Plasma lactate, insulin concentration and intestinal glycogen deposition responses to fructose infusion in male dogs

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Ingested carbohydrates digested to monosaccharides are absorbed directly into the bloodstream by the intestinal epithelial cells; recent research has shown intravenous infusion of fructose causes increase intestinal glucose uptake (IGU) and increase in blood glucose, however the fate of glucose has not been reported if it was metabolized or oxidized in the process. Ten fasted male anaesthetized adult dogs (Mongrel) were used for his experiment; the animals were divided into two groups (5 dogs per group). Control group received normal saline while the treatment group were infused with fructose (0.73 mg/kg/min). Through a midline laparotomy, the upper jejunum was cannulated for blood flow measurement and blood samples were obtained for measurement of glucose content of the arterial blood and venous blood from the upper jejunal segment. Mean intestinal glucose uptake (IGU) increased significantly from 38.86±2.44 (mg/dl) to 162.78±7.31 (mg/dl) in 30 min which was at peak uptake. Mean lactate concentration increased significantly from 66.45±2.02 (mg/dl) to 98.00±7.93 (mg/dl). However, there was a marginal increase in insulin concentration from 25.00±1.77 (mg/dl) to 27.25 (mg/dl) and no evidence of glycogen deposition in the small intestine. This study showed that intestinal glucose uptake increased in response to fructose infusion and probably the absorbed glucose after the infusion was converted to lactate with no trace of intestinal glycogen deposition.

Key words: Carbohydrates, Mongrel, laparotomy, metabolized, infused, cannulated.

INTRODUCTION

Fructose or fruit sugar is a simple ketonic monosaccharide found in many plants, often bonded to glucose to form the disaccharide sucrose. Discovered by French chemist Augustin-Pierre Dubrunfaut in 1847 (Fruton, 1972), it is one of the three dietary monosaccharaides, along with glucose and galactose, which are absorbed directly into the bloodstream during digestion and the sweetest of all naturally occurring...
carbohydrates (Shallenberger, 1993). During absorption, fructose transports across the apical membrane by GLUT5 and/or GLUT2 via facilitated diffusion (Schürmann, 2008). After absorption, monosaccharides are transported to the liver, which plays a key role in glucose homeostasis. Salman et al. (2014) found out that intravenous infusion of fructose caused about 67% increase in intestinal glucose uptake (IGU) and about 15% increase in blood glucose, so the question is what is the fate of the glucose that was absorbed by the intestine? Was it metabolized or oxidized? Was it converted to lactate in the blood or stored as glycogen in the small intestine? Therefore, this study aimed to determine the responses in insulin concentration, plasma lactate concentration, intestinal glucose uptake and tissue glycogen deposition following the infusion of fructose.

MATERIALS AND METHODS

Experimental animals and protocol

Ten male dogs (Mongrel) weighing between 8-15 kg were used for the study. The dogs were obtained from the Central Animal House, University of Ibadan, Nigeria. The animals were fed and given water ad libitum during acclimatization and the experiment. Housed in metal mesh cages under a photoperiod-controlled environment (12 h L: 12 h D cycles), the Mongrels were divided into two groups (5 dogs per group). Control group received normal saline while the treatment group were infused with fructose (0.73 mg/kg/min) during the experiment. All animals were cared for in accordance with the internationally accepted principles for laboratory animal use and care in the European Community (European Economic Community, 1986). Each animal was fasted for 18-24 h before the start of the experiment. Anesthesia was induced by an intravenous injection of 30 mg/kg – body weight of sodium thiopental. Light anaesthesia was maintained with supplementary doses of sodium thiopental as necessary. The animal was laid supine and firmly secured on the dissecting table. The trachea intubated with a Y-piece cannula, which allowed the animal to breathe room air spontaneously. The right and left femoral arteries and veins were exposed by incisions and cannulae were inserted into them. The cannula placed in the left femoral vein was connected to the infusion pump (Palmer, England) for infusion of the fructose. Arterial blood samples were collected from the right femoral artery while the right femoral vein was used for administration of the drugs or normal saline (Alada and Oyebola, 1996). Through a midline laparotomy, the jejunum identified and a vein draining the proximal segment of the jejunum was cannulated for blood flow measurement and blood samples were obtained for measurement of glucose content. At the end of the surgical procedure, sodium heparin 300 i.u was administered intravenously to prevent blood clotting and the abdomen was closed in two layers with interrupted sutures.

Experimental procedure

Following surgery, a period of 60 min was allowed for stabilization in all animals. After stabilization, basal measurements of jejunal venous blood flow, and glucose levels for arterial and venous blood glucose concentrations were made. After the basal measurements, the effects of a 30 min intravenous infusion of normal saline (0.2 ml/kg/min), fructose (22 mg/kg/min) were observed. These measurements were repeated at 5, 10, 15, 20, 25, 30, 45, 60, 75 and 90 min post-infusion of the fructose (Alada and Oyebola, 1996). Arterio-venous glucose difference was calculated as the difference between arterial and venous blood glucose concentrations while glucose uptake (mg/min) was calculated as the product of the arterio-venous glucose difference and jejunal blood flow per minute. Blood glucose was determined by the glucose oxidase method using a glucometer. Blood samples were collected at basal and 30 min respectively before and after infusion of fructose into lithium heparinized capillary tubes. Plasma was collected and preserved and -20°C.

Measurement of plasma biochemical parameters

Determination of insulin concentration

Plasma insulin assay was done using Enzyme-linked immunosorbent method with a clinical kit supplied by Monobind Inc. Lake Forest, CA, USA. The manufacturer’s instruction was used in carrying out the procedure. 0.05 ml of the appropriate calibrators, controls and samples were pipetted into the assigned wells with 0.1 ml of the insulin enzyme reagent added to each well. The microplates were swirled gently for 20-30 s to mix and covered with a plastic wrap. The samples were then incubated for 120 min at room temperature. After incubation, the contents of the microplates were discarded by decantation. Subsequently, 0.350 ml of wash buffer was added and decanted. 0.100 ml of working substrate solution was then added to all the wells. Another fifteen minutes of incubation was allowed for and further 0.050 ml of stop solution was added to each well and mixed gently for 15-20 s. Finally, after 20 min, the absorbance in each well was read at 450 nm in a microplate reader. A standard curve was generated by plotting the mean optical density value for each standard on the y-axis against the concentration on the x-axis. Corresponding concentration of insulin was then read.

Determination of lactate concentration

The concentration of lactate in the plasma was determined because L-lactate is oxidized to pyruvate and hydrogen peroxide by lactate oxidase (LOD).

\[
\text{L-Lactate} + \text{O}_2 \rightarrow \text{pyruvate} + \text{H}_2\text{O}_2
\]

Hydrogen peroxide reacted with p-Aminophenazone and p-Chlorophenol in the presence of peroxidase to form a red coloured chromogen.

\[
\text{H}_2\text{O}_2 + \text{p-Aminophenazone} + \text{p-Chlorophenol} \rightarrow \text{Chromogen} + \text{H}_2\text{O}_2
\]

The increase in absorption at 546 nm is proportional to the lactate concentration and can be measured photo-metrically.

Determination of glycogen deposition

Using Van der Kleij method of glycogen estimation (Van der Kleij, 1951), the extraction of glycogen from the small intestine tissue was done with tri-chloroacetyl acid (TCA). A 200 mg sample of small intestine tissue was finely grinded with 20 ml of 5%TCA in a mortar homogenizer. The precipitate was then mixed with iodine reagent (16-5 ml of lugol’s solution) in a calorimetric tube. After mixed, optical density was read on photometer at 650 nm against blank.
Table 1. Blood glucose levels of male dogs infused with fructose.

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>5 min</th>
<th>10min</th>
<th>15min</th>
<th>20min</th>
<th>25min</th>
<th>30min</th>
<th>45min</th>
<th>60min</th>
<th>75min</th>
<th>90min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial Glucose (mg/dl)</td>
<td>97.80 ± 8.09</td>
<td>114.40 ±8.09</td>
<td>118.20 ±9.20*</td>
<td>127.60 ±9.24*</td>
<td>130.20 ±9.05*</td>
<td>133.40 ±9.56*</td>
<td>136.60 ±10.43*</td>
<td>129.40 ±8.89*</td>
<td>129.20 ±8.07*</td>
<td>119.60 ±7.18*</td>
<td>114.00 ±7.67</td>
</tr>
<tr>
<td>Venous Glucose (mg/dl)</td>
<td>92.40 ±8.47</td>
<td>101.20 ±7.51</td>
<td>103.8 ±9.93</td>
<td>112.00 ±9.93*</td>
<td>114.60 ±8.5*</td>
<td>115.60 ±10.65*</td>
<td>119.80 ±11.49*</td>
<td>112.80 ±8.89*</td>
<td>109.60 ±7.68*</td>
<td>104.20 ±7.44</td>
<td>93.20 ±6.14</td>
</tr>
<tr>
<td>(A-V) diff</td>
<td>5.40 ±0.40</td>
<td>13.20 ±0.73*</td>
<td>14.40 ±0.81*</td>
<td>15.6 ±1.63*</td>
<td>15.60 ±1.43*</td>
<td>17.80 ±1.98*</td>
<td>16.80 ±2.21</td>
<td>16.60 ±1.02*</td>
<td>17.60 ±2.33*</td>
<td>15.40 ±1.43*</td>
<td>14.8 ±1.53*</td>
</tr>
</tbody>
</table>

Data expressed in mean ±SEM; n = 5. *Significant difference (p˂0.05) when compared with the basal blood glucose level at 0 min in male dogs.

Determined by adding 2 ml of 5% TCA to 3 ml of reagent in the same way. Glycogen concentration was then read on a calibration curve.

**Determination of blood glucose**

The determination of blood glucose was done using Accu-check glucometer and strips. The meter was checked against the glucose standard solution at regular intervals to ensure accuracy. Previous studies have shown that the values of blood glucose obtained using a glucometer correlate excellently with those from the use of standard biochemical methods. In this study, one large drop of blood (one microliters), was enough for blood glucose at each instance of measurement.

**Statistical analysis**

Data were expressed as Mean ± Standard Error of Mean (SEM). Results obtained from this study were analyzed using Statistical Package for Data Analysis (SPSS) version 17.0 for windows. Analysis of Variance (ANOVA) and students t-test was used to compare means, and values were compared at p<0.05.

**RESULTS**

**Effects of fructose infusion on blood glucose levels in male dogs**

Shown on Table 1 is the effect of fructose infusion on blood glucose level. There was significant (p<0.05) rise in the arterial blood glucose level of about 30% when it peaked at 30 min after infusion. It increased from basal level of 97.80 ± 8.09 (mg/dl) to 136.60 ± 10.43 (mg/dl) at peak level. There was significant (p<0.05) increase in the venous glucose at 15 to 45 min after infusion. The arterio-venous difference showed a significant increase at all instances except for 30 min of infusion.

**Effects of fructose infusion on blood flow in male dogs**

The effect of fructose infusion on blood flow in dogs is shown in Figure 1. There was a marginal increase in blood flow following the intravenous infusion of dogs with fructose (0.73 mg/kg/min). Peak flow was observed at 30 min.

**Effect of fructose infusion on intestinal glucose uptake (IGU) in male dogs**

The effect of intravenous fructose infusion on glucose uptake is shown in Figure 2. There was significant (p<0.05) increase in the mean intestinal glucose uptake from 38.86±2.44 (mg/dl) at basal level to 162.78±7.31 (mg/dl) at peak. The increase in intestinal glucose uptake was about 320% increase in uptake. Similarly, the increase observed which started at 5th min after infusion peaked at 30th min and reduced to almost basal level at 90th minute.

**Effect of fructose infusion on plasma insulin concentration in male dogs**

There was no significant change in plasma insulin concentration of the control and treated dogs when comparing basal values with other values after 30 min of infusion (Figure 3).

**Effect of fructose infusion on plasma lactate concentration of male dogs**

The effect of fructose infusion on plasma lactate concentration is shown in Figure 4. In the control group, a decrease from 73.65±3.86 (mg/dl) at basal to 68.7±2.42 (mg/dl) at 30 min which was not significant was observed. However, in the experimental group, a significant (p<0.05) increase from 66.45±6.11 (mg/dl) at basal to 98.0±7.93 (mg/dl) at 30 min respectively was observed. When control group was compared with experimental group at basal and 30 min respectively, there was marginal decrease at basal and a significant increase at 30 min respectively (Figure 5).

**DISCUSSION**

The intravenous infusion of fructose had no significant effect on intestinal blood flow, which
agrees with the work of Brundin and Wahhren (1993) as well as Salman et al. (2014), both of whom did not observe any effect of high fructose diet on blood flow. The hyperglycemia induced by fructose infusion is consistent with the well-established effect of administration of fructose in many reported studies (Hue, 1987; Annudi et al., 1987). Although, the level of increase in blood glucose during and after fructose infusion was not as high as that produced in glucose-induced hyperglycemia, which was reported by Salman et al. (2014).

The results of this study showed that intravenous infusion of fructose elicited increase in blood glucose level as well as increased intestinal glucose uptake...
The effects of fructose infusion on plasma insulin concentration of male dogs. Data expressed in Mean ± SEM; n = 5. *significant difference (p<0.05) compared with basal insulin concentration after 30 min of infusion.

Figure 3. The effects of fructose infusion on plasma insulin concentration of male dogs. Data expressed in Mean ± SEM; n = 5. *significant difference (p<0.05) compared with basal insulin concentration after 30 min of infusion.

IGU). This observation is in total agreement with the earlier conclusion of Alada and Oyebola (1996) that the intestine is capable of taking up large quantities of glucose, especially when blood glucose level increases. Levine and Haft (1970) and Butler and Rizza (1989) had reported that in both insulin sensitive and insulin-insensitive tissues, the blood glucose concentration is a key factor determining the rate of glucose uptake. The present result therefore leads to the conclusion that the gastrointestinal tract glucose uptake is largely in response to the blood level of glucose. If the blood glucose is very high, glucose uptake is very high.

The marginal increase in the plasma insulin concentration observed implies that insulin does not mediate the increased intestinal glucose uptake induced by fructose, which is similar to that observed in the hepatic uptake of glucose (Smith et al., 1953). After absorption in the gastrointestinal tract, fructose is transported via the portal circulation to the liver, where it enters hepatocytes via the glucose transporter GLUT5-independently of insulin - and is rapidly metabolized (Smith et al., 1953; Sato et al., 1996). In addition, phosphofructokinase, a hepatic enzyme that governs glycolysis in liver, negatively regulates glucose breakdown while fructose can evade this rate-limiting control mechanism and is metabolized into glycerol-3-phosphate and acetyl-coenzyme A. These two intermediate metabolites are then used as substrates for glyceride synthesis, contributing to very low-density lipoprotein (VLDL) triglyceride production in the liver [15].

Furthermore, due to the lack of GLUT5 expression in β-cells, fructose unlike glucose does not directly stimulate pancreatic insulin release. The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and triglyceride accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance (Qu et al., 2007).

In Figure 4, the significant increase in the Plasma lactate concentration observed suggests that the fate of glucose that has been taken up by the small intestine
Figure 4. Effect of fructose infusion on plasma lactate concentration of male dogs. n = 5. *Significant difference (p<0.05) compared with the basal blood lactate level at 0 min.

Figure 5. Glycogen deposition in the small intestine tissues of male dogs following fructose infusion. *significant difference (p<0.05) compared with tissue glycogen deposition in the control n = 5.
could be converted to lactate. It is well established that glycogen is made and stored primarily in the cells of the liver and the muscles, and functions as the secondary long-term energy storage. Therefore this study investigated the possibility of deposition of glycogen in the tissue of the small intestine using Van der Kleij method of glycogen estimation (Van der Kleij, 1951). With no observable traces of glycogen deposition in the tissues of the control and the experimental groups, which suggests that the absorbed glucose was not stored as glycogen in the tissues of the small intestine (Figure 5).

Conclusion

In this study, it was established that the intravenous infusion of fructose elicits increased intestinal glucose uptake (IGU) by about 32% and the fate of the absorbed glucose after infusion was converted to lactate. In addition, fructose-induced increased intestinal glucose uptake (IGU) was not insulin-dependent with no trace of intestinal tissue glycogen deposition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES