly present in plants, constitutes a staple component of our daily diet. Its sweetening properties render it a ubiquitous additive in an extensive array of consumer products. Clinically, glucose is defined as blood sugar, and its normal levels typically range between 65-110 mg/dL in the human bloodstream. Of paramount significance, glucose serves as a primary energy source, particularly for the brain, and thus plays a pivotal role in energy production and consumption a metabolic process that stands as the bedrock of life.

In light of these factors, glucose assumes a critical role as a vital nutrient molecule for the human body. Dietary carbohydrates undergo a sequential transformation initiated by the amylase enzyme in the mouth, furthered by amylase secretion from the pancreas within the intestinal lumen. This enzymatic cascade eventually converts carbohydrates into predominantly binary sugar derivatives, which, in turn, are refined into single sugar units by specific enzymes situated within the enterocyte lumen (Dashty 2013).

The processed glucose, now in its singular sugar form, is actively transported into enterocyte cells of the small intestine through the sodium-glucose symporter protein 1 located on the apical side. Subsequently, it is released into the bloodstream via glucose

transport-2 (GLUT2), orchestrating the seamless regulation of glucose uptake and distribution (see Figure 1.1).

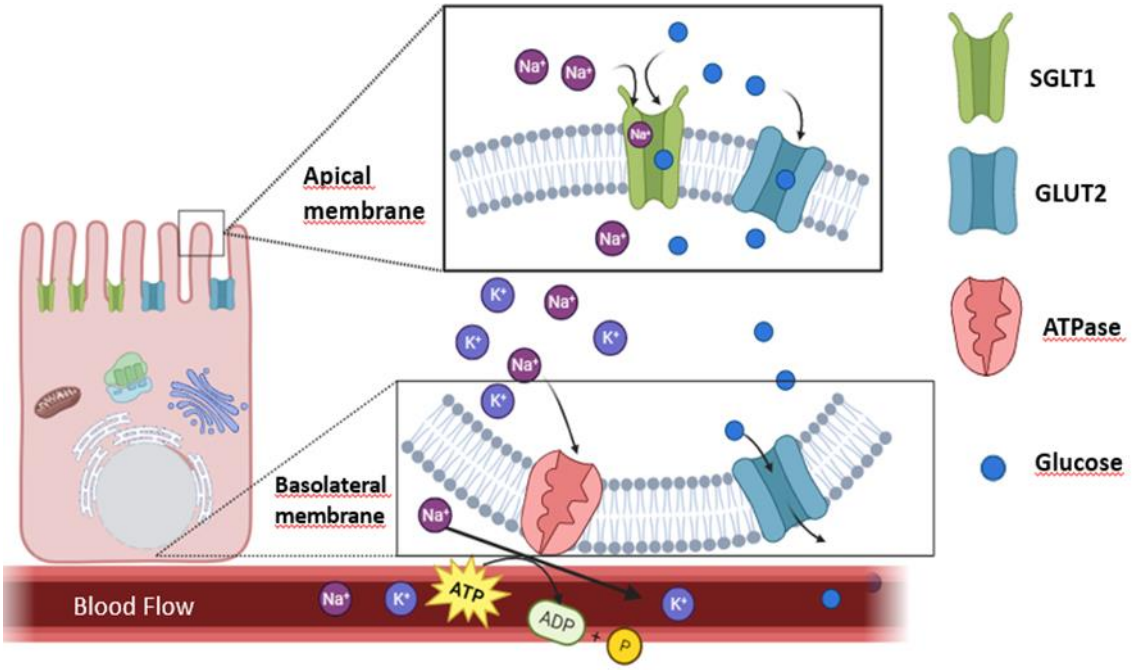

Figure 1.1. Glucose transport proteins in small intestine.

Following the World Health Organization's (WHO) guidelines, the determination of whether an individual falls into the category of obesity or not hinges on the calculation of their Body Mass Index (BMI). Those with a BMI equal to or greater than 30 are classified as obese. The root cause of obesity lies in the imbalance between the total calorie intake and the total calorie expenditure—wherein an excess of calorie consumption results in the accumulation of excess body fat (Scully 2014). Another perspective on obesity underscores the storage of surplus calories as fat (Scully 2014).

In the realm of normal physiology, adipose tissue comprises specialized cells that possess the capacity to store fat. This tissue plays a pivotal role in metabolic regulation through the secretion of the hormone leptin, which actively participates in signaling mechanisms that control food intake and satiety (Leibel et al., 1990). However, in cases of obesity, there is a notable increase in both glucose and insulin levels within the body (Bagdade et al., 1967; Pelleymounter et al., 1995).

The connection between glucose and obesity can be approached from two distinct angles. Firstly, excessive dietary glucose is stored as fat and contributes to weight gain within the body. When an abundance of glucose is introduced into the body, it is utilized in two primary ways: either as an immediate energy source if needed or it is converted into glycogen and fat within the liver, while in muscle tissue, it takes the form of fatty acids, ultimately becoming stored fat (Felber et al., 1981; Felber et al., 1988). The second perspective concerns the influence of obesity on glucose metabolism, potentially leading to the development of diabetes, particularly type 2 diabetes, due to the emergence of insulin resistance (Bloomgarden 2008). However, the precise molecular mechanisms underlying this relationship remain incompletely understood.

Thus, prudent dietary control with regard to foods rich in glucose is essential. Given the prevalence of carbohydrate-based nutrition in our country, it underscores the importance of monitoring glucose absorption within enterocyte cells and the influence of enterocyte-focused signaling pathways on glucose metabolism, particularly in the context of glucose-dependent metabolic disorders.

1.2. Small Intestinal Tissue and Glucose Metabolism

Nutrition entails the process of obtaining the body's requisite energy and essential nutrients from the foods we consume. Intricately woven into this process are the cellular and molecular control mechanisms that play a pivotal role in the uptake and metabolism of nutrients within the body. The effective operation of metabolic feedback mechanisms is essential to safeguard the body against the potential adverse effects that the intake of nutrients through food may precipitate. The disruption of this delicate equilibrium stands as a critical factor in the genesis of various diseases.

For instance, when food consumption reaches elevated levels, the hormone leptin, responsible for inducing a sensation of satiety, stimulates the hypothalamic cells in the brain, thereby creating a sense of fullness (Zhang et al., 1994). Conversely, in times of hunger, the ghrelin hormone, originating from the stomach, surges to significant levels, instigating the impulse to eat within the brain (Kojima et al., 1999). Another extensively studied protein, hepcidin, is released by the liver in response to an increased intake of iron, leading to a reduction in the absorption of dietary iron in the small intestine (Ganz

2003). These overarching examples underscore how the body operates within a metabolic framework that adapts to its nutritional requirements and allows different tissues to interact harmoniously.

As previously alluded to, glucose stands as the foremost nutritional factor intertwined with the pathogenesis of conditions such as obesity and diabetes. While the impact and molecular mechanisms of glucose in relation to these metabolic disorders have been extensively explored in tissues such as fat, liver, muscle, pancreas, and brain, there remains a gap in our understanding of the genes affected by glucose uptake within the small intestine, particularly in enterocyte cells, and the intricate metabolic pathways these genes govern.

One prominent aspect of metabolic regulation associated with intestinal tissue is the stimulation of the intestinal nervous system mediated by endocrine cells within the intestine (Grundy and Schemann, 2005). Furthermore, research suggests that enterocyte cells within the small intestine may play a crucial role in detecting dietary glucose (Lundgren et al., 1989). In a separate study, it was hypothesized that leptin, a protein synthesized in the stomach, may regulate liver glycogen metabolism via its receptor in the small intestine (Rasmussen et al., 2014). These studies collectively shed light on the potential influence of small intestinal tissue on glucose metabolism.

Obesity is a medical condition characterized by the excessive accumulation of fat in the body, leading to weight gain and a heightened risk of conditions like type 2 diabetes and other metabolic disorders. It is believed to impact the breakdown of nutrients, their absorption by enterocyte cells, and overall metabolism (Dailey, 2014). However, the precise influence of obesity on the mechanisms governing nutrient recognition by enterocyte cells, affecting the sensation and uptake of glucose, remains incompletely understood (Figure 1.2.).

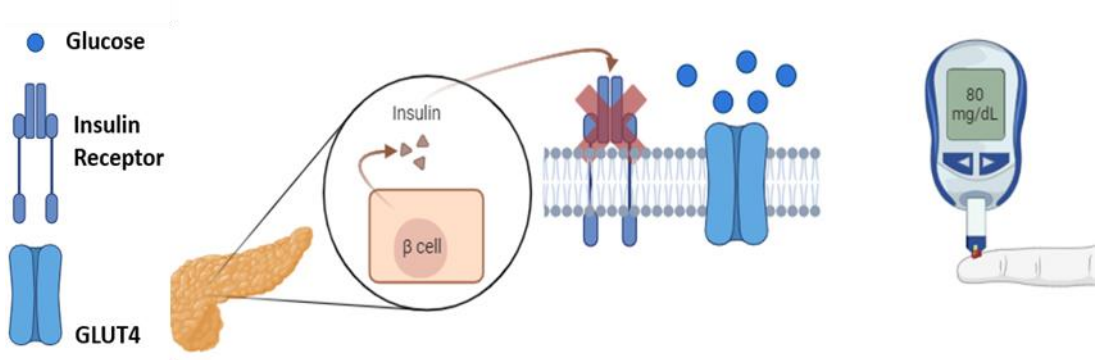

Figure 1.2. Glucose diabetes relation

The recognition and uptake of molecules by enterocyte cells, depending on their potential quantities, are primarily regulated by intracellular mechanisms. It is postulated that enterocyte cells possess specific control mechanisms for sensing the presence of glucose and regulating its absorption (Dyer et al., 2005). Yet, studies utilizing genetically modified mouse models with genes related to taste receptors (T1R2 and T1R3) located in the small intestine have not definitively demonstrated a direct impact of these taste receptors on enterocyte glucose metabolism. This suggests the existence of additional factors (Shirazi-Beechey et al., 2011; Simon et al., 2014).

Another noteworthy control mechanism unique to enterocyte cells is their ability to sense energy requirements and adjust eating patterns accordingly (Langhans, 2010). Increased glucose levels within the enterocyte cell can disturb intracellular energy balance and have adverse effects on the cell.

1.3. Small Intestinal Zinc Metabolism

Zinc, a fundamental element, plays a pivotal role in numerous biological processes within the human body (Maret, 2017). To minimize zinc loss and maintain a healthy zinc homeostasis, it is advisable to supplement this micronutrient on a daily basis (Rink and Gabriel, 2000). Zinc's homeostasis in the human body is predominantly regulated by its absorption at the intestinal level (Krebs, 2000). This absorption process involves the activation of zinc transporter receptors situated on the apical and basolateral

surfaces of enterocytes, with cellular and bodily zinc metabolism being overseen by metallothionein, a zinc-binding protein (King, 2011). Research efforts and data derived from these studies are contributing significantly to our understanding of the molecular mechanisms governing zinc absorption through the intestinal epithelium. It is important to note that zinc absorption depends not only on an adequate dietary intake but also on the physiology of the intestine, making the location of zinc absorption crucial.

This project has been designed to provide a deeper understanding of how factors such as dietary intake and intestinal physiology impact zinc absorption by the intestinal epithelium. Over the past five decades, the development of in vitro cell culture models has gained prominence as a means to replicate in vivo processes. This shift is attributed to the high costs associated with animal studies and the creation of a controlled microenvironment that facilitates ethical compliance while allowing for detailed cellular process investigations at the molecular level.

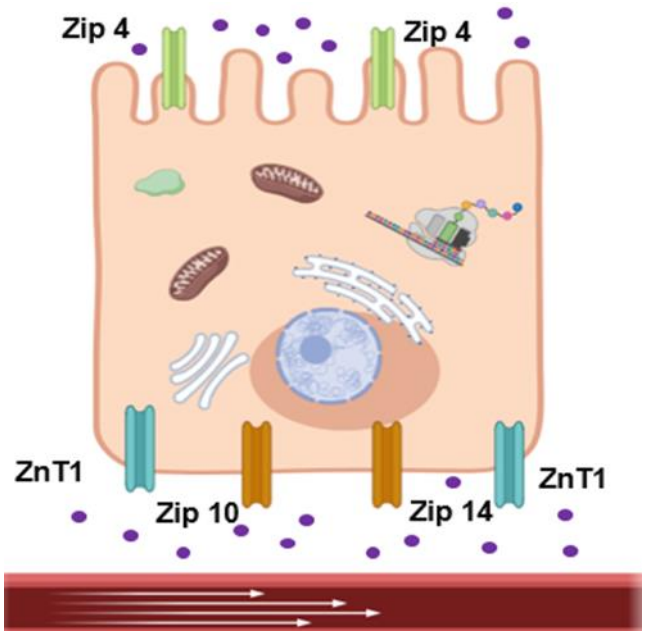

Figure 1.3. Illustration of zinc receptors on enterocyte cell.

The transfer of zinc from the intestinal lumen into enterocyte cells is facilitated by the ZIP4 protein located in the apical regions of enterocytes (Kury et al., 2002). Subsequently, the movement of zinc from enterocyte cells into the bloodstream is mediated by the ZnT-1 protein (Zhang et al., 2008). Moreover, zinc transport from the

bloodstream to enterocyte cells is orchestrated by the ZIP10 and ZIP14 transporters located in the basolateral region of enterocytes (Guthrie et al., 2015) (Figure 1.3.). Members of the ZIP transporter family work to increase cytoplasmic zinc concentrations by transporting zinc from intracellular organelles or the extracellular space, whereas ZnT proteins function to decrease cytoplasmic zinc levels by promoting cellular zinc efflux or sequestering zinc within subcellular compartments (Figure 1.4.).

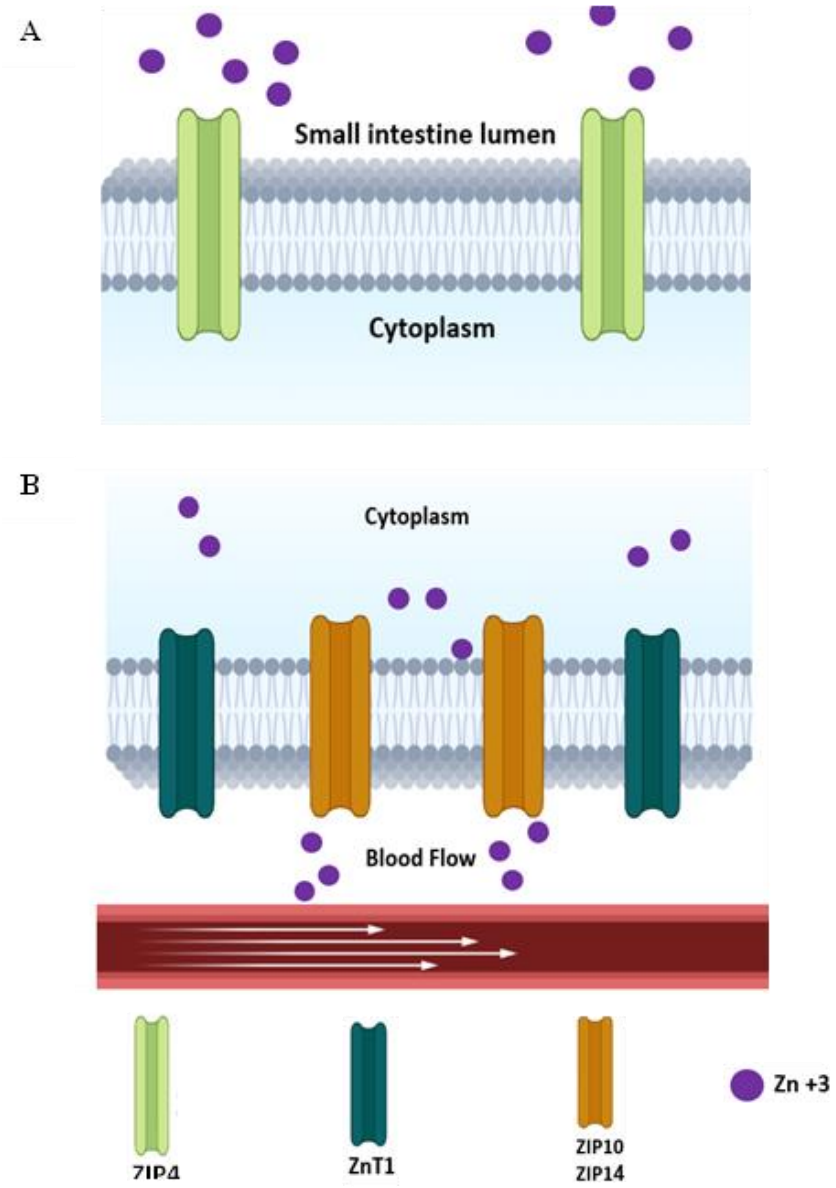

Figure 1.4. Cytoplasmic zinc efflux. (A) ZIP4 receptor, responsible for uptake zinc. (B) ZnT1 and ZIP10, ZIP14.

Another crucial group of proteins involved in maintaining cellular zinc homeostasis are metallothionein (MT). These proteins act as carriers, facilitating the movement of zinc to other zinc-binding proteins and enabling the uptake of zinc into the cell (Colvin et al., 2010).

1.4. Obesity – Zinc - Glucose Relations

In addition to their involvement in various critical functions such as cell division and immunity, trace minerals like zinc, iron, copper, and manganese play pivotal roles as co-factors in numerous metabolic pathways, including those governing carbohydrate, fat, and protein metabolism (Gaur and Agnihotri, 2017). Research studies have indicated that the levels of these trace minerals, namely zinc, iron, copper, and manganese, are impacted in the serum of individuals with obesity and type 2 diabetes (Blazewicz et al., 2013; Yerlikaya et al., 2013). Specifically, studies on obese individuals have revealed lower blood zinc levels (Di Martino et al., 1993; Rios-Lugo et al., 2020), prompting investigations into the potential influence of these mineral imbalances on the molecular and physiological mechanisms associated with obesity, an area of ongoing research.

Zinc, in particular, plays diverse roles within the body, participating in catalytic, regulatory, and structural functions. It is integral to many enzymes, notably those containing zinc finger motifs in transcription regulatory proteins (Frassinetti et al., 2006). Furthermore, in addition to the implementation of low-calorie diets, zinc supplementation has demonstrated effectiveness in ameliorating insulin resistance observed in obesity and type 2 diabetes (Khorsandi et al., 2019). A study conducted on 82,000 women in the United States found a 17% increased risk of diabetes associated with low zinc levels (Fukunaka and Fujitani, 2018).

There are 9 proteins, known as ZnTs, that act as zinc exporters, facilitating the transport of zinc out of intracellular organelles and cells. One of these proteins, ZnT8, is localized in granules within pancreatic beta cells responsible for insulin release and regulates zinc levels within these granules. Zinc is essential for synthesizing and stabilizing insulin when needed, as it binds to the insulin precursor protein (Dunn et al. 2005) (Figure 1.5). Functional abnormalities resulting from changes in the ZnT8 gene, particularly the conversion of the tryptophan-325 amino acid (rs13266634

polymorphism) to arginine, have been linked to reduced plasma zinc levels, thereby increasing the risk of glucose intolerance and type 2 diabetes (Sladek et al., 2007).

Zinc also plays a role in insulin-GLUT4 regulation by reducing the activity of PTP1B (protein tyrosine phosphatase 1B) in the insulin-GLUT4 signaling pathway, especially in adipose and muscle tissues. This regulation facilitates the transport of glucose from the blood into these tissues, thereby contributing to blood glucose control (Haase and Maret, 2005) (Figure 1.6). Furthermore, in a mouse model with the ZnT7 gene deleted, it has been demonstrated that the uptake of glucose into muscle tissue is compromised, negatively affecting glucose tolerance and insulin sensitivity in muscle tissue (Syring et al., 2016).

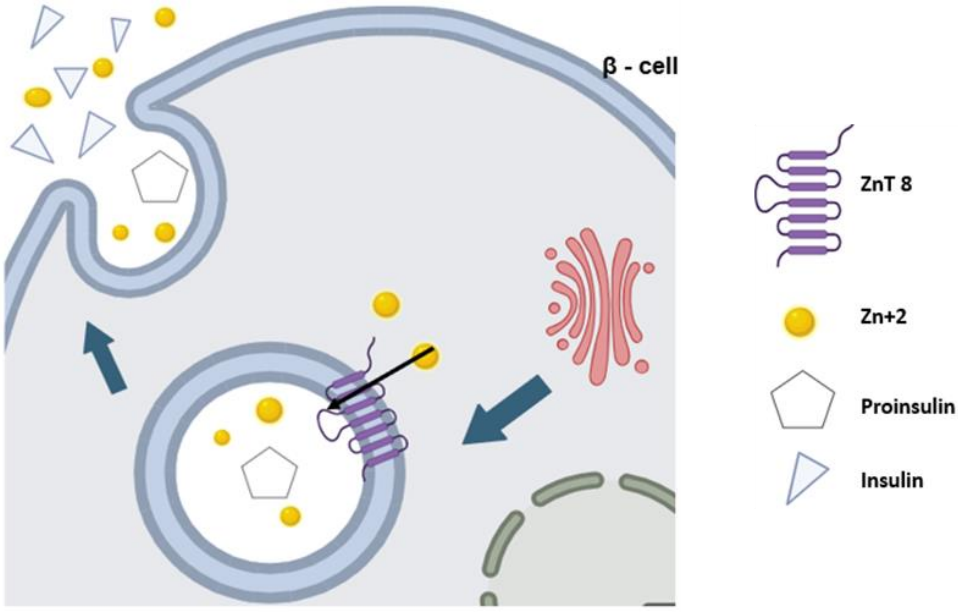

Figure 1.5. Insulin zinc interaction.

The regulation of zinc within the cell involves a group of 14 proteins known as ZIP transporters, which are found in various tissues and have been shown to have implications for glucose metabolism, obesity, and diabetes by controlling intracellular zinc levels (Fukunaka and Fujitani, 2018).

One such protein, ZIP13 (Zinc transport protein 13), plays a significant role in the formation and differentiation of fat cells by controlling zinc levels in adipose tissue (Fukunaka et al., 2017). The balance between brown adipose tissue and white adipose

tissue, both of which play distinct roles in energy metabolism, is crucial. White adipose tissue primarily stores fats, while brown adipose tissue is involved in energy expenditure. Increased intracellular zinc supports the development of precursor cells for white adipose tissue, whereas it hinders the formation of brown adipose tissue. Deleting the ZIP13 gene in a mouse model increased in gray adipose tissue, highlighting the role of intracellular zinc in fat metabolism, which is pertinent to obesity and type 2 diabetes.

ZIP7 (Zinc transport protein 7) is responsible for zinc uptake in skeletal muscle cells. Reducing ZIP7 levels has been shown to affect insulin receptor signaling through Akt protein phosphorylation, as demonstrated in a study using a mouse skeletal muscle cell line (C2C12) (Myers et al., November 2012; 2013).

ZIP14 (Zinc transport protein 14) is responsible for another aspect of zinc uptake, and studies have revealed increased levels of ZIP14 in obese individuals, which returned to normal following weight loss (Maxel et al., 2015). ZIP14 is believed to play a role in controlling the inflammatory response associated with the development of obesity and insulin resistance. In a mouse model with the ZIP14 gene deleted, it was found to increase the activities of the NF-kB and JAK2-STAT molecular pathways involved in inflammation, underscoring the importance of ZIP14 in reducing inflammation (Maxel et al., 2015).

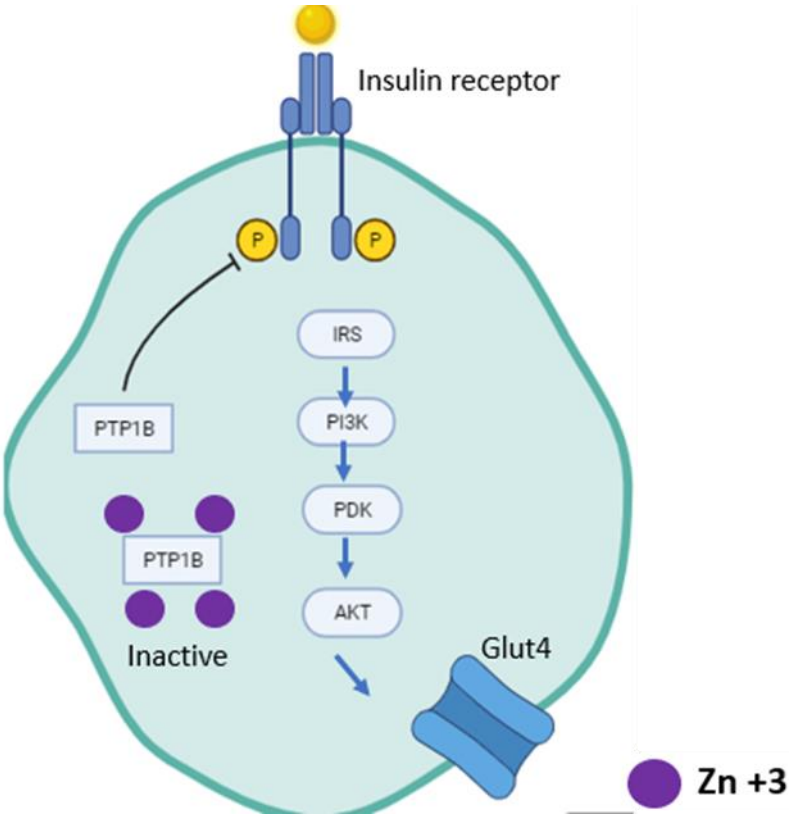

Figure 1.6. Zinc effected Glut4 activities indirectly.

In a study on ZIP14, it was observed that ZIP14 protein levels and zinc levels increased in the cell plasma membrane in response to elevated glucose uptake in liver cells (Aydemir et al., 2016). This indicates a physiological relationship between glucose ZIP14 and zinc in liver cells. The same study also noted that a reduction in zinc levels due to the deletion of the ZIP14 gene led to increased glycogen synthesis in liver cells, while gluconeogenesis and glycolysis pathways experienced a slowdown (Aydemir et al., 2016). These findings suggest that zinc functions as a sensor in intracellular glucose metabolism in the liver and influences carbohydrate metabolism through the ZIP14 protein.

Enterocyte cells in the small intestine play a pivotal role in regulating the body's mineral levels, constituting approximately 90% of the cells within the small intestine (Shirazi-Beechey et al., 2011). Considering this, the influence of nutrients from digested foods on enterocyte cells becomes a significant area of investigation. An illustrative example is the alteration in mRNA levels of 697 genes observed in response to increased glucose levels in a prior study. Among these genes, the changes in multiple genes

associated with zinc metabolism suggest a potential physiological connection between glucose, zinc, and enterocyte cells.

The primary proteins responsible for zinc uptake into enterocyte cells are ZIP4 (apical) and ZIP14 (basolateral), while the ZIP5 (basolateral) protein is also implicated in this process. Furthermore, there is a suggestion that other members of the ZIP protein family, such as ZIP10, ZIP11, and ZnT5, may have roles within enterocyte cells (Valentine et al., 2007). Information regarding the glucose-dependent regulation of mRNA levels for ZIP10 protein in enterocyte cells is currently lacking in the literature.

Despite the well-established link between zinc metabolism, mediated by ZnT and ZIP proteins, and metabolic disorders like obesity and diabetes, with demonstrated positive effects of zinc supplementation on various tissues such as the liver, fat, muscle, and pancreatic tissues, there is a dearth of information regarding a potential association with enterocyte cells in the small intestine. Building upon the findings of our previous study, it is hypothesized that such a relationship may indeed exist.

1.5. Aim of This Thesis

This thesis aims to explore the impact of zinc supplementation, administered in response to increased glucose intake in conditions such as diabetes and obesity, on the dynamics of enterocyte cells. The experimental design revolves around two primary hypotheses. The initial hypothesis aims to elucidate the modulation of genes responsible for zinc uptake and release within enterocyte cells under the influence of high glucose levels. This investigation extends to understanding the consequential effects on glucose absorption and release by these cells.

The second hypothesis delves into the potential role of zinc in regulating stress responses within enterocyte cells triggered by increased glucose concentrations. To interrogate this hypothesis, enterocyte cells were cultivated using an insert system, and the activities of genes associated with glucose metabolism were examined.

CHAPTER 2

METHODOLOGY

The second chapter explains the methodology that is followed in this thesis. Firstly, it explains how the human intestine's modeling works by Caco-2 cell culturing and how it is maintained properly. Also, how the intestinal transport system is formed due to Caco-2 polarization and how it is controlled with transepithelial resistance measurement. Then, high glucose examined in terms of intestinal zinc metabolism. Zinc effect on glucose uptake is mimicked by using 2-deoxy-glucose on the Caco-2 intestinal uptake system with glucose uptake colorimetric assay. Later, the investigation has demonstrated the influence of zinc on glucose release within the small intestine transport system, shedding light on its impact on the genes intricately linked to glucose metabolism. Consequently, the evaluation of zinc's effect on enterocyte cells amid increased glucose levels has been systemically conducted.

2.1. Modelling Human Intestine in Vitro

Caco-2 cells, originally identified in 1970 and subsequently utilized in cancer research models (Fogh et al., 1977), have played a critical role in advancing our understanding of the human intestinal system. The scarcity of non-cancerous small intestine enterocyte cells that can be directly obtained from human small intestine tissue and cultured has made it challenging to model the human intestinal system. Furthermore, the inability of these cells to effectively grow and polarize on specialized membranes has posed limitations on studying the human small intestine. Consequently, alternative cell models capable of being cultured and exhibiting metabolic activity under suitable conditions have become increasingly significant. To create in vitro small intestine cell models, it has been established that various growth and differentiation factors are necessary (Rousset et al., 1985).

When it comes to studying small intestine cell models, it has been observed that the Caco-2 cell model can closely mimic the functional, metabolic, and biological characteristics of enterocyte cells in the human body after approximately 21 days of cell culture growth (Sambuy et al., 2005). In comparison to other colon carcinoma cell lines, the Caco-2 cell line has demonstrated superior morphological and functional properties for investigating nutrient metabolism (Chantret et al., 1988). Notably, the Caco-2 cell model is the most widely used in vitro cell model for absorption studies of nutrient elements, drug-active substances, functional substances, and enterocyte mineral metabolism.

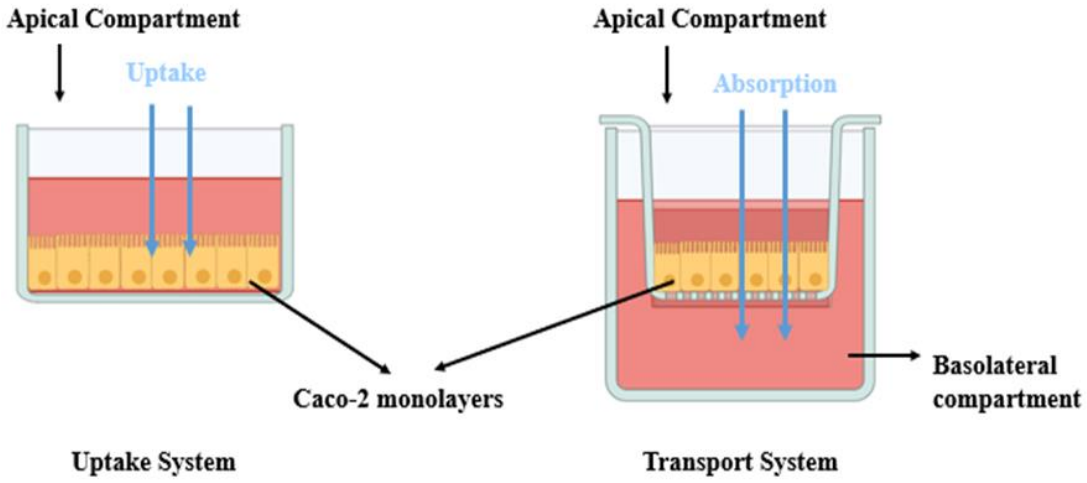

Figure 2.1. Uptake and transport system in Caco-2 cell.

One distinct advantage of working with Caco-2 cells is their capacity to polarize and differentiate when cultured on specialized membrane surfaces. Cell polarization involves the differentiation of apical (the luminal side where digested nutrients initially make contact) and basolateral (the side interfacing with the bloodstream) regions, closely resembling the human small intestinal system (Hidalgo et al., 1989). This polarization feature positions Caco-2 cells as the most suitable model for the human small intestinal system, enabling the investigation of nutrient metabolism and absorption. Furthermore, the Caco-2 cell line is frequently employed in studies focusing on intestinal glucose metabolism, thanks to its expression of proteins and molecular mechanisms involved in glucose metabolism (Bissonnette et al., 1996; Ravid et al., 2008; Grenier et al., 2013).

The presence of these crucial components in this cell line renders Caco-2 cells a valuable model for studying glucose metabolism.

2.2. Maintaining Caco-2 Cells and Simulating Small Intestine System

The human colon carcinoma cell line Caco-2 (HTB-37) was procured from the American Type Culture Collection (ATCC) as part of a previous research project supported by TUBITAK (Project Number: 214Z217). Subsequent investigations were conducted employing the available cell inventory.

Caco-2 cells were cultivated in a controlled cell culture environment within a CO2 incubator. This incubation facility was set to maintain a temperature of 37°C under a 5% CO2 atmosphere, with appropriate humidity levels.

Culturing was executed using a specialized medium that deviated from the traditional Minimum Essential Medium (MEM) (Sigma M4655). The medium composition consisted of 20% bovine serum (Hyclone, SV30160.03) 1% penicillin-streptomycin (Capricorn, PS-B), 1% non-essential amino acid (Multicell, 321-011-EL), and 1% sodium pyruvate (Capricorn, NPY-B). This medium formulation was thoughtfully designed to provide the requisite nutrients and conditions essential for the optimal growth and maintenance of Caco-2 cells.

To uphold the genetic and phenotypic integrity of the cell line, cells were regularly passaged. The choice to employ Caco-2 cells with a passage number ranging between 25 and 35 underscored a commitment to using early passages, thereby minimizing any potential genetic drift or phenotypic variation.

The meticulous execution of these techniques and protocols is paramount in ensuring the vitality of cell cultures and the attainment of dependable results in experimental investigations, aligning with the rigorous standards of professional and academic research.

Two distinct formulations of Dulbecco's Modified Eagle's Medium (DMEM) were employed, one containing a standard glucose concentration of 5.5 mM, and the other with an elevated glucose level of 25 mM. Prior to the utilization of these cell culture media, they were supplemented with 20% bovine serum and 1% penicillin-streptomycin.

Cells reaching 80% confluency were split 1:5 ratio before future cultivation. Cells were rinsed with PBS twice, and 2 ml tyripsin/EDTA was added into 75 cm^2 maintaining flask. The cells ere incubated at 37°C for 2-5 minutes. After detachment, cells were collected with 8ml maintaining medium, and 2 ml of collected cells were transferred into new 75 cm^2 flask. The medium replaces every three days. Generally, it takes 4-5 days for Caco-2 cells to reach 80% confluency. Cells were used between 25-35 passage for further experiments.

2.3. Polarizing Caco-2 Cells in Tissue Culture Inserts and Transepithelial Electrical Resistance (TEER) Measurement

The materials and methods employed in the preparation of the experimental cell line are succinctly illustrated in Figures 2.2. The cell expansion system is comprised of two components: the first component entails a 12 mm-diameter membrane crafted from polytetrafluoroethylene material, featuring 0.4-micron-sized pores and coated with collagen protein to facilitate cell adhesion. This membrane is housed within a dedicated cell culture flask.

During the preparation of the experimental cells, Caco-2 cells were initially seeded onto the membrane, with a starting cell count of 10,000. In this setup, 500 µL of the prepared culture medium was added to the upper compartment, while the lower compartment received 1.5 mL of the medium. The cells' growth progression was meticulously monitored under a microscope, with expectations that the cells would fully cover the entire membrane surface within approximately three days.

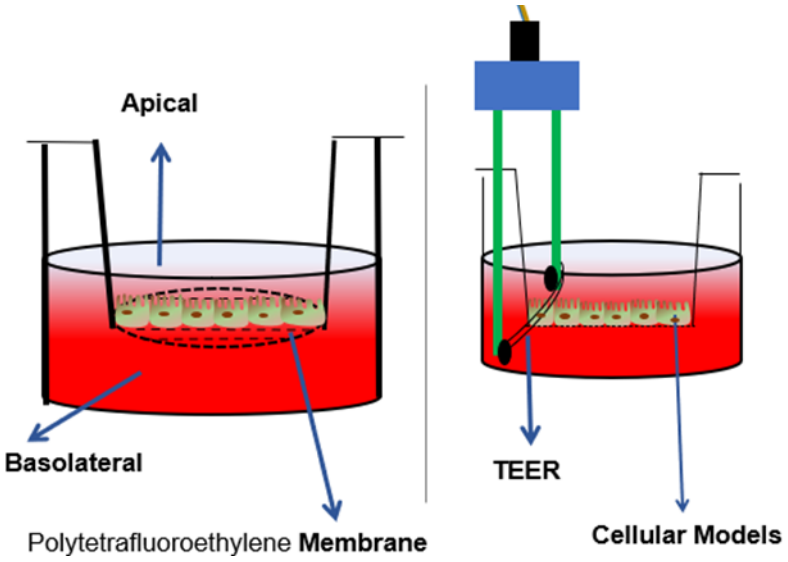

Figure 2.2. Preparation of experimental cell line.

Upon the attainment of this milestone, signifying the formation of a consistent single-layer (monolayer) and bipolar-polarized barrier system, the differentiation phase spanning 21 days commenced. During this period, the cells were subjected to regular medium changes, occurring at predefined intervals of every two days, with the specified volumes.

Following the establishment of a robust monolayer with full membrane coverage, glucose treatment was initiated, typically within 7-10 days. This model, emulating the human small intestine system with precision, designates the upper side as the apical aspect (the luminal part where nutrients initially interact with the intestine after ingestion) and the lower side as the basolateral facet (the region responsible for nutrient delivery to the bloodstream).

Notably, in this system, the glucose molecules introduced from the upper compartment are exclusively permitted to traverse the cellular barrier to reach the lower compartment, emulating the physiological processes observed in the human small intestine (Meunier et al., 1995; Delie and Rubas, 1997; Failla et al., 2008). This model ensures accurate replication of the intricate transport mechanisms intrinsic to the human small intestine.

2.4. Investigating The Effect of High Glucose on Zinc Metabolism

The cells were cultured on the membrane under two distinct glucose concentrations, namely 25 mM glucose(high glucose) and 5.5 mM glucose (control), over a period of 21 days. The primary rationale behind delivering glucose to both the apical and basolateral surfaces of the cells stems from two key observations associated with obesity. Firstly, the manifestation of excessive glucose consumption within enterocyte cells due to the influx of surplus glucose in obesity conditions and secondly, the augmented levels of blood glucose observed in obese individuals, thereby leading to heightened exposure of the basolateral aspect of enterocyte cells to glucose. A graphical representation of the experimental procedures employed in this section is provided in Figure 2.3 for clarity.

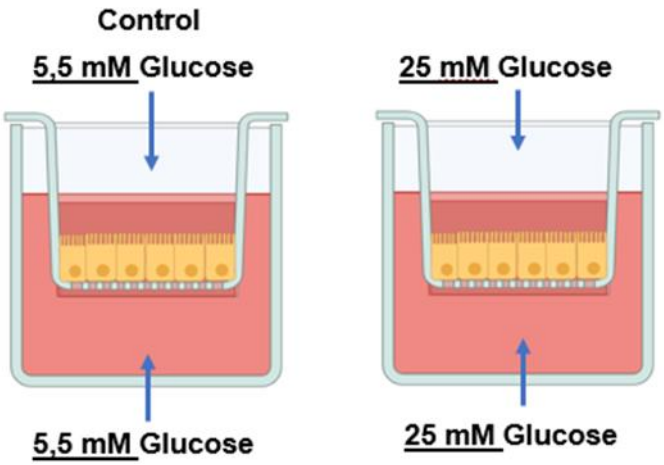

Figure 2.3. Polarized Caco-2 cell and glucose treatment.

2.4.1. Gene Regulations in Zinc Metabolism

In this section, the genes of interest for the study are ZIP4, ZIP14, and ZIP10, alongside Mt1 (Metallothionein1), a gene responsible for intracellular zinc transport and storage, regulated in response to zinc levels, as demonstrated by Ruttkay-Nedecky et al. in 2013. This comprehensive approach has yielded groundbreaking insights into the

potential interplay between glucose and zinc metabolism, with a focus on crucial gene regulatory processes in zinc homeostasis.

2.4.2. RNA Isolation

The RNA isolation process was executed using RNAZOLE chemical, following the manufacturer's protocol. In general, the cells were disrupted by treating them with 1 ml of RNAZOLE per sample, effectively severing their connection with the cellular membrane. Subsequently, ethanol was used to precipitate RNA from the RNAZOLE solution containing the fragmented cell population. The resulting RNA samples were further processed by washing with 75% ethanol and reconstitution in diethyl pyro-carbonate (DEPC) water. To assess RNA quality, the isolated RNA samples were subjected to electrophoresis in a 2% agarose gel. In brief, 2 µg of RNA per sample was combined with glyoxal chemical, incubated at 50°C for 30 minutes, and then loaded onto an agarose gel. The RNA bands were visualized under UV light, with the presence or absence of RNA degradation determined by the condition of the 18S and 5S RNA bands. RNA concentration in the samples was measured at a wavelength of 260 nanometers, and 1µg of RNA from each sample was used for the subsequent cDNA reaction.

2.4.3. cDNA Synthesis and Quantitative Polymerase Chain Reaction

The cDNA reaction, in essence, involved the synthesis of complementary DNA strands from the isolated single-stranded RNA fragments. In a simple process, RNA samples were reverse transcribed using a cDNA synthesis solution containing poly-A nucleotides and small six-base nucleotide primers in 20-µl volumes, with appropriate temperature adjustments. To assess the expression levels of specific genes, mRNA gene expression levels within the experimental groups were determined using the SYBR Green method, employing specific primers designed for the mRNA sequences in a qPCR device. The CT values obtained from the device were recorded for each sample, and subsequent analysis was conducted using the 2-ΔΔCt method to compare mRNA expression levels in

the samples. In the final step, expression levels were normalized to Cyclophilin A (a control gene) mRNA levels for accurate comparative analysis.

2.5. The Impact of Zinc Mineral on Glucose Absorption and Regulation Of Genes Involved In Glucose Metabolism

The study focuses on the distinctive role of zinc mineral administrated separately to the apical (the interface where enterocyte cells encounter nutrients) and basolateral (the interface enterocyte cells interact with blood) regions in intracellular glucose uptake, as well as its impact on the mRNA expression levels of genes integral to glucose metabolism. Furthermore, as part of these experimental procedures, control groups were incorporated to include samples untreated with zinc, thereby providing a basis for comparison.

2.5.1. Quantification of Cellular Glucose Uptake

Isotopes with radioactive properties, such as C14-Glucose, Fe59, and C14-Vit, have traditionally found use in nutrient absorption investigations (Johnston et al., 2005). Nevertheless, due to the challenges posed by workplace safety regulations and the high costs associated with these studies, alternative methods employing spectrophotometric or fluorometric techniques have been developed and are frequently employed.

In this particular research, a 2-deoxy-glucose (2DG) cell glucose absorption kit utilizing a spectrophotometric approach was adopted. The 2DG molecule is taken up by cells in a manner analogous to glucose absorption from food, and it is commonly employed in glucose uptake studies involving cells due to its non-metabolizable nature, which allows it to accumulate within the cell (Bissonnette et al., 1996; Goncalves et al., 2008; Lieder et al., 2017) (Figure 2.4).

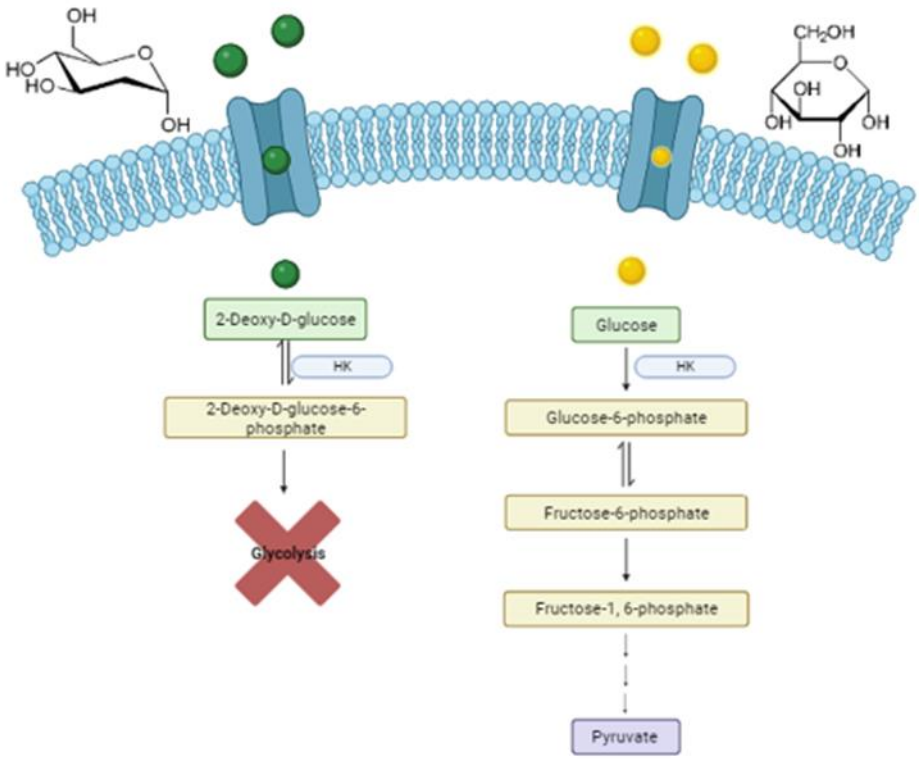

Figure 2.4. Working principle of 2 deoxy glucose

In this phase of the experiment, cells were cultured in 96-well cell culture plates and incubated for 14 days in a medium containing 5.5 mM glucose. Subsequently, the cells were subjected to mineral deprivation by exposing them to DMEM (supplemented with L-glutamine) without serum for 12 hours. After this period, the cells were treated with 200µM ZnSO4 in 15% FBS and incubated for another 12 hours. Following this incubation, the medium was aspirated, and the cells were washed twice with phosphate-buffered saline (PBS). A glucose-deprived medium (150µl/well) was then added, and the cells underwent a 1-hour glucose starvation phase. Afterward, they were rinsed twice with PBS and incubated in a 10 mm 2DG solution, as well as a transport solution (comprising 130 mmol/L NaCl, 10 mmol/L KCl, 1 mmol/L MgSO4-7H2O, and 50 mmol/L HEPES at pH 7.0), for a duration of 30 minutes.

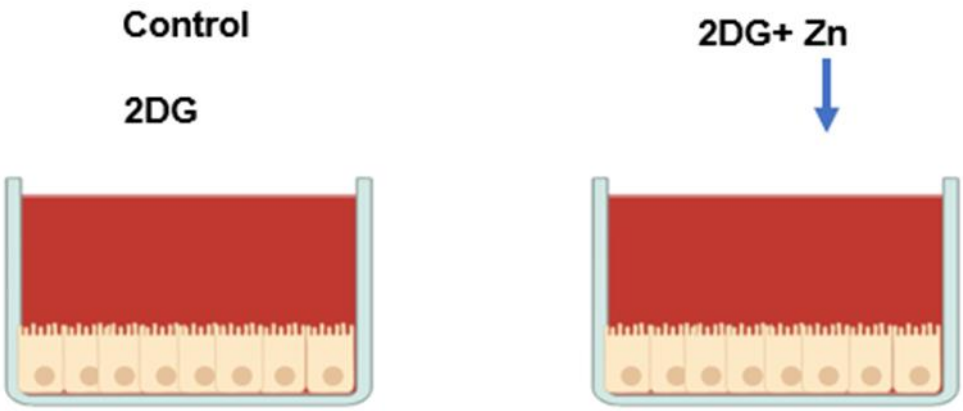

Figure 2.5. Glucose uptake assay.

Following the incubation, the cells were harvested. To eliminate any residual 2DGlc molecules that might remain unabsorbed on the cell surface, the cells were thoroughly washed with cold PBS. Subsequently, the cells were collected and treated with the extraction solution provided by the kit. The cellular breakdown was facilitated by subjecting them to a nitrogen-room temperature cycle for a brief duration, thereby releasing the intracellular 2DG (Figure 2.5.).

The quantification of zinc-dependent glucose uptake between the experimental groups was carried out spectrophotometrically, adhering to the procedures outlined in the manufacturer's protocol. The selection of incubation periods, cell glucose concentrations, and zinc treatments in this segment of the study was based on established ratios used in previous research (Maxel et al., 2015; Aydemir et al., 2016). When required, different incubation times, glucose levels, and zinc concentrations were employed, ensuring that experimental conditions remained physiologically relevant to the cells.

2.5.2. Assessment of Intracellular Glucose Release

To assess the release of glucose from cells triggered by elevated intracellular zinc levels, a 21-day growth period was initiated for the cells at the site designated for cell expansion, where a 5.5 mM glucose concentration was maintained within the membrane

system, as previously mentioned. Subsequently, the cells cultured on the membrane underwent the same experimental conditions as described in the cell glucose uptake experiment section.

The Trans-Epithelial Electrical Resistance (TEER) values of the cells were measured, and their polarization was carefully verified. Only cells exhibiting a TEER value of 250 Ω/cm2 or higher were included in the subsequent glucose absorption studies. Following the 21-day growth period, the cells were exposed to a serum-free DMEM medium for 12 hours and were subsequently subjected to zinc treatments. After this 12-hour incubation, 200 µM ZnSO4-7H2O was administered to both the apical and basolateral compartments of the insert system, and the cells were incubated for an additional 12 hours.

At this stage, TEER levels were measured once more. In the subsequent phase, instead of 2DG, 25 mM glucose was administered from the apical part of the system, and samples were collected from the basolateral compartment at 30-minute intervals.

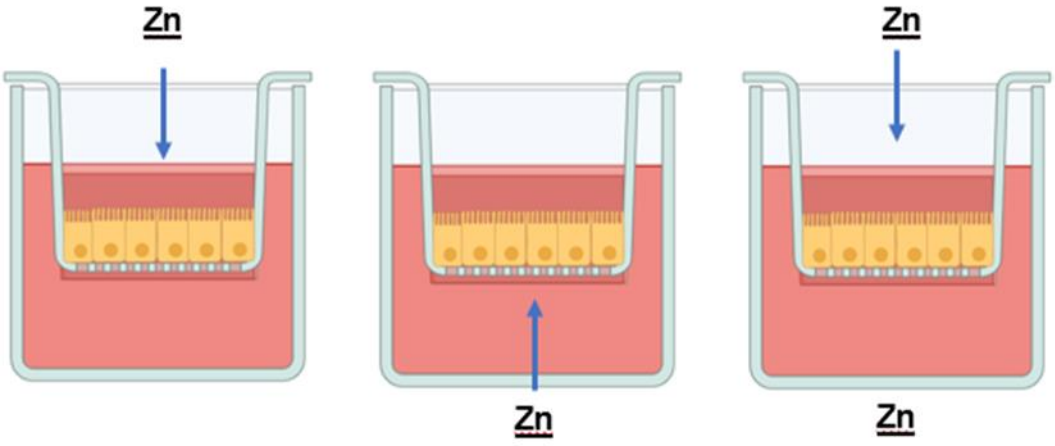

Figure 2.6. Zinc treatments in insert system.

The quantification of glucose within the collected samples was performed using a spectrophotometric approach, employing the provided glucose measurement kit. These measurements were subsequently normalized to the total cellular glucose concentrations, allowing for comparisons between the experimental groups. It's important to note that in this experimental design, 2DG was not utilized because it doesn't exit the cell once it has

been internalized, unlike glucose. Therefore, the form of glucose commonly found in normal carbohydrates was used in the glucose release experiments. Nonetheless, the intracellular glucose levels were measured and compared with the 2DG results obtained in the previous section of the study.

As outlined at the beginning of the method section, the cells were cultivated within the membrane system. Zinc treatments were administered to different parts of the cells, including the apical and basolateral compartments, the apical side alone, and the basolateral side alone. This approach was selected because treating distinct regions of enterocyte cells with the same nutrient may lead to differences in intracellular molecular and physiological responses. In other words, when zinc was delivered to the basolateral side, it could impact glucose absorption by influencing genetic regulation on the apical side. Previous studies focusing on various nutrients have demonstrated the existence of such regulatory mechanisms (Ravid et al., 2008; Grenier et al., 2013; Güleç, 2018)

2.5.3. Assessment of Gene Regulation in Glucose Metabolism

In this segment of the study, genetic investigations were conducted to examine the regulation of zinc-responsive mRNA associated with genes responsible for enterocyte glucose absorption and their role in intracellular glucose metabolism. This approach allowed us to elucidate both the impact of zinc on the cellular entry of glucose molecules and the modulation of genes responsible for glucose uptake.

For physiological insights, explored the zinc-dependent regulation of mRNA levels for the PEPCK (Phosphoenolpyruvate carboxykinase) and G6Pase (Glucose-6-phosphatase) enzymes, which play pivotal roles in the regulation of glycolysis-gluconeogenesis metabolism. The methods employed for RNA isolation, cDNA synthesis, and RT-qPCR were consistent with the procedures detailed in the preceding section. RT-qPCR analyses were executed using the appropriate primers designed for the specific mRNA sequences of the genes investigated in this phase of the study.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Effect of High Glucose on Zinc Metabolism

The primary objective of this thesis was to investigate the impact of apically and basolateral polarized Caco-2 cells on zinc metabolism in response to chronic treatment with 25 mM glucose. To achieve this, initially examined the mRNA regulation of genes related to zinc metabolism in enterocyte cells, including ZIP4 (responsible for zinc entry from the apical side), ZIP14 (associated with zinc uptake from the basolateral side), ZIP10 (involved in basolateral zinc uptake), and MT1a (associated with intracellular zinc binding and storage) (see Figure 3.1.).

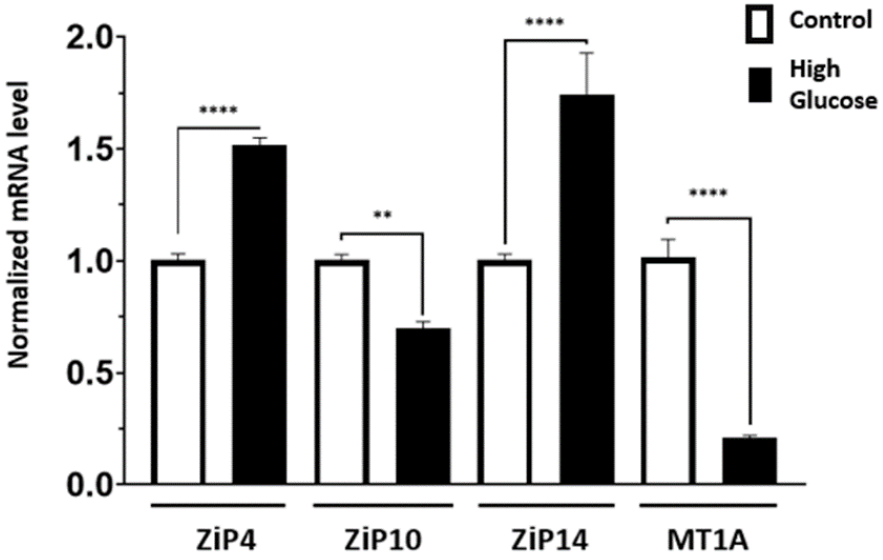

Figure 3.1. Illustrates the mRNA levels of genes implicated in zinc mineral metabolism. Following the determination of mRNA expression levels in the respective groups, CT values were normalized relative to Cyclophilin A (CypA) mRNA.

Upon evaluating the results, it became evident that treatment with 25 mM (high glucose) led to an increase in ZIP4 and ZIP14 mRNA levels compared to the group treated with 5.5 mM (low glucose). Conversely, the levels of ZIP10 and MT1a mRNA decreased in the high glucose group. These findings suggest that elevated glucose levels have a discernible impact on zinc metabolism and are associated with a reduction in intracellular zinc content. Notably, such physiological responses and alterations are observed when the small intestine enterocyte cells need to regulate the absorption of specific nutrients, with iron deficiency being one of the most illustrative examples of such a process.

In instances of iron deficiency, a remarkable adaptation is observed, where the volume of the intestinal villi expands to enhance the localization of transport proteins responsible for iron uptake into the cells (Gulec and Collins, 2014). When interpreting our results, the reduction in MT1a levels implies a decrease in intracellular zinc content, particularly in comparison to the control group. In light of this finding, the intracellular zinc levels were measured in cells cultured in both high and low-glucose environments, considering the decrease in MT1a mRNA. Our results have demonstrated a statistically significant decrease in intracellular zinc in cells cultivated in a high-glucose environment (see Figure 3.2).

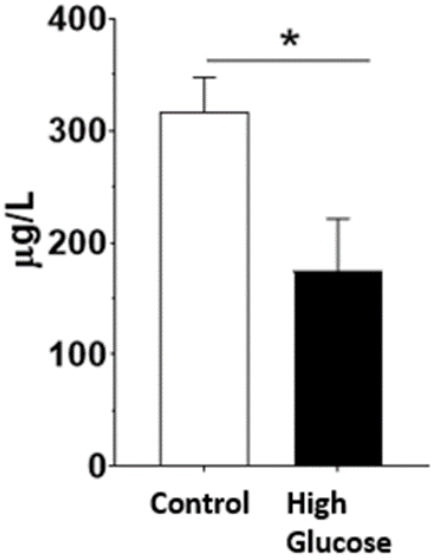

Figure 3.2. Illustrates the intracellular zinc levels in response to high glucose. The experiments were conducted using a minimum of n=2 samples per group, each experiment underwent three independent repetitions. Results are present as mean standart deviation (SD)

Intracellular zinc deficiency can be correlated with heightened levels of MT1a and mRNA related to the ZIP4 and ZIP14 genes, which are responsible for zinc uptake by the cell. Notably, when examining the literature's findings regarding ZIP14 regulation, an increase in ZIP14 expression in liver cells has been associated with intracellular glucose uptake (Aydemir et al., 2016). Furthermore, the deletion of the ZIP14 gene in mice has been shown to lead to an increase in glycogen content in liver cells. It has been reported that the expression level of ZIP4 increases in small intestinal enterocyte cells under conditions of low zinc (Liuzzi et al., 2009). In the same study, it was noted that the level of MT1a mRNA decreases in tandem with the reduction in intracellular zinc. It is worth mentioning that information concerning ZIP10 and its regulation in small intestinal enterocyte cells remains limited in the existing literature. ZIP10 is predominantly expressed in various tissues, especially in the brain, liver, blood, and epidermal cells (Jeong and Eide, 2013).

Information regarding ZIP10 and its regulation in small intestinal enterocyte cells is limited within the existing literature. ZIP10 is typically expressed prominently, particularly in brain, liver, blood, and epidermal cells (JeongveEide, 2013). To synthesize the findings on ZIP10, research in rat thin intestine tissue indicates that thyroid hormones elevate ZIP10 levels (Pawan et al., 2007). Examining zinc transport gene expression rates in various tissues within a pig animal model, administered zinc in varying proportions, revealed that ZIP10 gene expression is notably lower in samples obtained from jejunum and colon tissues compared to other tissues (Brugger et al., 2021).

In a study elucidating the role of zinc and zinc-specific receptors in the association between obesity and breast cancer, it was demonstrated that the growth of cancerous cells decelerates due to a decrease in ZIP10 gene activity in response to high glucose. Consequently, zinc and ZIP10 play a crucial role in impeding the growth of cancerous cells within a high-glucose environment (Takatani-Nakase et al., 2014).

Considering the collective body of research, an investigation was conducted to comprehend how the regulation of ZIP10 is influenced in the presence of high glucose and zinc in enterocyte cells. This was achieved by examining its expression in a high glucose-containing environment upon the administration of zinc (Figure 3.3).

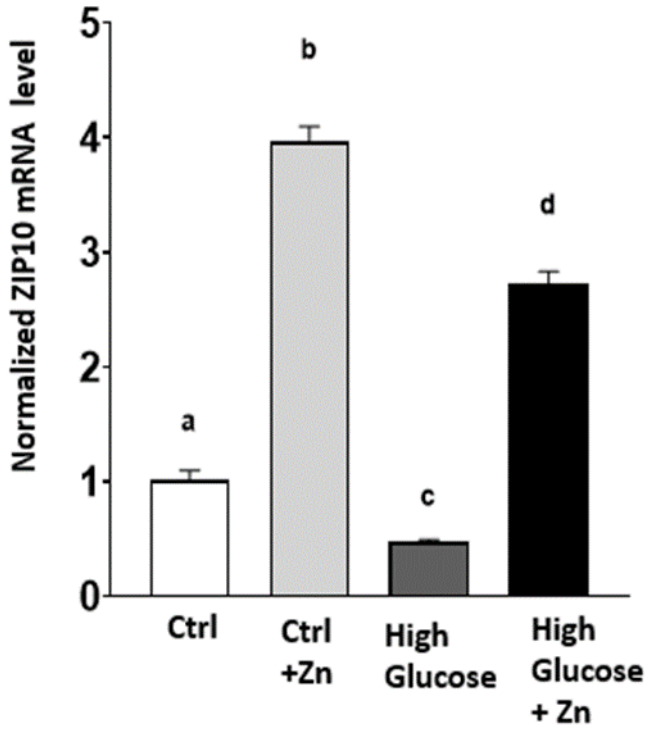

Figure 3.3. ZIP10 mRNA levels. After determining the mRNA expression levels of the samples in the groups, CT values were normalized according to Cyclophilin (CypA) mRNA.

Upon evaluating the results, it was observed that in the case of high glucose, ZIP10 mRNA levels, which initially decreased, increased significantly in the presence of zinc. This finding suggests that ZIP10 may play a pivotal physiological role, particularly under conditions of high glucose levels.

3.2. Investigation of the Zinc on Cell Glucose Uptake

Upon evaluating the results, it became evident that intracellular zinc levels are influenced by elevated glucose concentrations and may also be linked to the regulation of specific genes involved in zinc metabolism. In this context, a crucial question emerges concerning the potential association between the availability of zinc within the cells and glucose uptake. To investigate this, the most appropriate experimental model involves the use of a radioactively labeled carbon-marked glucose molecule. However, for cellular

glucose uptake experiments, 2-deoxy glucose (2DG) is the preferred choice due to the availability of specialized media and equipment for radioactivity studies, as well as regulatory compliance with government guidelines **(**Bissonnette et al., 1996).

After cell maintenance and differentiation, 12 hours was employed to induce zinc deficiency within the apical site, while keeping the cells in a serum-free environment. Following this, the cells were treated with 200 µM ZnSO4 in the presence of serum and incubated for an additional 12 hours. After this incubation, the cells were subjected to a glucose-deficient environment, and subsequently, a transport solution containing 2-deoxy glucose (2DG) was administered, allowing for a 30-minute treatment period. Following this procedure, the intracellular glucose levels were quantified through spectroscopic analysis.

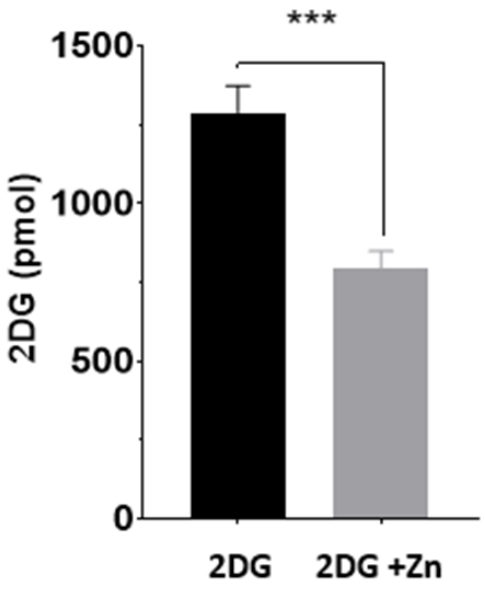

Figure 3.4. The effect of zinc given to cells on the uptake of 2 deoxy glucose.

The results indicated that the group receiving zinc exhibited a reduction in 2DG uptake into the cells (Figure 3.4). This outcome is hypothesized to stem from the influence of unknown molecular pathways at the cellular level, which may be a consequence of the increased intracellular zinc levels. In particular, the involvement of zinc in the formation of zinc finger motifs within numerous transcription factors suggests the potential for gene regulation in this process. Nevertheless, further investigations are required to substantiate this hypothesis.

3.3. The Effect of Zinc Given to Apical and Basolateral Parts of Cells on Glucose Metabolism in Enterocyte Cells

The primary physiological and structural characteristic of small intestine tissue is its polarization. This polarization designates one region of the small intestine tissue to specialize in nutrient absorption from digestion (the apical side) and the other region to serve as the interface with the circulatory system (blood) (the basolateral side). This polarization gives rise to distinctive intracellular signaling specializations and differentiation between the apical and basolateral regions.

Building upon this perspective, the impact of zinc supplementation on glucose metabolism was investigated in the apical, basolateral, and combined apical+basolateral regions. Initially, a membrane system was employed to culture cells for 21 days, creating a small intestine model. Subsequently, the trans-epithelial electrical resistance (TEER) value was assessed to ensure it reached at least 500 ohms/cm2. As mentioned earlier, 200 μM of zinc mineral was administered for a duration of 12 hours. RNA isolation was conducted from the cells, and samples were collected for the analysis of glucose levels in the basolateral region.

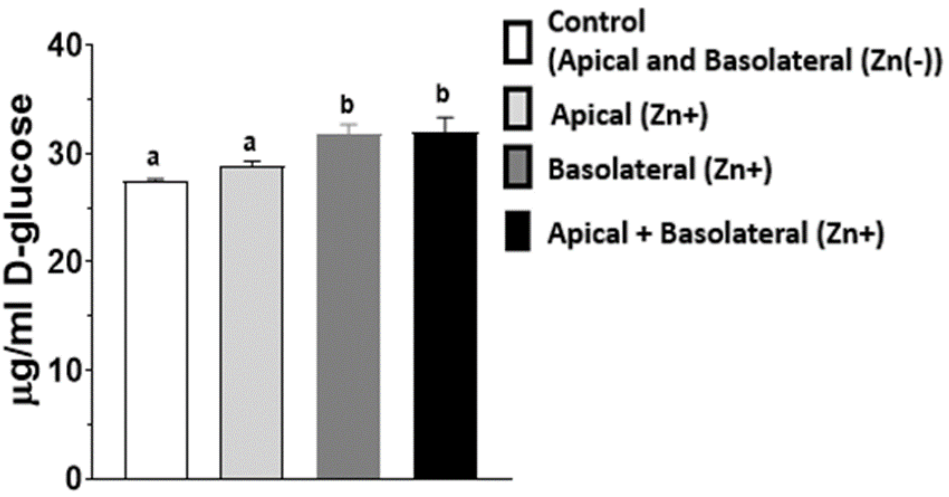

Figure 3.5. The impact of zinc administration to distinct regions of polarized Caco-2 cells on glucose release

In this section of the study, the comparison of glucose released from the cells and transferred to the basolateral part among different groups revealed a statistically significant increase in glucose levels only when zinc was administered to the basolateral and apical+basolateral regions. However, the increase observed was relatively modest, approximately 10% higher compared to the control and apical regions (as depicted in Figure 3.5). This suggests that zinc, when present in the basolateral region, tends to enhance the release of glucose from the cell. It is important to note that this effect may be attributed to the concurrent increase in intracellular zinc levels. This interpretation is supported by the noteworthy increase in MT1a expression levels, which was observed exclusively in the groups where zinc was administered to the basolateral and apical+basolateral regions (as shown in Figure 3.6).

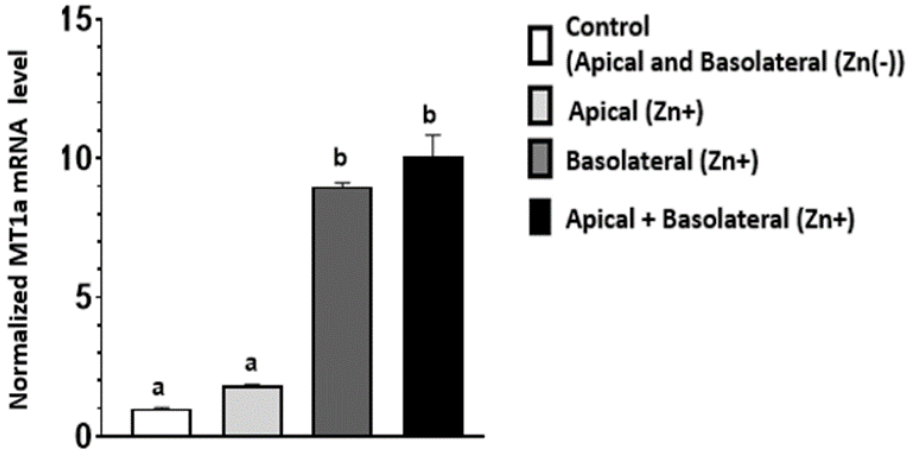

Figure 3.6. MT1a mRNA levels. After determining the mRNA expression levels of the samples in the groups, CT values were normalized according to Cyclophilin (CypA) mRNA

Another critical observation is the potential influence of zinc on the expression of genes associated with glucose metabolism. To explore this, the regulation of these genes was investigated using cells from the glucose release experiment mentioned previously. Specifically, the mRNA level of the glucose transport protein SGLT1, located in the apical region, decreased significantly as a result of zinc administration (as illustrated in Figure 3.7A). This reduction was consistent across all groups receiving zinc, regardless of the specific region.

Furthermore, the mRNA level of GLUT2, located in the basolateral region of enterocyte cells, increased notably when zinc was administered to the basolateral and apical+basolateral regions (as seen in Figure 3.7B). While these changes in the specified genes align with the entry and release of glucose into the cell, the regulation of these alterations at the protein level is of paramount importance.

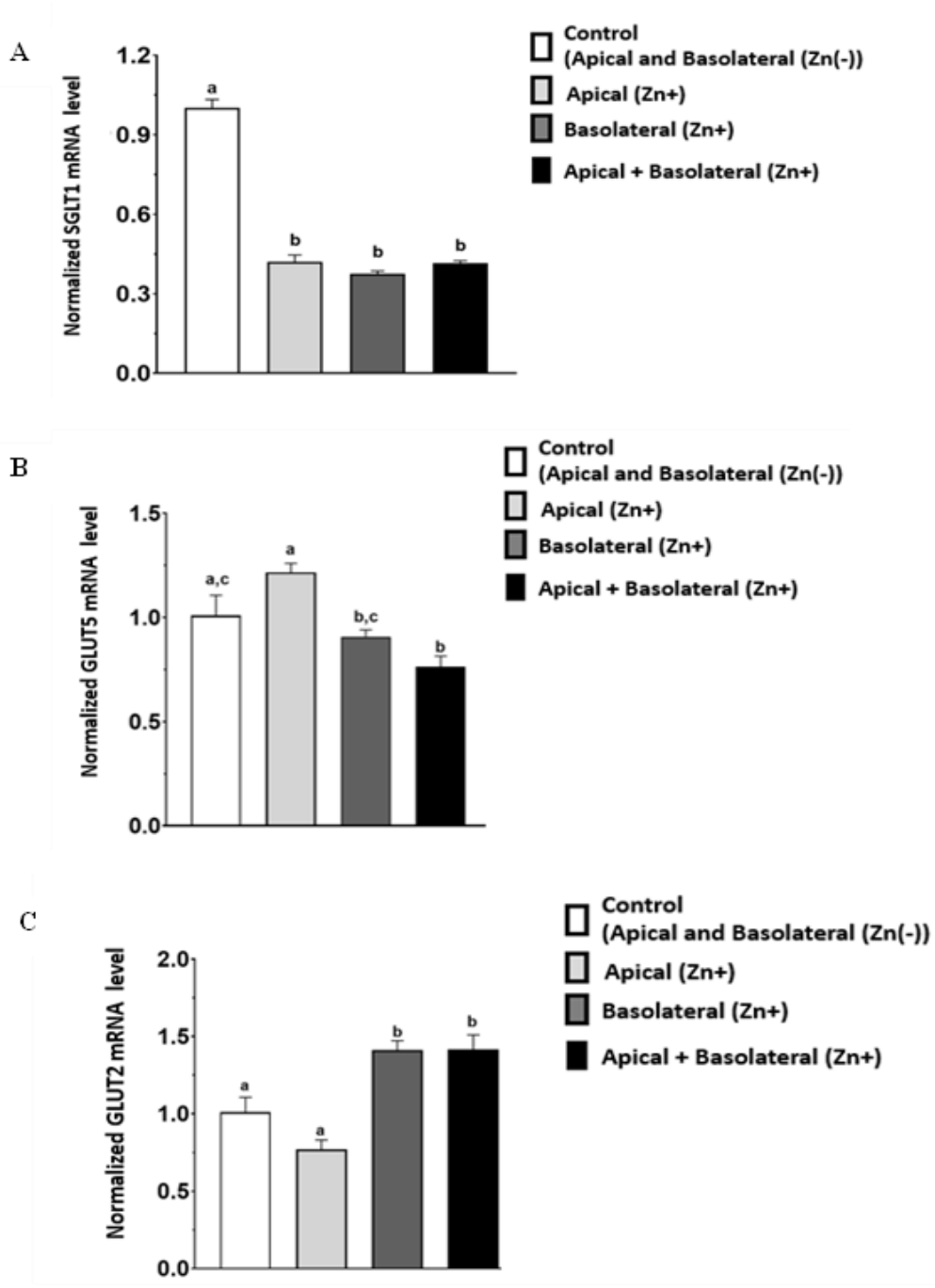

Figure 3.7. Zinc-dependent expression levels of genes involved in glucose metabolism

In addition to SGLT1 and GLUT2, the mRNA level of GLUT5, which is responsible for fructose uptake from enterocyte cells but is also believed to have the potential to transport glucose, was analyzed. The results indicated a regulation pattern similar to that observed for SGLT1 mRNA (Figure 3.7C).

PEPCK is a key enzyme in the gluconeogenesis pathway, which involves the synthesis of glucose from non-carbohydrate molecules in the absence of glucose. The presence of insulin or glucose in relevant tissues typically exerts an inhibitory effect on PEPCK (Quinn and Yeagley, 2005). Notably, the PEPCK mRNA level exhibited a statistically significant increase in the basolateral and apical+basolateral regions of the zinc-treated groups compared to the other groups (as depicted in Figure 3.8A). This increase could be linked to the observed trend of reducing intracellular glucose levels.

G6Pase is another pivotal enzyme involved in glucose production, particularly in the liver tissue, and plays a crucial role in regulating blood glucose levels (Van Schaftingen and Gerin, 2002). Additionally, it serves to protect red blood cells from oxidative damage. It has been reported that G6Pase mRNA and enzyme activity increase, especially in thin intestinal tissue during fasting, suggesting that the small intestinal tissue contributes to raising blood glucose levels by synthesizing glucose to meet systemic glucose demands (Rajas et al., 1999). However, it was observed that the level of G6Pase mRNA decreases when zinc is administered to the apical+basolateral regions of Caco-2 cells (Figure 3.8B).

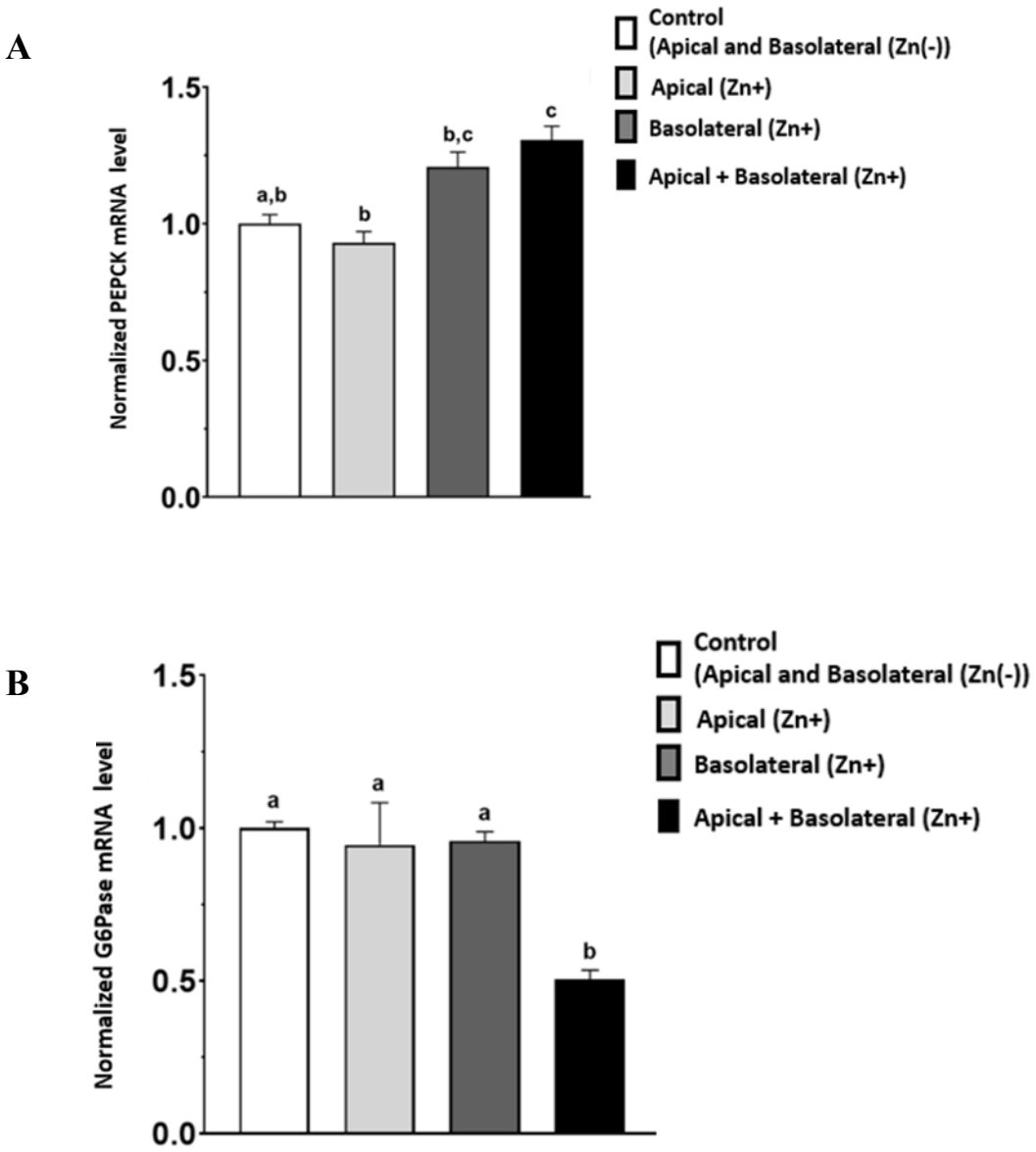

Figure 3.8. Zinc-dependent expression level of PEPCK and G6Pase.

When considered alongside other experimental findings, this result suggests that the presence of zinc results in both reduced glucose entry into the cell and increased glucose release from the cell. In terms of the regulatory role of G6Pase, it appears that glucose levels and enzyme activity change inversely. Nevertheless, the interpretation of this regulation in the context of zinc administration can be attributed to the antioxidant properties of zinc.

CHAPTER 4

CONCLUSION

Given the critical role of enterocyte cells in the small intestine, particularly in nutrient uptake and the simultaneous transport of numerous nutrients, it becomes imperative to scrutinize intercellular nutrient processes and unveil their potential implications on metabolic diseases. Research conducted within the framework of this project has revealed a plausible physiological correlation between high glucose levels and zinc in enterocyte cells. Additionally, it suggests that intracellular zinc, with particular emphasis on blood zinc levels, may emerge as influential factors in glucose metabolism within enterocyte cells. Another noteworthy consideration is the presence of various transcription factors, notably proteins featuring a zinc finger motif, operating in a zinc-dependent manner. From this perspective, heightened glucose levels in enterocyte cells can potentially modulate specific regulations associated with zinc-related molecular and genetic processes.

The application of high glucose to the cell leads to a reduction in the absorption of zinc, consequently resulting in a decrease in both intracellular zinc levels and the expression of MT1A within the cell. The diminished level of MT1A can be construed as a biomarker indicative of the necessity for increased zinc intake by the cell.

In essence, under high glucose conditions, the intracellular zinc content diminishes, MT1A expression decreases, and the ZIP4 receptor, responsible for zinc uptake into the cell, experiences an elevation. This prompts a cellular response involving an uptake of zinc.

To fulfill its zinc requirements, the cell amplifies the expression of its transport mechanisms. However, in the absence of a corresponding increase in zinc intake, the MT1A level diminishes. This underscores the inference that enterocyte cells require zinc in a high-glucose environment. Yet, the specific metabolic pathways regulated within the cell remain unidentified.

It can be postulated that zinc serves as a regulatory element in gene expression. Kruppel-like factor 4 (KLF4), a zinc transcription factor predominantly localized in the

small intestine, plays a pivotal role in this context. Notably, the zinc level in enterocyte cells exposed to high glucose diminishes. To compensate for the reduced zinc availability, the cell upregulates the expression of KLF4 and enhances the presence of the ZIP4 zinc receptor situated in the apical region. Consequently, due to zinc deficiency within the cell, the MT1A level decreases. KLF4 also contributes to the heightened expression of the SGLT1 receptor, responsible for glucose uptake into the cell.

Although an explicit zinc deficiency condition is not established within the cell in this scenario, in the presence of high glucose, the cell interprets this circumstance as a zinc deficiency. Consequently, it responds by elevating the level of KLF4 to increase the expression of ZIP4, facilitating zinc uptake into the cell. Upon evaluating these findings, it becomes apparent that the cell signals its need for zinc when exposed to increased glucose input. This underscores the role of zinc in regulating cellular balance under conditions of high glucose.

Under high glucose conditions, a notable observation is the reduction in expression of the ZIP10 receptor located in the basolateral region of enterocyte cells. This outcome contrasts with the behavior of other zinc-specific surface receptors. In an experiment designed to elucidate whether ZIP10 regulation is contingent upon zinc in a high glucose environment, results indicated a decrease in ZIP10 expression under high glucose conditions, with a subsequent increase upon the introduction of zinc into the environment. Consequently, the evaluation of ZIP10 regulation at the small intestine level reveals its dependency on zinc in the context of high glucose.

Moreover, the pivotal role of ZIP10 in epigenetic regulation within skin tissue, maintaining homeostasis via histone acetyltransferase in epidermal tissue, and its elevated expression in response to zinc deficiency in brain and liver tissues suggest that ZIP10 operates on distinct principles in various tissues.

The regulation of intracellular zinc levels is contingent upon various factors, including the presence of zinc receptors in both apical and basolateral regions in high glucose environments and the availability of zinc in the surrounding milieu. This condition emerges as a crucial regulator responsible for glucose metabolism. In the initial investigations into the role of zinc in glucose absorption, it was observed that the presence of zinc in the environment resulted in a reduction of glucose uptake by the cell. Conversely, in the absence of zinc, an increase in glucose entry into the cell was noted. This shift could potentially induce cellular stress or physiological effects such as alterations in intestinal permeability. To counteract this situation, zinc either diminishes

glucose uptake or amplifies glucose release from the cell, serving as a protective mechanism for cellular well-being.

Upon scrutinizing the results, it becomes conceivable to regard basolaterally administered zinc as analogous to zinc found in the bloodstream, leading to an augmentation in glucose release from the cell. Consequently, zinc emerges as a mitigating factor, potentially minimizing stress in enterocyte cells, particularly concerning intestinal metabolism, in scenarios involving high glucose concentrations. This observation supports the hypothesis that zinc may exert a regulatory influence at the small intestine level.

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