

## Physiology

**KEYWORDS:** Fructose-alloxan, Type-2 diabetic rat model, Fructose.

## A MODEL OF TYPE II DIABETES IN RAT USING LOW FRUCTOSE DIET AND ALLOXAN



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### ABSTRACT:

This study aimed at developing a type II diabetic animal model with low mortality, suitable for chronic studies using low fructose diet and a single injection of alloxan. Thirty male Wistar rats weighing between 150g-200g were equally divided into five groups. Group 1 (control) drank distilled water while groups 2 to 5 rats drank 20%w/v solution of fructose freely for 7 days. Thereafter, groups 2, 3 and 4 rats received 20mg/kg-i.v, 40mg/kg-i.v and 150mg/kg-i.p alloxan respectively. Fasting Blood Glucose (FBG) was measured after fructose administration then 24 hours, 6 weeks and 12 weeks after alloxan injection. Groups 2, 3 and 4 showed significant increase in FBG (124mg/dl,  $P < 0.05$ ; 286mg/dl,  $p < 0.01$ ; 419mg/dl,  $p < 0.001$ ) compared with control (60mg/dl). These findings showed that low fructose diet with a single injection of alloxan induces a diabetic condition of less severity, depending on the dose and/or route of administration of alloxan.

### INTRODUCTION

Fructose consumption has increased coincidentally with the worldwide epidemics of obesity and metabolic syndrome. Fructose is a primary contributor to human disease as it is metabolized in the liver differently to glucose, and is more akin to that of ethanol. D-fructose changes into glucose in the liver and intestine and is more rapidly glycolyzed by the liver than glucose. Fructose uptake requires a sodium-independent monosaccharide transporter (GLUT-5) for its absorption. Entry of fructose into cells is not insulin-dependent and in contrast to glucose, fructose does not promote the secretion of insulin. When consumed in large amounts, fructose promotes the same dose-dependent toxic effects as ethanol, promoting hypertension, hepatic and skeletal muscle insulin resistance, dyslipidaemia and fatty liver disease. Also similar to ethanol, through direct stimulation of the hedonic pathway and indirect stimulation of the starvation pathway, fructose induces alterations in the central nervous system energy signalling that leads to a vicious cycle of excessive consumption, with resultant morbidity and mortality (Lustig, 2010).

Fructose may induce insulin resistance in part by classic obesity-associated mechanisms thus long-term fructose feeding to the laboratory rats may result in the development of diabetes mellitus (Malik et al., 2010). High dose (about 60%) fructose feeding with streptozotocin has been used by researchers to mimic type II diabetes (Reed et al 2000, Bell et al., 2000). High fructose feed perturbs glucose metabolism causing enhanced rate of lipogenesis and triacylglycerol synthesis leading to insulin resistance (Basciano et al., 2005) while streptozotocin due to its beta cell toxicity, inhibits insulin secretion from pancreatic beta cells (Lenzen et al., 1996). This method has been used to induce type II diabetes in rats, pigs and primates (Dufrane et al., 2006). However, using high fructose diet and streptozotocin to induce type II diabetes usually give rise to severe diabetic condition in laboratory rats and, as such, the rats may not survive for long as most of them die before the end of the

study due to the severity of the diabetes (Williamson et al., 1996, Wilson and Islam 2012). This study aims at using low fructose diet and alloxan to induce type II diabetes of less severity and low mortality within a short period of time.

### METHODOLOGY

#### Fructose administration

D-fructose was procured from Lab Tech Chemicals with minimum assay of 99%. 500g of D-fructose was diluted in 2.5 litres of distilled water to make a solution of 20% fructose. The 20% fructose solution was administered freely to rats in groups two to five through gavage drinking for seven days while control received distilled water.

#### Induction of Diabetes Mellitus

Groups 2 and 3 rats received 20mg/kg-iv and 40mg/kg-i.v alloxan intravenously via the penile vein. The rats were first anaesthetized using 1g/kg Urethane, after which each rat was placed on a dissection board to expose the dorsal side. The penis was extruded by sliding the prepuce downwards while pressing at the base of the penis. The glans penis was held at the very tip while a 24-G scalpel vein injection needle was used to pierce the dorsal penis vein. Alloxan was then delivered through the dorsal penile vein via a syringe attached to the needle (Wynforth and Flecknell 1992). Group 4 was administered alloxan of 150mg/kg intraperitoneally. Group 1 (control) and group five (fructose only) were given 0.5ml of normal saline intraperitoneally.

#### Measurements

The weight and the length of the rats before and after fructose administration were measured from which their body mass index (BMI) and Lee index of obesity were calculated via the formulas below:

$$\text{BMI} = \frac{\text{body weight of rat (g)}}{\text{body length of rat}^2 (\text{cm}^2)}$$

$$\text{Lee index} = \frac{1000}{1} \times \frac{(\text{body weight of rat})^{1/3} (\text{g})}{\text{body length of rat (cm)}}$$

#### Fasting blood glucose (FBG)

Rats were fasted overnight for 16 hours. Drop of blood was collected from the tail vein and its glucose content measured using a glucometer.

#### Statistical Analysis

Data was analysed using ANOVA. P value  $< 0.05$  was considered significant.

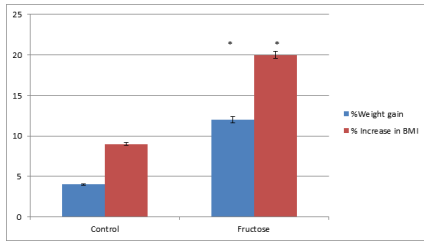
### RESULTS

Weight gain after fructose administration: There was significant ( $P < 0.01$ ) weight gain in the fructose fed rats (18kg) compared with the control (7.5kg). The percentage weight gain in the control rats was 4% while that of the fructose group was 12% (Figure 1).

Body mass index (BMI): The percentage increase in BMI of the rats on the 7th day of fructose administration was 20% as opposed to the 9% increase seen in control (fig. 1).

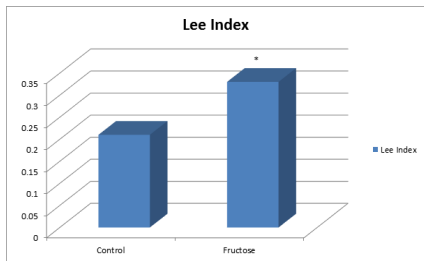
Weight gain and BMI were significantly ( $p < 0.05$ ) increased in fructose fed rats compared to control.

**Lee Index:** This is the index of obesity. As shown in figure 2, control had a Lee index of 0.21 while fructose fed rats had significantly ( $p < 0.05$ ) increased lee index of 0.33.



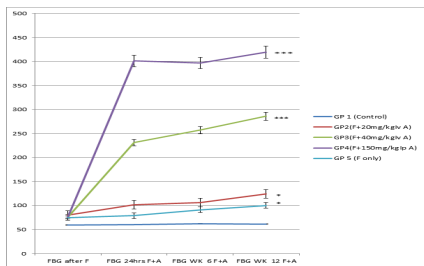
**Figure 1: Effect of fructose drinking on weight gain and body mass index (BMI)**

Values are expressed as mean  $\pm$  S.E.M \* $P < 0.05$



**Figure 2: Effect of Fructose drinking on Lee index**

Values are expressed as mean, \* $P < 0.05$



**Figure 3: Fasting blood glucose after fructose and alloxan administration**

Values are expressed as mean  $\pm$  S.E.M \* $P < 0.05$ , \*\*\* $p < 0.0001$

**Fasting blood glucose after fructose and alloxan administration**

The fasting blood glucose of rats after 20% fructose for seven days and a single injection of alloxan is illustrated in Figure 3. There was no significant increase in fasting blood glucose in group 1 (control). Fasting blood glucose (FBG) was slightly increased in wister rats after fructose drinking (fig. 3). In Group 2 (20mg/kgiv A), there was significant increase in FBG (102  $\pm$  3mg/dl) after 24hours of alloxan injection. The FBG rose slightly over the 12weeks study duration and was maintained at a moderately hyperglycemic value of 124  $\pm$  5mg/dl at the end of the study. There was significant increase ( $P < 0.001$ ) in FBG in Group 3 (40mg/kgiv A) 24 hours, 6 weeks and 12 weeks after alloxan injection i.e. from 76mg/dl after fructose feeding and before 40mg/kg i.v alloxan injection to 231 mg/dl 24hours later and 286mg/dl 12 weeks later. Group 4 (150mg/kgiv A) had a great significant increase ( $P < 0.0001$ ) in the FBG with a post-fructose pre-alloxan FBG of 76mg/dl and 401mg/dl 24hours after alloxan injection and 419mg/dl at the end of the study. In Group 5 (F only) there was no significant increase in fasting blood glucose after 24hours of administration but the FBG increased significantly ( $p < 0.05$ ) over a period of 12 weeks post-fructose administration, but not as high as that of the rats that received alloxan injection after fructose feeding.

**DISCUSSION**

This study has demonstrated that low dose fructose and a single injection of alloxan can be used to induce type II diabetes in rats. Effects of 20% fructose drinking for 7 days plus alloxan injection were increased weight, BMI and FBG elevation.

The weight gain observed in the fructose fed rats was due to rapid stimulation of lipogenesis and triglyceride accumulation (Basciano et al., 2005). If fructose is ingested for a prolonged period of time, it will lead to excessive weight gain (Chicco et al., 2003) and excessive increase in BMI. Twenty percent fructose drinking, together with normal feed ad libitum for 7 days, led to increased rate of weight gain and Lee index in rats. Lee index determination at the end of the fructose administration period revealed that the rats were obese as they had a mean index greater than 0.3. A lee index greater than 0.3, is an indication of obesity in rats. It is correlated with increased body fat (Bernardis and Patterson 1968; Bernardis 1970; Simson and Gold 1982). The adoption of hyper caloric diets has been used as a model to induce obesity in animals due to its similarity to the genesis and the metabolic responses arising from obesity in humans (Nascimento et al., 2008). Fructose (of non-natural sources) taken in diet is usually converted to fat in the liver via lipogenesis (Schwarz et al., 1994; Basciano et al., 2005), thus leading to the build-up of fat.

In this study, it was observed that 20% fructose intake for seven days increased fasting glycaemia. The increased fasting glycaemia and thus, the pre-diabetic state caused by fructose may be due to rapid stimulation of lipogenesis and triglyceride accumulation during fructose metabolism, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance (Basciano et al., 2005). The findings in this study was similar to that of Stanhope et al., (2008) who investigated in human participants the metabolic effects of consuming 25% fructose sweetened beverages with normal diet for 2 weeks and found out that it increases weight gain, visceral adiposity, dyslipidaemia and insulin resistance. These metabolic effects were also seen in rats fed 20% fructose for 7 days in this study.

Alloxan is a known  $\beta$ -cell toxin that has widely been used in inducing type II diabetes. Lenzen, (1991) have shown that alloxan selectively inhibits glucose-induced insulin secretion through its ability to specifically inhibit the glucokinase through oxidation of functionally essential thiol groups in this protein thereby impairing oxidative metabolism and glucose sensor function of this signalling enzyme of the beta cell. Administration of alloxan exacerbated the prediabetic hyperglycemia caused by short term 20% fructose drinking thus leading to a non-reversible diabetic state. The severity of the diabetes was observed to be dependent on the dose of alloxan, and the route of administration of alloxan and nutritional status of the animals. Considering that obesity developed in the rats prior to hyperglycaemia, the type of diabetes induced was most likely that of type-2. Reed et al., (2000) and Si et al., (2012) have successfully used high calorie diet and STZ dose as high as 50mg/kg intravenously to induce type 2 diabetes in laboratory rats. Wilson and Islam (2012) induced type 2 diabetes in Sprague-Dawley rats using 20-40% fructose and a single injection of streptozotocine (STZ). However, the rats in this study were able to survive the hyperglycaemia for the 12 weeks post alloxan period, unlike in the study of Wilson and Islam (2012) where rats fed with 20-40% fructose for two weeks before a single intraperitoneal injection of 40mg/kg STZ all died three weeks into the 11 weeks study due to the severity of the diabetes except the group given 10% fructose plus 40mg/kg STZ. In Wilson and Islam study, 10% fructose for 2 weeks and 40mg/dl i.v STZ resulted in a mean FBG as high as 450mg/dl however, in this study 20% fructose for one week and 40mg/dl iv alloxan resulted in moderate hyperglycemia with a mean FBG of 286mg/dl at the end of 12 weeks. Therefore, one may conclude that although the combination of high fat or fructose diet and STZ seem very compatible for type 2 diabetes induction, fructose and alloxan appear to be more efficient in inducing type 2 diabetes of less severity, longer tolerance and minimal mortality in

Wister rats.

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