CASE WESTERN RESERVE UNIVERSITY

Discussions

Volume 5 | Issue 2 Article 2

2024

Proxidant Injection Causes the Onset of Type 2 Diabetes in the Spontaneously Hypertensive Obese (SHROB/Kol) Rat

Maria Theodorou
Case Western Reserve University

Recommended Citation

Theodorou, Maria (2024) "Proxidant Injection Causes the Onset of Type 2 Diabetes in the Spontaneously Hypertensive Obese (SHROB/Kol) Rat," *Discussions*: Vol. 5: Iss. 2, Article 2.
DOI: https://doi.org/10.28953/2997-2582.1100
Available at: https://commons.case.edu/discussions/vol5/iss2/2

Proxidant Injection Causes the Onset of Type 2 Diabetes in the Spontaneously Hypertensive Obese (SHROB/Kol) Rat

-Maria Theodorou-

Maria is a fourth year student studying Spanish, Biology, and Chemistry at CWRU. She is active in the Office of Undergraduate Admissions, Greek Life, and is a volunteer at Rainbow Babies and Children's Hospital. Upon completion of her undergraduate studies, Maria plans to attend medical school. Maria enjoys traveling, downhill skiing, and Zumba.

-Acknowledgments-

I would like to thank my advisor Dr. Paul Ernsberger and our co-investigator Dr. Richard Koletsky for their guidance and support of my research endeavors. Thank you to Rachel Koletsky, Neema Patel, Alex Moore, Matt Koletsky, Liza Escobedo, Sabrina Jackson, and Simone Edwards for their help in conducting this research. I would like to thank the Dietrich Diabeties Research Institute of the Diabetes Association of Greater Cleveland and the SOURCE Office of Case Western Reserve University for their funding, and CWRU's Department of Nutrition for serving as home base for my research over the past three years.

ABSTRACT

The spontaneously hypertensive obese (SHROB/Kol) rat is a model of prediabetes characterized by normal fasting and high postprandial glucose and insulin resistance. Oxidative stress, through the damaging effects of oxygen radicals, may contribute to the onset of diabetes. Oxidative stress was induced in SHROB with a combination of a proxidant agent, hydroquinone, and a glutathione synthase inhibitor, L-buthionine sulfoximine (both 50 mg/kg ip). Following proxidant injections, plasma peroxide levels were increased within 1 h and returned to baseline within 24h. Diabetes, defined as basal blood glucose in excess of 126 mg/dL, appeared within two days of daily treatment. Morning blood glucose values averaged over 200 mg/dl in SHROB injected with pro-oxidant solution versus about 100 mg/dl in control SHROB injected with saline. Glucose fell to baseline within 3d after cessation of proxidant treatment, but glucose tolerance remained significantly impaired. Food intake and body weight were not significantly affected by proxidant treatment at any point. Oxidative stress was measured by assay of peroxides in plasma. The SHROB rat subjected to oxidative stress is a potential model to study the onset of Type 2 diabetes, and supports the hypothesis that oxidative stress may be a trigger for diabetes onset in susceptible individuals.

INTRODUCTION

Approximately 15 million Americans suffer from diabetes today (Candib, 2007). This serious illness exists in two forms, type 1 and type 2. Currently 5% of diabetics suffer from type 1, while 95% of diabetics suffer from type 2. Type 1 diabetes results from the autoimmune-mediated destruction of insulin-producing beta cells, which causes an absolute insulin deficiency. Multiple factors contribute to type 2 diabetes, including the combined influence of genetic susceptibility and environmental factors such as nutrition, obesity, age, and physical inactivity. In the early stages of the development of diabetes, prior to the onset of high glucose levels, these contributing factors result in insulin resistance compensated by insulin production that it adequate or even excessive (Candib, 2007). The next stage in the progression to diabetes is impaired glucose tolerance (Benjamin, Valdez, Geiss, Rolka, & Narayan, 2003). Human metabolic syndrome is characterized by a group of metabolic risk factors, including abdominal obesity, dyslipidemia, hypertension, insu-

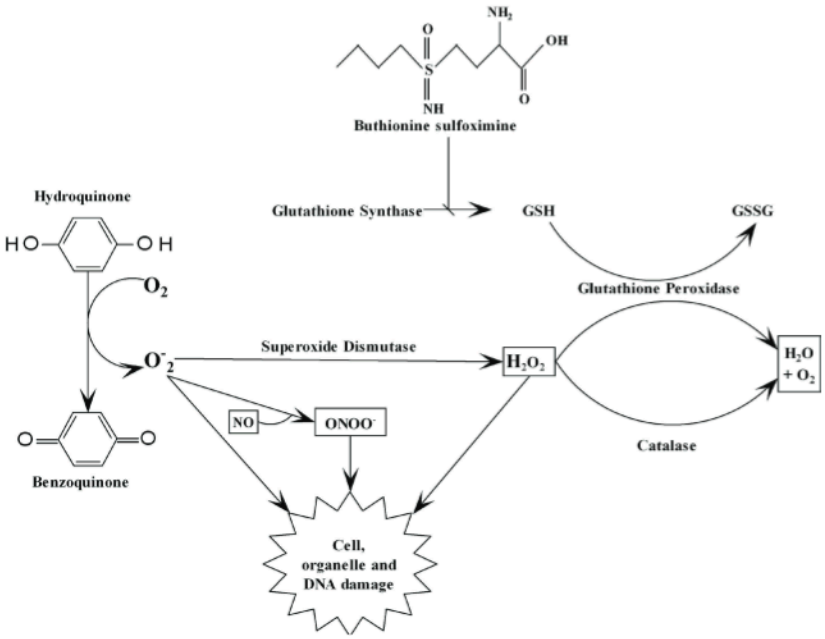

Figure 1: Experimental intervention in oxidative stress pathways. Rats were given hydroquinone, which yields superoxide when reduced to quinone. Additionally, the rats were given buthionine sulfoximine, a glutathione synthase inhibitor, which depletes the supply of this intracellular antioxidant. This reduces the breakdown of hydrogen peroxide and also interferes with other redox reactions in which glutathione participates. The combination of hydroquinone and buthionine sulfoximine leads to an imbalance of oxidative and antioxidant processes within cells, a condition known as oxidative stress.

lin resistance, and impaired glucose tolerance. Metabolic syndrome is thought to play a role in increasing the risk for type 2 diabetes.

Obesity contributes to all components of metabolic syndrome, especially in the presence of excess abdominal fat (Batsis, Nieto-Martinez, & Lopez-Jimenez, 2007). Increased weight, especially that caused by excess fat in the abdominal region, is associated with insulin resistance. For this reason, obesity is strongly associated with type 2 diabetes. However, this relationship is not absolute, as there are obese individuals who do not develop type 2 diabetes, as well as non-obese individuals who develop the disease (Karter et al., 2005). It is unclear why this is the case, leading to the question of which components in the body trigger diabetes in susceptible individuals.

A proposed mechanism for the insulin resistance implicated in metabolic syndrome, and subsequently in type 2 diabetes, is through oxidative stress. Oxidative stress is characterized by an imbalance between the production of reactive oxygen species and a biological system's ability to detoxify reactive intermediates or easily repair the damage caused by these intermediates (Haidara, Yassin, Rateb, Ammar, & Zorkani, 2006). The natural cell environment of all living systems is reducing and this state is, for the most part, maintained through the action of certain enzymes which constantly provide a source of metabolic energy. The production of reactive oxygen species (ROS) disturbs this normal redox state through the production of peroxides and free radicals, substances which have been shown to have a damaging effect on almost all components of the cell and which may ultimately lead to cell death. This process is described in Figure 1.

Superoxide is a major reactive oxygen species in cells. As indicated in Figure 1, it directly damages cells, and reacts with nitric oxide to form an additionally damaging peroxynitrite radical. Superoxide is rapidly acted upon by superoxide dismutase to produce hydrogen peroxide, which is also detrimental to cells. Hydrogen peroxide is subsequently broken down by two pathways. First, the enzyme catalase works directly on hydrogen peroxide to produce water and molecular oxygen. Additionally, glutathione peroxidase breaks down hydrogen peroxide to oxidized glutathione (GSSG, with a disulfide bridge) with the help of the reduced form of glutathione (GSH, with a free thiol group) (Figure 1). The chemical stimulation of these pathways through the injection of hydroquinone and buthionine sulfoximine leads to oxidative stress, the imbalance of oxidative and antioxidant processes within cells.

Oxidative stress has been implicated in the onset and progression of many diseases, including cancer, atherosclerosis, Parkinson's disease, Alzheimer's disease, and diabetes mellitus. In regards to diabetes, oxidative stress may contribute to pre-diabetic conditions such as insulin resistance and glucose intolerance, to diabetes itself, and to its complications such as retinopathy, nephropathy, neuropathy, stroke, and myocardial infarction (Keaney, Jr. et al., 2003). In this study, the effect of oxidative stress on blood glucose levels and the onset of diabetes was studied.

Antioxidants form part of the oxidative stress pathway and aid in the degradation of hydrogen peroxide to oxygen and water. Endogenous antioxidants include superoxide dismutase (SOD), glutathione, and catalase. An-

SHR 294g
Spontaneously
Hypertensive

SHROB 530g
Obese Spontaneously
Hypertensive

Figure 2: Pictures of typical SHROB and SHR female rats at 12 weeks of age

tioxidants can also be obtained exogenously from the diet, and are found in foods such as tomatoes, carrots, berries, garlic, and soy. Garlic's active principle, allicin, is thought to contribute significantly to the compound's antioxidant properties (Ernsberger, Johnson, Rosenthal, Mirelman, & Koletsky, 2007).

Antioxidants are thought to decrease components of metabolic syndrome, including blood pressure, fat content of the bloodstream, and insulin resistance. Additionally, measuring levels of antioxidants present in the cell is one way to measure the degree of oxidative stress, as an increased presence of antioxidants signifies an increased response to proxidant stress. It is hypothesized that the mechanism of the onset of diabetes may be caused by oxidative stress in combination with obesity and diet, especially a diet lacking antioxidants.

The SHROB rats used in this experiment exhibited metabolic syndrome (Figure 2). Metabolic syndrome is caused by genetic abnormalities and can be influenced by diet, physical activity, and medicines (Koletsky, Velliquette, & Ernsberger, 2007; Koletsky, Velliquette, & Ernsberger, 2003). This syndrome is characterized by abdominal obesity, dyslipidemia, hypertension, insulin resistance, and impaired glucose tolerance. These symptoms are all associated with, and often lead to, diabetes in humans; however, untreated SHROB rats remain in a prediabetic state. The obesity exhibited by this model results from a single point mutation leading to a premature stop codon in the leptin receptor, preventing translation of functional leptin receptors. Without these receptors, the effects of leptin on satiety are disrupted, and cause eating behaviors which lead to obesity. Other genetic mutations are responsible for the model's hypertension and dyslipidemia. However, these genes have not yet been fully identified. In combination, these genetic abnormalities form metabolic syndrome. Recently, a three-fold increase in plasma and organ lipid peroxides has been noted in SHROB relative to lean SHR littermates (Ernsberger, Theodorou, Edwards, & Koletsky, 2008). As such, we hypothesized that further increasing the elevated levels of oxidative stress in SHROB will be sufficient to trigger diabetes in this model.

METHODS

SHROB rats were obtained from Charles River at 10 weeks of age. Body weight and food intake were monitored 3 days per week throughout the study. Rats were administered inter-peritoneal injections for 7 days, were allowed to recover for 3 days, and then injected for 3 more days prior to oral glucose tolerance testing with either a proxidant solution or saline. Six rats received a proxidant solution of 50 mg/kg doses of both L-buthionine sulfoximine and hydroquinone. Six rats received saline injections. Tail blood was obtained under local anesthesia and tested for glucose using a meter and test strips (One-Touch Ultra). Blood samples were centrifuged and plasma was frozen immediately for later analysis. Oral glucose tolerance testing was conducted after the final day of injections. Rats were fasted 18h then gavaged with a 50% glucose solution at a dose of 6 g/kg. Rats were then injected with either saline or proxidant solution. Tail blood was obtained under local anesthesia at 0, 30, 60, 120, and 360 minutes after glucose loading. Rat sacrifice with organ harvest occurred within 5 days of oral glucose tolerance testing. Peroxides were measured as a marker of oxidative stress in plasma samples using the ferric orange xylenol (FOX) method (Sigma Chemicals; Peroxi-Detect kit).

RESULTS

Proxidant injection of L-buthionine sulfoximine and hydroquinone was found to increase blood glucose levels significantly over five days of injections (injected group baseline 87±5 to 234±29 mg/dL on day 5 versus vehicle group baseline 96±4 to 93±6 mg/dL on day 5; see Figure 3 on right).

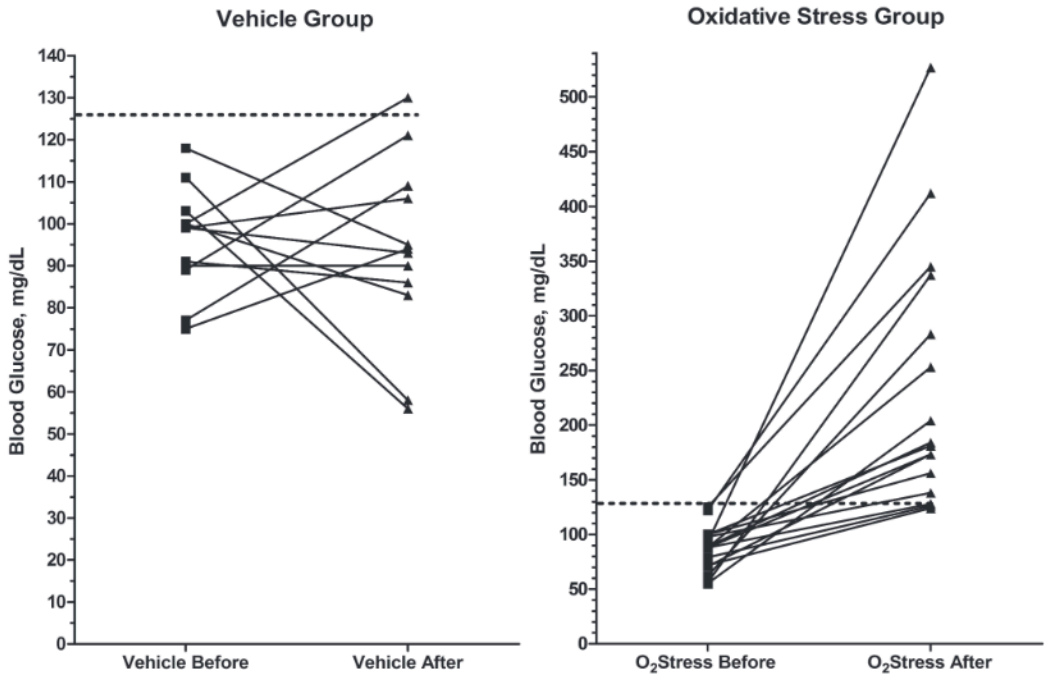

Figure 3: Morning blood glucose levels at the beginning of the study were all well below 126 mg/dL in both groups of SHROB (labeled Vehicle Before and O2Stress Before). Glucose levels remained relatively constant and below 126 mg/dL in SHROB receiving vehicle saline injection for 5 days (labeled Vehicle After). In contrast, SHROB subjected to oxidative stress through 5 days of proxidant injections showed marked hyperglycemia, with all but 3 of the 17 rats showing diabetic blood glucose levels above 126 mg/dL.

By the second day of proxidant injection, morning glucose rose significantly above 124 mg/dL, the threshold for diagnosing diabetes. Glucose levels remained elevated for the duration of injection and fell within 3 days upon cessation of injection. However, a re-initiation of proxidant injection in the days leading up to oral glucose tolerance testing resulted in an elevation of blood glucose levels. Control SHROB receiving saline solution remained in the pre-diabetic range (100-125 mg/dL glucose) throughout the course of injections.

Glucose tolerance was found to be impaired in proxidant treated SHROB relative to those receiving saline injection. Following 7 consecutive days of injections, glucose tolerance was measured and found to be impaired in proxidant treated SHROB relative to those receiving saline injection. Oxidative stress significantly increased glucose overall, with blood glucose levels remaining in the diabetic range 360 minutes after glucose challenge. However, upon re-initiation of injections for an additional 3 days following a 3 day recovery period, an oral glucose tolerance test found even greater impairment of glucose tolerance in proxidant treated SHROB relative to those receiving saline injection.

Body weight monitored continuously throughout the study found there to be no change in body weight during proxidant injections as compared to rats receiving control injections. Treatment groups did not differ in body weight by 2-way analysis of variance with repeated measures.

Oxidative stress was measured by assay of peroxides in plasma and the liver fraction of kidney and liver tissue. Proxidant injection increased plasma peroxide levels within 1h with a return to baseline levels within 24h . Baseline plasma peroxide levels were found to be 20±2 μmol/L, with elevation to 43±8 μmol/L at 1h, followed by a subsequent decrease to 31±6 μmol/L at 4h and 17±1 μmol/L at 24h.

DISCUSSION

The success of proxidant treatment in triggering the onset of diabetes in the Spontaneously Hypertensive Obese (SHROB/Kol) rat confirms the association between oxidative stress and the onset of diabetes in a model of metabolic syndrome. Hydroquinone acted to promote oxidative stress through up-regulation of the production of oxidative damage-causing free radicals. L-buthionine sulfoximine, a glutathione synthase inhibitor, served to prevent the break down of harmful free radicals to water and oxygen. The combination of upregulation of free radical production and inhibition of free radical breakdown maximized oxidative stress in the metabolic syndrome-exhibiting SHROB, thus leading to the onset of diabetes. These findings, in conjunction with the fact that saline-injected control SHROB did not develop diabetes, demonstrate that subjection to oxidative stress can cause type 2 diabetes in an at-risk, pre-diabetic model.

The transient elevation of morning blood glucose levels to above 125 mg/dl indicate that the regulation of oxidative damage in SHROB may have a mechanism for short-term recovery from oxidative stress. There are many potential points of regulation of oxidative damage in the SHROB, including the liver, pancreas, and peripheral muscle or fat tissue. Possible mechanisms may include increased glucose production from the liver, impaired insulin secretion from the pancreas, and receptor or post-receptor defects leading to insulin resistance in muscle and fat. Further studies will explore the effect of oxidative stress on each of these possible points of regulation, and seek to determine the possibility of adaptive measures to combat oxidative stress.

The impaired glucose tolerance observed in proxidant treated SHROB as compared to control SHROB receiving saline injection further demonstrates the effect of oxidative stress in this model. Impaired glucose tolerance is often associated with insulin resistance, a symptom seen in the pre-diabetic SHROB model. However, the further impairment of insulin resistance in the proxidant treated SHROB indicates the association between oxidative stress and the onset of diabetes.

Obesity is an underlying factor in prediabetes and diabetes. In this study, SHROB body weight remained unchanged despite the increased oxidative stress. This finding rules out the possibility that the onset of diabetes resulted indirectly from increased body weight. The lack of weight loss also suggests that the proxidant treatment is not simply toxic.

Elevated plasma peroxide levels within 1h of proxidant injection, with a return to baseline within 24h indicate the activity of internal mechanisms within SHROB for the control of oxidative stress. However, the fact that proxidant injections administered following a recovery period of three days lead to impaired glucose tolerance, and subsequently higher maximum glucose levels and longer recovery times in an oral glucose tolerance test, may indicate an underlying impairment of SHROB's ability to combat oxidative stress. Subsequent oxidative damage may contribute to insulin resistance in the liver as well as renal and hepatic dysfunction. These are characteristics of chronic type 2 diabetes.

Given the fact that oxidative stress can promote the onset of diabetes in a model of metabolic syndrome, further studies may be performed to explore the mechanism of this association and to investigate preventative treatments for those at risk for developing diabetes. These studies may include the exploration of the effects of diets containing varying levels of anti-oxidants in conjunction with proxidant treatment, as well as investigation of medications with the potential to inhibit oxidative stress. Anti-oxidant therapies alone or in combination with treatment for characteristics of metabolic syndrome, such as hypertension, may be beneficial in the prevention of the onset of type 2 diabetes in at-risk individuals. This has a widespread potential for benefit due to the increasing frequency of diabetes, obesity, and other characteristics of metabolic syndrome worldwide. Prevention and treatment of these aspects of disease is an important step in the improvement of the overall health of the international population, and future studies of the mechanism of the onset of diabetes through oxidative stress will work towards the achievement of this goal.

CONCLUSIONS

Induction of oxidative stress in prediabetic SHROB rats causes the onset of diabetes within two days of daily treatment, and further impairs glucose tolerance as measured by oral glucose tolerance testing. Weight gain is not a contributing factor to elevated the blood glucose levels or impaired glucose tolerance seen in proxidant treated SHROB. Plasma peroxide levels were found to be increased within 1h of proxidant injection with a return to baseline within 24h. The SHROB rat subjected to oxidative stress is a potential model to test the reduction of oxidative stress and subsequent diabetes prevention by antioxidants in individuals exhibiting metabolic syndrome.

REFERENCES

Batsis, J. A., Nieto-Martinez, R. E., & Lopez-Jimenez, F. (2007). Metabolic syndrome: from global epidemiology to ind vidualized medicine. Clin.Pharmacol.Ther., 82, 509-524.

Benjamin, S. M., Valdez, R., Geiss, L. S., Rolka, D. B., & Narayan, K. M. (2003). Estimated number of adults with prediabetes in the US in 2000: opportunities for prevention. Diabetes Care, 26, 645-649.

Candib, L. M. (2007). Obesity and diabetes in vulnerable populations: reflection on proximal and distal causes. Ann.Fam. Med, 5, 547-556.

Ernsberger, P., Johnson, J. L., Rosenthal, T., Mirelman, D., & Koletsky, R. J. (2007). Therapeutic actions of allylmercaptocaptopril and captopril in a rat model of metabolic syndrome. Am.J Hypertens, 20, 866-874.

Ernsberger, P., Theodorou, M., Edwards, S., & Koletsky, R. J. (2008). Effects of proxidant treatment on the progression of prediabetes in SHROB rats. The FASEB Journal, 22, 1153.

Haidara, M. A., Yassin, H. Z., Rateb, M., Ammar, H., & Zorkani, M. A. (2006). Role of oxidative stress in development of cardiovascular complications in diabetes mellitus. Curr.Vasc.Pharmacol., 4, 215-227.

Karter, A. J., D'Agostino, R. B., Jr., Mayer-Davis, E. J., Wagenknecht, L. E., Hanley, A. J., Hamman, R. F. et al. (2005). Abdominal obesity predicts declining insulin sensitivity in non-obese normoglycaemics: the Insulin Resistance Atherosclerosis Study (IRAS). Diabetes Obes.Metab, 7, 230-238.

Keaney, J. F., Jr., Larson, M. G., Vasan, R. S., Wilson, P. W., Lipinska, I., Corey, D. et al. (2003). Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arteriosclerosis, Thrombosis, and Vascular Biology, 23, 434-439.

Koletsky, R. J., Velliquette, R. A., & Ernsberger, P. (2003). The role of I(1)-imidazoline receptors and alpha(2)-adrenergic receptors in the modulation of glucose and lipid metabolism in the SHROB model of metabolic syndrome X. Annals of the New York Academy of Science, 1009, 251-261.

Koletsky, R. J., Velliquette, R. A., & Ernsberger, P. (2007). The SHROB (Koletsky) rat as a model for metabolic syndrome. In E.Shafrir (Ed.), Animal Models of Diabetes: Frontiers in Research (pp. 185-208). Boca Raton, FL: CRC Press.