The Effects of nutrient availability and O-GlcNAcylation on pericyte differentiation fate

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Abstract

Pericytes are mural cells on the surface of capillaries, which may have the capacity to differentiate into various lineages. However, the factors that could determine their differentiation fate remain to be determined. I hypothesized that nutrient availability, O-GlcNAcylation status, and metabolic stress could alter pericyte phenotype. In my experiments, I used mesenchymal cells from the skeletal muscle of C57B16/J mice as a model, due to the similarity in the characteristics of mesenchymal cells and pericytes. My data showed that Zfp423 protein levels are higher when exposed to normal glucose levels (5 mM), compared to high glucose levels (25 mM), which leads to higher fat droplet formation in mesenchymal cells. However, my data did not support the influence of O-GlcNAcylation on adipogenesis. Lastly, I observed that Transforming growth factor-β1 could potentially induce myocyte-looking cell formation. This finding provides an insight into how nutrient availability could influence the differentiation potential of pericytes.

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Statement of contributions

All chapters of this thesis were written by Barbod Azimikhiabani and further revised with recommendations from Dr. Tara Haas.

Most aspects of the study were conducted by me, although some of the cells were extracted by my research laboratory George Nader.

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List of Abbreviations:

0G: No Glucose

AMPK: Adenosine- Monophosphate- Activated Protein Kinase

Ang-1: Angiopoietin

BBB: Blood Brain Barrier

CD146: Cluster of Differentiation 146

C-EBPß: C-Enhance Binding Protein ß

EC: Endothelial Cell

EGF: Epithelial Growth Factor

FAO: Fatty Acid Oxidation

FAs: Fatty Acids

FBS: Fetal Bovine Serum

GFAT: Glutamine Fructose-6- Phosphate Amidotransferase

HBP: Hexosamine Biosynthesis Pathway

HG: High Glucose

HIFs: Hypoxic Inducible Factors

IGF-1: Insulin- Like Growth Factor- 1

IP: Immunoprecipitation

iPSCs: induced pluripotent stem cells

MPCs: Muscle Progenitor Cells

MSCs: Mesenchymal Stem Cells

NADH: Nicotinamide adenine dinucleotide

NG: Normal Glucose

NG2: Neural/glial antigen 2

NO: Nitric oxide

OGA: O-linked ß- N- acetylglucosaminidase

OGT: O-linked ß- N- acetylglucosamine transferase

OxPhos: Oxidative phosphorylation

PDGF: Platelet- derived growth factor

PPARγ: Peroxisome proliferator activated receptor gamma

qPCR: Quantitative polymerase chain reaction

ROS: Reactive oxygen species

RT: Reverse Transcription

SMA: Smooth muscle alpha- actin

TAG: Triglycerides

TCA: Tricaboxylic acid

Tbp: TATA-box binding protein

Tbx18: T-Box transcription factor 18

TGF-alpha: Transforming growth factor alpha

TGF-β1: Transforming growth factor-β1

TIMP-3: Tissue inhibitor of Metalloproteinase 3

VEGF: Vascular endothelial growth factor

VSMCs: Vascular smooth muscle cells

Zfp423: Zinc- Finger Protein 423

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Chapter 1. Literature review

1.1 Skeletal muscle structure and function

Skeletal muscle, which comprises approximately 40% of total body weight, is among the most plastic and dynamic tissues of the human body. Skeletal muscles are responsible for the generation of force and structural stability by converting chemical energy into mechanical force. Also, they play an important role in the metabolism of lipids, carbohydrates, and amino acids, in addition to their capacity for heat production to help maintain the core temperature (Wolfe, 2006). The structure of skeletal muscle is characterized by highly organized tissue containing multiple bundles of myofibers. Muscle tissues have high energy demand, which is supported by two major processes: the metabolism of glucose and the breakdown of fatty acids through ß-oxidation (Lieber, 2011; Mukund & Subramaniam, 2020; Eaton et al., 1996).

1.2 The microcirculation of skeletal muscle

The delivery of nutrient and oxygen-rich blood is possible through the function of a network of healthy blood vessels. Muscle tissues are highly vascularized to meet their metabolic needs. The microcirculation of skeletal muscles initiates from major arteries that branch into arterioles as they enter the tissue. Arterioles then supply the smallest forms of blood vessels called capillaries (Cunningham & Gotlieb, 2005).

1.2.1 Microvascular function in healthy adult skeletal muscle

Arterioles have the capacity to control the O_2 and nutrient supply to the working muscle and/ or organ by adjusting blood flow over 100-fold, depending on the local demand (Laughlin &

Armstrong, 1982; Andersen & Saltin, 1985). This adjustment in blood flow is achieved through multiple mechanisms. Under resting conditions, the spontaneous vasomotor tone is maintained by the skeletal muscle microvasculature with the help of the sympathetic nervous system (Duling & Damon, 1987; Segal, 2005; Segal et al., 1989). Arterial endothelial cells (ECs) are the main sensors of increased flow in skeletal muscles. Further, the endothelial release of vasodilatory agents such as prostaglandins and nitric oxide (NO) instruct SMCs to relax, which results in a decrease in resistance and an increase in the blood flow (Clifford, 2011; Welsh & Segal, 1998). One important avenue for matching the local blood flow demand is hypoxia-induced vasodilation. This hypoxia sensing mechanism, which can occur at the capillary levels, initiates a vasodilatory response that results in the augmentation of blood flow (Parthasarathi & Lipowsky, 1999; Jia et al., 2011). In addition, capillaries have the capacity to receive signals from the contracting muscle fibers, which is further transmitted by the capillary ECs to the arteriolar vasculature to increase the blood flow (Murrant et al., 2017).

1.2.2 Cellular organization of microcirculation in skeletal muscle

Endothelial cells (ECs) line the inner of all microvessels. Larger arterioles are covered with several layers of smooth muscle cells, whereas smaller arterioles are only wrapped and composed of a single layer of smooth muscle cells. At the capillary level, no smooth muscle layer exists but the ECs are partially covered by pericytes, which are important in the stabilization of capillaries (Emerson & Segal, 1997; Hudlicka, 2011) (Figure 1). Capillaries are the main site of gas, nutrient and waste exchange, but they also play important functions in metabolism, immune and hormonal homeostasis (Kissane et al., 2021). Capillary density, which is a measure of the number of capillaries, correlates with muscle health and skeletal muscle

oxidative capacity. A reduction in capillary density is seen in many vascular health complications such as peripheral artery disease (Ingjer, 1979). Capillary density in skeletal muscle is known to be dynamic and it can be manipulated in various conditions. The mechanism of angiogenesis (formation of new capillaries) increases capillary density, whereas capillary rarefaction refers to a reduction in capillary density.

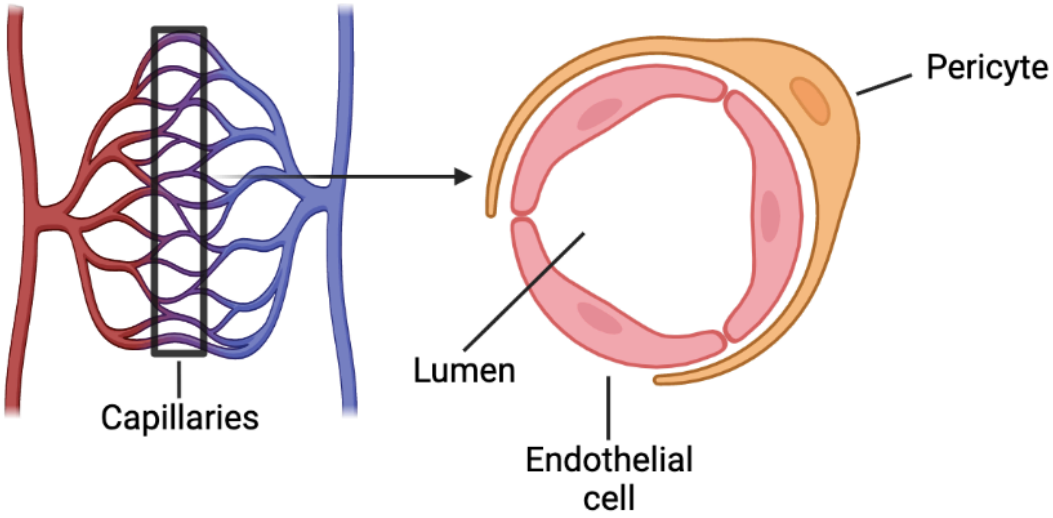


Figure 1.1. Endothelial cells are capillaries main cells. This diagram illustrates that a microvascular network is created with bigger blood vessels branching to smaller ones. Capillaries, the smallest form of blood vessels, are made up of endothelial cells that are partially wrapped by pericytes on their outer surface. Figure was made by myself, using Biorender website tools.

1.3 Pericytes

Pericytes are a heterogeneous population of mural vascular cells (Armulik et al., 2005). They reside on the abluminal surface of most capillaries, sharing the basement membrane with ECs. Pericytes are attracted to newly formed capillaries by the endothelial-secreted chemoattractant

platelet-derived growth factor (PDGF)- BB, which binds to PDGF- receptor beta (PDGFRβ) on pericytes (Armulik et al., 2010; Daneman et al., 2010).

1.3.1 Pericyte function and role

Studies on the brain have shown that pericytes are critical for vascular stability and structure (Geevarghese & Herman, 2014). Pericytes provide structural support for ECs, maintaining the integrity of the mature capillary network, restricting capillary expansion, and promoting ECs quiescence through secretion of paracrine factors such as Angiopoietin (Ang-1) and tissue inhibitor of Metalloproteinase 3 (TIMP-3) (Papapetropoulos et al., 2000; Saunders et al., 2006). In the brain, pericytes are responsible for the maturation and maintenance of the blood brain barrier (BBB), which is a diffusion barrier that blocks the entry of different molecules and toxins from the blood into the brain (Yao et al., 2014). Blood flow regulation and immunomodulation as well as contribution in various cellular processes involved in tissue homeostasis are some of other functions of pericytes in mature capillary networks (Bergers & Song, 2005; Armulik et al., 2011; Geevarghese & Herman, 2014).

Pericytes perform specific functions during sprouting angiogenesis. Pericyte detachment from ECs coincides with the activation of ECs and initiation of a sprout. The pericyte/ EC contact is re-established following sprouting, which re-stabilizes the capillary structure and causes ECs to re-enter a quiescent state, limiting further capillary formation (Carmeliet & Jain, 2011; Skalak et al., 1998; Volz et al., 2015).

1.3.2 Pericyte cellular markers and subtypes

Although no singular molecular marker has been established for pericytes, these cells are identified using a mixture of different approaches. Visualization of their location within the capillary basement membrane is one of the most accurate ways to identify pericytes. The molecular markers Neural/glial antigen 2 (NG2), cluster of differentiation 146 (CD146) and PDGFRß are expressed by most pericytes (Armulik et al., 2011; Holm et al., 2018). In addition, smooth muscle alpha- actin (SMA), vimentin (Bandopadhyay et al., 2001) and RGS5 (Mitchell et al., 2008) have been reported as pericyte cellular markers; however, their expression is dependent on the tissue location and species (Gerhardt & Betsholtz, 2003; Hellström et al., 1999; Hughes & Chan-Ling, 2004). *In vitro* studies by Birbrair and colleagues have shown that pericytes within skeletal muscle could be divided into two subtypes that are classified based on the molecular marker that they express (Birbrair et al., 2013). It was shown by these authors that Type-1 and type-2 pericytes express PDGFR alpha and Nestin, respectively (Birbrair et al., 2013). However, these findings have been defined only in isolated pericytes, and their distribution *in vivo* has not been validated.

1.3.3 Pericyte leptin production

Our lab recently reported that PDGFRβ+ pericytes in skeletal muscle increase leptin production when mice are fed with a high-fat diet for ~8 weeks to induce obesity/ insulin resistance (Nwadozi et al., 2019). Leptin is an adipocyte-derived hormone that controls the regulation of food intake and enhances fatty acid oxidation (Bjørbæk, 2009, Minokoshi et al., 2002). The increase in leptin levels by pericytes after exposure to high a fat diet may suggest that pericytes could act as local nutrient sensors. It is speculated that pericytes could react to nutrient availability and modify their function depending on the available nutrients. Also, the presence of

Zinc- Finger Protein 423 (Zfp423), a pre-adipocyte commitment factor in PDGFRβ+ pericytes increased following the high-fat diet (Nwadozi et al., 2019). The increased expression of leptin and Zfp423 following a high-fat diet could imply that exposure to a high-fat diet has driven the pericytes to adipocyte commitment lineage. However, complete differentiation did not occur as evidenced by the lack of detectable adipocytes in skeletal muscle by histology and lack of expression of peroxisome proliferator-activated receptor- gamma (PPARγ) (Nwadozi et al., 2019).

1.3.4 Stem cells

Stem cells are defined as undifferentiated cell types that have the potential to self-regenerate and differentiate into various functional cells (Hosseinkhani et al., 2014; Morelli et al., 2012). Adult stems cells are derived from various tissues and organs (Alison et al., 2002; Fu et al., 2011; Weissman, 2002; L. Li & Xie, 2005). They are classified as pluripotent, multipotent, or unipotent, based on their differentiation potential. Pluripotent cells can generate all cell types except an extra-embryonic trophoblast lineage (L. Li & Xie, 2005; Jaenisch & Young, 2008; Krampera et al., 2007). Multipotent cells differentiate into all cell types with a particular lineage, whereas, unipotent stem cells can differentiate into only a single lineage (Jaenisch & Young, 2008; Krampera et al., 2007).

Mesenchymal stem cells (MSCs) are multipotent stromal cells that have the capacity to self-renewal and differentiate into various lineages depending on their tissue of origin (Dennis et al., 2002; Torensma et al., 2003). These cells can be isolated from various tissues, including bone marrow, adipose tissues, umbilical cord and skeletal muscles (Ding et al., 2006; Ding et al.,

2007). *In vitro* studies have shown that MSCs can differentiate into mesodermal, ectodermal, and endodermal tissues such as bone, fat, muscle and neuronal cells, depending on the cell culture conditions and growth factors present (Kuo et al., 2009; Oishi et al., 2009; Bibber et al., 2013; Campagnoli et al., 2001). MSCs are also thought to contribute to tissue function through the expression of growth factors and chemokines, which can induce cell proliferation in adjacent cells and influence angiogenesis. Transforming growth factor alpha (TGF-alpha), TGF-β1, hepatocyte growth factor, epithelial growth factor (EGF), basic fibroblast growth factor, insulin-like growth factor- 1 (IGF-1), and vascular endothelial growth factor (VEGF) are some of the growth factors that are produced by MSCs (Haynesworth et al., 1996; Doorn et al., 2011; Caplan & Bruder, 2001; L. Chen et al., 2008). MSCs are sometimes used to study pericytes, due to similarities in their multipotency characteristics (De Bari et al., 2003; Klimczak et al., 2018) and because of the technical challenges of extraction and purification of specific pericyte population.

1.3.5 Pericyte multipotency

Although pericytes are deemed as predominantly quiescent in an established microvascular network, pathological conditions can result in their activation and, in unique circumstances, may initiate their differentiation into other cell types. Capillary-associated pericytes have the possibility to undergo differentiation into smooth muscle cells, helping to convert capillaries into arterioles (Skalak et al., 1998; Peirce & Skalak, 2003; Volz et al., 2015). In a cell culture environment, pericytes have been shown to differentiate into multiple lineages such as osteoblasts, chondrocytes and adipocytes (Crisan et al., 2008; Geevarghese & Herman, 2014) (Figure 2).

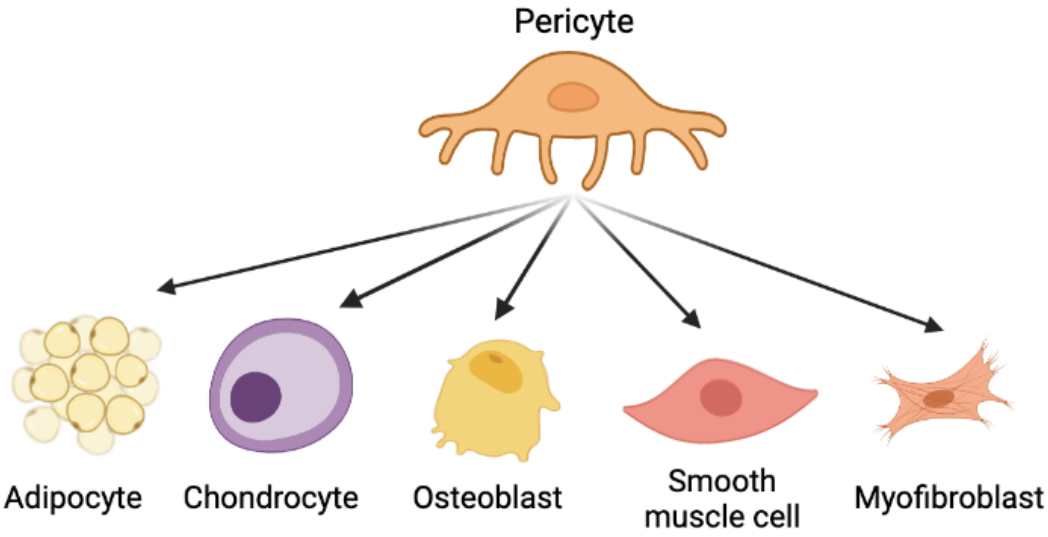


Figure 1.2. *In vitro* studies suggest pericytes are multipotent cells. Under the right environmental cues, pericytes can differentiate into various cell types, such as adipocytes, chondrocytes, osteoblasts, smooth muscle cells, and myofibroblasts. The figure was made by myself, using Biorender website tools.

1.3.6 Pericyte adipogenesis

Studies on lung and brain pericytes have provided evidence for the expression of the transcription factor CCAAT/enhance binding protein ß (C/EBPß) in these pericyte population (He et al., 2018; Vanlandewijck et al., 2018). C/EBPß, an enhancer binding protein, is responsible for regulation of target genes associated with adipogenesis (Siersbæk et al., 2014). The interaction of C/EBPß with PPARγ in lung and brain pericytes results in responsiveness to adipogenic cues (Lefterova et al., 2014). It has been hypothesized that only a limited population of pericytes that express PPARγ have adipogenic potential, and can differentiate into fat cells (Gupta et al., 2010). PPARγ is a transcription factor known as a master regulator of pre-adipocyte differentiation. The increase in PPARγ expression is associated with an augmented

level of adipocytes and fat tissue in cells that have pre-commitment adipogenic potential (Farrington-Rock et al., 2004; Lefterova et al., 2014).

Zfp423 transcription factor is a pre-adipocyte commitment factor that is required in the initial steps of the formation of both brown and white adipocytes in vivo. Gupta's work has shown that Zfp423 can regulate PPARγ gene expression, leading undifferentiated fibroblasts to undergo adipocyte differentiation (Gupta et al., 2010). Further, Vishvanath and colleagues observed that Zfp423- positive mural cells (PDGFRß+) in adipose tissues, could differentiate into adipocytes (Vishvanath et al., 2016). These results show that pericytes could only differentiate into adipocytes if they have a pre-commitment to this specific lineage. As mentioned earlier, our laboratory found that a high-fat diet in mice could increase levels of Zfp423 in pericytes from the skeletal muscle vasculature (Nwadozi et al., 2020), suggesting that this transcription factor could contribute towards adipocyte differentiation.

1.3.7 Pericyte Myofibroblast differentiation

Transforming growth factor-β1 (TGF-β1) is a growth factor that regulates cellular processes such as proliferation, apoptosis, migration and differentiation, and promote collagen formation, which can contribute to tissue fibrosis (Massagué et al., 2000; Blobe et al., 2000). It has been shown that following tissue injury in lung and kidney, pericyte conversion to myofibroblasts occurs through TGF-β1, Notch and Wnt signaling pathways (Kirton et al., 2006; Cheng et al., 2008; Andersson-Sjöland et al., 2016). It has also been observed, that myofibroblasts differentiation is associated with a decline in adipogenic markers, such as PPARγ (Mann et al., 2010; Perugorria et al., 2012; Zeybel et al., 2017).

1.3.8 Pericyte smooth muscle cell differentiation

Vascular smooth muscle cells (VSMCs) are one of the major cellular constituents of arterioles and arteries, and their contractile properties enable them to regulate blood pressure and the blood flow between organs (Lacolley et al., 2012; Brozovich et al., 2016). Skalak and colleagues have shown that pericytes and smooth muscle cells share NG2 as a cellular marker and it has been suggested that these cells probably share a similar origin during the development (Murfee et al., 2005). *In vitro* studies on adipose-derived and bone marrow-derived mesenchymal stromal cells have shown that the addition of TGF-β1and angiotensin II to the cell culture environment promotes the differentiation of these cells into VSMCs (C. Wang et al., 2010; Kim et al., 2008; M.-K. Wang, 2012).

1.3.9 Tissue specificity of pericyte fate

The differentiation fate of pericytes is suspected to be related to the origin of these cells, as reported on studies on brain and lung pericytes (He et al., 2018; Vanlandewijck et al., 2018). Researchers proposed that differentiation potential may correspond with pre- programmed commitment to certain cell types and that the pericytes would only differentiate into that specific lineage (Birbrair et al., 2013; Sacchetti et al., 2016;). It is noteworthy, that *in vivo* investigation in multiple tissues such as skeletal, cardiac and adipose tissues have revealed that pericytes that express T-Box transcription factor 18 (Tbx18) fail to possess trans- differentiation capacity (Guimarães-Camboa et al., 2017). Others have debated the interpretation of this outcome, suggesting that the absence of this transcription factor is a sign of differentiation potential in pericytes (Birbrair et al., 2017; Wörsdörfer & Ergün, 2018).

1.4 Role of metabolic activity and nutrient availability in stem cells

Our lab has been interested in studying the effect of nutrient manipulation and metabolic pathways on microvascular cell functions. Cells adjust their metabolic state in response to nutrient availability, extracellular cues, and differentiation signals. For instance, higher nutrient sources of glucose and insulin might favor glycolysis, whereas an increase in fatty acids and amino acids could result in a shift towards oxidative phosphorylation (OxPhos) (Guo et al., 2012). Recent studies have advanced our understanding of multipotent stem cell differentiation fate and their response to changes in metabolic activity and nutrient availability. Glycolysis and OxPhos are the two major metabolic pathways that can alter the self-renewal or differentiation fate of multipotent cells by regulating the intrinsic cell cues (Shyh-Chang & Ng, 2017). Generally, it is recognized that stem cells need different metabolic pathways along their process of differentiation. For instance, naïve quiescent stem cells rely heavily on OxPhos, whereas they rely on glycolysis when they are activated and proliferating (Zhou et al., 2012; Takashima et al., 2014; Sperber et al., 2015). For example, multipotent mesenchymal stem cells are known to rely heavily on Oxphos when they are in their native, quiescent stage and have low proliferation activities (Sbrana et al., 2016; Katajisto et al., 2015). Studies have shown that introducing hMSC to a nutrient-rich environment, could shift their metabolic dependence on OxPhos to support their energy demand (Sherr & DePinho, 2000; Pattappa et al., 2011; Y. Liu et al., 2015). Lastly, oxygen availability shifts human MSC towards OxPhos metabolism, which could affect their differentiation fate by increasing the reactive oxygen species (ROS) due to tricarboxylic acid (TCA) cycle activity (Shyh-Chang et al., 2013)

1.4.1 Glycolysis and stem cells

Glycolysis is a series of redox reactions through which a glucose molecule gets broken down into two pyruvate molecules and results in the production of two molecules of ATP. Although glycolysis is a fast ATP-producing reaction and it can occur in the absence of oxygen, it is the least efficient source of energy. The proliferation of stem cells is generally supported by glycolysis (Shyh-Chang & Ng, 2017; Leese, 1995; Zhou et al., 2012). For example, it has been observed that glycolysis is essential for the proliferation of muscle progenitor cells (MPCs), since in the absence of glucose, proliferation is dramatically reduced (Chen et al., 2019). Also, the absence of glucose is associated with a reduction in ATP levels available for MPCs, suggesting that glycolysis is essential for energy production in proliferating MPCs (Yucel et al., 2019). In summary, glycolysis plays an important role in transition of naïve cells to a primed and proliferative state.

1.4.2 Oxidative phosphorylation (OxPhos) and stem cells

Oxidative phosphorylation (OxPhos) is the main and most efficient source of energy for mammalian cells. OxPhos is an oxygen- consuming series of reactions in electron transport chain and ATP synthase complex of mitochondria. The final product of OxPhos are ATP molecules that are produced by the oxidation of nicotinamide adenine dinucleotide (NADH) (Shyh-Chang & Ng, 2017). OxPhos also actively promotes the differentiation of stem cells (Zhou et al., 2012; Takashima et al., 2014; Sperber et al., 2015). The activity of the mitochondrial TCA cycle in OxPhos leads to the production of dioxygenase which can affect stem cells and lead them to initiate differentiation. Dioxygenase alters stem cells by erasing multiple repressive chromatin modifications that can result in the initiation of differentiation processes (Shyh-Chang & Ng, 2017).

1.4.3 Lipid metabolism and stem cells

Lipids are responsible for a vast variety of roles inside the cells (Fahy et al., 2011). For example, various biological processes, including cell proliferation, cell migration, cell death, gene expression and immune reactions are influenced by lipids (Wymann & Schneiter, 2008). Fatty acids (FAs), stored in the form of triglycerides (TAG), are an important source of energy. Lipids are oxidized by first breaking down of TAGs through lipolysis to release FAs, which then undergo ß- oxidation in the mitochondria (Houten & Wanders, 2010). The role of lipid oxidation in stem cell behaviors has been an area of interest. Studies on stem cells have shown that differentiated cells generally have increased levels of saturated free FAs and higher fatty acid transport into mitochondria which results in a shift of metabolism towards OxPhos and influences the differentiation of stem cells (Yanes et al., 2010). It has been observed that differentiation capabilities of stem cells are maintained by high levels of unsaturated FAs (Yanes et al., 2010). Lastly, evidences from studies on human embryonic stem cells and induced pluripotent stem cells (iPSCs) have shown the importance of lipid profile on the multipotency of stem cells (Kiamehr et al., 2017).

1.4.4 Glucose and the Hexosamine Biosynthesis pathway

The hexosamine biosynthesis pathway (HBP) is an alternative branch of glycolysis that gets activated when glucose availability overwhelms the glycolytic pathway, thus it acts as a nutrient sensor for the cell. A greater influx of glucose, as occurs with hyperglycemia, along with ROS and hyperlipidemia, are the main contributors to increase flux through the HBP (Liu et al., 2000; Du et al., 2000; Nissen & Wolski, 2007; Gerstein et al., 2008). Increases in glucose, acetyl- CoA,

fatty acids, glutamine, and nucleotide (UTP) lead to the upregulation of glutamine fructose-6-phosphate amidotransferase (GFAT), which is the rate-limiting enzyme responsible for the production of UDP-N-acetylglucosamine (UDP-GlcNAc) (Semba et al., 2014; Vasconcelos-dos-Santos et al., 2018). O-GlcNAcylation, the addition of N- acetylglucosamine (GlcNAc) from UDP-GlcNAc to serine and threonine amino acids of intracellular proteins is a post-translational modification that occurs as part of the HBP (Hart et al., 2007) (Figure 3). O-GlcNAcylation is responsible for altering many cellular processes such as calcium signaling, insulin signaling, mitochondrial homeostasis, gluconeogenesis and apoptosis and it affects various functions of proteins, such as enzymatic activity, protein stability and subcellular localization, thus leading to a modification of cell signaling and metabolism (Bond & Hanover, 2013; Hart et al., 2007). To date, up to around 4000 proteins have been identified to be O-GlcNAcylated (Ma & Hart, 2014).

The process of O-GlcNAcylation is modulated by two key enzymes, O-linked ß- N-acetylglucosamine transferase (OGT) and O-linked ß- N- acetylglucosaminidase (OGA). OGT is responsible for catalyzing the addition of GlcNAc to proteins, while the enzyme OGA removes the modifications from proteins (Hart et al., 2007) (Figure 3). Experimentally, O-GlcNAcylation levels can be manipulated by modifying OGA activity. PUGNAc is an inhibitor of OGA, thus it is used experimentally to increase cellular O-GlcNAcylation levels (Figure 3). Physiological disruption of OGT and OGA can have pathological implications in various diseases, such as cancer, neurological disease, and diabetes (Bond & Hanover, 2013).

OGT and OGA receive information about nutrient changes inside the cell from key nutrient-sensitive signaling pathways and respond by either increasing or decreasing the levels of O-

GlcNAcylation. For instance, adenosine- monophosphate-activated protein kinase (AMPK), which regulates cellular energy levels in response to nutrient availability and cellular stress, phosphorylates OGT, inhibiting O-GlcNAcylation (Janetzko & Walker, 2014; Bullen et al., 2014; Xu et al., 2014). Overall, the effects of AMPK on OGT regulates the activity of proteins involved cellular growth, metabolism and proliferation (Bullen et al., 2014).

O-GlcNAcylation modifications, play an important role in stem cell fate determination, regulation of pluripotency as well as gene silencing (Speakman et al., 2014; Vella et al., 2013). An elevation in O-GlcNAcylation levels is associated with a maintenance of pluripotency and its marker genes such as OCT4 and SOX2, whereas lowering levels of O-GlcNAcylation is linked to differentiation (Jang et al., 2012). For example, studies have shown that increasing O-GlcNAcylation results in delay in differentiation of mouse embryonic stem cells and decreasing O-GlcNAcylation promoted differentiation in neuronal cells (Shi et al., 2013; Constable et al., 2017).

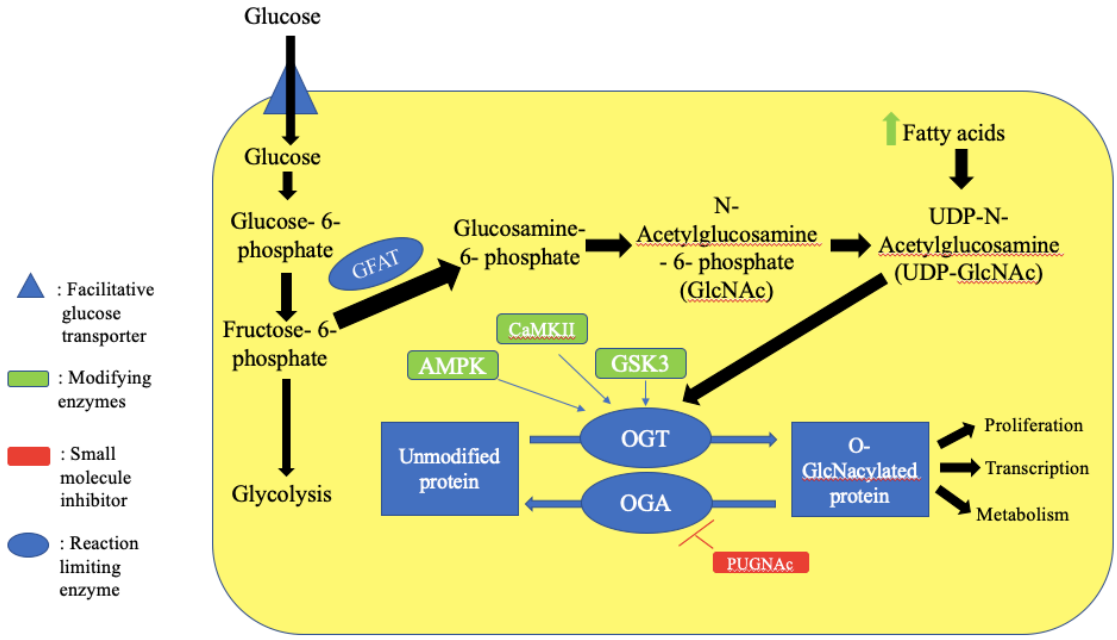


Figure 1.3. Hexosamine Biosynthesis Pathway and O-GlcNAcylation processing glucose. This diagram illustrates the fate of glucose as it enters the cell through Glut1 transporters and some are diverted through the HBP pathway. Fatty acids also can increase UDP-GlcNAc and O-GlcNAcylation. GFAT: glutamine fructose-6- phosphate amidotransferase, OGT: O-GlcNAc Transferase, OGA: O-GlcNAcase, AMPK: adenosine-monophosphate-activated protein kinase, CaMKII: calcium/ calmodulin-dependent protein kinase II, GSK3: insulin-regulated mitotic protein glycogen synthase kinase.

1.4.5 Effects of oxygen availability on stem cells functions

Since oxygen is required for OxPhos, hypoxic conditions shift metabolism from OxPhos to glycolysis. Hypoxia also stabilizes hypoxic inducible factors (HIFs) and inactivates p53. This can influence somatic cell differentiation ability, and re-enter these cells to pluripotency, since p53 has been shown to stop stem cell generation (Marión et al., 2009; Hong et al., 2009; Yoshida et al., 2009). Alternatively, some studies show that hypoxia could promote stem cells differentiation process as there is evidence that it promote pluripotent stem cells differentiation

into retinal or lung progenitors (Takahashi et al., 2020; Pimton et al., 2015), and induces upregulation of myoD in skeletal muscle-derived stem cells to promote myogenesis (Elashry et al., 2022). Lastly, there is evidence that mesenchymal stem cells shift toward osteogenesis in a hypoxic environment (Delorme et al., 2006; Cipolleschi et al., 1993).Therefore, hypoxia could be an environmental driver that modifies the metabolism of stem cells and influence the differentiation fate of these cells. However, these findings might be unique in different cell types, therefore it is important to study the population of interest.

1.4.6 Metabolism of pericytes

Although pericytes have similar characteristics as mesenchymal cells, there is a very little direct investigation of their metabolism to date. Research on human and murine brain pericytes has shown that glucose uptake is mediated by insulin-dependent GLUT1 and GLUT2 glucose transporters (Castro et al., 2018; Vanlandewijck et al., 2018). One study that characterized metabolic pathway use in cultured pericytes reported that proliferating pericytes rely on glycolysis to meet about 85% of their energy demand, while the other 15% is dependent on fatty acid oxidation (Cantelmo et al., 2016). It is possible that a shift in metabolism from a glycolytic pathway to OxPhos could affect pericyte differentiation fate, similar to other stem cells. Notably, the influx of glucose provides a greater opportunity for activation of the HBP. It is thought that the HBP could mediate pericytes' self-renewal by sustaining proliferation and re-establishing the quiescent state in pericytes (Wellen et al., 2010). Despite the recent findings about pericyte metabolism, our understanding of their differentiation potential remains limited.

1.5 Knowledge gap in understanding of pericytes behavior

There remains an extensive knowledge gap regarding how pericytes interact with their molecular environment and how they can be induced to differentiate into specific cell types. To date, most of our knowledge of pericyte differentiation is based on the observations of experiments done on other types of stem cells. The differentiation of pericytes into various cell types and their abnormalities could contribute to various health complications. For example, pericytes can turn into undesired fibroblasts or adipocytes after a muscle injury, which could negatively affect the recovery of the muscle tissue. Based on knowledge gained from studies of other cell types, nutrient availability, and metabolic shift are critical variables that could affect pericyte phenotype and differentiation fate. Therefore, it is important to study the effect of various nutrient availability on the pericyte phenotype.

Chapter 2. Rationale and objectives

The availability of various nutrients and oxygen can shift a cell's reliance on distinct metabolic pathways. If pericytes are multipotent cells, this could provoke them to differentiate into various lineages. Thus, the metabolic activity of the cell could act as a decision-maker for the initiation of the phenotype change and differentiation fate. Also, O-GlcNAc modifications have the potential to affect pericyte phenotype. However, testing factors that evoke phenotype shifts in pericytes in vivo is very challenging, because many variables are altered simultaneously by ischemia or by diet changes. Instead, cultured cell experiments enable the testing of specific parameters of interest, while excluding confounding factors such as inflammation. Therefore, manipulation of nutrient availability (excess and nutrient deficiency) in vitro will be used in this thesis as a strategy to study factors that could promote shifts in pericyte phenotype. For this purpose, mesenchymal stem cells are used as a proxy for pericytes since these cells display similar characteristics and phenotypes and are easier to obtain.

2.1 Hypothesis:

Mesenchymal cells phenotype can be altered by nutrient availability and metabolic stress.

2.2 Objectives:

2.2.1 Objective 1: To test the effects of nutrient excess on mesenchymal cells O-GlcNAcylation and phenotype in culture.

2.2.2 Objective 2: To test the effects of ischemia- mimetic conditions on mesenchymal cells O-GlcNAcylation and phenotype in culture.

Chapter 3. Methods

3.1 Ethical approval

Animal studies were approved by the York University Committee on Animal Care (#2017-19R3, #2017-20R3).

3.2 Isolation of mouse skeletal muscle mesenchymal cells:

Hindlimb muscles were extracted from C57B16/J mice, minced, and digested by 2 mg/ml collagenase D (Millipore Sigma #11088882001). The digested fraction then was washed once with alpha MEM. The fraction was resuspended with dispase (Sigma #D4693) solution and incubated at 37C for 5 mins then was diluted with Alpha MEM. Then it was passed through a cell strainer, then washed, and resuspended in High glucose (25 mM) DMEM (Gibco # 11960044) with 10% Fetal Bovine Serum (FBS) (Gibco #10082147), and plated on Matrigel-coated flasks overnight. For mesenchymal stromal cell culture media, DMEM with 25 mM glucose, 10% FBS, glutamine (1X) (Gibco #35050061), sodium pyruvate (1 mM) (Gibco #11360070), and Penicillin-Streptomycin (10 U/mL) (Gibco #15140163) was used to grow cells. For experiments, pericytes were treated with various conditions (described below) and then lysed for protein and mRNA extraction.

3.3 Cell line confirmation experiments:

The 10T1/2 cells line was used to confirm some of the results that were observed on mesenchymal cells. 10T1/2 cells are immortalized mesenchymal stem cells. These cells were cultured in high glucose (25 mM) DMEM media with 10% FBS.

3.4 Nutrient modifications:

To test the effect of nutrient excess on mesenchymal cells, cells were treated for 48 hours or 72 hours with high glucose (25 mM) DMEM, normal glucose (5 mM) DMEM (Gibco #11885084) media, Insulin (1 μM) (Humalog #02229704), palmitate (Sigma #P1585) (200 μM; conjugated to BSA) or Oleic Acid (Sigma #03008) (200 μM; conjugated to BSA). The treatment groups were as follows: normal glucose (control); high glucose; high glucose+ Insulin+ Palmitate; high glucose+ Insulin+ Oleic Acid

3.4.1 To test the effects of **nutrient and oxygen deficiency** as occurs in ischemia, cells were treated for 24, 48 or 72 hours with no glucose DMEM (Gibco #11966052) and cobalt chloride (1 mM; Sigma #5862). The treatment groups were as follows: normal glucose (control); no glucose; no glucose+ Cobalt Chloride.

3.4.2 To test the **role of O-GlcNAcylation**, some cells were treated with 100 μM PUGNAc (Toronto Research Chemicals #A157250) for 48 hours.

3.5 Differentiation potential:

To identify possible changes in phenotype that might be observed in pericytes under various nutrient conditions, the indicators of adipogenic, myogenic and fibrotic lineages were induced by following treatments and cells were assessed by qPCR and morphological analysis.

3.5.1 To induce adipogenesis, cells were treated with adipogenic cocktail (1µM dexamethasone, 125nM indomethacin, 1 nM triiodothyronine (T3), 1µM rosiglitazone, 850 nM insulin, 0.5 mM 3-isobutyl-1-methylxanthine) for 9 days and monitored for lipid accumulation using Bodipy stain (Invitrogen #D3823).

3.5.2 To stimulate myogenesis, cells were cultured in media containing 5% horse serum (Gibco # 16050122) for 9 days and monitored for myotube formation.

3.5.3 TGF-β1 (Biolegend #580702) treatment (10 ng/mL) for 5 days was used to stimulate fibrosis.

3.6 Morphological imaging:

Following the above treatments, the cells were observed under the light microscope and their phenotype changes were examined and images captured using the software called "Capture Image". The images were collected at 10X and 20X magnification.

3.7 Mesenchymal cells fat deposition staining:

To detect fat droplets following adipogenic treatments, the cells were washed 3 times with 1X PBS and then fixed with 4% paraformaldehyde. Then cells were stained with bodipy (1:100 for 10 minutes, Invitrogen #D3823). After one wash with 1X PBS, cells were re-stained using DAPI (1:1000 for 5 minutes). The cells were observed and imaged under the fluorescent microscope using a constant camera exposure time and settings. For each condition, 6 fields of view pictures were taken. Images were quantified by first setting a specific pixel threshold to demarcate

adipocytes, and then calculating the mean of the thresholded features on each image using ImageJ software. For each condition, the 6 fields of view were averaged to generate a single value (n=1) for each treatment.

3.8 qPCR:

RNA was extracted from cultured cells using Qiazol (Qiagen #79306) and Reverse Transcribed (RT) to synthesize cDNA using dNTP (New England Biolabs #N0447S), Oligo dT (Invitrogen #100002344), Ribolock RNase inhibitor (Thermo Fisher Scientific #EO038), Random primers (Invitrogen #100026484), M-MuLV reverse transcriptase and buffer (New England Biolabs). cDNA was diluted by adding 80 µl of double distilled water. cDNA was analyzed by real-time polymerase chain reaction (qPCR) using Taqman© fast advanced master mix (Applied Biosystems, Thermo Fisher Scientific #4444557) probes. Gene expression of the adipocyte markers *Zfp423* (a marker of adipocyte precursors) and *PPARγ* (a marker of mature adipocyte) was measured to study the phenotype shift of mesenchymal cells to adipocytes. Gene expression of collagen type 1 and *TGF-β1*, which are markers of a fibroblast phenotype, was measured to assess the shift of pericytes phenotype to fibroblasts. Lastly, the phenotype shift to skeletal myocyte was studied by assessing the gene expression of *Myf5* and *MyoG* (markers of myoblasts). All RNA probes were normalized to TATA-box binding protein (*Tbp*) mRNA since it has a relatively consistent expression profile across all treatments.

3.9 Western Blot:

Cells were lysed for protein analysis using RIPA buffer (50 mM Tris base, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing phosSTOP (Sigma #04906837001)

and cOmplete Mini (Sigma #11836153001) and the protein concentration was measured by BCA. Western Blot was used to measure the protein levels in each treatment. Depending on the molecular weight of the protein of interest, cell lysates were loaded on 8% to 15% SDS-Polyacrylamide gels. Proteins were transferred from the gel to a PVDF membrane using a wet transfer protocol. The membrane then was incubated with 5% milk in TTBS to block non-specific binding. Later, the membrane was incubated overnight with the primary antibody in TTBS containing 5% BSA [anti-Zfp423 antibody (1:1000; abcam #AB216970), anti- O-GlcNAc antibody (1:1000; Cell Signaling Technology #82332S)]. On the second day, membranes were incubated with a secondary antibody (diluted in 5% BSA; 1:10000) for an hour. Next, the membrane was imaged and the pixel intensity of each protein band was analyzed using ImageJ software. The intensity of each band was normalized to that of β-actin (1:1000) (Cell signaling #4967) for the same condition.

3.10 Immunoprecipitation (IP):

Cells were lysed using IP lysis buffer which consists of 50 mM Tris pH 7.5, 10 mM MgCl2, 0.3 M NaCl, 2% NP-40. The protein levels were quantified by BCA protocol using IP lysis buffer. Then the lysate was incubated overnight with primary antibody (anti-Zpf423) (ABclonal #A15795) and with normal IgG (as the negative control). The day after, lysates were incubated with protein A/G magnetic beads (Bimake #B23202) to isolate purified Zfp423 and IgG proteins. Western blot analysis using anti- O-GlcNAc (Cell Signaling Technology #82332S) antibody was used to assess the O-GlcNAcylation status of Zfp423 proteins.

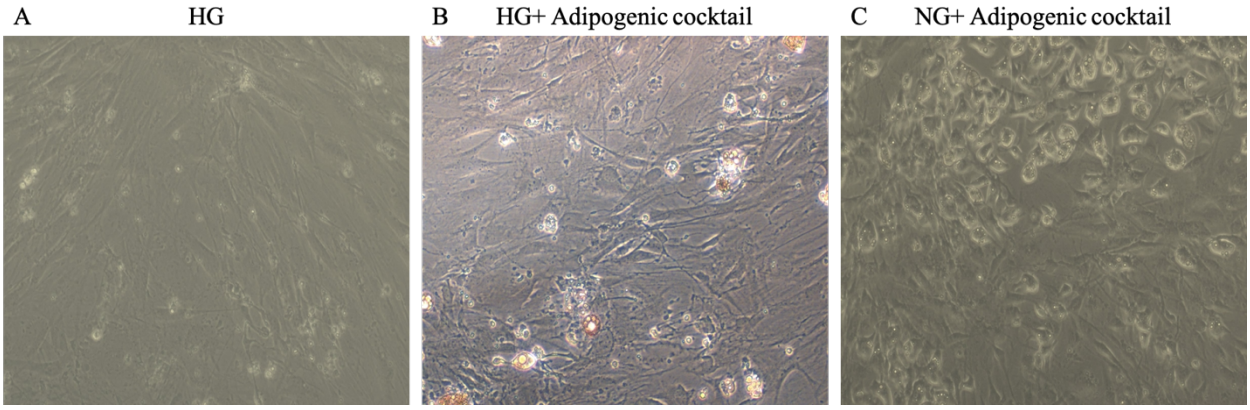
3.11 Statistical analysis

Results are presented as showing individual (n) values with bars representing ± standard deviation. One-way ANOVA repeated measures followed by Bonferroni post hoc tests were used to analyze the statistical results for all of the qPCR, Western blot analysis, and Bodipy quantification using Prism software (Prism4; Graphpad Software Inc.). However, Two- way ANOVA was used to analyze the qPCR quantifications for comparing the *Zfp423* and the *Col1a1* genes in the PUGNAc experiment, and *Myh11* gene in the TGF-β1 of the experiment. A $P<0.05$ was considered to represent statistical significance.

Chapter 4. Results

4.1 Mesenchymal cell adipogenic potential

Mesenchymal cells can be initiated towards either adipogenic or fibrotic differentiation, depending on the environmental cues. I first investigated whether glucose levels can alter adipogenic differentiation. A 9-day treatment with adipogenic cocktail results in cells with extensive lipid formation (Figure 4.1 A-C). Bodipy staining (to detect non-polar lipids) was used to quantify the intercellular lipid content. Lipid droplet formation was observed in cells cultured with both high and normal levels of glucose, in combination with the adipogenic cocktail (Figure 4.1 E, F). Lipid quantification showed that normal glucose treated cells had significantly higher levels of lipid accumulation compared to control. Also, there was no statistical significance observed between high glucose (25 mM) adipogenic cocktail and control, and high glucose adipogenic cocktail and normal glucose (5 mM) adipogenic cocktail. These results show that cells treated with normal glucose are more efficient at undergoing adipogenesis.



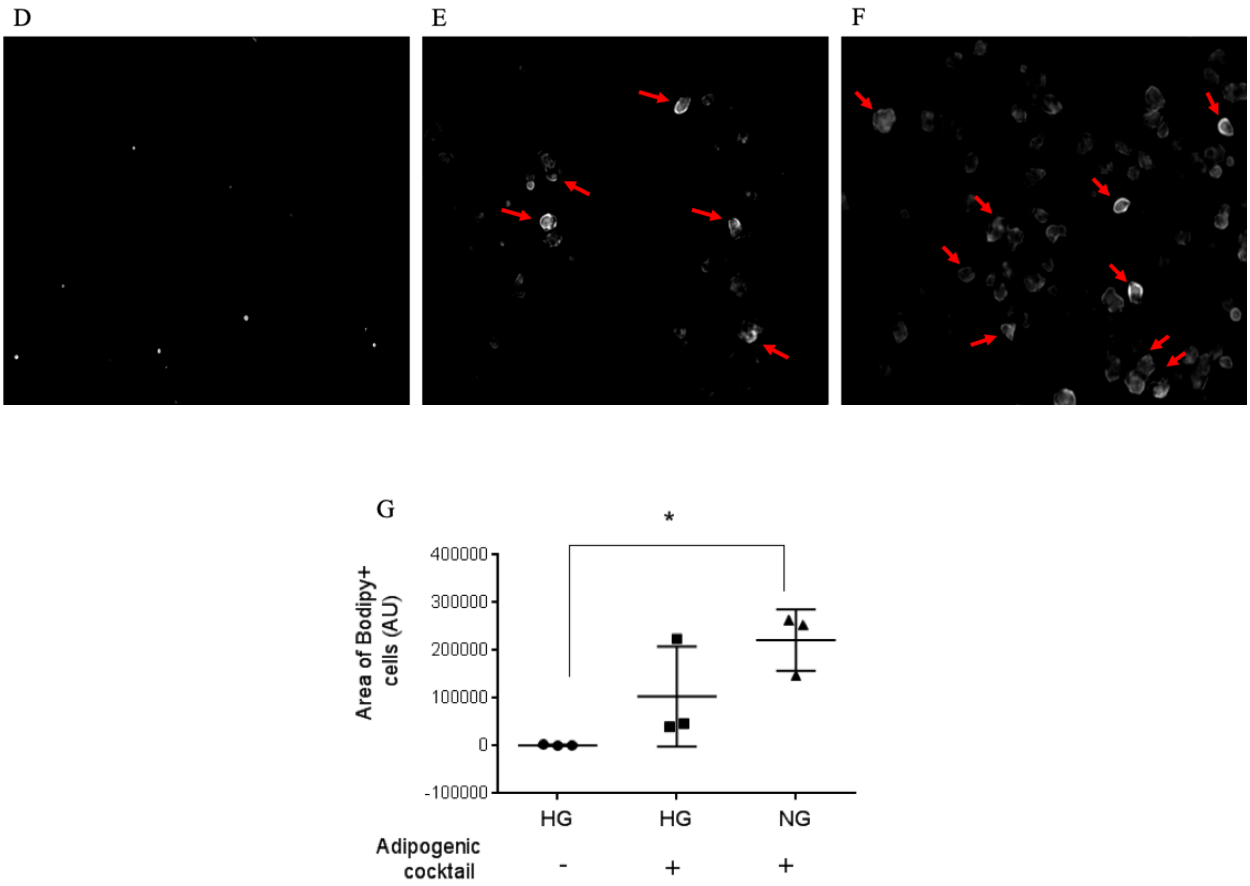


Figure 4.1. Effect of glucose availability on adipogenesis. Mesenchymal cells were treated with either high glucose (25 mM) (HG) or normal glucose (5.5 mM) media (NG) and adipogenic cocktail for 9 days to investigate their adipogenic capacity. The images captured by light microscope from mesenchymal cells treated for 9 days with A) HG B) HG+ adipogenic cocktail and C) NG+ adipogenic cocktail and their corresponding Bodipy staining images captured by the fluorescent microscope, highlighting the fat droplets formed in treated mesenchymal cells. G) the intensity of Bodipy stain was quantified using ImageJ and the area of the Bodipy+ cells (per field of view) was plotted for each condition. Data are shown as independent experiments (n=3), with lines representing mean ± standard deviation. *P<0.05.

Further, I investigated if glucose and palmitate exposure alter adipogenic or fibrotic potential in mesenchymal cells by assessing the RNA for *Zfp423* and *Col1a1*, respectively. After 72 hours of treatment, mesenchymal cells treated with normal glucose did not alter *Zfp423* mRNA significantly compared to the no-glucose control. In fact, cells treated with high levels of glucose exhibited a significantly higher levels of *Zfp423* mRNA compared to all other treatment groups (Figure 4.2 A). Although there was no significant difference in *Col1a1* between treatment groups after 72 hours (Figure 4.2 B), a trend was observed suggesting that increasing glucose levels could increase the Col1a1 levels. Also, palmitate/ insulin treatment did not affect the *Zfp423* or *Col1a1* levels in these experiments.

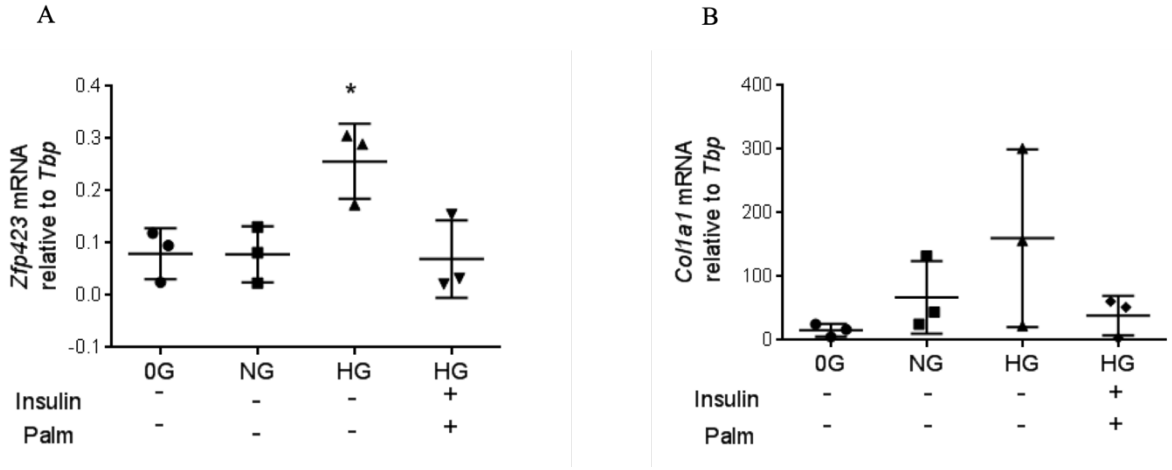


Figure 4.2. Effect of glucose availability on *Zfp423* and *Col1a1* gene expression. Mesenchymal cells were cultured with no glucose media (0 mM; 0G), normal glucose media (5.5 mM; NG) and high glucose media (25 mM; HG), or HG with insulin (1 µM) and Palmitate (200 µM) (HG+ Ins+ Palm) for 72 hours. Then RNA was extracted and analyzed by qPCR. The expression of A) *Zfp423* mRNA and B) *Col1a1* mRNA were measured using qPCR analysis, normalizing to the housekeeping gene *Tbp*. Data are shown as independent experiments (n=3), with lines representing mean ± standard deviation. * $P<0.05$ represents a statistical significance compared to all other treatment groups.

4.2 Modification of O-GlcNAc in various conditions

In order to examine the effect of nutrient availability, I tested the O-GlcNAcylation levels, in mouse mesenchymal cell treated with normal glucose media, high glucose media, PUGNAc and Palmitate for 48 hours. After performing western blot analysis on the extracted lysates from these cells (Figure 4.3 A,C), no significant difference was observed among these groups. However, there was a trend for an increase in O-GlcNAcylation in cells treated with PUGNAc and Palmitate (Figure 4.3 E). Also, I treated mesenchymal cells with PUGNAc (100 μM) and either high or normal glucose levels to investigate whether their phenotype would change. After 48 hours of treatment, no apparent changes were observed in these cells with PUGNAc treatment in either high and normal glucose (Figure 4.4 A-D).

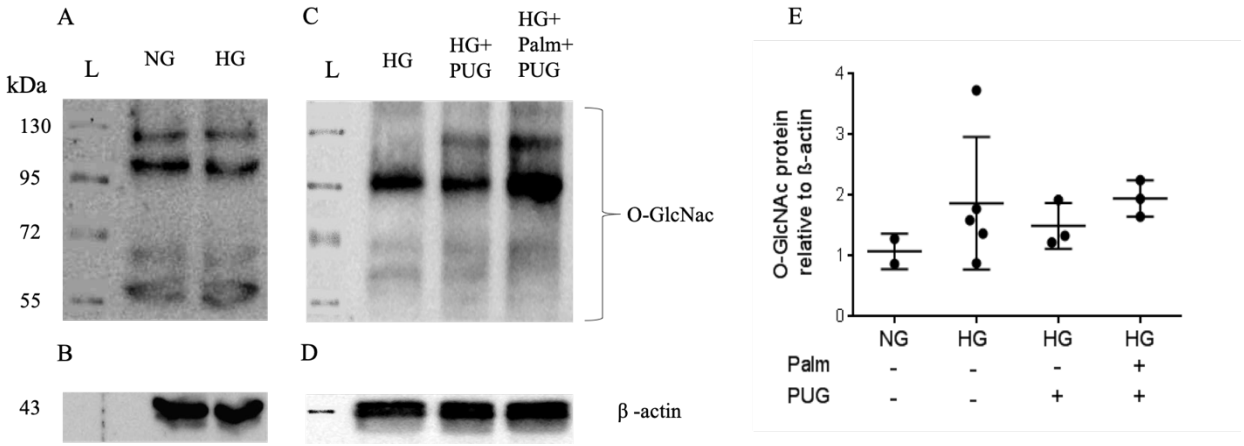


Figure 4.3. Effect of nutrient availability on O-GlcNAcylation. Mesenchymal cells were treated with various glucose levels (high and normal) with PUGNAc and Palmitate for 48 hours and the levels of O-GlcNAcylation was measured. A) Western blot showing O-GlcNAc proteins in extracts from mesenchymal cells treated with normal glucose DMEM (NG), high glucose DMEM (HG), and B) the corresponding β-actin

blot. C) O-GlcNAc was assessed by Western blot for cells treated with high glucose DMEM (HG) high glucose DMEM + PUGNAc and high glucose DMEM+ palmitate+ PUGNAc and D) corresponding β-actin blot. E) The O-GlcNAc band intensities were quantified and normalized to each corresponding β-actin band using ImageJ. Data are shown as independent experiments (n=2-5), with lines representing mean ± standard deviation.

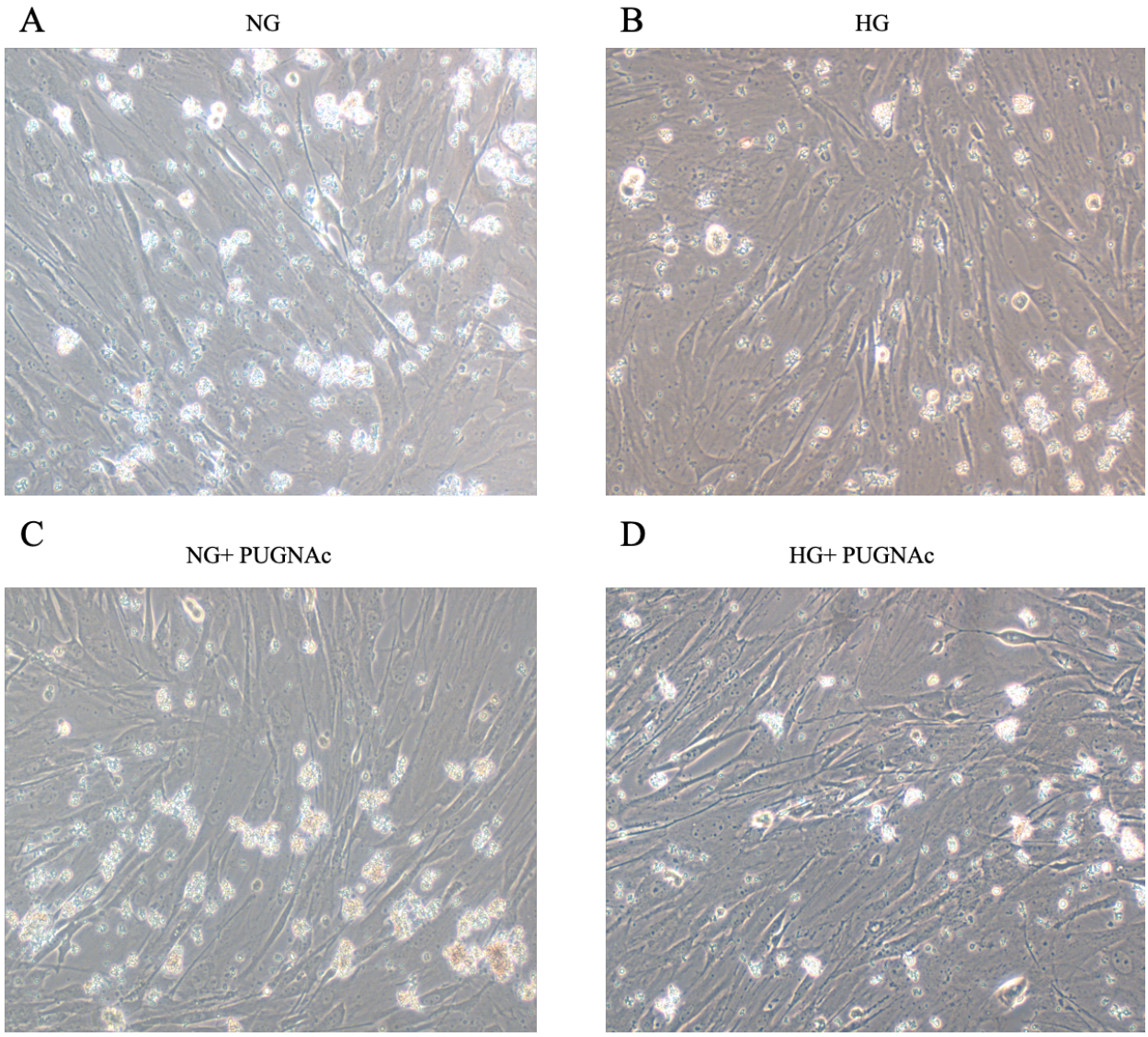


Figure 4.4. Effect of glucose availability and O-GlcNAcylation on mesenchymal cell phenotype. Mesenchymal cells were treated with either high glucose (25 mM) or normal glucose (5.5 mM) media and PUGNAc (100 µM) for 48 hours to investigate the phenotype change. The images were captured by light microscope from mesenchymal cells treated for 48 hours with A) normal glucose DMEM (NG) B) normal

glucose DMEM treated with PUGNAc (NG+ PUGNAc); C) high glucose DMEM (HG) D) high glucose DMEM treated with PUGNAc (HG+ PUGNAc).

4.3 Metabolic disturbance of 10T1/2 cells

To investigate the effect of metabolic disturbances on O-GlcNAc levels in various conditions, I used the immortalized mesenchymal stem cell line 10T1/2, which has similar properties as pericytes and primary mesenchymal cells and assessed the O-GlcNAcylation levels by western blotting (Figure 4.5 A). O-GlcNAc levels were quantified and normalized to β-actin levels in each sample (Figure 4.5 B), then values were normalized to that of HG alone in each experiment (Figure 4.5 C). Basal levels of O-GlcNAc were increased by PUGNAc in cells treated with both PUGNAc alone and PUGNAc and palmitate, and there was a significant difference between the group treated with PUGNAc compared to the control group.

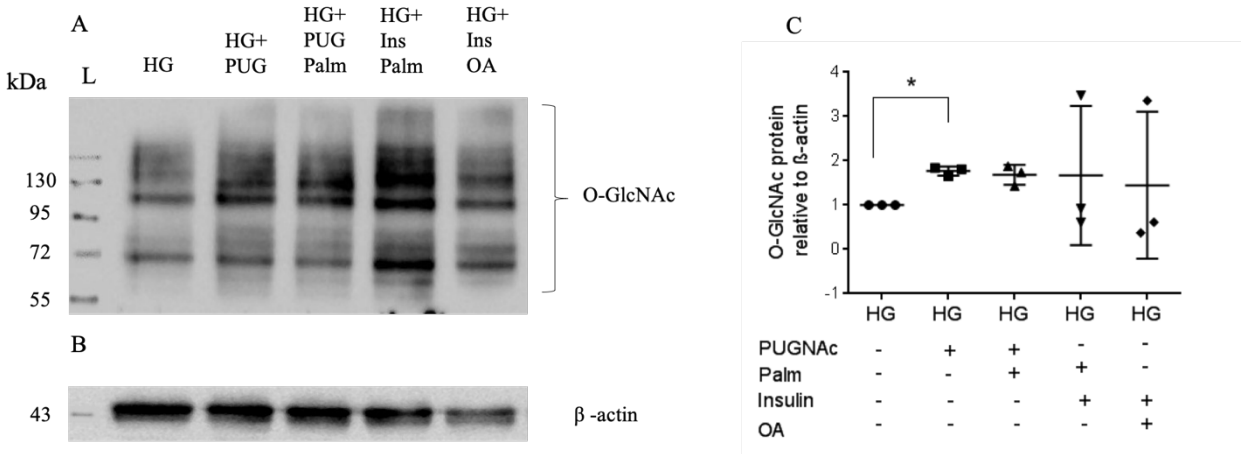


Figure 4.5. Effect of metabolic disturbances on 10T1/2 cells on O-GlcNAcylation. 10T1/2 cells were treated with various treatments for 48 hours to investigate the O-GlcNAcylation levels in these cells in the presence of various treatments such as PUGNAc, Palmitate, Insulin and Oleic Acid. A) Western blot comparing O-GlcNAc levels in extracted proteins from 10T1/2 conditioned with high glucose DMEM +

PUGNAc (HG+ PUGNAc), high glucose DMEM + PUGNAc + palmitate (HG+ PUG+ Palm), high glucose DMEM + insulin+ palmitate (HG+ Ins+ Palm) and high glucose DMEM + insulin+ oleic acid (HG+ Ins+ Oleic) treated for 48 hours. B) Western blot of β-actin in the same experimental groups. C) The O-GlcNAc bands intensities were quantified and normalized to each corresponding β-actin band; Data are shown as independent experiments (n=3), with lines representing mean ± standard deviation. *P<0.05.

4.4 Zfp423 is an O-GlcNAcylated protein

To investigate whether Zfp423 could be O-GlcNAcylated, I treated 10T1/2 cells for 24 hours with 100 µM PUGNAc to amplify O-GlcNAcylation. Then, using the immunoprecipitation technique, Zfp423 proteins were pulled down from the lysate extract. Western blot with anti-O-GlcNAc antibody enabled the detection of a high-intensity band at the molecular weight of Zfp423 (Figure 4.6). No signal was seen in the IgG control immunoprecipitation. This suggests that Zfp423 is an O-GlcNAcylated protein (Figure 4.6).

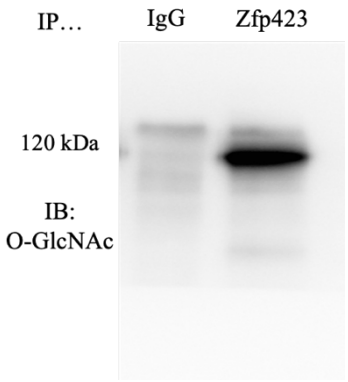


Figure 4.6. O-GlcNAcylation of Zfp423. 10T1/2 cells were treated for 24 hours with PUGNAc (100 µM) to induce O-GlcNAcylation and then the proteins were extracted from these cells. Then the lysates were immunoprecipitated (IP) with anti- Zfp423 antibody or IgG as a negative control and Western blotted for O-GlcNAc.

4.5 Modification of Zfp423 in various nutrient availability

I investigated the effect of various glucose concentrations, palmitate and, PUGNAc on Zfp423 protein levels. I observed that the expression of the Zfp423 protein was significantly higher in mesenchymal cells that were treated with normal glucose levels for 48 hours compared to cells that were treated at the same time with high glucose. However, Zfp423 levels in cells that were treated with PUGNAc or with Palmitate and PUGNAc, were not significantly different compared to other treatment groups (Figure 4.7).

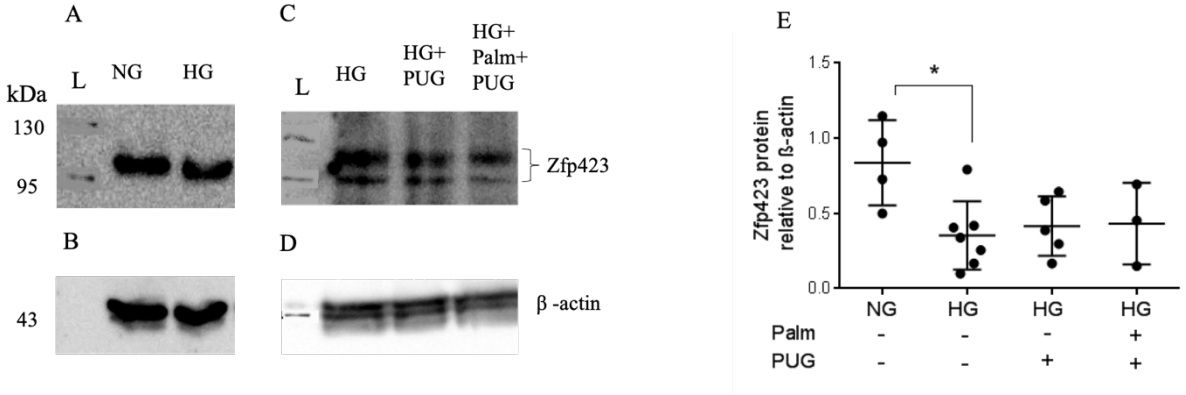


Figure 4.7. Effect of nutrient availability on Zfp423. Mesenchymal cells were treated with various glucose levels (high and normal) with PUGNAc and Palmitate for 48 hours and the levels of Zfp423 protein was measured a) Western blot showing Zfp423 proteins in extracts from mesenchymal cells treated with normal glucose DMEM (NG), high glucose DMEM (HG), b) β-actin blot for NG and HG c) Zfp423 blot for high glucose DMEM (HG) high glucose DMEM + PUGNAc and high glucose DMEM+ palmitate+ PUGNAc d) β-actin blot for HG, HG+ PUG and HG+ Palm+ PUG e) The Zfp423 bands intensities were quantified and normalized to each corresponding β-actin band using ImageJ. Data are shown as independent experiments (n=3-7), with lines representing mean ± standard deviation. *P<0.05.

4.6 O-GlcNAcylation of mesenchymal cells

To study the effect of O-GlcNAcylation on the differentiation fate of mesenchymal cells, I cultured these cells with variable levels of glucose and treated some cells with PUGNAc to amplify the O-GlcNAcylation levels. Neither *Zfp423* nor *Col1a1* mRNA levels were altered by glucose concentration or by PUGNAc treatment (Figure 4.8 A, B).

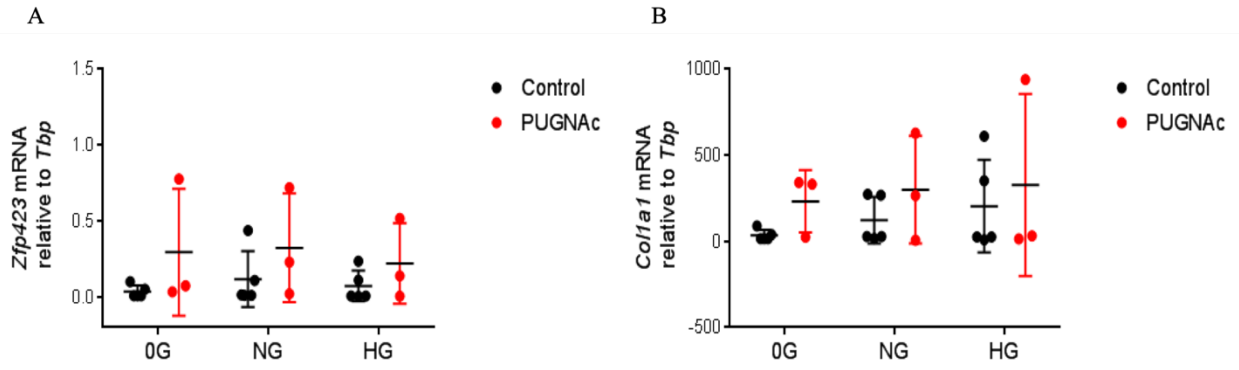
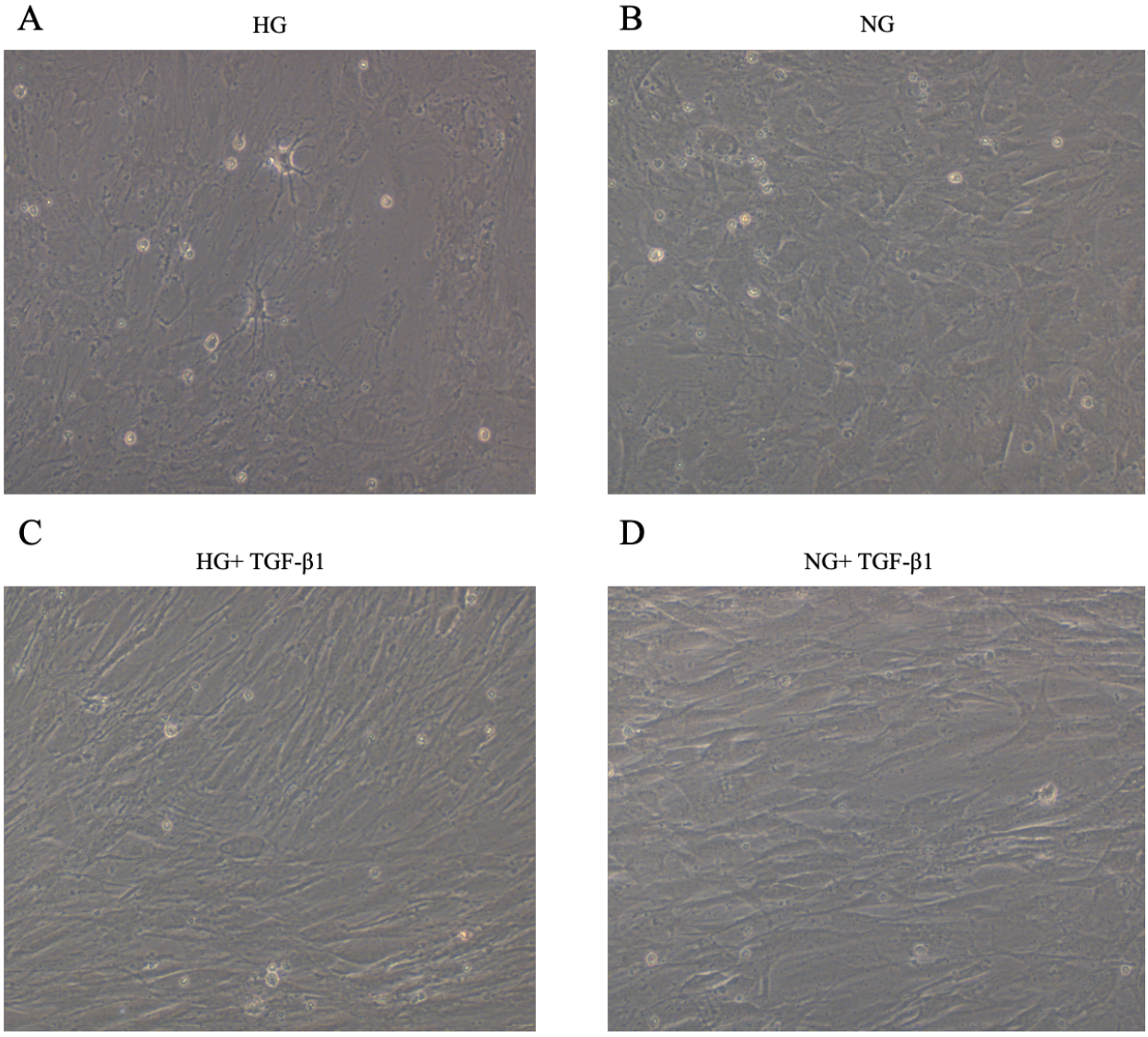


Figure 4.8. Effect of PUGNAc and glucose availability on gene expression of *Zfp423* and *Col1a1*. Mesenchymal cells were cultured with no glucose media (0 mM. 0G), normal glucose media (5.5 mM, NG) and high glucose media (25 mM, HG) and 100µM PUGNAc for 48 hours and the RNA was extracted to perform QPCR analysis to compare the control groups with the treatment groups. The expression of A) *Zfp423* RNA and B) collagen RNA (*Col1a1*) was measured and quantified using qPCR analysis normalizing to the housekeeping gene *Tbp* for mesenchymal cells. Data are shown as independent experiments (n=3), with lines representing mean ± standard deviation. **P*<0.05.

4.7 Myogenic potential of mesenchymal cells

Lastly, TGF-β1 is known to promote fibrosis and the expression of smooth muscle genes. To test these outcomes in mouse mesenchymal cells, I cultured cells with either normal or high glucose DMEM for 2 days and subsequently treated them with TGF-β1 (100 ng/ml) for 5 days, comparing them with a time-matched control group. I observed that the appearance of cells

treated with TGF-β1 was altered with cells becoming elongated and looking more like myocytes (Figure 4.9). This suggests that TGF-β1 could induce the formation of smooth muscle cells in mesenchymal cells. RNA analysis of these cells indicated the presence of *Myh11*, which encodes smooth muscle myosin, though, there was no specific trend observed in the expression of *Mhy11* mRNA in various groups (Figure 4.9 E).



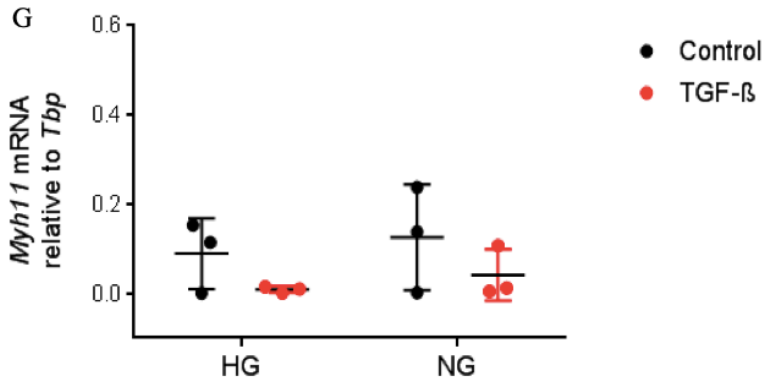


Figure 4.9. Effect of glucose availability on mesenchymal cells phenotype shift towards myogenesis. Mesenchymal cells were cultured with either high glucose (25 mM, HG) or normal glucose (5.5 mM, NG) media for 2 days and with 100ng/ml TGF-β1 for 5 days to investigate the myotube formation in these cells. The phenotype shift in mesenchymal cells towards myocyte- like cells was observed and images were captured using light microscope and Capture software in cells treated with A) high glucose DMEM (HG) B) normal glucose DMEM (NG) C) HG+ TGF-β1 D) NG+ TGF-β1. E) The expression of *Myh11* RNA was measured and quantified using qPCR analysis normalizing to the housekeeping gene *Tbp* for mesenchymal cells. Data are shown as independent experiments (n=3), with lines representing mean ± standard deviation.

Chapter 5. Discussion

5.1 Overview of findings

The goal of this study was to test the effects of normal and high glucose concentration on O-GlcNAcylation and pericyte phenotype by studying mesenchymal cell phenotype shift. I observed greater formation of adipocytes by these cells when they are cultured in normal compared to high glucose conditions. Levels of Zfp423 protein also were significantly higher in mesenchymal cells cultured in 5.5 mM glucose levels. In contrast, *Zfp423* mRNA was significantly higher in mesenchymal cells treated with 25 mM glucose. I established that Zfp423 protein is an O-GlcNAcylated protein. However, an increase in O-GlcNAcylation was unable to induce a phenotype change independently of the adipogenic stimulus. Lastly, I observed that higher dosage of TGF-β1 could induce phenotype changes in mesenchymal cells and transfer them into myocyte looking cells. These studies show promise that manipulation of nutrient status (glucose) may be a means to manipulate their differentiation capacity. The figure 5.1 summarizes the hypothesis as well as the findings of this study.

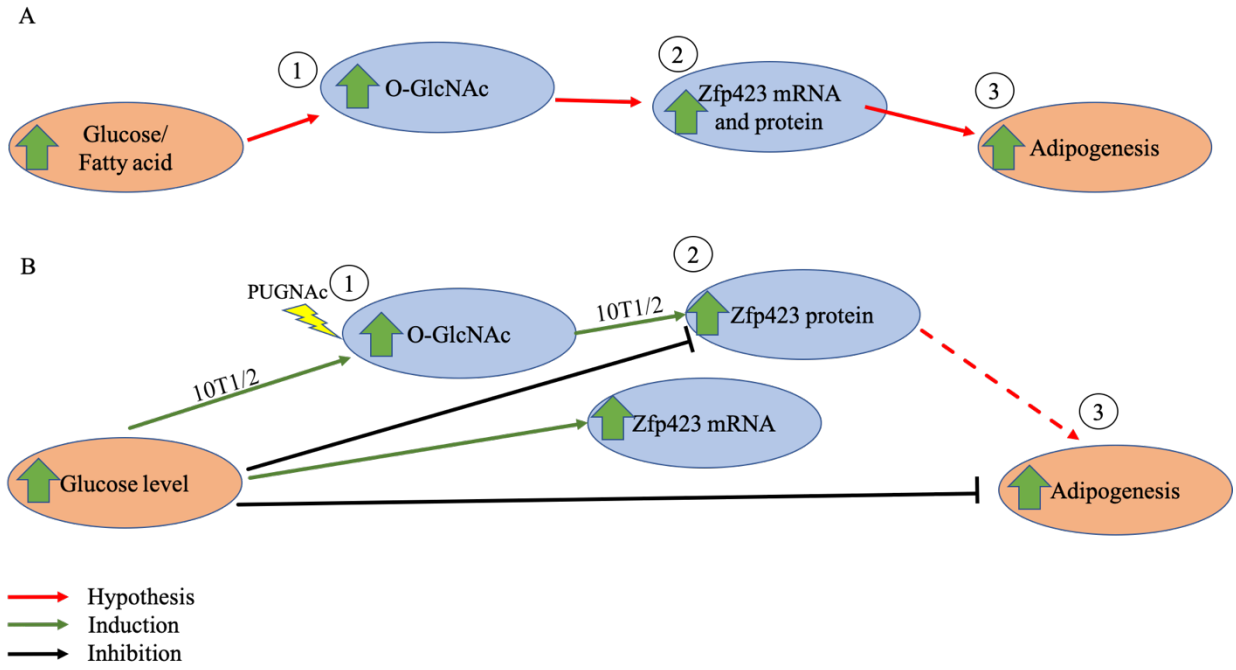


Figure 5.1. Summary of hypothesis and findings. A) 1- Initially, I hypothesized that an increase in glucose/fatty acid levels can increase the O-GlcNAcylation levels. 2- I hypothesized that an increase in glucose availability can increase the Zfp423 protein and mRNA levels through O-GlcNAcylation. 3- I hypothesized that an increase in Zfp423 mRNA and protein levels can increase the adipogenesis of mesenchymal cells. B) 1- My hypothesis of the effect of glucose increase on O-GlcNAcylation was only true in 10T1/2 cells that were treated with PUGNAc. The O-GlcNAcylation was increased in these cells following an increase in glucose levels. 2- After conducting my experiments, I observed that in 10T1/2 cells O-GlcNAcylation can increase the Zfp423 protein levels. However, in mesenchymal cells, I observed that an increase in glucose levels inhibits the Zfp423 protein and increases the Zfp423 mRNA level. 3- I did not test the relation between Zfp423 protein increase and adipogenesis of mesenchymal cells.

5.2 Adipogenesis in mesenchymal cells

In measuring the effects of nutrient availability on the phenotype shift of mesenchymal cells, I found that adipogenesis occurred to a greater extent in the presence of normal glucose levels. This suggests that a physiological normal glucose concentration is optimal for increasing pre-

adipocytes differentiation and the formation of fat droplets following exposure to the adipogenic cocktail. This observation appears to conflict with previous literature, as high glucose availability (25 mM glucose) led to higher adipogenic ability in 3T3-L1 pre-adipocytes after 9 days (Jackson et al., 2017). However, the use of different cell types could explain the difference in our observation.

Zfp423 is a transcription factor that is enriched in pre-adipocytes and regulates adipogenesis through the expression of PPARγ (Gupta et al., 2010; Longo et al., 2019). My finding that Zfp423 protein levels were higher in normal glucose conditions fits with the greater adipogenic seen in normal glucose. Interestingly, I detected opposing effects of glucose levels on *Zfp423* mRNA and protein. Protein levels can be regulated independently of mRNA by controlling the degradation rate. One possibility for this discrepancy could be that in the normal glucose environment, there is lower degradation of Zfp423 proteins compared to a high glucose environment. Also, evaluating the PPARγ levels could be helpful in understanding Zfp423 activity, since PPARγ is a downstream target of Zfp423.

Mesenchymal cells can either undergo adipogenesis or fibrogenesis. These two processes appear to have inverse relation meaning that the occurrence of one, results in the inhibition of other differentiation cascades (Shen et al., 2022). This is evident since an increase in PPARγ has been seen to associate with a decrease in fibrosis (Buechler et al., 2015). While I expected to see a similar inverse relationship in my studies, I was not able to detect an inverse relationship between the levels of *Zfp423* mRNA and *Col1a1* mRNA, when mesenchymal cells were treated with various glucose levels.

5.3 The effect of O-GlcNAcylation and mesenchymal cell phenotype

I hypothesized that O-GlcNAcylation levels would vary dependent on glucose availability. Manipulation of glucose levels on 10T1/2 cells indicated that high glucose availability is associated with higher levels of O-GlcNAcylation in these cells. While this relationship could be seen in 10T1/2 cells, I did not detect an effect of glucose on the O-GlcNAcylation of mesenchymal cells. Also, I utilized palmitate and oleic acid treatment to test the effect of fatty acid excess on O-GlcNAcylation. Although the literature provides evidence for the role of excess fatty acid in increasing the hexosamine biosynthesis pathway (Wong et al., 2022), my data did not support any induction in O-GlcNAcylation. One possible explanation could be that HBP flux was already maximal in these cells and thus adding palmitate had no additional effect. The independent effect of fatty acids could be re-tested in a normal glucose environment.

Multiple studies have shown that O-GlcNAcylation levels increase during adipogenesis. For example, following adipocyte differentiation, multiple proteins such as Vimentin and Nucleoporins were heavily O-GlcNAcylated (Sun et al., 2016; Franke et al., 1987). Also, the pharmacological inhibition of the HBP pathway was shown to impair the adipogenesis differentiation (Hsieh et al., 2012). Based on this literature, I hypothesized that an increase in O-GlcNAcylation levels increase in Zfp423 protein and in adipogenesis. This concept was supported by my observations in 10T1/2 cells that Zfp423 can be O-GlcNAcylated and that Zfp423 protein increases following PUGNAc treatment. I had assumed that treatment of primary mesenchymal cells would result in similar outcomes as in 10T1/2 cells. However, since I was not able to detect an increase in Zfp423 protein levels or in adipocyte formation, in mesenchymal

cells treated with PUGNAc, I cannot interpret the role of O-GlcNAcylation on adipogenesis in mesenchymal cells. One possibility is that PUGNAc treatment failed to increase O-GlcNAcylation in the mesenchymal cells. Another possibility is that the enzyme target of PUGNAc, OGT, is already minimally active in mesenchymal cells such that the addition of PUGNAc does not result in further inhibition of this enzyme. Lastly, O-GlcNAcylation could also alter protein activity and its cellular localization, independent of its levels (Issad et al., 2022). There is a possibility that the function of Zfp423 protein gets altered following O-GlcNAcylation, regardless of its levels, altering the role of this protein in adipogenesis. Therefore, there is a need for further investigation of O-GlcNAcylation in these cells.

5.4 Response of mesenchymal cells to TGF-β1

TGF-β1 is pro fibrotic by enhancing collagen production (de Larco & Todaro, 1978) via the action of Smad3 (Verrecchia & Mauviel, 2007). TGF-β1 also regulates the differentiation process of multipotent cells through Smad and non- Smad pathways (Zhang, 2009)(Rahimi & Leof, 2007). TGF-β1 is known to promote the differentiation of smooth muscle cells, chondrocytes, immature cardiomyocytes and to inhibit the differentiation of stem cells into adipocytes, endothelial cells, myotubes, and natural killer cells (M.-K. Wang, 2012). The literature shows that there is an optimal concentration of TGF-β1 for promotion of fibrosis which is about 5ng/ml and high doses of TGF-β1 might inhibit collagen formation (Fragiadaki et al., 2011). However, I was not able to observe any phenotypic changes to the mesenchymal cells following the treatment with 5ng/ml of TGF-β1. Also, there is no evidence in the literature that supports the shift in phenotype of mesenchymal cells into smooth muscle- like cells following the treatment of high doses of TGF-β1 (100ng/ml). This failure to increase production of *Col1a1*

could be due to the efficacy of the recombinant TGF-β1 that I have used. This recombinant protein should be tested in future to testing whether it is still functional, or multiple thaw/ freeze cycles has affected its function. In order to test the efficacy of the TGF-β1 treatment, phosphorylated Smads levels could be measured. The phosphorylation of Smads protein levels should increase if the drug is functioning properly. However, it is important to add that I only investigated *Col1a1* gene which is only one of many collagen genes. It is possible that other collagens were increased in response to TGF-β1.

5.5 Limitations of my thesis

5.5.1 Cell culture

My experiments were conducted *in vitro*, which eliminates all the factors that cells are exposed to in their natural environment. *In vitro* experiments are beneficial since they provide a cost-efficient insight about a topic which could later be tested *in vivo*. However, applying the observations from *in vitro* experiments to *in vivo* is always a challenge. The *in vitro* treatment conditions may not resemble the physiological *in vivo* conditions. For example, we culture our cells in high glucose DMEM which has 25 mM glucose and resembles that seen under hyperglycemic conditions *in vivo* such as in individuals living with poorly controlled type 1 or type 2 diabetes. Therefore, the cells would behave differently *in vivo* due to the different in glucose levels. Also, mesenchymal cells may alter their phenotypic characteristics as they are propagated in culture (Kwist et al., 2016). These changes in their characteristic could result in inconsistent responses to the treatments, which could be one potential reason for the inconsistency of some of my qPCR data. One ideal solution to eliminate this confounding

variable is to use only one specific passage number for all the experiments. However, this is not the time or cost-efficient.

5.5.2 PUGNAc treatment

I used PUGNAc treatment to induce O-GlcNAcylation in my cells, as it is an established method to increase O-GlcNAc levels through the inhibition of the OGA enzyme. However, I did not observe a consistent increase in O-GlcNAC levels when using PUGNAc. One possibility for this result could be high basal levels of O-GlcNAcylation in these cells that make it impossible to increase the O-GlcNAcylation levels further. This could be tested by reducing the O-GlcNAcylation levels by cellular starvation. Another factor affecting PUGNAc could be drug efficacy, which could have degraded following freeze/thaw cycles.

5.6 Future work

I have used mesenchymal cells as a model to study the effects of nutrient availability on pericyte phenotype shift. Mesenchymal cells are a very good model to study pericytes since they are a heterogeneous population of cells containing muscle-resident pericytes that are also easy to extract. However, it is important to repeat these experiments on a pure population of pericytes, to confirm that they will exhibit similar responses to glucose availability.

I have established that Zfp423 is an O-GlcNAcylated protein. However, my experiments do not prove a causal role for Zfp423 in the shift of mesenchymal cell phenotype. To determine the role of Zfp423 in adipogenesis, it will be necessary to manipulate its levels and investigate the consequences of changes in the adipogenic process and fat droplet formation. One possible way

to test the effect of Zfp423 would be to silence its gene using siRNA and observe the effect of its absence on adipogenesis.

I studied O-GlcNAcylation as a factor that could affect Zfp423 levels and potentially modifies adipose tissue formation. However, various factors can affect Zfp423 levels and O-GlcNAcylation is only one factor to study. Also, the degradation rate of Zfp423 is an important mean that determines the levels of this transcription factor. Reduction in the degradation rate of Zfp423 results in an increase in the levels of this protein. Therefore, it is important to investigate the factors that potentially affect the degradation rate of Zfp423, influencing its levels.
I have observed the change in the morphology of mesenchymal cells to smooth muscle cell-like cells after the treatment with TGF-β1. It would be beneficial to further treat these cells with 5% horse serum, which is a technique used to induce myocyte formation and investigate whether it leads to further change in the morphology of mesenchymal cells. Lastly, to conclude whether the formation of myocytes occurred and was significant in my treatments, they need to be quantified and analyzed to compare the treatment groups with the controls.

5.7 Conclusion and significance

In recent years, interest in the use of pericytes as a means of cell-based therapy has grown. However, our understanding of their differentiation potential and the factors that could induce these processes is incomplete. My thesis project provided evidence for the initiation of the differentiation process in mesenchymal cells, such as adipogenesis in the presence of an adipogenic cocktail and myogenesis in the presence of high dosages of TGF-β1. Also, I have shown that an increase in O-GlcNAcylation might affect levels of Zfp423 protein, which could

lead to an increase in adipogenesis, considering that Zfp423 is a transcriptional regulator of adipogenic genes such as PPARγ. In addition, I observed that a high glucose concentration environment results in an increase in *Zfp423* mRNA levels. These findings are a starting point in understanding the effect of nutrient availability on mesenchymal cell phenotype shift, which can later be translated to the study of pericytes. Also, these findings contribute to the understanding of how mesenchymal cells could negatively impact the process of recovery in case of injury by shifting their phenotype to unwanted outcomes. For example, the formation of adipocytes or fibrotic cells negatively impacts the muscle recovery process. On the other hand, the formation of smooth muscle cells could be beneficial in blood vessel formation and reperfusion of injured skeletal muscle. Therefore, by understanding the effect of nutrient availability on mesenchymal cells, we could better understand how the local environment will affect the mesenchymal cell phenotype and use this information to prevent their differentiation into cells that negatively impact muscle recovery.

References:

Alison, M. R., Poulsom, R., Forbes, S., & Wright, N. A. (2002). An introduction to stem cells. *The Journal of Pathology*, *197*(4), 419–423. https://doi.org/10.1002/path.1187

Andersen, P., & Saltin, B. (1985). Maximal perfusion of skeletal muscle in man. *The Journal of Physiology*, *366*(1), 233–249. https://doi.org/10.1113/jphysiol.1985.sp015794

Andersson-Sjöland, A., Karlsson, J. C., & Rydell-Törmänen, K. (2016). ROS-induced endothelial stress contributes to pulmonary fibrosis through pericytes and Wnt signaling. *Laboratory Investigation*, *96*(2), 206–217. https://doi.org/10.1038/labinvest.2015.100

Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/Pericyte Interactions. *Circulation Research*, *97*(6), 512–523. https://doi.org/10.1161/01.RES.0000182903.16652.d7

Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: Developmental, Physiological, and Pathological Perspectives, Problems, and Promises. *Developmental Cell*, *21*(2), 193–215. https://doi.org/10.1016/j.devcel.2011.07.001

Armulik, A., Genové, G., Mäe, M., Nisancioglu, M. H., Wallgard, E., Niaudet, C., He, L., Norlin, J., Lindblom, P., Strittmatter, K., Johansson, B. R., & Betsholtz, C. (2010). Pericytes regulate the blood–brain barrier. *Nature*, *468*(7323), 557–561. https://doi.org/10.1038/nature09522

Bandopadhyay, R., Orte, C., Lawrenson, J. G., Reid, A. R., De Silva, S., & Allt, G. (2001). [No title found]. *Journal of Neurocytology*, *30*(1), 35–44. https://doi.org/10.1023/A:1011965307612

Bergers, G., & Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro-Oncology*, *7*(4), 452–464. https://doi.org/10.1215/S1152851705000232

Bibber, B., Sinha, G., Lobba, A. R. M., Greco, S. J., & Rameshwar, P. (2013). A review of stem cell translation and potential confounds by cancer stem cells. *Stem Cells International*, *2013*, 241048. https://doi.org/10.1155/2013/241048

Birbrair, A., Borges, I. da T., Gilson Sena, I. F., Almeida, G. G., da Silva Meirelles, L., Gonçalves, R., Mintz, A., & Delbono, O. (2017). How Plastic Are Pericytes? *Stem Cells and Development*, *26*(14), 1013–1019. https://doi.org/10.1089/scd.2017.0044

Birbrair, A., Zhang, T., Wang, Z.-M., Messi, M. L., Enikolopov, G. N., Mintz, A., & Delbono, O. (2013). Role of Pericytes in Skeletal Muscle Regeneration and Fat Accumulation. *Stem Cells and Development*, *22*(16), 2298–2314. https://doi.org/10.1089/scd.2012.0647

Bjørbæk, C. (2009). Central Leptin Receptor Action and Resistance in Obesity. *Journal of Investigative Medicine*, *57*(7), 789–794. https://doi.org/10.2310/JIM.0b013e3181bb0d49

Blobe, G. C., Schiemann, W. P., & Lodish, H. F. (2000). Role of transforming growth factor beta in human disease. *The New England Journal of Medicine*, *342*(18), 1350–1358. https://doi.org/10.1056/NEJM200005043421807

Bond, M. R., & Hanover, J. A. (2013). O-GlcNAc cycling: A link between metabolism and chronic disease. *Annual Review of Nutrition*, *33*, 205–229. https://doi.org/10.1146/annurev-nutr-071812-161240

Brozovich, F. V., Nicholson, C. J., Degen, C. V., Gao, Y. Z., Aggarwal, M., & Morgan, K. G. (2016). Mechanisms of Vascular Smooth Muscle Contraction and the Basis for Pharmacologic Treatment of Smooth Muscle Disorders. *Pharmacological Reviews*, *68*(2), 476–532. https://doi.org/10.1124/pr.115.010652

Buechler, C., Krautbauer, S., & Eisinger, K. (2015). Adipose tissue fibrosis. *World Journal of Diabetes*, *6*(4), 548–553. https://doi.org/10.4239/wjd.v6.i4.548

Bullen, J. W., Balsbaugh, J. L., Chanda, D., Shabanowitz, J., Hunt, D. F., Neumann, D., & Hart, G. W. (2014). Cross-talk between Two Essential Nutrient-sensitive Enzymes. *Journal of Biological Chemistry*, *289*(15), 10592–10606. https://doi.org/10.1074/jbc.M113.523068

Campagnoli, C., Roberts, I. A., Kumar, S., Bennett, P. R., Bellantuono, I., & Fisk, N. M. (2001). Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood*, *98*(8), 2396–2402. https://doi.org/10.1182/blood.v98.8.2396

Cantelmo, A. R., Conradi, L.-C., Brajic, A., Goveia, J., Kalucka, J., Pircher, A., Chaturvedi, P., Hol, J., Thienpont, B., Teuwen, L.-A., Schoors, S., Boeckx, B., Vriens, J., Kuchnio, A., Veys, K., Cruys, B., Finotto, L., Treps, L., Stav-Noraas, T. E., … Carmeliet, P. (2016). Inhibition of the Glycolytic Activator PFKFB3 in Endothelium Induces Tumor Vessel Normalization, Impairs Metastasis, and Improves Chemotherapy. *Cancer Cell*, *30*(6), 968–985. https://doi.org/10.1016/j.ccell.2016.10.006

Caplan, A. I., & Bruder, S. P. (2001). Mesenchymal stem cells: Building blocks for molecular medicine in the 21st century. *Trends in Molecular Medicine*, *7*(6), 259–264. https://doi.org/10.1016/s1471-4914(01)02016-0

Carmeliet, P., & Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature*, *473*(7347), 298–307. https://doi.org/10.1038/nature10144

Castro, V., Skowronska, M., Lombardi, J., He, J., Seth, N., Velichkovska, M., & Toborek, M. (2018). Occludin regulates glucose uptake and ATP production in pericytes by

influencing AMP-activated protein kinase activity. *Journal of Cerebral Blood Flow & Metabolism*, *38*(2), 317–332. https://doi.org/10.1177/0271678X17720816

Chen, F., Zhou, J., Li, Y., Zhao, Y., Yuan, J., Cao, Y., Wang, L., Zhang, Z., Zhang, B., Wang, C. C., Cheung, T. H., Wu, Z., Wong, C. C.-L., Sun, H., & Wang, H. (2019). YY1 regulates skeletal muscle regeneration through controlling metabolic reprogramming of satellite cells. *The EMBO Journal*, *38*(10), e99727. https://doi.org/10.15252/embj.201899727

Chen, L., Tredget, E. E., Wu, P. Y. G., & Wu, Y. (2008). Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PloS One*, *3*(4), e1886. https://doi.org/10.1371/journal.pone.0001886

Cheng, J. H., She, H., Han, Y.-P., Wang, J., Xiong, S., Asahina, K., & Tsukamoto, H. (2008). Wnt antagonism inhibits hepatic stellate cell activation and liver fibrosis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *294*(1), G39–G49. https://doi.org/10.1152/ajpgi.00263.2007

Cipolleschi, M. G., Dello Sbarba, P., & Olivotto, M. (1993). The role of hypoxia in the maintenance of hematopoietic stem cells. *Blood*, *82*(7), 2031–2037.

Clifford, P. S. (2011). Local control of blood flow. *Advances in Physiology Education*, *35*(1), 5–15. https://doi.org/10.1152/advan.00074.2010

Constable, S., Lim, J.-M., Vaidyanathan, K., & Wells, L. (2017). O-GlcNAc transferase regulates transcriptional activity of human Oct4. *Glycobiology*, *27*(10), 927–937. https://doi.org/10.1093/glycob/cwx055

Crisan, M., Yap, S., Casteilla, L., Chen, C.-W., Corselli, M., Park, T. S., Andriolo, G., Sun, B., Zheng, B., Zhang, L., Norotte, C., Teng, P.-N., Traas, J., Schugar, R., Deasy, B. M., Badylak, S., Bűhring, H.-J., Giacobino, J.-P., Lazzari, L., … Péault, B. (2008). A

Perivascular Origin for Mesenchymal Stem Cells in Multiple Human Organs. *Cell Stem Cell*, *3*(3), 301–313. https://doi.org/10.1016/j.stem.2008.07.003

Cunningham, K. S., & Gotlieb, A. I. (2005). The role of shear stress in the pathogenesis of atherosclerosis. *Laboratory Investigation*, *85*(1), 9–23. https://doi.org/10.1038/labinvest.3700215

Daneman, R., Zhou, L., Kebede, A. A., & Barres, B. A. (2010). Pericytes are required for blood–brain barrier integrity during embryogenesis. *Nature*, *468*(7323), 562–566. https://doi.org/10.1038/nature09513

De Bari, C., Dell'Accio, F., Vandenabeele, F., Vermeesch, J. R., Raymackers, J.-M., & Luyten, F. P. (2003). Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *Journal of Cell Biology*, *160*(6), 909–918. https://doi.org/10.1083/jcb.200212064

de Larco, J. E., & Todaro, G. J. (1978). Growth factors from murine sarcoma virus-transformed cells. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(8), 4001–4005. https://doi.org/10.1073/pnas.75.8.4001

Delorme, B., Chateauvieux, S., & Charbord, P. (2006). The concept of mesenchymal stem cells. *Regenerative Medicine*, *1*(4), 497–509. https://doi.org/10.2217/17460751.1.4.497

Dennis, J. E., Carbillet, J.-P., Caplan, A. I., & Charbord, P. (2002). The STRO-1+ marrow cell population is multipotential. *Cells, Tissues, Organs*, *170*(2–3), 73–82. https://doi.org/10.1159/000046182

Ding, D.-C., Shyu, W.-C., Lin, S.-Z., & Li, H. (2006). Current concepts in adult stem cell therapy for stroke. *Current Medicinal Chemistry*, *13*(29), 3565–3574. https://doi.org/10.2174/092986706779026237

Ding, D.-C., Shyu, W.-C., Lin, S.-Z., & Li, H. (2007). The role of endothelial progenitor cells in ischemic cerebral and heart diseases. *Cell Transplantation*, *16*(3), 273–284. https://doi.org/10.3727/000000007783464777

Doorn, J., van de Peppel, J., van Leeuwen, J. P. T. M., Groen, N., van Blitterswijk, C. A., & de Boer, J. (2011). Pro-osteogenic trophic effects by PKA activation in human mesenchymal stromal cells. *Biomaterials*, *32*(26), 6089–6098. https://doi.org/10.1016/j.biomaterials.2011.05.010

Du, X. L., Edelstein, D., Rossetti, L., Fantus, I. G., Goldberg, H., Ziyadeh, F., Wu, J., & Brownlee, M. (2000). Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(22), 12222–12226. https://doi.org/10.1073/pnas.97.22.12222

Duling, B. R., & Damon, D. H. (1987). An examination of the measurement of flow heterogeneity in striated muscle. *Circulation Research*, *60*(1), 1–13. https://doi.org/10.1161/01.RES.60.1.1

Eaton, S., Bartlett, K., & Pourfarzam, M. (1996). Mammalian mitochondrial beta-oxidation. *The Biochemical Journal*, *320 (Pt 2)*, 345–357. https://doi.org/10.1042/bj3200345

Elashry, M. I., Kinde, M., Klymiuk, M. C., Eldaey, A., Wenisch, S., & Arnhold, S. (2022). The effect of hypoxia on myogenic differentiation and multipotency of the skeletal muscle-derived stem cells in mice. *Stem Cell Research & Therapy*, *13*(1), 56. https://doi.org/10.1186/s13287-022-02730-5

Emerson, G. G., & Segal, S. S. (1997). Alignment of microvascular units along skeletal muscle fibers of hamster retractor. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *82*(1), 42–48. https://doi.org/10.1152/jappl.1997.82.1.42

Fahy, E., Cotter, D., Sud, M., & Subramaniam, S. (2011). Lipid classification, structures and tools. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1811*(11), 637–647. https://doi.org/10.1016/j.bbalip.2011.06.009

Farrington-Rock, C., Crofts, N. J., Doherty, M. J., Ashton, B. A., Griffin-Jones, C., & Canfield, A. E. (2004). Chondrogenic and Adipogenic Potential of Microvascular Pericytes. *Circulation*, *110*(15), 2226–2232. https://doi.org/10.1161/01.CIR.0000144457.55518.E5

Fragiadaki, M., Ikeda, T., Witherden, A., Mason, R. M., Abraham, D., & Bou-Gharios, G. (2011). High doses of TGF-β potently suppress type I collagen via the transcription factor CUX1. *Molecular Biology of the Cell*, *22*(11), 1836–1844. https://doi.org/10.1091/mbc.E10-08-0669

Franke, W. W., Hergt, M., & Grund, C. (1987). Rearrangement of the vimentin cytoskeleton during adipose conversion: Formation of an intermediate filament cage around lipid globules. *Cell*, *49*(1), 131–141. https://doi.org/10.1016/0092-8674(87)90763-X

Fu, R.-H., Wang, Y.-C., Liu, S.-P., Huang, C.-M., Kang, Y.-H., Tsai, C.-H., Shyu, W.-C., & Lin, S.-Z. (2011). Differentiation of stem cells: Strategies for modifying surface biomaterials. *Cell Transplantation*, *20*(1), 37–47. https://doi.org/10.3727/096368910X532756

Geevarghese, A., & Herman, I. M. (2014). Pericyte-endothelial crosstalk: Implications and opportunities for advanced cellular therapies. *Translational Research*, *163*(4), 296–306. https://doi.org/10.1016/j.trsl.2014.01.011

Gerhardt, H., & Betsholtz, C. (2003). Endothelial-pericyte interactions in angiogenesis. *Cell and Tissue Research*, *314*(1), 15–23. https://doi.org/10.1007/s00441-003-0745-x

Gerstein, H. C., Miller, M. E., Byington, R. P., Goff, D. C., Bigger, J. T., Buse, J. B., Cushman, W. C., Genuth, S., Ismail-Beigi, F., Grimm, R. H., Probstfield, J. L., Simons-Morton, D. G., & Friedewald, W. T. (2008). Effects of intensive glucose lowering in type 2 diabetes. *The New England Journal of Medicine*, *358*(24), 2545–2559. https://doi.org/10.1056/NEJMoa0802743

Guimarães-Camboa, N., Cattaneo, P., Sun, Y., Moore-Morris, T., Gu, Y., Dalton, N. D., Rockenstein, E., Masliah, E., Peterson, K. L., Stallcup, W. B., Chen, J., & Evans, S. M. (2017). Pericytes of Multiple Organs Do Not Behave as Mesenchymal Stem Cells In Vivo. *Cell Stem Cell*, *20*(3), 345-359.e5. https://doi.org/10.1016/j.stem.2016.12.006

Guo, X., Li, H., Xu, H., Woo, S., Dong, H., Lu, F., Lange, A. J., & Wu, C. (2012). Glycolysis in the control of blood glucose homeostasis. *Acta Pharmaceutica Sinica B*, *2*(4), 358–367. https://doi.org/10.1016/j.apsb.2012.06.002

Gupta, R. K., Arany, Z., Seale, P., Mepani, R. J., Ye, L., Conroe, H. M., Roby, Y. A., Kulaga, H., Reed, R. R., & Spiegelman, B. M. (2010). Transcriptional control of preadipocyte determination by Zfp423. *Nature*, *464*(7288), 619–623. https://doi.org/10.1038/nature08816

Hart, G. W., Housley, M. P., & Slawson, C. (2007). Cycling of O-linked β-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature*, *446*(7139), 1017–1022. https://doi.org/10.1038/nature05815

Haynesworth, S. E., Baber, M. A., & Caplan, A. I. (1996). Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: Effects of dexamethasone and IL-

1 alpha. *Journal of Cellular Physiology*, *166*(3), 585–592. https://doi.org/10.1002/(SICI)1097-4652(199603)166:3<585::AID-JCP13>3.0.CO;2-6

He, L., Vanlandewijck, M., Mäe, M. A., Andrae, J., Ando, K., Del Gaudio, F., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., Sun, Y., Raschperger, E., Segerstolpe, Å., Liu, J., Gustafsson, S., Räsänen, M., Zarb, Y., Mochizuki, N., Keller, A., … Betsholtz, C. (2018). Single-cell RNA sequencing of mouse brain and lung vascular and vessel-associated cell types. *Scientific Data*, *5*(1), 180160. https://doi.org/10.1038/sdata.2018.160

Hellström, M., Kalén, M., Lindahl, P., Abramsson, A., & Betsholtz, C. (1999). Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development (Cambridge, England)*, *126*(14), 3047–3055. https://doi.org/10.1242/dev.126.14.3047

Holm, A., Heumann, T., & Augustin, H. G. (2018). Microvascular Mural Cell Organotypic Heterogeneity and Functional Plasticity. *Trends in Cell Biology*, *28*(4), 302–316. https://doi.org/10.1016/j.tcb.2017.12.002

Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., Okita, K., & Yamanaka, S. (2009). Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature*, *460*(7259), 1132–1135. https://doi.org/10.1038/nature08235

Hosseinkhani, M., Mehrabani, D., Karimfar, M. H., Bakhtiyari, S., Manafi, A., & Shirazi, R. (2014). Tissue engineered scaffolds in regenerative medicine. *World Journal of Plastic Surgery*, *3*(1), 3–7.

Houten, S. M., & Wanders, R. J. A. (2010). A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. *Journal of Inherited Metabolic Disease*, *33*(5), 469–477. https://doi.org/10.1007/s10545-010-9061-2

Hsieh, T.-J., Lin, T., Hsieh, P.-C., Liao, M.-C., & Shin, S.-J. (2012). Suppression of Glutamine:Fructose-6-phosphate amidotransferase-1 inhibits adipogenesis in 3T3-L1 adipocytes. *Journal of Cellular Physiology*, *227*(1), 108–115. https://doi.org/10.1002/jcp.22707

Hudlicka, O. (2011). Microcirculation in skeletal muscle. *Muscles, Ligaments and Tendons Journal*, *1*(1), 3–11.

Hughes, S., & Chan-Ling, T. (2004). Characterization of smooth muscle cell and pericyte differentiation in the rat retina in vivo. *Investigative Ophthalmology & Visual Science*, *45*(8), 2795–2806. https://doi.org/10.1167/iovs.03-1312

Ingjer, F. (1979). Effects of endurance training on muscle fibre ATP-ase activity, capillary supply and mitochondrial content in man. *The Journal of Physiology*, *294*(1), 419–432. https://doi.org/10.1113/jphysiol.1979.sp012938

Issad, T., Al-Mukh, H., Bouaboud, A., & Pagesy, P. (2022). Protein O-GlcNAcylation and the regulation of energy homeostasis: Lessons from knock-out mouse models. *Journal of Biomedical Science*, *29*(1), 64. https://doi.org/10.1186/s12929-022-00851-w

Jackson, R. M., Griesel, B. A., Gurley, J. M., Szweda, L. I., & Olson, A. L. (2017). Glucose availability controls adipogenesis in mouse 3T3-L1 adipocytes via up-regulation of nicotinamide metabolism. *Journal of Biological Chemistry*, *292*(45), 18556–18564. https://doi.org/10.1074/jbc.M117.791970

Jaenisch, R., & Young, R. (2008). Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*, *132*(4), 567–582. https://doi.org/10.1016/j.cell.2008.01.015

Janetzko, J., & Walker, S. (2014). The Making of a Sweet Modification: Structure and Function of O-GlcNAc Transferase. *Journal of Biological Chemistry*, *289*(50), 34424–34432. https://doi.org/10.1074/jbc.R114.604405

Jang, H., Kim, T. W., Yoon, S., Choi, S.-Y., Kang, T.-W., Kim, S.-Y., Kwon, Y.-W., Cho, E.-J., & Youn, H.-D. (2012). O-GlcNAc regulates pluripotency and reprogramming by directly acting on core components of the pluripotency network. *Cell Stem Cell*, *11*(1), 62–74. https://doi.org/10.1016/j.stem.2012.03.001

Jia, Y., Li, P., Dziennis, S., & Wang, R. K. (2011). Responses of Peripheral Blood Flow to Acute Hypoxia and Hyperoxia as Measured by Optical Microangiography. *PLoS ONE*, *6*(10), e26802. https://doi.org/10.1371/journal.pone.0026802

Katajisto, P., Döhla, J., Chaffer, C. L., Pentinmikko, N., Marjanovic, N., Iqbal, S., Zoncu, R., Chen, W., Weinberg, R. A., & Sabatini, D. M. (2015). Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. *Science*, *348*(6232), 340–343. https://doi.org/10.1126/science.1260384

Kiamehr, M., Viiri, L. E., Vihervaara, T., Koistinen, K. M., Hilvo, M., Ekroos, K., Käkelä, R., & Aalto-Setälä, K. (2017). Lipidomic profiling of patient-specific induced pluripotent stem cell-derived hepatocyte-like cells. *Disease Models & Mechanisms*, dmm.030841. https://doi.org/10.1242/dmm.030841

Kim, Y. M., Jeon, E. S., Kim, M. R., Jho, S. K., Ryu, S. W., & Kim, J. H. (2008). Angiotensin II-induced differentiation of adipose tissue-derived mesenchymal stem cells to smooth

muscle-like cells. *The International Journal of Biochemistry & Cell Biology*, *40*(11), 2482–2491. https://doi.org/10.1016/j.biocel.2008.04.016

Kirton, J. P., Wilkinson, F. L., Canfield, A. E., & Alexander, M. Y. (2006). Dexamethasone Downregulates Calcification-Inhibitor Molecules and Accelerates Osteogenic Differentiation of Vascular Pericytes: Implications for Vascular Calcification. *Circulation Research*, *98*(10), 1264–1272. https://doi.org/10.1161/01.RES.0000223056.68892.8b

Kissane, R. W. P., Al-Shammari, A. A., & Egginton, S. (2021). The importance of capillary distribution in supporting muscle function, building on Krogh's seminal ideas. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *254*, 110889. https://doi.org/10.1016/j.cbpa.2020.110889

Klimczak, A., Kozlowska, U., & Kurpisz, M. (2018). Muscle Stem/Progenitor Cells and Mesenchymal Stem Cells of Bone Marrow Origin for Skeletal Muscle Regeneration in Muscular Dystrophies. *Archivum Immunologiae et Therapiae Experimentalis*, *66*(5), 341–354. https://doi.org/10.1007/s00005-018-0509-7

Krampera, M., Franchini, M., Pizzolo, G., & Aprili, G. (2007). Mesenchymal stem cells: From biology to clinical use. *Blood Transfusion = Trasfusione Del Sangue*, *5*(3), 120–129. https://doi.org/10.2450/2007.0029-07

Kuo, T. K., Ho, J. H., & Lee, O. K. (2009). Mesenchymal stem cell therapy for nonmusculoskeletal diseases: Emerging applications. *Cell Transplantation*, *18*(9), 1013–1028. https://doi.org/10.3727/096368909X471206

Kwist, K., Bridges, W. C., & Burg, K. J. L. (2016). The effect of cell passage number on osteogenic and adipogenic characteristics of D1 cells. *Cytotechnology*, *68*(4), 1661–1667. https://doi.org/10.1007/s10616-015-9883-8

Lacolley, P., Regnault, V., Nicoletti, A., Li, Z., & Michel, J.-B. (2012). The vascular smooth muscle cell in arterial pathology: A cell that can take on multiple roles. *Cardiovascular Research*, *95*(2), 194–204. https://doi.org/10.1093/cvr/cvs135

Laughlin, M. H., & Armstrong, R. B. (1982). Muscular blood flow distribution patterns as a function of running speed in rats. *American Journal of Physiology-Heart and Circulatory Physiology*, *243*(2), H296–H306. https://doi.org/10.1152/ajpheart.1982.243.2.H296

Leese, H. J. (1995). Metabolic control during preimplantation mammalian development. *Human Reproduction Update*, *1*(1), 63–72. https://doi.org/10.1093/humupd/1.1.63

Lefterova, M. I., Haakonsson, A. K., Lazar, M. A., & Mandrup, S. (2014). PPARγ and the global map of adipogenesis and beyond. *Trends in Endocrinology & Metabolism*, *25*(6), 293–302. https://doi.org/10.1016/j.tem.2014.04.001

Li, L., & Xie, T. (2005). Stem cell niche: Structure and function. *Annual Review of Cell and Developmental Biology*, *21*, 605–631. https://doi.org/10.1146/annurev.cellbio.21.012704.131525

Lieber, R. (2011). *Skeletal Muscle Structure, Function, and Plasticity*.

Liu, K., Paterson, A. J., Chin, E., & Kudlow, J. E. (2000). Glucose stimulates protein modification by O-linked GlcNAc in pancreatic beta cells: Linkage of O-linked GlcNAc to beta cell death. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(6), 2820–2825. https://doi.org/10.1073/pnas.97.6.2820

Liu, Y., Muñoz, N., Bunnell, B. A., Logan, T. M., & Ma, T. (2015). Density-Dependent Metabolic Heterogeneity in Human Mesenchymal Stem Cells. *Stem Cells*, *33*(11), 3368–3381. https://doi.org/10.1002/stem.2097

Longo, M., Zatterale, F., Naderi, J., Parrillo, L., Formisano, P., Raciti, G. A., Beguinot, F., & Miele, C. (2019). Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *International Journal of Molecular Sciences*, *20*(9), E2358. https://doi.org/10.3390/ijms20092358

Ma, J., & Hart, G. W. (2014). O-GlcNAc profiling: From proteins to proteomes. *Clinical Proteomics*, *11*(1), 8. https://doi.org/10.1186/1559-0275-11-8

Mann, J., Chu, D. C. K., Maxwell, A., Oakley, F., Zhu, N., Tsukamoto, H., & Mann, D. A. (2010). MeCP2 Controls an Epigenetic Pathway That Promotes Myofibroblast Transdifferentiation and Fibrosis. *Gastroenterology*, *138*(2), 705-714.e4. https://doi.org/10.1053/j.gastro.2009.10.002

Marión, R. M., Strati, K., Li, H., Murga, M., Blanco, R., Ortega, S., Fernandez-Capetillo, O., Serrano, M., & Blasco, M. A. (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature*, *460*(7259), 1149–1153. https://doi.org/10.1038/nature08287

Massagué, J., Blain, S. W., & Lo, R. S. (2000). TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell*, *103*(2), 295–309. https://doi.org/10.1016/s0092-8674(00)00121-5

Minokoshi, Y., Kim, Y.-B., Peroni, O. D., Fryer, L. G. D., Müller, C., Carling, D., & Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, *415*(6869), 339–343. https://doi.org/10.1038/415339a

Mitchell, T. S., Bradley, J., Robinson, G. S., Shima, D. T., & Ng, Y.-S. (2008). RGS5 expression is a quantitative measure of pericyte coverage of blood vessels. *Angiogenesis*, *11*(2), 141–151. https://doi.org/10.1007/s10456-007-9085-x

Morelli, S. S., Yi, P., & Goldsmith, L. T. (2012). Endometrial stem cells and reproduction. *Obstetrics and Gynecology International*, *2012*, 851367. https://doi.org/10.1155/2012/851367

Mukund, K., & Subramaniam, S. (2020). Skeletal muscle: A review of molecular structure and function, in health and disease. *WIREs Systems Biology and Medicine*, *12*(1). https://doi.org/10.1002/wsbm.1462

Murfee, W. L., Skalak, T. C., & Peirce, S. M. (2005). Differential arterial/venous expression of NG2 proteoglycan in perivascular cells along microvessels: Identifying a venule-specific phenotype. *Microcirculation (New York, N.Y.: 1994)*, *12*(2), 151–160. https://doi.org/10.1080/10739680590904955

Murrant, C. L., Lamb, I. R., & Novielli, N. M. (2017). Capillary endothelial cells as coordinators of skeletal muscle blood flow during active hyperemia. *Microcirculation*, *24*(3), e12348. https://doi.org/10.1111/micc.12348

Nissen, S. E., & Wolski, K. (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *The New England Journal of Medicine*, *356*(24), 2457–2471. https://doi.org/10.1056/NEJMoa072761

Nwadozi, E., Ng, A., Strömberg, A., Liu, H., Olsson, K., Gustafsson, T., & Haas, T. L. (2019). Leptin is a physiological regulator of skeletal muscle angiogenesis and is locally produced by PDGFRα and PDGFRβ expressing perivascular cells. *Angiogenesis*, *22*(1), 103–115. https://doi.org/10.1007/s10456-018-9641-6

Nwadozi, E., Rudnicki, M., De Ciantis, M., Milkovich, S., Pulbere, A., Roudier, E., Birot, O., Gustafsson, T., Ellis, C. G., & Haas, T. L. (2020). High-fat diet pre-conditioning improves microvascular remodelling during regeneration of ischaemic mouse skeletal muscle. *Acta Physiologica (Oxford, England)*, *229*(1), e13449. https://doi.org/10.1111/apha.13449

Oishi, K., Noguchi, H., Yukawa, H., & Hayashi, S. (2009). Differential ability of somatic stem cells. *Cell Transplantation*, *18*(5), 581–589. https://doi.org/10.1177/096368970901805-614

Papapetropoulos, A., Fulton, D., Mahboubi, K., Kalb, R. G., O'Connor, D. S., Li, F., Altieri, D. C., & Sessa, W. C. (2000). Angiopoietin-1 Inhibits Endothelial Cell Apoptosis via the Akt/Survivin Pathway. *Journal of Biological Chemistry*, *275*(13), 9102–9105. https://doi.org/10.1074/jbc.275.13.9102

Parthasarathi, K., & Lipowsky, H. H. (1999). Capillary recruitment in response to tissue hypoxia and its dependence on red blood cell deformability. *American Journal of Physiology-Heart and Circulatory Physiology*, *277*(6), H2145–H2157. https://doi.org/10.1152/ajpheart.1999.277.6.H2145

Pattappa, G., Heywood, H. K., de Bruijn, J. D., & Lee, D. A. (2011). The metabolism of human mesenchymal stem cells during proliferation and differentiation. *Journal of Cellular Physiology*, *226*(10), 2562–2570. https://doi.org/10.1002/jcp.22605

Peirce, S., & Skalak, T. (2003). Microvascular Remodeling: A Complex Continuum Spanning Angiogenesis to Arteriogenesis. *Microcirculation*, *10*(1), 99–111. https://doi.org/10.1080/713773592

Perugorria, M. J., Wilson, C. L., Zeybel, M., Walsh, M., Amin, S., Robinson, S., White, S. A., Burt, A. D., Oakley, F., Tsukamoto, H., Mann, D. A., & Mann, J. (2012). Histone methyltransferase ASH1 orchestrates fibrogenic gene transcription during myofibroblast transdifferentiation. *Hepatology*, *56*(3), 1129–1139. https://doi.org/10.1002/hep.25754

Pimton, P., Lecht, S., Stabler, C. T., Johannes, G., Schulman, E. S., & Lelkes, P. I. (2015). Hypoxia enhances differentiation of mouse embryonic stem cells into definitive endoderm and distal lung cells. *Stem Cells and Development*, *24*(5), 663–676. https://doi.org/10.1089/scd.2014.0343

Rahimi, R. A., & Leof, E. B. (2007). TGF-beta signaling: A tale of two responses. *Journal of Cellular Biochemistry*, *102*(3), 593–608. https://doi.org/10.1002/jcb.21501

Sacchetti, B., Funari, A., Remoli, C., Giannicola, G., Kogler, G., Liedtke, S., Cossu, G., Serafini, M., Sampaolesi, M., Tagliafico, E., Tenedini, E., Saggio, I., Robey, P. G., Riminucci, M., & Bianco, P. (2016). No Identical "Mesenchymal Stem Cells" at Different Times and Sites: Human Committed Progenitors of Distinct Origin and Differentiation Potential Are Incorporated as Adventitial Cells in Microvessels. *Stem Cell Reports*, *6*(6), 897–913. https://doi.org/10.1016/j.stemcr.2016.05.011

Saunders, W. B., Bohnsack, B. L., Faske, J. B., Anthis, N. J., Bayless, K. J., Hirschi, K. K., & Davis, G. E. (2006). Coregulation of vascular tube stabilization by endothelial cell TIMP-2 and pericyte TIMP-3. *Journal of Cell Biology*, *175*(1), 179–191. https://doi.org/10.1083/jcb.200603176

Sbrana, F. V., Cortini, M., Avnet, S., Perut, F., Columbaro, M., De Milito, A., & Baldini, N. (2016). The Role of Autophagy in the Maintenance of Stemness and Differentiation of

Mesenchymal Stem Cells. *Stem Cell Reviews and Reports*, *12*(6), 621–633. https://doi.org/10.1007/s12015-016-9690-4

Segal, S. S. (2005). Regulation of Blood Flow in the Microcirculation. *Microcirculation*, *12*(1), 33–45. https://doi.org/10.1080/10739680590895028

Segal, S. S., Damon, D. N., & Duling, B. R. (1989). Propagation of vasomotor responses coordinates arteriolar resistances. *American Journal of Physiology-Heart and Circulatory Physiology*, *256*(3), H832–H837. https://doi.org/10.1152/ajpheart.1989.256.3.H832

Semba, R. D., Huang, H., Lutty, G. A., Van Eyk, J. E., & Hart, G. W. (2014). The role of *O* -GlcNAc signaling in the pathogenesis of diabetic retinopathy. *PROTEOMICS - Clinical Applications*, *8*(3–4), 218–231. https://doi.org/10.1002/prca.201300076

Shen, H., Huang, X., Zhao, Y., Wu, D., Xue, K., Yao, J., Wang, Y., Tang, N., & Qiu, Y. (2022). The Hippo pathway links adipocyte plasticity to adipose tissue fibrosis. *Nature Communications*, *13*(1), 6030. https://doi.org/10.1038/s41467-022-33800-0

Sherr, C. J., & DePinho, R. A. (2000). Cellular Senescence. *Cell*, *102*(4), 407–410. https://doi.org/10.1016/S0092-8674(00)00046-5

Shi, F.-T., Kim, H., Lu, W., He, Q., Liu, D., Goodell, M. A., Wan, M., & Songyang, Z. (2013). Ten-eleven translocation 1 (Tet1) is regulated by O-linked N-acetylglucosamine transferase (Ogt) for target gene repression in mouse embryonic stem cells. *The Journal of Biological Chemistry*, *288*(29), 20776–20784. https://doi.org/10.1074/jbc.M113.460386

Shyh-Chang, N., Daley, G. Q., & Cantley, L. C. (2013). Stem cell metabolism in tissue development and aging. *Development*, *140*(12), 2535–2547. https://doi.org/10.1242/dev.091777

Shyh-Chang, N., & Ng, H.-H. (2017). The metabolic programming of stem cells. *Genes & Development*, *31*(4), 336–346. https://doi.org/10.1101/gad.293167.116

Siersbæk, R., Rabiee, A., Nielsen, R., Sidoli, S., Traynor, S., Loft, A., Poulsen, L. L. C., Rogowska-Wrzesinska, A., Jensen, O. N., & Mandrup, S. (2014). Transcription factor cooperativity in early adipogenic hotspots and super-enhancers. *Cell Reports*, *7*(5), 1443–1455. https://doi.org/10.1016/j.celrep.2014.04.042

Skalak, T. C., Price, R. J., & Zeller, P. J. (1998). Where Do New Arterioles Come From? Mechanical Forces and Microvessel Adaptation. *Microcirculation*, *5*(2–3), 91–94. https://doi.org/10.1038/sj.mn.7300022

Speakman, C. M., Domke, T. C. E., Wongpaiboonwattana, W., Sanders, K., Mudaliar, M., van Aalten, D. M. F., Barton, G. J., & Stavridis, M. P. (2014). Elevated O-GlcNAc levels activate epigenetically repressed genes and delay mouse ESC differentiation without affecting naïve to primed cell transition. *Stem Cells (Dayton, Ohio)*, *32*(10), 2605–2615. https://doi.org/10.1002/stem.1761

Sperber, H., Mathieu, J., Wang, Y., Ferreccio, A., Hesson, J., Xu, Z., Fischer, K. A., Devi, A., Detraux, D., Gu, H., Battle, S. L., Showalter, M., Valensisi, C., Bielas, J. H., Ericson, N. G., Margaretha, L., Robitaille, A. M., Margineantu, D., Fiehn, O., … Ruohola-Baker, H. (2015). The metabolome regulates the epigenetic landscape during naive-to-primed human embryonic stem cell transition. *Nature Cell Biology*, *17*(12), 1523–1535. https://doi.org/10.1038/ncb3264

Sun, C., Shang, J., Yao, Y., Yin, X., Liu, M., Liu, H., & Zhou, Y. (2016). O-Glc NA cylation: A bridge between glucose and cell differentiation. *Journal of Cellular and Molecular Medicine*, *20*(5), 769–781. https://doi.org/10.1111/jcmm.12807

Takahashi, T., Aoki, K., Ashikaga, S., Chang, W.-C., Hamada, E., Honda, R., Ichikawa, M., Ikeno, M., Kajikawa, S., Kanno, K., Kawama, D., Kondo, T., Lin, C.-S., Lin, C.-H., Morino, Y., Murakami, T., Nakai, W., Nakasuga, S., Naruki, M., … Yokkaichi, S. (2020). *Data acquisition system in Run-0a for the J-PARC E16 experiment* (arXiv:2011.0225). arXiv. http://arxiv.org/abs/2011.00225

Takashima, Y., Guo, G., Loos, R., Nichols, J., Ficz, G., Krueger, F., Oxley, D., Santos, F., Clarke, J., Mansfield, W., Reik, W., Bertone, P., & Smith, A. (2014). Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell*, *158*(6), 1254–1269. https://doi.org/10.1016/j.cell.2014.08.029

Torensma, R., ter Brugge, P. J., Jansen, J. A., & Figdor, C. G. (2003). Ceramic hydroxyapatite coating on titanium implants drives selective bone marrow stromal cell adhesion. *Clinical Oral Implants Research*, *14*(5), 569–577. https://doi.org/10.1034/j.1600-0501.2003.00949.x

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Del Gaudio, F., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., Sun, Y., Raschperger, E., Räsänen, M., Zarb, Y., Mochizuki, N., Keller, A., Lendahl, U., & Betsholtz, C. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature*, *554*(7693), 475–480. https://doi.org/10.1038/nature25739

Vasconcelos-dos-Santos, A., de Queiroz, R. M., da Costa Rodrigues, B., Todeschini, A. R., & Dias, W. B. (2018). Hyperglycemia and aberrant O-GlcNAcylation: Contributions to tumor progression. *Journal of Bioenergetics and Biomembranes*, *50*(3), 175–187. https://doi.org/10.1007/s10863-017-9740-x

Vella, P., Scelfo, A., Jammula, S., Chiacchiera, F., Williams, K., Cuomo, A., Roberto, A., Christensen, J., Bonaldi, T., Helin, K., & Pasini, D. (2013). Tet proteins connect the O-linked N-acetylglucosamine transferase Ogt to chromatin in embryonic stem cells. *Molecular Cell*, *49*(4), 645–656. https://doi.org/10.1016/j.molcel.2012.12.019

Verrecchia, F., & Mauviel, A. (2007). Transforming growth factor-beta and fibrosis. *World Journal of Gastroenterology*, *13*(22), 3056–3062. https://doi.org/10.3748/wjg.v13.i22.3056

Vishvanath, L., MacPherson, K. A., Hepler, C., Wang, Q. A., Shao, M., Spurgin, S. B., Wang, M. Y., Kusminski, C. M., Morley, T. S., & Gupta, R. K. (2016). Pdgfrβ+ Mural Preadipocytes Contribute to Adipocyte Hyperplasia Induced by High-Fat-Diet Feeding and Prolonged Cold Exposure in Adult Mice. *Cell Metabolism*, *23*(2), 350–359. https://doi.org/10.1016/j.cmet.2015.10.018

Volz, K. S., Jacobs, A. H., Chen, H. I., Poduri, A., McKay, A. S., Riordan, D. P., Kofler, N., Kitajewski, J., Weissman, I., & Red-Horse, K. (2015). Pericytes are progenitors for coronary artery smooth muscle. *ELife*, *4*, e10036. https://doi.org/10.7554/eLife.10036

Wang, C., Yin, S., Cen, L., Liu, Q., Liu, W., Cao, Y., & Cui, L. (2010). Differentiation of adipose-derived stem cells into contractile smooth muscle cells induced by transforming growth factor-beta1 and bone morphogenetic protein-4. *Tissue Engineering. Part A*, *16*(4), 1201–1213. https://doi.org/10.1089/ten.TEA.2009.0303

Wang, M.-K. (2012). Different roles of TGF-β in the multi-lineage differentiation of stem cells. *World Journal of Stem Cells*, *4*(5), 28. https://doi.org/10.4252/wjsc.v4.i5.28

Weissman, I. L. (2002). Stem cells—Scientific, medical, and political issues. *The New England Journal of Medicine*, *346*(20), 1576–1579. https://doi.org/10.1056/NEJMsb020693

Wellen, K. E., Lu, C., Mancuso, A., Lemons, J. M. S., Ryczko, M., Dennis, J. W., Rabinowitz, J. D., Coller, H. A., & Thompson, C. B. (2010). The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes & Development*, *24*(24), 2784–2799. https://doi.org/10.1101/gad.1985910

Welsh, D. G., & Segal, S. S. (1998). Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. *American Journal of Physiology-Heart and Circulatory Physiology*, *274*(1), H178–H186. https://doi.org/10.1152/ajpheart.1998.274.1.H178

Wolfe, R. R. (2006). The underappreciated role of muscle in health and disease. *The American Journal of Clinical Nutrition*, *84*(3), 475–482. https://doi.org/10.1093/ajcn/84.3.475

Wong, Y.-K., Wang, J., Lim, T. K., Lin, Q., Yap, C. T., & Shen, H.-M. (2022). O-GlcNAcylation promotes fatty acid synthase activity under nutritional stress as a pro-survival mechanism in cancer cells. *Proteomics*, *22*(9), e2100175. https://doi.org/10.1002/pmic.202100175

Wörsdörfer, P., & Ergün, S. (2018). Do Vascular Mural Cells Possess Endogenous Plasticity In Vivo? *Stem Cell Reviews and Reports*, *14*(1), 144–147. https://doi.org/10.1007/s12015-017-9791-8

Wymann, M. P., & Schneiter, R. (2008). Lipid signalling in disease. *Nature Reviews Molecular Cell Biology*, *9*(2), 162–176. https://doi.org/10.1038/nrm2335

Xu, Q., Yang, C., Du, Y., Chen, Y., Liu, H., Deng, M., Zhang, H., Zhang, L., Liu, T., Liu, Q., Wang, L., Lou, Z., & Pei, H. (2014). AMPK regulates histone H2B O-GlcNAcylation. *Nucleic Acids Research*, *42*(9), 5594–5604. https://doi.org/10.1093/nar/gku236

Yanes, O., Clark, J., Wong, D. M., Patti, G. J., Sánchez-Ruiz, A., Benton, H. P., Trauger, S. A., Desponts, C., Ding, S., & Siuzdak, G. (2010). Metabolic oxidation regulates embryonic

stem cell differentiation. *Nature Chemical Biology*, *6*(6), 411–417. https://doi.org/10.1038/nchembio.364

Yao, Y., Chen, Z.-L., Norris, E. H., & Strickland, S. (2014). Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nature Communications*, *5*(1), 3413. https://doi.org/10.1038/ncomms4413

Yoshida, Y., Takahashi, K., Okita, K., Ichisaka, T., & Yamanaka, S. (2009). Hypoxia Enhances the Generation of Induced Pluripotent Stem Cells. *Cell Stem Cell*, *5*(3), 237–241. https://doi.org/10.1016/j.stem.2009.08.001

Yucel, N., Wang, Y. X., Mai, T., Porpiglia, E., Lund, P. J., Markov, G., Garcia, B. A., Bendall, S. C., Angelo, M., & Blau, H. M. (2019). Glucose Metabolism Drives Histone Acetylation Landscape Transitions that Dictate Muscle Stem Cell Function. *Cell Reports*, *27*(13), 3939-3955.e6. https://doi.org/10.1016/j.celrep.2019.05.092

Zeybel, M., Luli, S., Sabater, L., Hardy, T., Oakley, F., Leslie, J., Page, A., Moran Salvador, E., Sharkey, V., Tsukamoto, H., Chu, D. C. K., Singh, U. S., Ponzoni, M., Perri, P., Di Paolo, D., Mendivil, E. J., Mann, J., & Mann, D. A. (2017). A Proof-of-Concept for Epigenetic Therapy of Tissue Fibrosis: Inhibition of Liver Fibrosis Progression by 3-Deazaneplanocin A. *Molecular Therapy*, *25*(1), 218–231. https://doi.org/10.1016/j.ymthe.2016.10.004

Zhang, Y. E. (2009). Non-Smad pathways in TGF-beta signaling. *Cell Research*, *19*(1), 128–139. https://doi.org/10.1038/cr.2008.328

Zhou, W., Choi, M., Margineantu, D., Margaretha, L., Hesson, J., Cavanaugh, C., Blau, C. A., Horwitz, M. S., Hockenbery, D., Ware, C., & Ruohola-Baker, H. (2012). HIF1α induced switch from bivalent to exclusively glycolytic metabolism during ESC-to-

EpiSC/hESC transition. *The EMBO Journal*, *31*(9), 2103–2116.

https://doi.org/10.1038/emboj.2012.71