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RESEARCH ARTICLE

DEVELOPMENT OF A CHRONIC HYPERGLYCEMIC AND HYPERTENSIVE RAT MODEL THROUGH REPETITIVE INTRAPERITONEAL AND ORAL GLUCOSE LOADING

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ABSTRACT

Hyperglycemia in animals can be induced by different methods: namely, chemical treatment (eg. streptozotocin, alloxan), dietary intervention (eg. excess feeding of sucrose/fructose/fat) with/without chemical treatment, pancreas removal (pancreatectomy), and genetic modifications (eg. transgenic animals). The current study test if a two-week sequence of oral and intraperitoneal glucose loading can sustain hyperglycemia inSprague Dawley rat.A dose of 80 $gm.kg^{-1}BW$ (feed), 2 $gm.kg^{-1}BW.ml^{-1}$ (drinking water), 2.25 $gm.kg^{-1}BW.ml^{-1}$(IP) (first week) and increased in the second week to 80 $gm.kg^{-1}BW$, 2.5 $gm.kg^{-1}BW.ml^{-1}$, 2.75 $gm.kg^{-1}BW.ml^{-1}$ of glucose in feed, drinking water, and injection (two times/day) respectively, was selected from 15 different administered doses. A first week moderate increase in plamsa glucose was observed followed by a sustained elevation in plasma glucose levels in the second week. Plasma glucose levels increased on average to 265 $mg.dl^{-1}$ and 371 $mg.dl^{-1}$ on day 9 and day 12, respectively. Moreover, the observed hyperglycemia was sustained through day 14 of the study. In addition, plasma insulin levels also significantly increased from 8.92 $\mu lU.ml^{-1}$ in control to 27.24 $\mu lU.ml^{-1}$ in the glucose-treated group. Hyperglycemia and the resulting hyperinsulinemia promoted a significant increase in mean areterial pressure (MAP) throughout the entire study.The stated dose is effective to develop a lean hypertensive hyperglycemic Sprague Dawley rat model, thereby excluding the confounding factors of obesity and chemical treatements, when studying cardiovascular, renal, and diabetic complications.

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INTRODUCTION

Diabetes is a metabolic disease marked by persistent hyperglycemia. Hyperglycemia, which is characterized by high plasma glucose levels (usually ≥126 mg/dl in the fasting state), poses severe physiological complications ranging from cardiovascular abnormalities to renal failure. Diabetes is classified into 2 board classes namely type 1 insulin dependent and type 2 insulin independent diabetes. In type 1 diabetes, persistent hyperglycemia results from absolute insulin insufficiency caused by immunological destruction of pancreatic beta cells, which produce and secrete insulin (American Diabetes Association, 2015; Dell Guerra *et al*., 2005). Type 2 diabetes is marked by persistent hyperglycemia resulting from a progressive decline of insulin action (insulin resistance) in liver and peripheral tissues coupled with the inability of beta cells to compensate for insulin resistance (American Diabetes Association, 2015; Wang, 2013). Blood glucose levels begin to rise about 10 min after the start of a meal as a result of the absorption of dietary carbohydrates which are the major sources of circulatory glucose in healthy subjects. Insulin is secreted from beta cells in response to elevated blood glucose that is above a tight normal physiological range. Though insulin can also be secreted in response to various hormones and neural inputs as well as other nutrients such as amino acids and fatty acids (Chandra and Liddle, 2009; Wilcox, 2005), glucose is by far the most potent and physiologically important regulator of β-cell function through coordinated stimulation of insulin gene transcription, proinsulin biosynthesis, and insulin secretion from beta cells (Cerf, 2013). Secreted insulin plays an important role in glucose homeostasis through suppression of hepatic glucose production, and stimulation of peripheral glucose uptake by insulin-sensitive tissues (eg. adipose tissues and skeletal muscles), thus insulin prevents excessive postprandial glucose excursion, without causing hypoglycemia (Rolla, 2004). The beta cells efficiently regulate glucose homeostasis through

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secretion of insulin in a biphasic pattern, where an initial rapid phase (first-phase) of insulin secretion occurs in response to glucose stimulus followed by a less intense but more sustained release of the hormone (Bratanova-Tochkova *et al*., 2002). The first-phase rapid insulin response at mealtime is important in maintaining normal physiological glucose levels after a meal through prompt inhibition of endogenous glucose production by liver and increase in glucose disposal. The first-phase insulin response in turn restricts a potential mealtime rise in glucose, which eventually prevents late hyperinsulinemia and prolonged secretory stress on beta cells (Rolla, 2004).

During an oral glucose tolerance test (OGTT) or intravenous glucose tolerance test (IVGTT), a bolus dose of glucose is given to a patient. Patients with an impaired glucose tolerance (IGT) overwhelm the disposal capacity of first-phase insulin given that there is a decrease in initial insulin secretion (Matsumoto *et al*., 1997), leading to sustained compensatory insulin secretion during the second phase that not only prolongs secretory stress on beta cells, but also delays glucose disposal. This results in sustained hyperglycemia and hyperinsulinemia in these patients (Prato, 2003; Toschi *et al*., 2002). Further prolongation of hyperglycemia at a point can start to play a deleterious role on beta cell function by reducing insulin gene expression, insulin contents, glucose-stimulated insulin secretion, and/or by promoting beta cell apoptosis resulting in a vicious cycle of hyperglycemia (Poitout and Robertson, 2008). In addition, many studies show that high glucose can promote induction of reactive oxygen species, which leads to development of oxidative stress in β-cells resulting in decreased insulin biosynthesis, insulin content and secretion, and increased beta cell apoptosis (Poitout and Robertson, 2008; Tanaka *et al*., 1999; Zhang *et al*., 2010). Interestingly, high glucose-induced ROS can also contribute to the development of insulin resistance in beta cells and peripheral insulin sensitive tissues leading to impairment in insulin secretion and glucose disposal, respectively (Eriksson, 2007). At the outset of type 2 diabetes (T2D), beta cells can compensate for attenuated glucose disposal in peripheral tissues via a gradual increase in insulin secretion. However, insulin secretion gradually decreases due to persistent hyperinsulinemia- and hyperglycemia-induced impairment in diverse signaling pathways leading to β-cell dysfunction and apoptosis. Moreover, sustained hyperinsulinemia induces insulin resistance in various tissues including beta cells (Shanik *et al*., 2008), liver, muscle (Kanety *et al*., 1994), adipose tissues (Pederson *et al*., 2001), and lymphocytes (Gavin III *et al*., 1974) through downregulating insulin receptors, abating receptor binding affinity, and/or impairing activation of insulin receptor substrates IRS-1 and IRS-2, as well as downstream insulin-signaling molecules (Shanik, 2008; Kanety, 1994; Pederson, 2001; Gavin III *et al*., 1974; Heaton and Gelehrter, 1981; Paz *et al*., 1997), leading to an aggravated hyperglycemic state followed by T2D.

Based on the previously delineated studies, we hypothesized that excessive oral glucose with or without intraperitoneal glucose loading for two weeks in normal rats will impair beta cell function as well as glucose disposal promoting a sustained hyperglycemic and hypertensive state. The underlying mechanism is that a maximum dose of glucose can be administered to exhaust insulin contents causing acute hyperglycemia which may promote a chronic hyperglycemic condition in response to repeated high doses of glucose through hyperglycemia-mediated deleterious effects on beta cells, the liver, and peripheral tissues. A growing body of evidence supports the premise that feeding animals a high nutrient-enriched (sugar, fructose, fat or glucose) diet for a period of time can promote sustained hyperglycemia and concomitant insulin resistance (Lai *et al*., 2014; Wang and Liao, 2012). For example, studies show that Sprague Dawley rats fed a fructose-enriched diet for a period of 8 weeks have significantly increased plasma glucose and insulin levels in comparison to regular chow-fed rats. This implies that a high fructose diet can promote progressive development of hyperglycemia and insulin resistance (D'Angelo *et al*., 2005; Suwannaphet *et al*., 2010). Similarly, a high-fat diet can also induce hyperglycemia and insulin resistance as demonstrated by other studies (Buettner*et al*., 2000). Although an increasing number of studies demonstrated pro-hyperinsulinemic and hyperglycemic effects of fructose, fat, and sucrose-enriched diets, studies employing glucose-enriched diets to develop hyperglycemia or hyperinsulinemia are rare. In addition, Reaven and Ho (1991) observed an increase in insulin levels in Sprague-Dawley rats fed glucose-enriched diets for a period of two weeks. However, there was no evidence of hyperglycemia development in their study. Moreover, drinking 10% glucose water over a period of 3 weeks was shown to induce hypertension with a significant but mild increase in plasma glucose. The observed hypertension was attenuated by an antioxidant supplement, α-lipoic acid, indicating involvement of glucose-induced ROS in the development of hypertension (Midaoui and de Champlain, 2002).

A growing body of evidence suggests that hyperglycemia can be induced by different methods: namely, chemical treatment (eg. streptozotocin, alloxan), dietary intervention (eg. excess feeding of sucrose/fructose/fat) with or without chemical treatment, removal of the pancreas (pancreatectomy), and genetic modifications (eg. transgenic animals). However, the various pathophysiological characteristics of different diabetic types still require the development of additionalsuitable animal models of diabetes to study its complications in cardiovascular and renal functions.

MATERIALS AND METHODS

D-(+)-Glucose and inactin (thiobutabarbital sodium) were purchased from Sigma-Aldrich Inc, Saint Louis, MO, USA. Rat insulin elisa kit was obtained from Life Technologies, Frederick, Maryland, USA. Blood glucose test strips were purchased from Nipro Diagnostics, For Lauderdale, Florida, USA. Regular rat chow (in powder form) was bought from Harlan, Indianapolis. All solutions and glucose-enriched dietswere freshly made daily throughout the study and glucose water for injection was made by dissolving glucose into saline containing 0.9% NaCl (w/v).

Animals

Eight-week-old Male Sprague Dawley rats (250-320g, Harlan, Indianapolis, IN) were housed at room temperature with 12/12-hours light/dark cycle. Each group of animals (n =3) was allowed to acclimatize for at least one week before the study was begun. All animals had free access to food and water (modified according to protocol) throughout the experiment. All animal experiments were approved by the University of Louisiana at Monroe Institutional Animal Care and Use Committee (IACUC).

Experimental Protocol

Prior to the study, animals were housed individually and divided into two groups: glucose-treated (n = 6) and control (n = 3). Food and/or glucose water were prepared (on daily basis) according to the doses given in table 1(feed: 80 gm.kg^{-1}BW, drinking water: 2-2.5 gm.kg^{-1}BWml^{-1}, injection: 2.25-2.75 gm.kg^{-1}BW.ml^{-1}) (Table 1). For groups treated with glucose-enriched food and/or glucose water, glucose levels were monitored by a TRUE test glucose meter in the morning (8-9 am) and evening (7-8 pm) and the average glucose levels of two readings were recorded. In addition, to achieve sustained blood glucose levels of greater than 170 mg.dl^{-1} for two weeks, various doses of intraperitoneal (IP) glucose injections were employed (table 1). Animals were treated initially with a single glucose injection, however, the desired glucose levels were not realized, therefore adouble glucose injection (eg. morning and afternoon) separated by an 8 hour span was administered. The double glucose injection groups received glucose-enriched food and/or glucose water. In each of the glucose treated groups, blood glucose was measured in the morning before the injection, and 3 hours after the injection in the afternoon and the average of two glucose measures was used for both time points. However, for the single dose injection (given in the morning) group, no post injection measurement of glucose levels was made until the afternoon time point.

glucose treatment groups. Food was freshly prepared daily by mixing the required glucose amount with standard rat chow (powder form) and reconstituted into a ball with a small quantity of tape water. Based on previous studies in our lab, rats were determined to consume an average of 50 gm of food daily, therefore the glucose doses were calculated based on the animal's weight to provide a daily allowance of at least 50gm. On the other hand, glucose-enriched drinking water was prepared by simply dissolving the required amount of glucose in water (Table 1).

Table 1: Description of doses. A total of 15 different doses were used and D-15 was selected as the most effective dose. Glucose doses mentioned in the chart is in feed, drinking water, and injection sequentially. Four control groups were used to volume match with the different treatment groups. For example, control-1 was used to compare D1-D11 dose groups with no IP injection involved. However, different control groups namely, control-2, 3, and 4 were used by providing regular rat chow, normal drinking water along with variable saline injection volumes and/or injection frequency to compare with the respective glucose-treatment groups. Comparison was made as follows: control-1 vs D1-D11, control-2 vs D-12, control-3 vs D13-D14, and control-4 vs D-15. This should be legend of table 1.

Table 1. Description of Glucose Doses

Doses	Description
Control-1	Control to compare with treated animals corresponding to D1-D11 doses
D-1	40 gm.kg^{-1}BW, normal water
D-2	80 gm.kg^{-1}BW, normal water
D-3	120 gm.kg^{-1}BW, normal water
D-4	160 gm.kg^{-1}BW, normal water
D-5	80 gm.kg^{-1}BW,1gm.kg^{-1}BW.ml^{-1} glucose water
D-6	120 gm.kg^{-1}BW, 1 gm.kg^{-1}BW.ml^{-1}glucose water
D-7	160 gm.kg^{-1}BW, 1 gm.kg^{-1}BW.ml^{-1}glucose water
D-8	100 gm.kg^{-1}BW, 2 gm.kg^{-1}BW.ml^{-1}glucose water
D-9	125 gm.kg^{-1}BW, 2 gm.kg^{-1}BW.ml^{-1}glucose water
D-10	80 gm.kg^{-1}BW, 3 gm.kg^{-1}BW.ml^{-1}glucose water
D-11	120 gm.kg^{-1}BW, 3 gm.kg^{-1}BW.ml^{-1}glucose water
Control-2	Control provided with regular rat chow, normal water, and normal saline (IP)
D-12	Regular rat chow, 2.5 gm.kg^{-1}BW.ml^{-1}, injection: 2 gm.kg^{-1}BW.ml^{-1}, single dose/day. All injection doses were calculated as per the following formula: *Intended injection volume: 4 ml, initial glucose concentration: 45% w/v (.45gm.ml^{-1}). V1*S1 =V2*S2 (V1= 4 ml, S1= 0.25*2gm, given that rat's weight is 250 gm, S2 = 0 .45gm.ml^{-1}).*
Control-3	Control provided with regular rat chow, normal water, and normal saline (IP)
D-13	80 gm.kg^{-1}BW, normal water, injection: 1gm.kg^{-1}BW.ml^{-1}, double dose/day
D-14	Regular rat chow, 2 gm.kg^{-1}BW.ml^{-1}, injection: 1.5 gm.kg^{-1}BW.ml^{-1}, double dose/day
Control-4	Control provided with regular rat chow, normal water, and normal saline (IP)
D-15	80 gm.kg^{-1}BW, 2 gm.kg^{-1}BW.ml^{-1}, injection: 2.25 gm.kg^{-1}BW.ml^{-1}, double dose/day (First Week), 80 gm.kg^{-1}BW, 2.5 gm.kg^{-1}BW.ml^{-1}, injection: 2.75 gm.kg^{-1}BW.ml^{-1}, double dose/day (Second Week)

The previously delineated glucose treatments were administered for a two week period. Glucose levels were monitored on days 0, 3, 6, 9, 12, 13, and 14. On days 12 – 14, consecutive glucose measurements were made to determine the stability of high glucose levels over time in the later part of the study. The injection volume of a maximum 20 ml/kg^{-1} for an IP injection was derived from a study by Diehl *et al*. (2001). Doses were calculated daily based on the animal's weight and intended volume of 4ml to be injected (Table 1). As recommended by Diehl *et al*., the glucose concentrations (%w/v) were selected in such a way that the total volume to be injected should be less than 20 ml.kg^{-1}. Therefore, 45% and 50% w/v glucose solutions were selected for the first and second week respectively given the fact that the maximum glucose dose of 20 ml.kg^{-1} was lethal in several rats. Volume matched control groups were studied in concert to each of the

Blood Pressure (BP) Measurement

Throughout the treatment period, blood pressure was monitored on days 0, 3, 6, and 12 via tail cuff plethysmography. At the end of the final study day (on 15th day), in-line blood pressure measurements were determined for comparison as described in the surgical procedure.

Surgical Procedure

On day 15, rats were weighed, anesthetized with a single dose of thiobutabarbital (Inactin, 120 mg.kg^{-1} IP) and a carotid arterial catheter (PE-50, Becton Dickinson, Sparks, MD) was implanted for acute determination of blood pressure and heart rate. In order to facilitate breathing, rats were intubated with a tracheal cannula (PE-240, Becton Dickinson, Sparks, MD). The

carotid catheter was connected to a pressure transducer which is coupled with a polygraph system (Biopac Systems) and a personal computer. Throughout the treatment period, blood pressure was monitored on days 0, 3, 6, and 12 via tail cuff plethysmography. At the end of the final study day (on 15th day), in-line blood pressure measurements were determined for comparison as described in the surgical procedure.

Statistics

Data were expressed as mean ± SE and analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test when appropriate (INSTAT 3). Some data were also analyzed using one-way ANOVA followed by Dunnette tests or unpaired student's t-test when appropriate. $P < 0.05$ was considered significant.

RESULTS

Food, Water Intake and Body Weight

Glucose-enriched food as well as water consumption and body weight were recorded, as the average of the first and second week values. Food and water intake as well as body weight were significantly decreased for many of the selected doses. Moreover, body weight, and food and water consumption were decreased at the maximum dose (D-15), where a sustained elevation in blood glucose was observed.

Table 2 Effect of glucose treatment on food, water intake, and body weight. *P<0.05represents food and water consumption for glucose treatments in the first and second week as compared to the volume matched controls (control-1 vs D1-D11, control-2 vs D-12, control-3 vs D13-D14) (one-way ANOVA followed by Dunnett test or student's t-test). *P<0.05also indicates significant level for body weight in the first and second week when compared to the initial weight of corresponding group. Values are Mean±SEM (n = 3 for each group). This should be legend of Table 2.

Table 3 Blood glucose measurement. Blood samples were obtained from the tail vein in each of the treatment groups (saline treated, n=3, and glucose treated, n=3). Blood glucose levels were erratically changed and no significant increase in glucose levels with time was observed for the entire period of treatment. Values are expressed as mean ± SEM.*P < 0.05 for the glucose treated groupson day 0, day 3, day 6, day 9, and day 12 when compared to the volume matched control as follows: control-1 vs. D1-D11, control-2 vs. D-12, control-3 vs. D13-D14 (two-way ANOVA followed by Bonferroni tests). This should be the legend of Table 3.

Blood Glucose Measurements

Doses 1 – 14 (Table 1) produced an increase in blood glucose (Table 3); however this elevation was not maintained throughout the two week study period. As noted, the study goal was to achieve a sustained increase in blood glucose (>170 $mg.dl^{-1}$) over the entire study period. D-15 produced

Table 2. Effect of Glucose Treatment on food intake, water intake and body weight

Dose/ Control	Food Intake		Tap/Glucose water intake (in ml)		Body Weight (in gram)		
	1st week	2nd week	1st week	2nd week	Initial Weight	1st week	2nd week
Control-1	22.76±1.65	24.76±1.25	24.76±0.83	25.76±1.80	273.33±6.57	291.6.83	329.14±8.10*
D-1	31.24±0.78*	34.9±0.039*	42±0.57	34.38±0.45	259.33±2.91	269.81±2.81	301.52±4.49*
D-2	25.24±0.83	33.62±1.40*	72.95±10.10*	34.19±1.72	260±5.77	261.81±9.6	285.24±6.53
D-3	20.67±0.74	38.57±3.40*	76.71±3.90*	51.05±1.95*	259.33±2.91	245.14±5.12	234.76±3.93*
D-4	22.67±0.34	28.86±1.30	73.33±4.10*	31.71±3.88	254.67±3.53	245.33±3.56	242±5.20
D-5	24.38±0.78	22±0.17	24.48±2.93	25±1.67	292±6.11	297.24±5.80	308.95±4.83
D-6	26.86±1.35	25.48±0.84	16.33±0.88*	14.9±0.42*	266.67±3.33	269.14±5.02	278.28±4.68
D-7	22.95±0.53	20.67±0.74	15.95±1.28*	14.38±0.74*	254.67±6.67	250.85±10.42	255.24±13.30
D-8	20.29±1.08	20.86±1.03	21.67±0.19*	26.76±1.34	290.67±4.81	282.19±2.56	285.33±6.64
D-9	21.86±1.08	24.1±1.82	20.62±0.47*	19.86±2.00	286.67±8.82	283.24±7.36	278.86±7.73
D-10	22.57±0.59	25.9±1.81	24±0.49	21.05±2.10	262.67±4.70	264.38±4.81	276.48±5.24
D-11	23.9±0.42	24.95±3.56	13.05±0.17*	12.33±0.94*	254±4.20	242.19±3.16	243.52±1.33
Control-2	22.48±1.42	20.29±1.31	26.43±0.76	22.14±1.59	274.67±5.70	282.29±5.95	305.71±8.65*
D-12	10.29±0.33*	9.81±1.40*	25.48±2.04	28.95±2.93	284.67±14.44	272.76±8.39	263.62±8.35
Control-3	23.62±1.49	21.43±1.03	34.95±5.70	32.76±1.16	273.33±8.82	280±9.32	293.9±9.40
D-13	7.33±0.67*	10.24±0.45*	29±3.65	27.29±4.16	292±6.43	267.43±3.57*	270.48±3.89*
D-14	9.43±0.49*	10±0.76*	27.29±6.60	23±7.22	282±14.42	265.62±11.38	255.71±16.26
Control-4	24.95±0.62	25.9±0.99	35.33±5.31	47.81±7.16	289.33±8.67	289.24±9.76	298.86±14.37
D-15	9.14±0.44*	9.52±1.40*	20.48±2.18	26.1±4.99	302±1.15	286.1±2.06*	269.81±5.63*

Table 3.

Doses/ Control	Glucose Levels (Mean±SEM) in $mg.dl^{-1}$				
	Day 0	Day 3	Day 6	Day 9	Day 12
Control-1	69±4.51	70±1.73	76±3.22	76.67±2.96	89±5.00
D-1	86±1.00	74.33±0.33	73.33±1.33	71±3.46	76±6.00
D-2	76±3.06	80.67±2.67	80±4.73	83±6.11	86.33±5.24
D-3	78.67±4.98	70.67±0.88	79.67±9.17	81.33±4.1	94.33±8.41
D-4	77.33±2.67	68.33±1.76	73.33±3.18	70.33±6.33	70±1.73
D-5	72.67±1.86	77.67±3.38	81.67±6.44	187.33±52.23*	125±30.53
D-6	76.67±4.41	115±20.52	91.67±6.69	71±4.36	109±4.51
D-7	80.67±2.96	111.67±22.26	85.33±0.67	80±13.12	100±4.51
D-8	78.67±3.71	113.33±15.51	96.67±10.67	114±10.00	85±2.00
D-9	79±5.69	115.33±29.34	93.33±1.76	78.67±1.33	119.33±21
D-10	80.67±5.81	87±6.56	95±13.80	126.67±1.20	87.33±14.33
D-11	78.67±3.28	95±4.61	129±24.43	151.33±35*	132.33±55.87
Control-2	71.67±1.67	73±1.53	81.33±5.70	75.33±2.03	73.67±1.20
D-12	73.33±3.53	151.33±63.19	132.67±33.77	112.33±8.51	123.33±25.26
Control-3	71±1.53	75.33±1.20	74.67±0.33	84±3.79	75.33±4.33
D-13	71.33±1.33	91±7.21	83±2.31	99.67±10.14	99±11.02
D-14	75.33±2.91	91±11.14	82.33±9.39	200.67±44.08*	110±12.49

a sustained increase in blood glucose throughout the two week study period. Blood glucose was also measured on day 13 and 14 with no significant difference in plasma glucose was observed between day 13 and day 14 of the study. However, albeit many doses showed significant dose and/or time effects on increment of glucose levels in the overall two-way ANOVA test, there were no significant interactions shown in the statistical test. Thus, no posttest statistical analysis was required when no interaction between dose and time effect was present. On the other hand, Bonferroni posttests were administered when an interaction between dose and time effect was identified. D-5, D-11, and D-14 had significantly enhanced glucose levels to 187.33, 151.33, and 200.67 $mg.dl^{-1}$ only on day 9 with no significant increase in glucose levels at any of the later time points (data for day 13 and 14 not shown).Therefore the doses in table 3 were excluded from further study given that a sustained elevation in blood glucose was not observed.

Although 14 doses were found to be nonsignificant, dose-15 (D-15, details in table 1) was able to establish a persistent and stable hyperglycemia over the 2 week study period. In the D-15 group glucose levels started to significantly but moderately increase from day 3 through day 6, and progressively increase from day 9 (265 $mg.dl^{-1}$) today 12 (371 $mg.dl^{-1}$). Moreover, measurements in a small subset of animals from day 13 showed a similar glucose level to day 14, indicating that a peak and sustained elevation in blood glucose level was achieved. It is noteworthy that post-injection glucose levels in the D-15 group on day 12, 13 and 14 in most of the animals could not be determined, because they exceeded the glucose meter's maximum measurement capacity of 600 $mg.dl^{-1}$. Therefore, it is possible that the glucose levels may go up far beyond 600 $mg.dl^{-1}$ during later time points (after 3 hours of injection) of our study period.

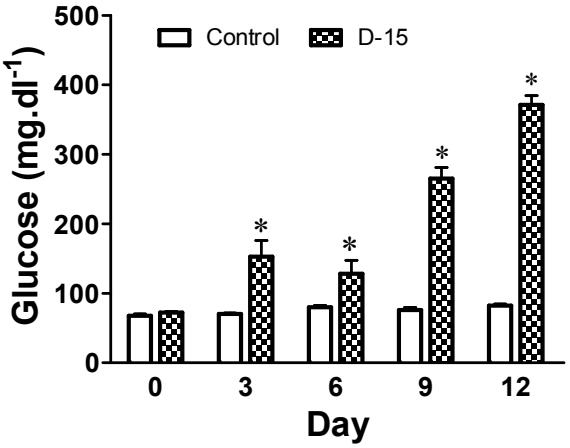

Fig.1. Blood glucose levels in the D-15 group of animals. Values are expressed as mean ± SEM. *P <0.05 in the D-15 glucose treated group when compared to the control-4 group (Two-way ANOVA followed by Bonferroni tests)

Blood Pressure Measurement

Tail-cuff method

Blood pressure was measured in the afternoon of days 0, 3, 6, 9, and 12 before administration of the IP glucose injections. MAP was significantly increased in a dose dependent manner in all of the study groups (D1 – D15) with no present significant interaction between time and dose factor.

Table 4: Blood pressure measurement. Blood pressure was measured via tail cuff plethysmography for each of the groups (saline treated, n=3, and glucose treated, n=3). Values are expressed as mean ± SEM. *P <0.05 for the glucose treated groups on day 0, day 3, day 6, day 9, and day 12 when compared to the control as follows: control-1 vs. D1-D11, control-2 vs. D-12, control-3 vs. D13-14 (two-way ANOVA followed by Bonferroni tests). Table 4 has been excluded. Table 4 should be included from the originally sent manuscript. This highlighted text should be the legend of Table 4.

In the D-15 group there was a significant increase in MAP beginning on day 3 with a consistent elevation in MAP throughout the two week study period. Interestingly, MAP was not persistently raised in concert with the glucose levels though it showed significant increments of 33, 27, and 29 on day 6, 9 and 12 respectively when compared to control (Figure 2). Thus, demonstrating that once hyperglycemia is established there is concurrent maximal sustained elevation in MAP.

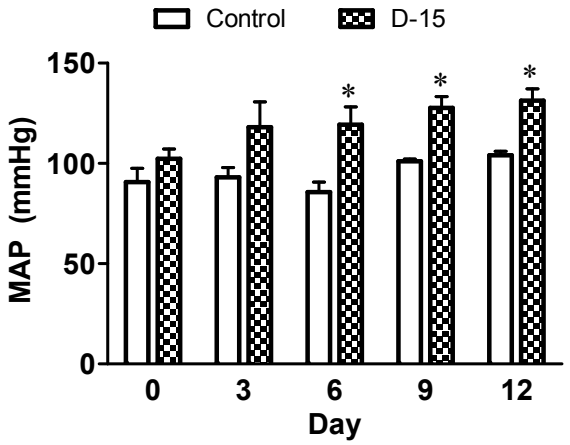

Fig.2. Blood pressure measurement in the D-15 treatment group. Values are expressed as mean ± SEM. *P <0.05 for the D-15 glucose treated group on day 0, day 3, day 6, day 9, and day 12 when compared to the control-4 (Two-way ANOVA followed by Bonferroni tests)

In-line BP measurement via Biopac Systems

BP and heart rate was monitored on day 14 in anesthetized rats to verify the tail cuff results. MAP was significantly increased by 18 mmHg when compared to control (Figure 3A), where a much larger difference was measured via tail cuff. The differences in MAP observed in the tail cuff and Biopac results are more than likely due to awake vs anesthetized animal comparison. However, both tail cuff and Biopac BP were elevated in comparison to control. No significant differences in heart rate were observed (Figure 3B).

Plasma Insulin Levels

Chronic glucose feeding combined with IP injections resulted in a slower blood glucose disposal response by endogenous insulin leading to sustained hyperglycemia. To determine if the observed hyperglycemia was due to impaired glucose disposal, decreased insulin release, or insulin resistance; plasma insulin was measured on day 14 and a significant increase in insulin

(8.92 vs. 27.24 in control vs. glucose-treated group respectively) was observed (Figure 4).

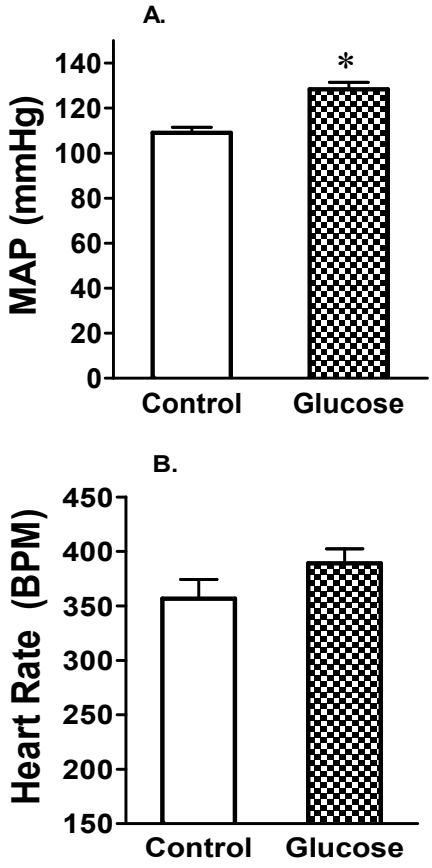

Fig.3. A. In-line blood pressure measurement in the D-15 group of animals. Values are expressed as mean ± SEM. *P < 0.05 for the glucose treated group compared to the corresponding control (Student's t-test). B. Heart rate: There was no significant difference in HR between control and the glucose-treated group

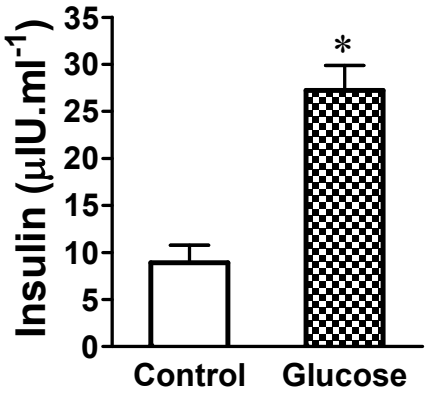

Fig.4. A. Plasma insulin levels in the D-15 group of animals. Values are expressed as mean ± SEM. *P < 0.05 for the glucose treated group compared to the corresponding control (Student's t-test)

DISCUSSION

Postprandial handling of nutrients such as glucose, amino acids, and triglycerides/fatty acids is solely controlled by beta cells of the pancreas through insulin synthetization and secretion. Among all nutrients, glucose is the predominant determinant of insulin secretion, where secreted insulin subsequently stimulates the liver and peripheral tissue to uptake glucose, thus maintaining glucose homeostasis.According to the American Diabetes Association, (2001), postprandial blood glucose usually returns to normal physiologic levels in nondiabetic individuals within 2-3 hours after the start of a meal. However, prosprandial glucose levels can be controlled by many factors including glycemic index, quantity and composition of the meal (American Diabetes Association, 2001). For example, consumption of a high carbohydrate meal is likely to delay glucose disposal as it requires increased amounts of insulin to be secreted. In the current study, insulin's ability to facilitate pheripheral disposal or metabolism of glucose in animals was compromised by administering high doses of glucose in feed, water, and via IP injections. Glucose levels rose moderately in the early days of the study followed by a robust elevation to 265mg.dl^{-1} by day 9 and 371mg.dl^{-1} by day 12. The observed increase in blood glucose levels was maintained on day 13 and day 14. Even as the morning pre glucose loading levels remained moderate (approx. 130 mg.dl^{-1}), the majority of the post-injection (>3h) glucose levels from day 12 through day 14 were beyond maximum capacity (≤600 mg.dl^{-1}) of measurement by the glucose meter. Thus, suggesting that beta cells' insulin secretory actions is either exhausted or a significnat insulin resistance is present. To address this very interesting question, plasma insulin was measured and determined to be siginicantly elevated. Therefore, given that hyperglycemia and hyperinsulemia were concurrently present confirms the development of peripheral insulin resistance. Interestingly, hyperinsulinemia itself can greatly contribute to insulin resistance as evident in numerous studies, which indicates that hyperinsulinemia should not only be considered as a compensatory response to persistent hyperglycemia, but also as a self-perpetuating cause of the present defect in insulin action (Del Prato *et al*., 1994; Shanik *et al*., 2008; Kanety *et al*., 1994; Pederson *et al*., 2001; Gavin III *et al*., 1974; Heaton and Gelehrter, 1981; Paz *et al*., 1997).

Several studies show that animals fed a high sugar content (fructose, sucrose, glucose), and fat-enriched diet have a significantly increase in blood pressure, and insulin resistance with a concurrent hyperglycemia, which confers the presence of the metabolic syndromes of type 2 diabetes (Wang *et al*., 2012; D'Angelo *et al*., 2005; Reaven and Ho, 1991; Martinez *et al*., 1994; Gajda *et al*., 2007). Among carbohydrates, fructose and sucrose are increasingly involved in insulin resistance, hypertension, obesity, and hypertriglyceridemia all of which are consistent with the metabolic syndrome (Gajda *et al*., 2007; Bascian*oet al*., 2005; Malik *et al*., 2010). However, studies involving glucose-fed animals are scarce and limited to only feeding of glucose with no intraperitoneal loading and demonstrate either no or modest increases in glucose levels with varying production of hyperinsulinemia and hypertension (Reaven and Ho, 1991). The current study employed the strategy of intraperitoneal glucose injection along with oral loading, where incremental increases in blood glucose were observed throughout the study. Consistent with current literature, a significat hyperinsulinemia was present in the current study as well (Reaven and Ho, 1991; Midaoui and de Champlain, 2002). In the fructose- and sucrose-fed models, obesity concomitantly develops with insulin resistance making it difficult to know the exact cause of IR as obesity per se can impair insulin sensitivity. In our study, the obesity factor is not present since all animals are lean. In our hyperglycemic model, emergence of hyperinsulinemia can be explained by the fact

that beta cells can undergo hyperplasia/ hypertrophy in the early stages of sustained hyperglycemia in response to increasing demand of insulin to maintain glucose homeostasis. This idea has been supported by studies where sustained insulin secretory overload elicited by either obesity-induced or dietary-induced insulin resistant state concomitantly caused β-cell hyperplasia or increase in β-cell density within the early stages of persistent hyperglycemia development (Tomita *et al*., 1992; Zotto *et al*., 2004).

In line with the development of sustained hyperglycemia, our model has developed hypertension throughout the entire study period. The current data support the premise that hypertension can be developed during mild increases in blood glucose and sustained by a consistent increase in blood glucose and insulin resistance. This observation is supported by other findings where BP was significantly raised resulting from glucose-enriched diet without the development of hyperglycemia (Reaven and Ho, 1991). It is possible that glucose and insulin both together or individually play a role in raising BP through sympathetic activation as previously reported, however, the literature suppots that hyperinsulinemia is demonstrated to be the predominant player in causing hypertension in glucose-induced hypertensive rats (Meehan *et al*., 1994). However, high glucose can also be increasingly involved in causing hypertension, which is exhibited by animals that are fed glucose-enriched diets, where the obeserved hypertension is suggested to be due to an increase in sympathetic nervous system (SNS) activity (Kaufman *et al*., 1991). In addition, high glucose can promote oxidative stress through multiple potential pathways (eg. NADPH oxidase, AGEs, mitochondrial electron transport chain, uncoupled eNOS) (Fakhruddin *et al*., 2017) in heart, vasculature, and kidney that may promote hypertension. For example, glucose-fed rats had an increase in aorta superoxide anion (O_2^-) production leading to elevated blood pressure which was normalized by antioxidant, α-lipoic acid, treatement suggesting that oxidative stress was involved in the observed hypertension (Midaoui and de Champlain, 2002). Blood pressure can also be upregulated via increased local production of renal angiotensin II and oxidative stress in hyperglycemic individuals (Suzaki *et al*., 2007; Abdo *et al*., 2014). Hypertension is one of the most complex cardiavascular abnormalities given the present interplay of a diverse number of factors. Thus this new chronic hyperglycemic and hypertensive lean rat model can be employed to study many different diabetic as well as cardiovascular complications.

Conclusion

The current study has developed a chronic hyperglycemic and hypertensive rat model through glucose-enriched dietary intervention along with intraperitoneal glucose loading. This strategy enables glucose levels to be gradually increased to considerably high levels at the later part of the study. Increased blood glucose levels were also accompanied with high plasma insulin levels, where both promoted a significant elevation in blood pressure. This model can be used to investigate different complexities, which occur in diabetes and hypertensive patients without complications that arise due to surgery or drug treatments.

Conflict of Interest

The authors declare no conflicts of interest.

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