Adipokine Regulation of Human Placental GLUT-1 Transporter

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Abstract

In the United States, 48% of women start their pregnancies being overweight or obese. There is a well-known association between maternal obesity and adverse pregnancy outcomes. Fetal macrosomia occurs 2 to 3 times more frequently in infants of obese women than in those from healthy-weight women, and the risk of fetal macrosomia increases with increasing maternal BMI and maternal blood glucose concentration. Adipose tissue is responsible for the production of adipokines. TNF-alpha and leptin are two adipokines that have been implicated in the pathogenesis of maternal diabetes and obesity and fetal growth abnormalities and metabolic dysfunction. Maternofetal glucose transport is primarily mediated by the GLUT proteins family, in particular GLUT-1. Despite our current understanding of human placental GLUT-1, little is known about its regulation. In this experiment, we hypothesize that the adipokines leptin and TNF-alpha increase human placental GLUT-1 protein expression. BeWo human choriocarcinoma cells were treated with leptin (10, 50, and 100 ng/mL) and TNF-alpha (0.1, 10, 50 ng/mL) for 24 hours, and cell protein was extracted in RIPA buffer. Total protein concentration was measured using BCA assay, and the total cell membrane GLUT-1 protein was quantified. There appears to be a positive dose-dependent relationship between the concentration of leptin and GLUT-1 expression and the concentration of TNF-alpha and GLUT-1 expression. The *in vitro* expression of GLUT-1 protein is upregulated by adipokines leptin and TNF-alpha.

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Introduction

More than two thirds of adults in the United States are overweight (BMI between 25 and 30 kg/m^2) or obese (BMI greater than 30 kg/m^2) ("Overweight & Obesity," 2018). Compared to those of healthy weight, those classified as overweight or obese have an increased risk for many diseases and detrimental chronic health conditions such as high blood pressure, type 2 diabetes, coronary heart disease, certain types of cancer, osteoarthritis, and others ("The Health Effects of Overweight and Obesity," 2015). In fact, 90% of those with type 2 diabetes are obese, and overall mortality and morbidity increase directly with increasing BMI (Mitchell, Catenacci, Wyatt, & Hill, 2011; Kolotkin, Meter, & Williams, 2001).

Moreover, obesity has negative effects on overall quality of life and places an economic burden on the individual and society. Obesity increases the risk for mental health disorders like depression and anxiety, and it may also lead to body pains that negatively affect daily life ("The Health Effects of Overweight and Obesity," 2015). In 2014, the global economic cost of obesity was estimated to be $2 trillion (Tremmel, Gerdtham, Nilsson, & Saha, 2017). In the United States, the estimated medical cost of being overweight is $266 and obese is $1723 per person, which in total sums to about $113.9 billion, or 5 to 10% of U.S. healthcare expenditures (Tsai, Williamson, & Glick, 2011). In addition to these direct healthcare costs, obesity may also lead to losses in economic growth as a result of lost work days, decreased productivity at work, and increased risk for mortality and morbidity (Tremmel, et al., 2017).

In the United States, 48% of women start their pregnancies being overweight or obese (Dudenhausen, Grünebaum, & Kirschner, 2015). There is a well-known association between maternal obesity and adverse pregnancy outcomes. Women who are obese have an increased risk

of pregnancy loss and recurrent early miscarriages (Lashen, Fear, & Sturdee, 2004). Additionally, babies born to obese women have a higher risk for birth defects like spina bifida (Watkins, Rasmussen, Honein, Botto, & Moore, 2003). For each additional 1-unit increase in maternal BMI past 30 kg/m^2, the risk of fetal neural tube defect increases by 7% (Watkins, et al., 2003). One of the most concerning complications during pregnancy is fetal macrosomia, where fetal birth weight is predicted to be greater than or equal to 4500g (Leddy, Power, & Schulkin, 2008). Fetal macrosomia occurs 2 to 3 times more frequently in infants of obese women than in those from healthy-weight women, and the risk of fetal macrosomia increases with increasing maternal BMI and maternal blood glucose concentration (Ehrenberg, Mercer, & Catalano, 2003; Scholl, Sowers, Chen, & Lenders, 2001). Macrosomic infants are more likely to experience intrapartum complications such as birth asphyxia and shoulder dystocia, as well as neonatal complications such as respiratory distress, hypoglycemia, and increased neonatal mortality (Said & Manji, 2016). Mothers of macrosomic infants are also at risk for complications such as prolonged labor and postpartum hemorrhage (Said & Manji, 2016). Additionally, there is increasing literature to support the correlation between increased infant birth weight and future metabolic disease including obesity, glucose intolerance, and diabetes (Oken & Gillman, 2003). This correlation portends potential increased risks of future health complications and expenditures for these children born to obese mothers.

Adipose tissue is a noteworthy endocrine and paracrine organ, especially in obesity. It is responsible for the production of adipokines, or cytokines that play important roles in lipid degradation, blood pressure control, insulin sensitivity, angiogenesis, as well as many other functions (Hajagos-Tóth, Ducza, Samavati, Vari, & Gaspar, 2017). Specifically, and of particular interest in the regulation of maternofetal glucose transfer, TNF-alpha and leptin are two

adipokines that have been implicated in the pathogenesis of maternal diabetes and obesity and fetal growth abnormalities and metabolic dysfunction (Patrick, Williams, Orlando, Wasserfall, & Gregg, 2018). The production of both TNF-alpha and leptin increases with increasing adiposity (Tzanavari, Giannogonas, & Karalis, 2010; Klein, Coppack, Mohamed-Ali, & Landt, 1996). TNF-alpha's primary role in the body is in the inflammatory response where it acts as a proinflammatory cytokine (Tzanavari, et al., 2010). TNF-alpha mRNA and protein have been found in the placental villi, with a significant presence in the synctiotrophoblast (Cvitic, Desoye, & Hiden, 2014). Leptin's role in the body is to regulate hunger and satiety as well as to exert growth factor and proangiogenic actions (Cvitic, et al., 2014). In the placenta, it is expressed in cytotrophoblasts, syncytiotrophoblast, and the fetoplacental endothelial cells (Cvitic, et al., 2014).

Due to the absence of significant gluconeogenesis in the fetus, the primary source of energy for fetus growth and metabolism is maternal plasma glucose (Baumann, Deborde, & Illsley, 2002). Maternofetal glucose transport is dependent on maternal plasma glucose concentration, rate of glucose transport, and placental metabolism (Takata, Kasahara, Kasahara, Ezaki, & Hirano, 1992). Maternofetal glucose transport is primarily mediated by the GLUT proteins family, in particular GLUT-1. GLUT-1 proteins are embedded throughout syncytiotrophoblast microvillous membranes (maternal-facing) and synctiotrophoblast basal membranes (fetal-facing) (Baumann, et al., 2002). The syncytiotrophoblast is the main placental barrier layer, and the density of GLUT-1 is higher in the microvillous membrane than in the basal membrane of the synctiotrophoblast (Baumann, et al., 2002). This suggests that the basal membrane is the rate-limiting step in the transplacental transport of glucose to the fetus. Thus, changes in the basal expression of GLUT-1 will lead to proportional changes in the glucose flux

to the fetus (Baumann, et al., 2002). It has also been discovered that the microvillous expression of GLUT-1 remains relatively unchanged throughout pregnancy, but that basal membrane GLUT-1 increases by about 50% over the late second and third trimesters of pregnancy (Baumann, et al., 2002). This discovery is supported by the finding that supply of glucose to the fetus is significantly increased over the latter half of pregnancy, which parallels the increased growth of the fetus (Baumann, et al., 2002).

The BeWo cell line, initiated from a malignant gestational choriocarcinoma of the placenta, is a well-accepted cell culture model of human syncytiotrophoblast ("BeWo Cell Line," n.d.; Baumann, et al., 2002). Moreover, the BeWo cell line has also been shown to be an appropriate model to investigate glucose transport, as GLUT-1 expression and activity are similar in BeWo cells and human term placenta (Baumann, et al., 2002; Baumann, Zamudio, & Illsley, 2007).

Despite our current understanding of human placental GLUT-1, little is known about its regulation. In this experiment, we hypothesize that the adipokines leptin and TNF-alpha increase human placental GLUT-1 protein expression. As such, our objective is to investigate the effects of leptin and TNF-alpha on GLUT-1 protein expression in BeWo cells.

Methods

BeWo human choriocarcinoma cells, from the laboratory of Dr. Kirk Conrad, M.D. at the University of Florida, were cultured in cell growth media composed of Gibco F-12K Nutrient Mixture (Kaighn's Modification) supplemented with 10% fetal bovine serum. The cells were contained in T-75 flasks and were kept in an incubator held at a constant 37.0 ℃ and 5% CO_2. Every 24-48 hours, the growth media in the flasks was replaced with fresh media warmed to 37.0 ℃. At each media replacement, cell confluence was monitored under a light microscope. At 70-80% cell confluence, the cells were tripsinized to unstick them from the flask and transferred to 6-well plates for experimentation. These plates contained serum-free Dulbecco's Modified Eagle's medium with 5 mM glucose and 0.5% fetal bovine serum, and cells were incubated for 24 hours. Next, the cells were treated with leptin (10, 50, and 100 ng/mL) and TNF-alpha (0.1, 10, 50 ng/mL) for 24 hours, with each of the 6 wells receiving one treatment condition. A separate well contained a control and remained untreated. After the 24 hour time period, cell protein was extracted in RIPA buffer, and total protein concentration was measured using BCA assay. The total cell membrane GLUT-1 protein was quantified using the area under the curve of an electropherogram at a peak of 63 kDa (Protein Simple Wes immunoassay, San Jose, California). All samples were run in triplicate, and data was statistically analyzed with a $p < 0.05$ considered significant.

Results

As shown in Figure 1, there appears to be a positive dose-dependent relationship between the concentration of leptin and GLUT-1 expression and the concentration of TNF-alpha and GLUT-1 expression. As the concentration of leptin and TNF-alpha increased, so did the expression of GLUT-1, where TNF-alpha at 50 ng/mL resulted in the largest expression of GLUT-1 protein.

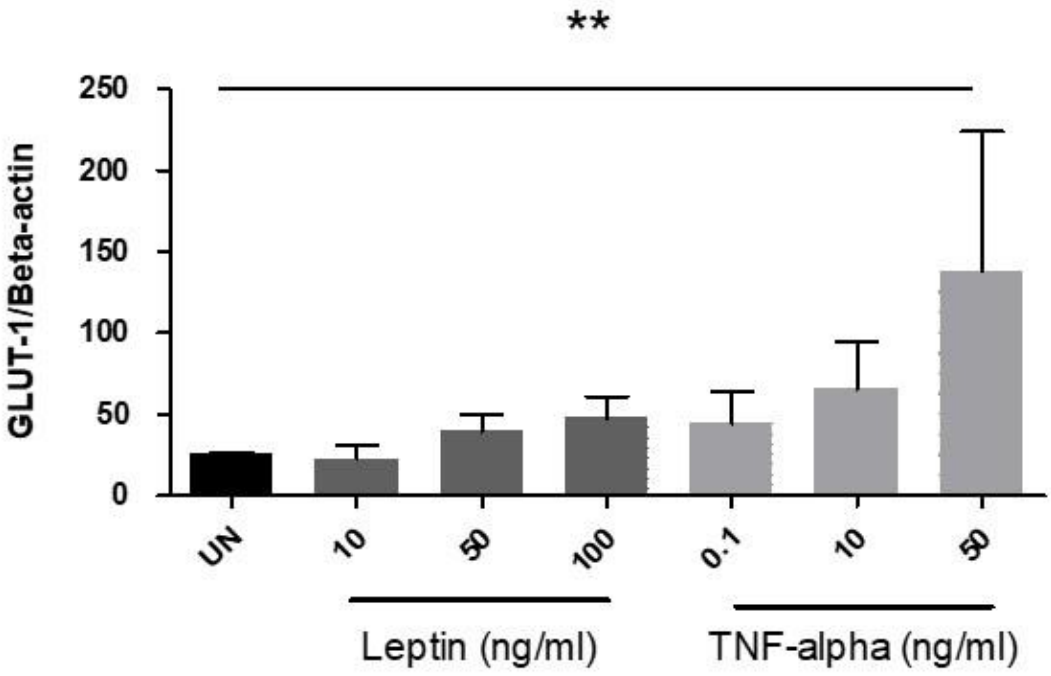

Figure 1. GLUT-1 protein expression in BeWo cells after 24 hours of treatment. UN, unstimulated. **, $P < 0.01$.

Conclusion

The *in vitro* expression of GLUT-1 protein is upregulated by adipokines leptin and TNF-alpha. This finding could potentially explain the linkage between maternal BMI and increased incidence of fetal macrosomia (Ehrenberg, et al., 2003). Additionally, it could partially explain the correlation between increased infant birth weight and future metabolic disease such as obesity, glucose intolerance, and diabetes (Oken & Gillman, 2003). This finding will provide maternal-fetal medicine researchers with future directions in the investigation of the role of obesity on fetal health and provide insight into a solution as the obesity rate continues to increase in developed countries.

Future experiments are planned to examine the effect of adipokine IL-6 on GLUT-1 protein expression, as well as repeat experiments with measurements of transcriptional activity using qPCR.

References

Baumann, M. U., Deborde, S., & Illsley, N. P. (2002). Placental Glucose Transfer and Fetal Growth. *Endocrine, 19*(1), 13-22. doi:10.1385/endo:19:1:13

Baumann, M. U., Zamudio, S., & Illsley, N. P. (2007). Hypoxic upregulation of glucose transporters in BeWo choriocarcinoma cells is mediated by hypoxia-inducible factor-1. *American Journal of Physiology. Cell Physiology*, *293*(1), C477–C485. http://doi.org/10.1152/ajpcell.00075.2007

BeWo Cell Line human CB_86082803. (n.d.). Retrieved April 16, 2018, from https://www.sigmaaldrich.com/catalog/product/sigma/cb_86082803?lang=en®ion=US

Cvitic, S., Desoye, G., & Hiden, U. (2014). Glucose, Insulin, and Oxygen Interplay in Placental Hypervascularisation in Diabetes Mellitus. *BioMed Research International, 2014*, 1-12. doi:10.1155/2014/145846

Dudenhausen, J. W., Grünebaum, A., & Kirschner, W. (2015). Prepregnancy body weight and gestational weight gain—recommendations and reality in the USA and in Germany. *American Journal of Obstetrics and Gynecology, 213*(4), 591-592.

Ehrenberg, H., Mercer, B., & Catalano, P. (2003). The influence of obesity and diabetes on neonatal macrosomia. *American Journal of Obstetrics and Gynecology, 189*(6). doi:10.1016/j.ajog.2003.10.156

Gunderson, E. P. (2009). Childbearing and Obesity in Women: Weight Before, During, and After Pregnancy. *Obstetrics and Gynecology Clinics of North America, 36*(2), 317-332. doi:10.1016/j.ogc.2009.04.001

Hajagos-Tóth, J., Ducza, E., Samavati, R., Vari, S. G., & Gaspar, R. (2017). Obesity in pregnancy: a novel concept on the roles of adipokines in uterine contractility. *Croatian Medical Journal*, *58*(2), 96–104. http://doi.org/10.3325/cmj.2017.58.96

Jansson, T., Wennergren, M., & Illsley, N. P. (1993). Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. *Journal of Clinical Endocrinology & Metabolism, 77*(6), 1554-1562. doi:10.1210/jc.77.6.1554

Jung, U. J., & Choi, M.-S. (2014). Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. *International Journal of Molecular Sciences*, *15*(4), 6184–6223. http://doi.org/10.3390/ijms15046184

Klein, S., Coppack, S. W., Mohamed-Ali, V., & Landt, M. (1996). Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes, 45*(7), 984-987. doi:10.2337/diabetes.45.7.984

Kolotkin, R. L., Meter, K., & Williams, G. R. (2001). Quality of life and obesity. *Obesity Reviews, 2*(4), 219-229. doi:10.1046/j.1467-789x.2001.00040.x

Lashen, H., Fear, K., & Sturdee, D. W. (2004). Obesity is associated with increased risk of first trimester and recurrent miscarriage: Matched case-control study. *Human Reproduction,19*(7), 1644-1646. doi:10.1093/humrep/deh277

Leddy, M. A., Power, M. L., & Schulkin, J. (2008). The Impact of Maternal Obesity on Maternal and Fetal Health. *Reviews in Obstetrics and Gynecology*, *1*(4), 170–178.

Mitchell, N., Catenacci, V., Wyatt, H. R., & Hill, J. O. (2011). OBESITY: OVERVIEW OF AN EPIDEMIC. *The Psychiatric Clinics of North America*, *34*(4), 717–732. http://doi.org/10.1016/j.psc.2011.08.005

Oken, E., & Gillman, M. W. (2003). Fetal Origins of Obesity. *Obesity Research, 11*(4), 496-506. doi:10.1038/oby.2003.69

Overweight & Obesity. (2018, March 05). Retrieved April 16, 2018, from https://www.cdc.gov/obesity/data/adult.html

Patrick, K., Williams, M., Orlando, M., Wasserfall, C., & Gregg, A. (2018). Adipokine Regulation of Human Placental GLUT-1 Transporter. *Socieyt for Reproductive Investigation*. Retrieved from http://www.abstractsonline.com/pp8/#!/4592/presentation/3887

Said, A. S., & Manji, K. P. (2016). Risk factors and outcomes of fetal macrosomia in a tertiary centre in Tanzania: a case-control study. *BMC Pregnancy and Childbirth*, *16*(1), 243. http://doi.org/10.1186/s12884-016-1044-3

Scholl, T. O., Sowers, M., Chen, X., & Lenders, C. (2001). Maternal Glucose Concentration Influences Fetal Growth, Gestation, and Pregnancy Complications. *American Journal of Epidemiology, 154*(6), 514-520. doi:10.1093/aje/154.6.514

Takata, K., Kasahara, T., Kasahara, M., Ezaki, O., & Hirano, H. (1992). Localization of erythrocyte/HepG2-type glucose transporter (GLUT1) in human placental villi. *Cell & Tissue Research, 267*(3), 407-412. doi:10.1007/bf00319362

The Health Effects of Overweight and Obesity. (2015, June 05). Retrieved April 16, 2018, from https://www.cdc.gov/healthyweight/effects/index.html

Tremmel, M., Gerdtham, U.-G., Nilsson, P. M., & Saha, S. (2017). Economic Burden of Obesity: A Systematic Literature Review. *International Journal of Environmental Research and Public Health*, *14*(4), 435. http://doi.org/10.3390/ijerph14040435

Tsai, A. G., Williamson, D. F., & Glick, H. A. (2011). Direct medical cost of overweight and obesity in the United States: a quantitative systematic review. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, *12*(1), 50–61. http://doi.org/10.1111/j.1467-789X.2009.00708.x

Tzanavari, T., Giannogonas, P., & Karalis, K. P. (2010). TNF-alpha and Obesity. *Current Directions in Autoimmunity TNF Pathophysiology,* 145-156. doi:10.1159/000289203

Watkins, M. L., Rasmussen, S. A., Honein, M. A., Botto, L. D., & Moore, C. A. (2003). Maternal Obesity and Risk for Birth Defects. *Pediatrics, 111*(1), 1152-1158. Retrieved from http://pediatrics.aappublications.org/content/111/Supplement_1/1152