UNC PEMBROKE | MAYNOR HONORS COLLEGE

Glucose Metabolism through Pentose Phosphate Pathway: Effects on the Development of Congenital Heart Defects

Senior Project

In partial fulfillment of the requirements for
The Esther G. Maynor Honors College
University of North Carolina at Pembroke

By

Olivia A Spaulding
Biology Department
May 1, 2019

_____	_____
[Type your name here and sign above] Honors College Scholar	Date
_____	_____
[Type your mentor's name] Faculty Mentor	Date
_____	_____
Teagan Decker, Ph.D. Senior Project Coordinator	Date

Acknowledgements

I would like to thank the Honors College for giving me this opportunity to explore my interests.

Abstract

Several studies have shown that genes related to cardiac muscle and function thrive in low glucose concentrations, whereas cells over actively replicate and do not reach full maturation in high glucose concentrations. Data suggests that blocking the pentose phosphate pathway induces cardiac maturation. The pentose phosphate pathway is responsible for generating ribose sugars that contribute to making nucleotides and NADP+/NADPH. Prolonged activation of the pentose phosphate pathway leads to excess nucleotide synthesis resulting in immature cardiomyocytes leading to congenital heart defects. This paper discussed how the pentose phosphate pathway is involved in the inhibition of fetal cardiomyocytes in high glucose conditions.

Glucose Metabolism through Pentose Phosphate Pathway: Effects on the Development of Congenital Heart Defects

Glucose in Healthy Pregnancy

In a healthy pregnancy, blood glucose levels remain stable in utero. A healthy pregnancy means that the blood glucose levels are normal. Hyperglycemia increases the likelihood by 2-5 fold in the developing congenital heart defects independent of genetic factors (Nakano, 2017). Maternal blood glucose levels reflect the blood glucose levels of the fetus. In the US, roughly 4000 children are born every year with a congenital heart defect, CHD (Mccommis et al, 2013). Some of the most common CHD include ventricular septal defect and tetralogy of Fallot.

Embryonic stem cell cardiac differentiation has shown that cells switch from glycolysis to oxidation phosphorylation during the latter stages of gestation (Rao, 2013). The switch to oxidative metabolism is due to the more efficient production of ATP. The maturing heart has a higher energy demand. Compromising this switch causes deficiencies in sarcomere formation and contractile force.

In a healthy fetus, transcript levels of aldolase and transketolase are reduced (Chung et al, 2010). Aldolase and transketolase are enzymes in the reductive phase of the pentose phosphate pathway. They help to shunt glucose away from glycolysis. The transcript levels of aldolase and transketolase would suggest that during cardiogenesis, there is an uncoupling of glycolysis from the pentose phosphate pathway. There is also an increase in oxygen consumption in healthy fetus hearts.

Glucose Metabolism and Maturation of Cardiomyocytes

To determine the role of glucose metabolism in the maturation of human embryonic stem cells cardiomyocytes, hESC-CMs researchers performed six methods with cells cultured in various concentrations of glucose (Nakano, 2017). The hESC-CMs originate from inner cell mass of blastocysts from donated fertilized eggs.

The first method included hESC-CMs stained with JC-1, a green fluorescent dye that turns red upon detection of active mitochondria. In low glucose media, a higher level of JC-1 was significantly more present meaning, a higher abundance of active mitochondria detected. In the second method, intracellular lactate levels of hESC-CMs were measured using a lactate nanosensor, Laconic. With both lactate and differentiated hESC-CMs in standard 25 mM glucose plate, there is an increase in intracellular lactate levels. Along with the addition of pyruvate and sodium cyanide (NaCN), an inhibitor of mitochondrial respiration, a minimal increase can be seen. The minimum increase would suggest that glucose does not actively metabolize pyruvate alone. However, when pyruvate and NaCN are added to hESC-CMs in low glucose media, there was a significant increase in intracellular lactate levels. In the third method, cellular respiration of the hESC-CMs was studied using XF24 Extracellular Flux Analyzer. This tool measures the oxygen consumption rate, ATP linked respiration and maximum respiration capacity presented higher in low glucose conditions. The fourth method measured hESC-CMs calcium kinetics using Ca^{2+} transient assay. The maximum upstroke (V_{max}) were significantly faster in low glucose media. The Vmax of calcium kinetics measurements suggests that high glucose concentrations have some role in inhibiting the maturation of hESC-CMs. The fifth method, assessed electrophysiological properties of the hESC-CMs using a multi-electrode array culture plate. This tool was used to measure maximum

upstroke velocity (dV/dt_{max}) of field potential. In glucose-restricted media, there was a significant increase in maximum upstroke velocity. The sixth concluding method examined cell contractility using digital imaging with the MotionGUI program. The average contraction and relaxation speed were higher in hESC-CMs in 0 mM glucose media.

Nakano et al (2017) research suggests that mitochondria can metabolize pyruvate in low glucose conditions as opposed to glucose enriched environments. The methods used by the researchers show that hESC-CMs can reach functional maturation in low glucose conditions.

Pentose Phosphate Pathway

A review of Stincone et al (2015) discussed the discovery, biochemistry and regulation of the pentose phosphate pathway (PPP) in the context of deficiencies causing metabolic disease and the role of the pathway in neurons, stem cell potency and cancer metabolism. PPP, also known as the pentose shunt and the hexose monophosphate shunt, provides precursors for nucleotide biosynthesis and reducing molecules for anabolism and protecting against oxidative stress.

The pathway occurs in the cytosol of the cell. It is an alternate route for glucose oxidation without direct consumption of ATP. Glucose-6-phosphate can either continue through glycolysis or be shunted off to the pentose phosphate pathway. The glucose-6-phosphate path is directed depending on the current needs of the cell and the concentration of NADP+ in the cytosol. The oxidative phase of the pathway generates ribulose while the reductive phase generates NADPH.

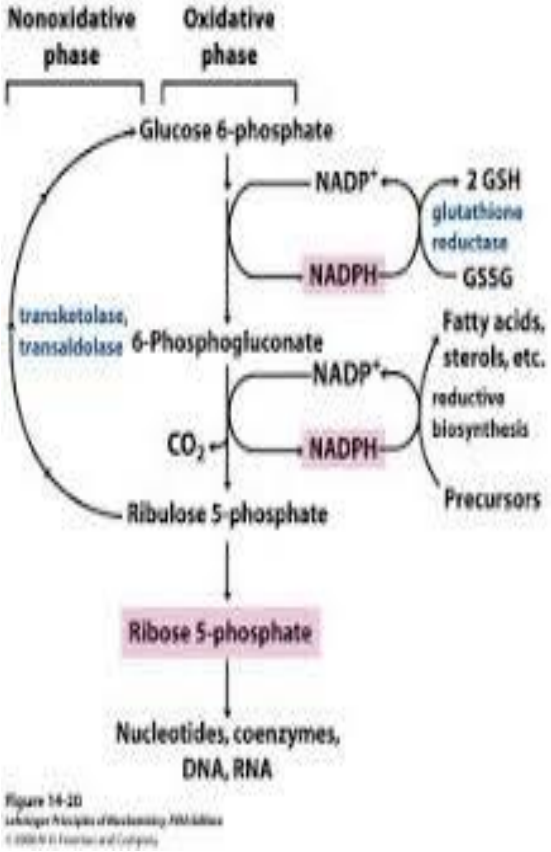

Figure 1: Pictured above is the Pentose Phosphate Pathway. Opperoes F. Kinetoplastid Metabolism. 5.6. Pentose-phosphate pathway. 2016 [accessed 2019 Apr 30]. http://big.icp.ucl.ac.be/~opperd/metabolism/Kinetoplastida/Blog/Entries/2016/1/31_5.6._Pentose-

PPP is an important modulator of the overall redox state of the cell through reduction of NADP+ to NADPH, a cofactor for several key cellular enzymes (Larsen and Gutterman, 2006). NADP+ maintains reducing equivalents that are important to counteract oxidative damage and specific reductive synthesis reactions. Oxidative damage includes free radicals flowing inside of a cell that can lead to apoptosis. The conversion of ribonucleotides to deoxyribonucleotides requires NADPH as the electron source. This conversion implies that any cell that is rapidly multiplying needs a lot of NADPH.

Role of Pentose Phosphate Pathway in Congenital Heart Defects

Ventricular septal defect (VSD) is a common CHD. The wall or septum separating the left and right ventricles is missing in the VSD. This in turn interrupts the flow of oxygen rich blood. The oxygen rich blood then flows back into the lungs. Adverse effects include heart failure and pulmonary hypertension due to the excessive workload of the heart. Small ventricular septal defects may close on their own while larger ones may require surgery (Mayo Clinic Staff, 2018). Ventricular septal defect can be seen in *Figure 2* along with an image of a normal heart.

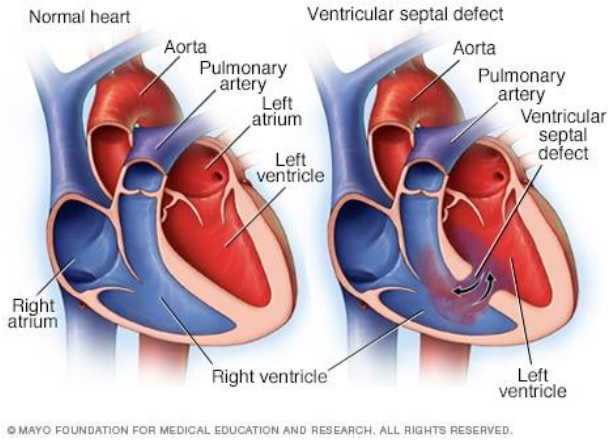

Figure 2: Depicts a normal heart and a heart with a ventricular septal defect. Mayo Clinic Staff. Ventricular septal defect (VSD). Mayo Clinic. 2018 Mar 9 [accessed 2019 Apr 30]. https://www.mayoclinic.org/diseases-conditions/ventricular-septal-

Another common congenital heart defect is tetralogy of Fallot. As the name suggests, this includes four birth defects: pulmonary valve stenosis, VSD, overriding aorta and right ventricular hypertrophy. Unlike, VSD tetralogy of Fallot involves the interruption of oxygen poor blood flow. This defect causes oxygen poor blood to circulate throughout the body. As a result, infants may present with cyanosis due to lack of oxygen. Pulmonary valve stenosis reduces blood flow to the lungs due to the constriction of the pulmonary valve. VSD was mentioned in the preceding paragraph. The overriding aorta combines oxygen rich blood and oxygen poor blood from the right ventricle and

left ventricle, respectively. The combining of the blood is due to the shifted position of the aorta in tetralogy of Fallot. Right ventricular hypertrophy is the thickening of the right ventricle wall. The heart stiffens and in untreated conditions, fails. Tetralogy of Fallot requires surgery and continued medical care throughout life (Mayo Clinic Staff, 2018).

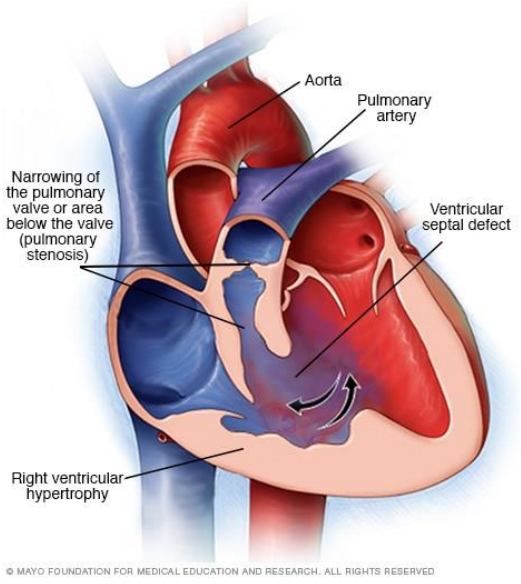

Figure 3: Tetralogy of Fallot. Mayo Clinic Staff. Tetralogy of Fallot. Mayo Clinic. 2018 Mar 9 [accessed 2019 Apr 30]. https://www.mayoclinic.org/diseases-conditions/tetralogy-of-fallot/symptoms-causes/syc-

Larsen and Gutterman (2006) conducted a study to relate hypoxia, coronary dilation and the pentose phosphate pathway. Since Gupte and Wolin (2005) have reported that hypoxia promotes relaxation in the absence of the endothelium. The current researchers used endothelium-denuded bovine coronary artery (BCA) to demonstrate that hypoxia promotes the oxidation of NADPH. Hypoxic vasorelaxation of arteries is a mechanism in which oxygenation in cardiac tissue is maintained. Vasorelaxation is enhanced in glucose free conditions with the addition of pyruvate, showing consistency with sensitivity to the PPP. The researchers explained that inhibition of the PPP reduces reactive oxygen species (ROS) generation in the BCA, and that ROS can inhibit potassium channels and other proteins involved in vasodilation. It is important to note that too much flow through the PPP is not conductive up an efficient energy supply for the fetal heart but too little flow through the PPP can be equally

harmful as the PPP is needed for the production of nucleotides and protection against oxidative stress of the cell.

Several studies have demonstrated the effects of the PPP on the cardiac differentiation of stem cells.

Chung et al (2007) conducted a study to establish a relationship between mitochondrial oxidative metabolism and the differentiation of cardiomyocytes. Using embryonic stem cells, researchers were able to show that mitochondrial oxidative metabolism is required for the differentiation and specification of cardiomyocytes. The mitochondrial oxidation perform a sufficient amount of ATP to supply the increasing energy demands of the constant contracting heart. The study showed that glycolytic metabolism was not efficient enough but that mitochondrial oxidation metabolism must take place to convert the stem cells into a functional cardiac phenotype.

Mccommis et al (2013) designed a study to determine how overexpression of hexokinase 2 leads to hypertrophy by increasing the flux through the PPP. The researchers used neonatal rat ventricular myocytes infected with a hexokinase 2 adenovirus and treated them with phenylephrine. The study found that overexpression of hexokinase 2 decreased hypertrophy in neonatal rat ventricular myocytes. Hexokinase 2 increases the activity of glucose-6-phosphate dehydrogenase within the PPP. The increased flux through the PPP reduces ROS accumulation.

Nakano et al (2017) studied cardiac maturation through glucose metabolism. The researchers used stem cells in varying concentrations of glucose to better understand how cardiac maturation is inhibited in high levels of glucose. They found that the pentose phosphate pathway was responsible for the increased number of immature

cardiomyocytes in high glucose media. It is important to note that glucose comes from the maternal source. Thus the glucose deprivation experienced in the latter stages may be from isoform switching (Nakano, 2017). They connected this as a possible underlying mechanism for the development of CHD.

Helle et al (2017) studied how first trimester plasma glucose levels may be an indicator for the risk of the development of CHD in children of mothers with and without diabetes. In a case-control study, researchers measured random samples of plasma glucose levels in expectant mothers. The researchers found that higher plasma glucose levels corresponded with an increased risk for CHD. It was also reported that children of diabetic mothers with controlled glucose levels still had an increased risk of developing CHD. This would suggest that the variation in plasma glucose levels may be the underlying mechanism for the increased likelihood of the development of CHD. Variation in plasma glucose levels are higher in mothers with diabetes (Helle et al 2018). The researchers concluded that measuring random plasma glucose levels during the first trimester could be a better indicator for the likelihood of developing CHD.

Malandraki-Miller et al (2018) studied the oxidative environment of the cardiomyocytes and how this impacts the cell's ability to become differentiated. Using cardiac progenitor cells, researchers observed changes made in development in an oxidative state. Transplanted stem cells in vivo can display one or a combination of three characteristics: (1) replicate themselves and/or differentiate into mature cardiomyocytes, (2) regenerate cardiomyocytes, (3) through paracrine mechanisms apply benefits to the cell, (Malandraki- Miller, 2018). They found that oxidative metabolism contributed to the differentiation of progenitor cells to mature cardiomyocytes.

An increased glucose utilization and decreased fatty acid metabolism is accompanied by cardiac hypertrophy (Mccommis et al, 2013). Flux through the PPP increases during this time. Glucose metabolism is initiated by uptake by glucose transporters and glucose phosphorylation by a hexokinase leading to the formation of glucose-6-phosphate. A particular hexokinase, named hexokinase 2 is regulated by insulin and hypoxia. This enzyme was studied in neonatal rat ventricular myocytes. An overexpression of hexokinase 2 decreased hypertrophy in the neonate rats (Mccommis et al, 2013). Hexokinase 2 works to increase the flux through the PPP by increasing G6PDH activity. A result of hypertrophy, ROS accumulation is reduced with the increase G6PDH activity. Glucose shuttling to the PPP may be used to reduce CHD in neonatal rats with the overexpression of hexokinase 2.

Mass spectrometry showed that glucose deprivation led to a significant decline in levels of metabolites in purine metabolism, pyrimidine metabolism, the pentose phosphate pathway, the hexosamine pathway and glycolysis (Nakano, 2017). Each pathway was inhibited to determine which route was inhibiting cardiac maturation in the fetus. The results show that when 6AN (6-[cyclohexa-2, 5-dien-1-ylideneamino] naphthalene-2-sulfonate) and DHEA (didehydroepiandrosterone), inhibitors of G6PD, were added cardiomyocytes matured (Nakano, 2017). This maturation showed that the pentose phosphate pathway was involved in inhibiting cardiomyocyte maturation.

To test if cardiac maturation is inhibited by NADPH or the pentose sugars produced that aid in nucleotide synthesis, excess thymidine was added to hESC-CMs (Nakano, 2017). Excess thymidine blocks nucleotide synthesis by forming deoxycytidine. The excess thymidine blocks resulted in an increase in TNNT2 and NKX2-5 expression

(Nakano, 2017). TNNT2 is a gene that codes for cardiac troponin T. NKX2-5 codes for a homeobox-containing transcription factor that is important in heart formation and development. This increase would suggest that nucleotide metabolism regulates maturation as opposed to the concentration of NADPH.

The formation of the heart is regulated by non-genetic factors more intensely during late stage of cardiogenesis in the womb (Helle et al 2017). The placenta and fetal liver work together to meet the fetal metabolism needs. During continued levels of high maternal blood glucose levels, the fetus can develop an obesity- induced insulin resistance leading to a defective oxidative phosphorylation (Rao, 2013). The defective oxidative phosphorylation takes rise due to increased flux through the PPP. Increased flux through the PPP inhibits the abundance of mature mitochondria. This factor does not allow the cardiomyocytes to sustain themselves and the cardiovascular system as a whole. Essentially, differentiation and maturation of cardiomyocytes is parallel with increased oxidative metabolism.

Further Studies/ Conclusion

With the advances of the understanding of glucose metabolism through the pentose phosphate pathway, the numbers of those affected by CHD can decrease drastically. This understanding will allow scientists to understand the proliferating cardiomyocytes better and possibly induce maturation. These studies could also be useful in studying cancerous cells and treatment.

Glucose metabolism through the pentose phosphate pathway causes the rapid proliferation of fetal cardiomyocytes. This rapid proliferation leads to many immature cells contributing to the development of congenital heart defects. Through extensive

testing, conclusions can be made that when G6PD is inhibited, the cardiomyocytes mature more specifically with the blocking of nucleotide synthesis through the pentose phosphate pathway. The pentose phosphate pathway serves as an oxygen sensor in vascular muscle that regulates hypoxic coronary vasodilation. From these results, an underlying mechanism for the high rates of CHD may be determined. Another treatment that could be further tested is stem cell therapy. Stem cell therapy is a great option because it promotes repair of injured tissue by generating new healthy cells. This could replace the need and wait of heart transplants. However, there are some cons with this approach including ethical concerns and the possibly of triggering an immune response if the body recognizes the stem cells as foreign. This approach is already being used in other medical endeavors and proving to be an alternative to transplants.

References

1. Chung S, Arrell DK, Faustino RS, Terzic A, Dzeja PP. Glycolytic network restructuring integral to the energetics of embryonic stem cell cardiac differentiation. Journal of Molecular and Cellular Cardiology. 2010; 48(4):725–734. doi:10.1016/j.yjmcc.2009.12.014
2. Chung S, Dzeja PP, Faustino RS, Perez-Terzic C, Behfar A, Terzic A. Mitochondrial oxidative metabolism is required for the cardiac differentiation of stem cells. Nature Clinical Practice Cardiovascular Medicine. 2007; 4(S1):60–67. doi:10.1038/ncpcardio0766
3. Helle EI, Biegley P, Knowles JW, Leader JB, Pendergrass S, Yang W, Reaven GR, Shaw GM, Ritchie M, Priest JR. First Trimester Plasma Glucose Values in Women without Diabetes are Associated with Risk for Congenital Heart Disease in Offspring. The Journal of Pediatrics. 2018; 195:275–278. doi:10.1016/j.jpeds.2017.10.046
4. Katare R, Oikawa A, Cesselli D, Beltrami AP, Avolio E, Muthukrishnan D, Munasinghe PE, Angelini G, Emanueli C, Madeddu P. Boosting the pentose phosphate pathway restores cardiac progenitor cell availability in diabetes. Cardiovascular Research. 2012; 97(1):55–65. doi:10.1093/cvr/cvs291
5. Larsen BT, Gutterman DD. Hypoxia, coronary dilation, and the pentose phosphate pathway. American journal of physiology. Heart and circulatory physiology. 2006 Jun [accessed 2019 Apr 28]. https://www.ncbi.nlm.nih.gov/pubmed/16687606
6. Malandraki-Miller S, Lopez CA, Al-Siddiqi H, Carr CA. Changing Metabolism in Differentiating Cardiac Progenitor Cells—Can Stem Cells Become Metabolically Flexible Cardiomyocytes? Frontiers in Cardiovascular Medicine. 2018; 5. doi:10.3389/fcvm.2018.00119
7. Mayo Clinic Staff. Tetralogy of Fallot. Mayo Clinic. 2018 Mar 9 [accessed 2019 Apr 30]. https://www.mayoclinic.org/diseases-conditions/tetralogy-of-fallot/symptoms-causes/syc-20353477
8. Mayo Clinic Staff. Ventricular septal defect (VSD). Mayo Clinic. 2018 Mar 9 [accessed 2019 Apr 30]. https://www.mayoclinic.org/diseases-conditions/ventricular-septal-defect/symptoms-causes/syc-20353495
9. Mccommis KS, Douglas DL, Krenz M, Baines CP. Cardiac-specific Hexokinase 2 Overexpression Attenuates Hypertrophy by Increasing Pentose Phosphate Pathway Flux. Journal of the American Heart Association. 2013; 2(6). doi:10.1161/jaha.113.000355
10. Nakano H, Minami I, Braas D, Pappoe H, Wu X, Sagadevan A, Vergnes L, Fu K, Morselli M, Dunham C, et al. Glucose inhibits cardiac muscle maturation through nucleotide biosynthesis. eLife. 2017; 6. doi:10.7554/elife.29330
11. Opperoes F. Kinetoplastid Metabolism. 5.6. Pentose-phosphate pathway. 2016 [accessed 2019 Apr 30]. http://big.icp.ucl.ac.be/~opperd/metabolism/Kinetoplastida/Blog/Entries/2016/1/31_5.6._Pentose-phosphate_pathway.html

12. Rao PN, Shashidhar A, Ashok C. In utero fuel homeostasis: Lessons for a clinician. Indian Journal of Endocrinology and Metabolism. 2013; 17(1):60. doi:10.4103/2230-8210.107851
13. Stincone A, Prigione A, Cramer T, Wamelink MMC, Campbell K, Cheung E, Olin-Sandoval V, Grüning N-M, Krüger A, Alam MT, et al. The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. Biological Reviews. 2014; 90(3):927–963. doi:10.1111/brv.12140