



ORIGINAL ARTICLE

The effect of combined inositol hexakisphosphate and inositol supplement in streptozotocin-induced type 2 diabetic rats

Shadae R. Foster*, Felix O. Omoruyi[†], Juan Bustamante[‡], Ruby L. A. Lindo* and Lowell L. Dilworth[§]

*Biochemistry Section, Department of Basic Medical Sciences, The University of the West Indies, Mona, Jamaica, [†]Department of Life Sciences, Texas A&M University, Corpus Christi, TX, USA, [‡]Rangel College of Pharmacy, Texas A&M University, Kingsville, TX, USA and [§]Department of Pathology, the University of the West Indies, Mona, Jamaica

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SUMMARY

Inositol hexakisphosphate (IP6) and inositol both regulate insulin secretion, but their combined use in the management of diabetes deserves investigation. The combined effects of IP6 and inositol supplementation were investigated in streptozotocin-induced type 2 diabetic rats. The following groups of rats were studied for 8 weeks: non-diabetic control, non-diabetic high-fat diet control, diabetic untreated, diabetic rats treated with the combination of IP6 and inositol (650 mg/kg bw) and diabetic rats treated with glibenclamide (10 mg/kg bw). High-fat diet and streptozotocin were used to induce type 2 diabetes mellitus in Sprague–Dawley rats. Body weight, blood glucose, glycated haemoglobin, insulin, serum leptin, HOMA-insulin resistance scores, intestinal amylase activity, serum and faecal lipids and food and fluid consumption were measured. Treatment with the combination significantly reduced blood glucose (306 ± 53 mg/dl) and insulin resistance score (1.93 ± 0.45) compared with diabetic controls (522 ± 24 mg/dl and 5.1 ± 0.69 respectively). Serum leptin (2.8 ± 0.6 ng/dl) and faecal triglycerides (108 ± 8 mg/dl) were significantly increased in rats treated with the combination compared with the diabetic control (1.8 ± 0.06 ng/dl and 86 ± 4 mg/dl). Serum triglyceride (47 ± 5.1 mg/dl), total cholesterol (98 ± 3.2 mg/dl) and food intake (26 ± 0.3 g) were significantly reduced by 45%, 25% and 25%, respectively, in rats treated with the combination compared with the diabetic control. Inositol and IP6 combined supplementation may be effective in the management of type 2 diabetes mellitus and related metabolic disorders by regulating some aspects of lipid and carbohydrate metabolism.

Keywords

diabetes mellitus, inositol hexakisphosphate, leptin, lipid, phytic acid

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Correspondence:

Lowell L. Dilworth
Department of Pathology
The University of the West Indies
Mona Campus
Mona
Jamaica
Tel.: 1 876 927 1410
Fax: 1 876 977 1811
E-mail: Lowell.dilworth02@uwimona.edu.jm

Diabetes mellitus is a group of endocrine or metabolic diseases characterized by impaired glucose utilization that leads to chronic hyperglycaemia which is a direct result of a relative or absolute deficiency of insulin. It is characterized by the disruption of carbohydrate, protein and fat metabolism. The metabolic disorders associated with diabetes mellitus development involve several pathogenic processes which are the basis of secondary pathophysiological changes in multiple organs. Type 2 diabetes mellitus is the most prominent form of diabetes, affecting approximately 90% of individuals diagnosed with the disease, and is often asymptomatic (World Health Organization [WHO] 2014). Obesity and physical inactivity are the main contributing factors

associated with the development of type 2 diabetes mellitus, and deaths associated with the disease are very high globally (WHO 2014). This high rate of morbidity and mortality among patients with type 2 diabetes is a result of abnormalities in blood lipid profile which contribute to the development of cardiovascular complications (Ginsberg 2000).

Inositol, also known as myo-inositol, is a pseudovitamin, with a hexacarbon carbohydrate structure. It is found in mammalian cell membranes attached to lipids, as phosphatidylinositol (a signal transduction molecule). Inositol hexakisphosphate (IP6 or InsP6), also known as phytic acid, is a polyphosphorylated inositol derivative. It exists as a phosphorylated carbohydrate in plant and mammalian

cells. Inositol and its derivatives are also common constituents of legumes and whole grains (Vucenik & Shamsuddin 2003). Shamsuddin *et al.* (1989) demonstrated that when IP6 and inositol are combined in the appropriate ratio, it produces two inositol 1,4,5-trisphosphate (Ins (1,4,5)P3 or IP3) signalling molecules, an important cellular regulator.

Inositol compounds play significant roles in the regulation of insulin secretion from pancreatic beta cells (Barker *et al.* 2002, 2009; Vanderlinden & Vucenik 2004; Berggren & Barker 2008). IP6 is shown to function by regulating the mobilization of Ca²⁺ into the cell via the voltage-dependent Ca²⁺ channels (Yang & Berggren 2005). IP6 enhances Ca²⁺-gated channel activity by increasing beta cell phosphorylation state (Larsson *et al.* 1997). This occurs through deactivation of serine/threonine protein phosphatases as well as the activation of serine/threonine kinases. Studies of hippocampal neurons demonstrated that IP6 indirectly activates protein kinase A (PKA) by activating adenylyl cyclase which resulted in increased cyclic adenosine monophosphate (cAMP) production (Yang *et al.* 2001). Other studies demonstrated that protein kinase C (PKC) is directly activated by IP6 in the pancreatic beta cells (Efanov *et al.* 1997; Høy *et al.* 2003). PKC and PKA promote Ca²⁺-induced exocytosis through serine/threonine phosphorylation. The final steps of insulin exocytosis are driven by Ca²⁺ influx (Berggren *et al.* 1993). According to Høy *et al.* (2002), the mode of action of IP6 does involve not only exocytosis but also endocytosis where a more significant effect is seen. The latter is achieved through the activation of calcineurin which dephosphorylates dynamin 1 and accounts for both the activation of PKC and inhibition of the phosphoinositide phosphatase synaptojanin and ultimately phosphatidylinositol 4,5-bisphosphate production (Høy *et al.* 2002).

Shamsuddin *et al.* (1988) hypothesized that IP6 in cells dephosphorylates to form lower inositol phosphates (IP1–IP5). Preliminary studies have shown that IP6 is transported into the gastrointestinal epithelial cells intact where it is dephosphorylated (Sakamoto *et al.* 1993). Other studies have shown that intracellular concentration of lower inositol phosphates increased with IP6 treatment (Vucenik & Shamsuddin 1994; Grases *et al.* 2002). We propose that one of the several ways in which the combination works is by acting as precursors for the formation of lower inositol phosphates and phosphatidylinositols, thereby enhancing its antidiabetic effects. Treatment with the IP6/inositol combination can therefore result in increased inositol IP3 cellular concentration. IP3 plays vital regulatory roles in Ca²⁺ mobilization (Berridge & Irvine 1984; Irvine & Schell 2001; Irvine 2005) and therefore insulin secretion.

The safety of IP6 consumption as well as how efficiently it is absorbed from the digestive tract is still under debate among researchers (Sakamoto *et al.* 1993; Shamsuddin & Vucenik 1999; Grases *et al.* 2001). Ullah and Shamsuddin (1990) reported that IP6 displays no apparent toxicity and is thus deemed safe for consumption, even with long-term

administration. The debate continues, however, with recent research suggesting that IP6 may not be beneficial to humans as it is not thought to be found in mammalian tissue (Wilson *et al.* 2015). This is compounded by the theory that chronic use may be ill-advised owing to risk of trivalent cation deficiency in individuals who are prone to mineral deficiencies (Wilson *et al.* 2015). Nevertheless, important multicellular functions including cell proliferation, signal transduction and differentiation are believed to be regulated by IP6 (Vucenik & Shamsuddin 2006). Studies have reported antidiabetic properties of IP6 in diabetic rats (Lee *et al.* 2006; Kuppasamy *et al.* 2011). These studies have provided valuable information to date; however, the use of an IP6/inositol combination in the treatment of type 2 diabetes has not been studied. It is theorized that the combined IP6/inositol product may be more effective in regulating serum lipids and carbohydrates via various cellular regulatory mechanisms compared with IP6 alone. Studies of this nature are important, as there is no cure for diabetes mellitus and current drug therapies for the management of the disease are costly and have undesirable side effects. As a result, there is emphasis on potential natural alternative antihyperglycaemic remedies with minimal or no side effects. We hypothesized that consumption of an IP6 and inositol combined supplement in a predetermined ratio will be effective in the management of some metabolic disturbances associated with type 2 diabetes mellitus.

Materials and methods

Animal care

Healthy adult male Sprague–Dawley rats (168 ± 5.9 g) were procured from Harlan Laboratories Inc. (Indianapolis, IN, USA). The rats were housed in polypropylene cages with a solid floor and bedding materials. The room was maintained at controlled temperature (22 ± 2°C), with humidity (45 ± 5%) and 12/12-hour light/dark cycle. The animals had free access to standard rat laboratory diet (PicoLab[®] Rodent Diet 20; 5053) or high-fat diet consisting 45% fat as a percentage of total kcal (D12451; Research Diets Inc. New Brunswick, NJ, USA). They were also given clean drinking water *ad libitum*.

Ethical approval statement

All animal experiments were in accordance with and approved in advance by the Institutional Animal Care and Use Committee (IACUC) of the Institute of Biosciences and Technology, Texas A&M Health Sciences Center, Houston, USA (protocol number 011645).

Induction of type 2 diabetes

Induction of type 2 diabetes was carried out according to a modified version of previously used protocols (Danda *et al.* 2005; Srinivasan *et al.* 2005). The rats were fed 45% high-

fat diet for 4 weeks. At the end of week 2, type 2 diabetes was induced by a single intraperitoneal (i.p.) administration of 35 mg/kg body weight of streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.1 M of cold citrate buffer, pH 4.5. This solution was freshly prepared, shielded from light and administered within 5 min of preparation. Control rats were only injected with an equivalent volume of citrate buffer. One week later, the non-fasting blood glucose level was measured and animals with a blood glucose ≥ 300 mg/dl were considered diabetic. To further confirm type 2 diabetes, an antidiabetic drug response test was carried out (briefly described below). The rats were classified as being type 2 DM based on a positive response to glibenclamide.

Experimental design

This animal trial was an 8-week study. For the first 4 weeks of the experiment, six rats were fed a normal basal diet (PicoLab[®] Rodent Diet 20; 5053) and 24 rats were fed 45% high-fat diet. Diabetes was induced in 18 of the rats on the high-fat diet. At the end of week 4, the rats were assigned the following five groups (six rats per group): normal control (NC; non-diabetic rats fed basal diets), high-fat control (HFC; high-fat diet and non-diabetic), diabetic untreated control (DC), diabetic rats treated with combined IP6 and inositol supplement (hereafter referred to as the combination; 650 mg/kg body weight/day) and glibenclamide positive control group (10 mg/kg body weight/day). During the treatment period, all the rats were fed basal diet along with their respective treatment regime as outlined above. The experiment was designed to administer a dosage of 1% IP6 and inositol combination which is equivalent to 650 mg/kg body weight at a ratio of 220:800. The IP6 and inositol used were extracted from rice and supplied by Vita-Tech International Inc. (Tustin, CA, USA). Glibenclamide (pharmaceutical grade) and the combined IP6 and inositol were dissolved in 1% sodium carboxymethyl cellulose (Na-CMC) and were administered once daily via oral gavage. The control groups (NC, HFC and DC) received Na-CMC daily. Glibenclamide is slightly soluble in water, so to improve the solubility and ultimately the bioavailability of this drug, Na-CMC was used as the transport medium.

Antidiabetic drug response test

To confirm the validity and suitability of the type 2 diabetes animal models, its response to the conventional antidiabetic drug (glibenclamide) was tested. Fasting blood glucose concentrations of the diabetic rats were measured. Following this, 10 mg/kg body weight of glibenclamide was administered orally using intubation needles in 1% sodium carboxymethyl cellulose (Na-CMC) at a dose of 0.5 ml/kg body weight. The blood glucose concentration of each rat was then measured 120 min after the administration of glibenclamide.

Blood glucose concentration and body weight measurements

Non-fasting blood glucose levels of each group of rats were measured with the ReliOn blood glucose monitoring system on the following days: 14, 21, 28, 42, 49 and 56. At the start of the experiment, initial body weights were recorded. Rats were weighed once weekly throughout the study.

Fluid and food intake

The amount of food consumed and that of fluid consumed were calculated from the daily unconsumed rat chow and deionized water.

Intestinal analysis

The intestine of each rat was excised, weighed and sectioned into proximal (duodenum) and distal (jejunum and ileum) portions. Sodium chloride (0.9%) solution was used to flush the intestinal lumen several times. The scraped mucosa was homogenized and centrifuged (5000 g), and the supernatant was frozen until required for assays (Percival & Schneeman 1979). Wotton (1964) and Bradford (1976) methods were used to determine intestinal amylase activity and the protein concentration of the intestinal homogenate respectively.

Faecal lipid profile analyses

The Folch *et al.* (1957) method was used for lipid extraction from the faecal samples. The faecal samples were collected, dried for 1 h at 70°C, weighed and homogenized with a chloroform-methanol mixture (2:1, v/v) (20 ml per gram of faeces). The mixtures were incubated for 60 min at 60°C with constant agitation in an orbital shaking incubator. The homogenates were then centrifuged for 5 min at 3000 g to recover the supernatants. The supernatants were thoroughly washed with 0.2 ml of its volume of 0.9% sodium chloride solution by vortexing. The mixtures were allowed to separate into two phases by low-speed centrifugation (1000 g for 10 min). The upper phases were removed without disturbing the lower chloroform phases using a pipette. The upper aqueous layers were discarded, while the lower layers containing the purified lipids were retained, and the lipid contents were determined. The Zlatkis *et al.* (1953) method was used to determine the cholesterol levels, while the method of Gottfried and Rosenberg (1973) was used to determine triglyceride levels.

Biochemical analysis

The rats were fasted overnight and euthanized by decapitation at the end of the 8-week period. Blood samples were collected and stored in the appropriate vacutainer tubes. The insulin and glycohaemoglobin (HbA_{1c}) levels were measured using the Mercodia Rat Insulin Elisa kit (Mercodia, Uppsala, Sweden) and the Stanbio Glycohaemoglobin kit (Stanbio

Laboratory, Boerne, TX, USA) respectively. The protocols were carried out according to the manufacturers' instructions. Total cholesterol, triglyceride and HDL-cholesterol levels were determined using the Stanbio Sirus Clinical Chemistry Analyzer. Insulin resistance was estimated using the Matthews *et al.* (1985) homoeostasis model assessment (HOMA-IR) equation:

$$\text{Insulin resistance score (HOMA-IR)} = \frac{[\text{Insulin (U/l)} \times \text{Fasting blood glucose (mmol/l)}]}{22.5}$$

Blood glucose: 1 mmol/l = 18 mg/dl.

Statistical analysis

All data were analysed using the Statistical Package for the Social Sciences (SPSS) version 20 software (SPSS Inc., Chicago, IL, USA). Variation among test groups was evaluated using the one-way ANOVA. *Post hoc* analysis was carried out using Duncan's multiple-range test to assess the significant difference among the means ($P < 0.05$). For tables and figures, different superscript letters indicate significant difference among test groups. Paired Student's *t*-test was used to determine the significant difference in blood glucose levels before and after the administration of glibenclamide ($P < 0.05$). The results were expressed as mean \pm SEM.

Results

Weekly non-fasting blood glucose levels

Figure 1 shows the non-fasting blood glucose (NFBG) concentrations in diabetic rats treated with the combination or glibenclamide. Before induction of type 2 diabetes, there were no significant differences in NFBG concentration among the groups ($P = 0.76$). However, 1 week after

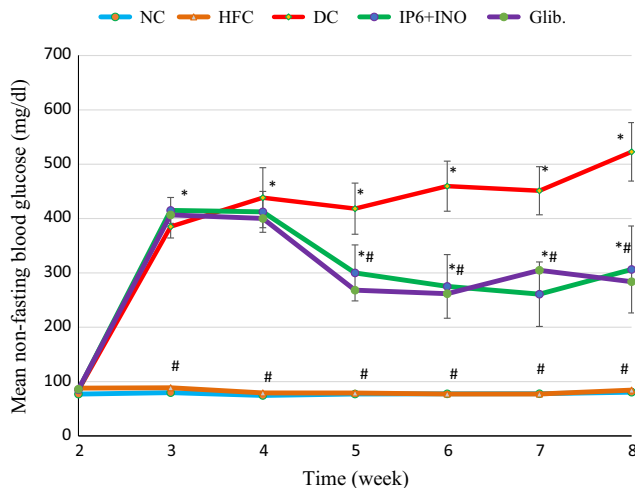


Figure 1 Non-fasting blood glucose concentration in diabetic rats treated with combined IP6 & inositol supplement or glibenclamide. *indicates a significant difference at $p < 0.05$ compared with NC at the same time. # indicates a significant difference at $p < 0.05$ compared with DC at the same time period.

induction, a significant increase in the NFBG level was noted in the STZ-induced diabetic groups when compared with the non-diabetic groups ($P < 0.05$). Following the treatment of the diabetic groups, there was a significant reduction in the NFBG concentration in the combination or glibenclamide-treated group when compared with the diabetic control group ($P < 0.05$).

Change in body weight

Figure 2 shows the change in body weight in diabetic rats treated with the combination or glibenclamide. For the first two weeks of the experiment, there was no significant difference in body weight among the groups. The untreated diabetic group (DC) gained less weight compared with the other groups. Although diabetic rats treated with the combination showed an increase in body weight throughout the feeding trial, the gain in body weight was significantly less than that of the diabetic rats treated with glibenclamide ($P < 0.05$).

Fluid intake

Table 1 shows the mean daily fluid consumption in diabetic rats treated with combination or glibenclamide. The untreated diabetic group consumed more fluid when compared with the other groups. There was no significant difference in fluid consumption between diabetic rats treated with the combination or glibenclamide at weeks 5 ($P = 0.74$) and 6 ($P = 0.98$) of the experiment. However, it was noted in the final two weeks of the experiment that there was a significant reduction in fluid consumption in the combination-treated group when compared with the glibenclamide-treated group ($P < 0.05$).

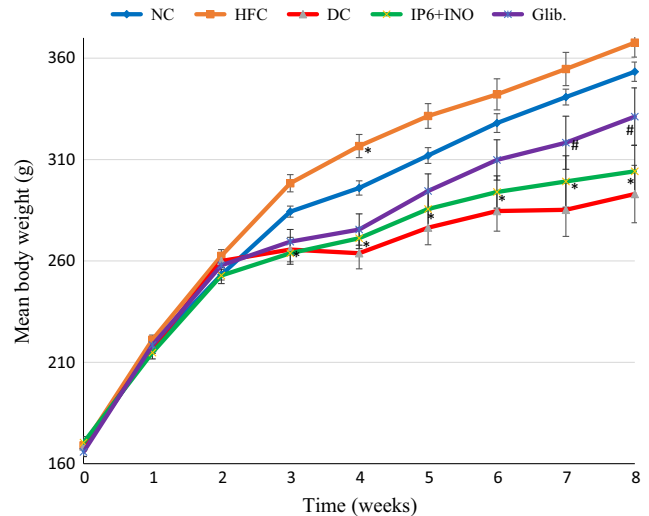


Figure 2 Mean body weight changes in diabetic rats treated with combined IP6 and inositol supplement or glibenclamide. * indicates a significant difference at $p < 0.05$ compared with NC at the same time. # indicates a significant difference at $p < 0.05$ compared with DC at the same time period.

Food intake

Table 2 shows the mean daily food consumed over the last four weeks of treatment. There were no significant differences observed in food consumption between the normal and HFC groups throughout the last 4 weeks of the experiment (week 5: $P = 1.00$; week 6: $P = 0.41$; week 7: $P = 0.26$; week 8: $P = 0.38$). The diabetic control group consumed significantly more food at weeks 6, 7 and 8 than all the treatment groups ($P < 0.05$). There was no significant difference in food consumption among the groups treated with the combination or glibenclamide by weeks 5 ($P = 0.55$) and 6 ($P = 0.89$) of the feeding trial. However, food consumption was significantly lower in the combination-treated group compared with the glibenclamide-treated group ($P < 0.05$).

Intestinal amylase activity

Figure 3 shows the proximal and distal intestinal amylase activity in diabetic rats treated with the combination or glibenclamide. There was no significant difference in the distal intestinal amylase activities among the groups. However, there was a significant reduction in the proximal intestinal amylase activity in the group treated with the combination compared with the group treated with glibenclamide ($P < 0.05$).

Faecal lipid levels

Figure 4 shows faecal total cholesterol and triglyceride levels in diabetic rats treated with the combination or glibenclamide. Faecal triglyceride and total cholesterol

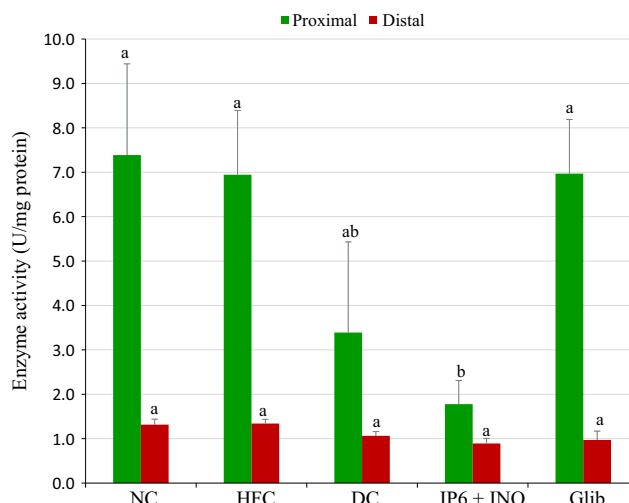


Figure 3 Amylase activity in the proximal and distal intestinal mucosa of diabetic rats treated with combined IP6 and inositol supplement or glibenclamide. Data are shown as mean \pm S.E.M. The figures with different superscript letters are significantly different at $p < 0.05$

concentrations were significantly increased in diabetic rats treated with the combination compared with the NC ($P < 0.05$).

Serum leptin

Figure 5 shows the serum leptin levels in diabetic rats treated with the combination or glibenclamide. Serum leptin level was significantly increased in the combination-treated group compared with the other groups ($P < 0.05$).

Table 1 Mean daily fluid intake for type 2 diabetic rats treated with combined IP6 and inositol supplement or glibenclamide

Time (week)	Fluid intake per day (ml/day)				
	NC	HFC	DC	IP6 + INO	Glib.
5	34.6 \pm 0.5 ^a	33.3 \pm 1.3 ^a	147.6 \pm 4.2 ^b	81.2 \pm 3.4 ^c	70.6 \pm 5.5 ^c
6	39 \pm 2.3 ^a	34 \pm 3.0 ^a	150 \pm 4.1 ^b	83 \pm 9.3 ^c	83 \pm 10 ^c
7	32.8 \pm 0.3 ^a	33.1 \pm 0.7 ^a	156.4 \pm 8.7 ^b	72.3 \pm 7.8 ^c	90.1 \pm 4.7 ^d
8	29.6 \pm 2.1 ^a	30 \pm 0.1 ^a	147.2 \pm 0.12 ^b	70.4 \pm 8.9 ^c	82.5 \pm 4.6 ^d

Data are shown as mean \pm SEM. The figures with different superscript letters are significantly different at $P < 0.05$. NC, normal control; HFC, high-fat control; DC, diabetic untreated control; IP6+INO, combined IP6 and inositol.

Table 2 Mean daily food intake for type 2 diabetic rats treated with combined IP6 and inositol supplement or glibenclamide

Time (week)	Food intake per day (g/d)				
	NC	HFC	DC	IP6 + INO	Glib.
5	19.7 \pm 0.3 ^a	19.6 \pm 0.2 ^a	24.4 \pm 2.5 ^b	25 \pm 1.1 ^b	25.7 \pm 1.7 ^b
6	17 \pm 0.4 ^a	17.6 \pm 0.9 ^a	34.4 \pm 4 ^c	22.6 \pm 4 ^b	24 \pm 0.3 ^b
7	20.5 \pm 0.3 ^a	19.3 \pm 0.1 ^a	33.7 \pm 1.6 ^b	23.8 \pm 0.3 ^c	28.8 \pm 0.7 ^d
8	21.1 \pm 0.1 ^a	20.6 \pm 0.02 ^a	35.9 \pm 0.8 ^b	26 \pm 0.3 ^c	28.5 \pm 0.2 ^d

Data are shown as mean \pm SEM. The figures with different superscript letters are significantly different at $P < 0.05$. NC, normal control; HFC, high-fat control; DC, diabetic untreated control; IP6+INO, combined IP6 and inositol.

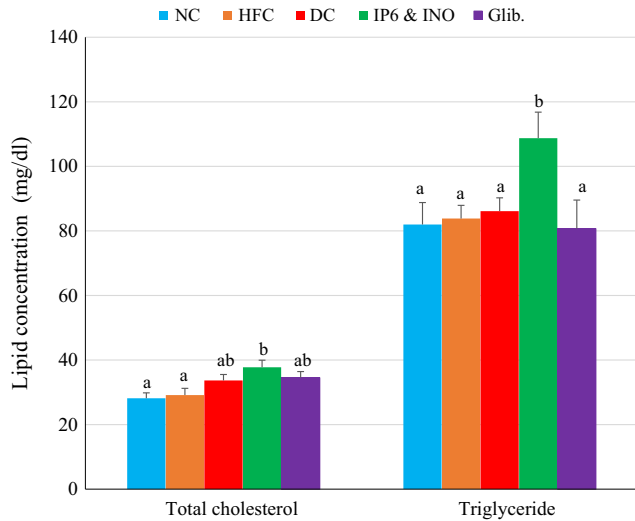


Figure 4 Fecal lipid profile in diabetic rats treated with combined IP6 and inositol supplement or glibenclamide. Data are shown as mean ± S.E.M. The figures with different superscript letters are significantly different at $p < 0.05$.

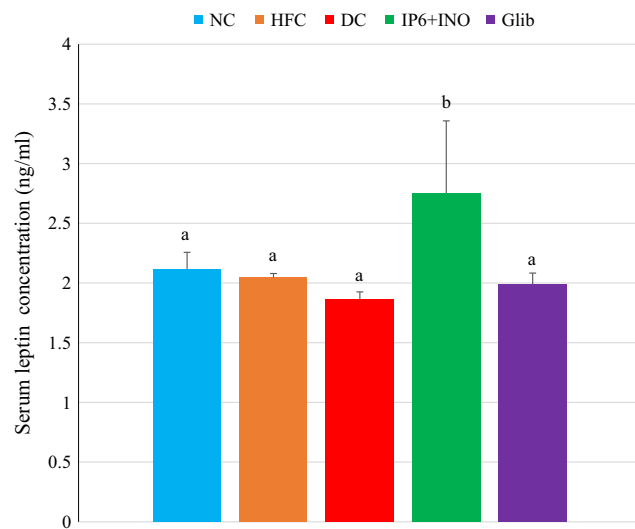


Figure 5 Serum leptin concentration of diabetic rats treated with combined IP6 and inositol or glibenclamide. Data are shown as mean ± S.E.M. The figures with different superscript letters are significantly different at $p < 0.05$.

Serum insulin, HOMA-IR, glycated haemoglobin concentration and intestinal weight

Table 3 shows glycated haemoglobin levels, insulin concentration, intestinal weight and homeostatic model assessment for insulin resistance (HOMA-IR). There was a significant increase in the HbA_{1c} % among the untreated and treated diabetic groups compared with the non-diabetic groups ($P < 0.05$). There were no significant differences in the insulin levels among the groups ($P = 0.5$). The homeostatic model assessment for insulin resistance (HOMA-IR) score in the diabetic control group was significantly higher when compared with the other groups ($P = 0.002$). Intestinal weight was significantly elevated in the diabetic control and glibenclamide-treated group compared with the other groups ($P = 0.005$). However, there was no significant difference in intestinal weight between the NC and the combination-treated group.

Serum lipids

Figure 6 shows the serum lipid profile in diabetic rats treated with the combination supplement or glibenclamide. The

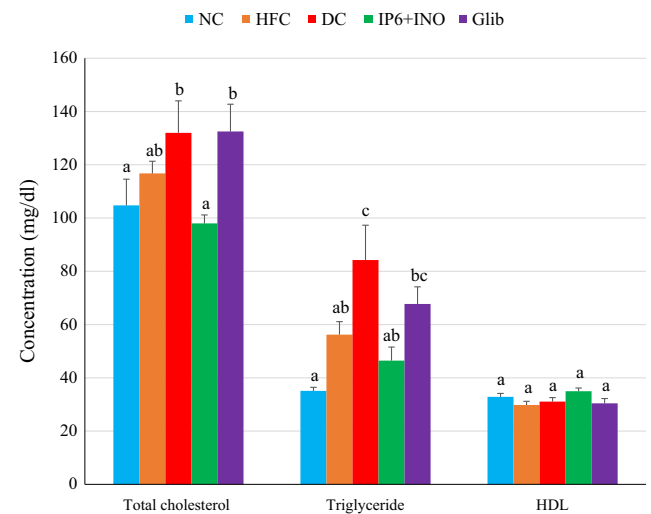


Figure 6 Serum lipid profile in diabetic rats treated with glibenclamide or combined IP6 and inositol supplement. Data are shown as mean ± S.E.M. The figures with different superscript letters are significantly different at $p < 0.05$.

Table 3 Glycated haemoglobin levels, fasting insulin levels and homeostatic model assessment for insulin resistance score and intestinal weight in diabetic rats treated with combined IP6 and inositol supplement or glibenclamide

Parameters	NC	HFC	DC	IP6 + INO	Glib.
HbA _{1c} (%)	4.22 ± 0.21 ^a	4.41 ± 0.08 ^a	6.33 ± 0.15 ^b	5.91 ± 0.36 ^b	5.98 ± 0.2 ^b
Fasting insulin (U/l)	4.8 ± 0.20 ^a	5.0 ± 0.23 ^a	5.2 ± 0.15 ^a	4.9 ± 0.12 ^a	4.8 ± 0.19 ^a
HOMA-IR	0.86 ± 0.75 ^a	0.89 ± 0.45 ^a	5.1 ± 0.69 ^b	1.93 ± 0.45 ^a	1.7 ± 0.64 ^a
Intestinal wt. (g)	7.89 ± 0.38 ^a	7.99 ± 0.47 ^a	13.9 ± 0.58 ^b	9.3 ± 1.16 ^a	12.38 ± 3.4 ^b

Values that share different superscript letters horizontally are significantly different ($P < 0.05$)

NC, normal control; HFC, high-fat control; DC, diabetic untreated control; IP6+INO, combined IP6 and inositol.

group treated with combination had significantly lower serum total cholesterol levels when compared with the diabetic control and glibenclamide groups ($P < 0.05$). The serum triglyceride levels were significantly elevated in the diabetic control and the glibenclamide-treated group compared with the NC ($P < 0.05$). Serum HDL was mildly higher in the group treated with the combination, but was not significantly different compared with the other groups.

Discussion

In this study, 45% high-fat diet followed by streptozotocin administration (35 mg/kg body weight) resulted in successful induction of type 2 diabetes in rats. Previous studies have demonstrated that the consumption of high-fat diet for 2 weeks or more may result in insulin resistance in rats without elevated blood glucose (Danda *et al.* 2005; Srinivasan *et al.* 2005; Zhang *et al.* 2008). Insulin resistance precedes hyperglycaemia in humans with type 2 diabetes mellitus. The administration of a low dose of STZ in rats induces a mild impairment of insulin secretion that results in a hyperglycaemic condition. To confirm whether a type 2 diabetic model was achieved in this study, the sensitivity of the diabetic rat model was evaluated with a conventional antidiabetic drug (glibenclamide). Stocks *et al.* (1988) reported that glibenclamide had no effect on blood glucose concentration in type 1 diabetic models of the disease. Therefore, a positive response to this test suggests that a type 2 diabetic model was achieved. There was a significant reduction in blood glucose concentration after the administration of a single oral dose of glibenclamide. The data showed no significant difference in fasting serum insulin levels between the diabetic and non-diabetic groups, and this was reported to be characteristic of type 2 diabetes (Reed *et al.* 2000). Interestingly, no significant increase was observed in fasting insulin concentration of diabetic rats treated with glibenclamide or combined IP6 and inositol, although the blood glucose concentrations in these groups were significantly reduced. Studies showing the effect of glibenclamide on the beta cell function in patients with type 2 diabetes have found similar results (Shapiro *et al.* 1989; O'Meara *et al.* 1990). These and other studies have concluded that the insulin secretagogue effect of glibenclamide occurred by increasing the pancreatic beta cells' responsiveness to glucose (Shapiro *et al.* 1989; O'Meara *et al.* 1990; Patanè *et al.* 2000). It was proposed that this may be the predominant mechanism of action of glibenclamide and this effect may have been concealed by the fasting conditions (Shapiro *et al.* 1989; O'Meara *et al.* 1990). Studies by Lee *et al.* (2006) also found that phytic acid reduced the blood glucose concentration in diabetic KK mice without affecting the fasting insulin concentration. The results obtained in this study are in line with these findings and suggest that the IP6/inositol combination may function similar to glibenclamide in this respect. The calculated homeostatic model assessment for insulin resistance (HOMA-IR) score showed that there was

insulin resistance in the diabetic control group. However, the administration of the combination or glibenclamide to the diabetic rats significantly reduced insulin resistance to normal levels.

Previous research (Dilworth *et al.* 2005; Lee *et al.* 2006; Kuppusamy *et al.* 2011; Omoruyi *et al.* 2013) has demonstrated the hypoglycaemic effects of phytic acid alone in diabetic and non-diabetic rats. In this study however, the main focus was on the treatment of type 2 diabetic rats with an IP6/inositol combination product that we theorized may be more effective than IP6 or inositol alone. It was found that the treatment of diabetic rats with the combination or glibenclamide resulted in a significant reduction in the non-fasting blood glucose concentration compared with the diabetic untreated group. We observed that serum glycated haemoglobin concentrations were not significantly altered among the diabetic groups. This was possibly attributed to the short-term duration of the study. Further investigations are therefore needed to assess the long-term effect of the combination supplementation on glycated haemoglobin levels in type 2 diabetic rats.

Intestinal weight significantly increased in the diabetic control and the glibenclamide-treated group compared with the NC group. Several studies have reported similar increases in intestinal weight in streptozotocin-induced diabetic rats (Jervis & Levin 1966; Schedl & Wilson 1971). It was proposed that increased intestinal weight may be due to modifications to the brush border cell enzyme activities geared at increasing intestinal monosaccharide absorption. However, in the diabetic state, increased monosaccharide absorption leads to increased blood glucose concentration which can further complicate the pathophysiology of the disease. In this study, there was significant reduction in intestinal weight in the diabetic rats treated with the combination compared with the diabetic control group. This may be as a result of the combination's role in altering the activities of some enzymes involved in carbohydrate absorption. Thompson (1988) suggested that phytic acid reduces blood glucose concentration by impairing starch digestion. Similarly, Liu *et al.* (2008) reported a reduction in endogenous carbohydrase activity and digestive capability in animal model fed a phytate-supplemented diet. Similarly, Deshpande and Cheryan (1984) reported a reduction in amylase activity when minor quantities of phytate were introduced to the diet. In this study, we noted a significant reduction in the proximal intestinal amylase activity in diabetic rats treated with the combination compared with the diabetic group treated with glibenclamide and the non-diabetic control group. This suggests that administration of the combination may lead to a reduction in the rate of intestinal carbohydrate digestion. This in turn leads to reduced blood glucose concentration. As mentioned above, reduced carbohydrase activity (in this case amylase) could be one of the factors contributing to the decreased intestinal weight in the diabetic rats treated with the combination.

The data on body weight gain showed that the treatment of type 2 diabetes with glibenclamide may provide

fewer benefits due to the significant increase in body weight observed in this group compared with the combination-treated group. Leptin is a hormone that plays significant roles in the regulation of body weight and food intake (Klok *et al.* 2006). Activation of the leptin receptor initiates various signalling cascades that result in alterations in food consumption (Schwartz 2001; Sahu 2004). Any reduction in leptin action results in an increased drive for food, reduced satiety and energy utilization (Ahima *et al.* 1996). Reduced leptin action has also been reported to diminish liver insulin sensitivity, while increasing liver glucose production, decreasing glucose uptake into muscle and reducing the overall metabolic rate (Myers & Olson 2012; Schwartz *et al.* 2013). Leptin released from the adipose tissue conveys information to the brain about the body's energy status with the resultant decrease in food intake and subsequent increase in energy utilization to maintain body fat stores (Halaas *et al.* 1995; Jørgensen *et al.* 1998; Farooqi *et al.* 2001; Jeon *et al.* 2003). Obese patients have been reported to be leptin-resistant; hence, treatment must be geared towards overcoming leptin insensitivity. In this study, a significant increase in the serum leptin levels of the diabetic rats treated with the combination was noted. This increase in serum leptin could be a possible mechanism by which food intake and body weight gain were significantly reduced in the combination-treated group. However, there are conflicting reports on the role of leptin in diabetes in humans and animal models. Previous reports indicate that leptin increases insulin sensitivity, reduces hepatic glucose production and decreases glucagon levels, while insulin is known to upregulate leptin production and secretion in adipose tissue (Seufert 2004). Further studies showed that leptin is able to ameliorate hyperglycaemia by increasing tissue glucose uptake independent of insulin and by suppressing hepatic glucose production (German *et al.* 2011). On the contrary, Söderberg *et al.* (2007) reported that high leptin levels are associated with the future development of diabetes in Mauritian men. However, other research indicates that leptin therapy improves hyperglycaemia and insulin resistance in a mouse model of type 2 diabetes (Toyoshima *et al.* 2005). Leptin gene therapy has been reported to improve type 1 and type 2 diabetes and diet-induced obesity in animal models (Yaspelkis *et al.* 2001; Yu *et al.* 2008; Fujikawa *et al.* 2010). This is the first study highlighting the effect of IP6/inositol combination on serum leptin concentration in diabetic animals. We hypothesized that the observed significant increase in the serum leptin level may increase hepatic insulin sensitivity. This in addition to the observed significant decrease in insulin resistance and reduced food intake may account for the hypoglycaemic activity of the combined IP6 and inositol supplement treatment. It is theorized that IP6 and inositol consumption may be beneficial to diabetics in regard to reducing the risks associated with obesity and other downstream pathological complications. The link between type 2 diabetes and obesity has been well established for decades, and the main

basis for this connection is insulin resistance. Studies have found that IP6 and myo-inositol reduced insulin resistance through the activation of peroxisome proliferator-activated nuclear receptors (PPARs) in a dose-dependent manner thereby inhibiting lipolysis, increasing glucose uptake and increasing lipid storage in differentiated adipocytes (Kim *et al.* 2014; Malarvizhi *et al.* 2016). The reduction in serum lipids along with reduced blood glucose and enhanced insulin sensitivity noted in this study is consistent with these *in vitro* findings, suggesting that the mechanism of action for these outcomes may be mediated through activation of PPARs. In this regard, treatment with the combined IP6 and inositol supplement may be effective in controlling dyslipidaemia and obesity which are common features of type 2 diabetes mellitus.

There was a significant increase in serum triglyceride and total cholesterol concentrations in the diabetic control group compared with the NC group. However, treatment with the combination significantly reduced serum total cholesterol and triglyceride levels compared with the diabetic control group. This may be due to the reported increase in intestinal lipase activity in rats treated with phytic acid (Dilworth *et al.* 2015). There were no significant changes observed in serum lipids in diabetic rats treated with glibenclamide compared with the untreated diabetic control group. Mughal *et al.* (1999) reported that patients with type 2 diabetes treated with glibenclamide showed no significant changes in plasma lipoprotein and lipids. Sulphonylureas stimulate insulin production and can result in excess insulin, which causes increased carbohydrate and fat storage with resultant weight gain. Increased weight gain was noted in the diabetic group treated with glibenclamide compared with the other diabetic groups. Increased faecal cholesterol and triglyceride concentrations were also noted in the diabetic group treated with the combination. This may account for the observed reductions in serum cholesterol and triglyceride concentrations in these animals. Omoruyi *et al.* (2013) highlighted a positive association between decreased body weight and increased HDL in diabetic rats fed phytic acid. It is however not known whether it is increased lipid uptake or increased utilization that may be affecting serum HDL in these animals. In the present study, similar trends were noted in the group treated with the combined IP6 and inositol supplement. However, the observed reduction in weight gain in the diabetic control did not result in elevated serum HDL-cholesterol. Overall, reduced cholesterol and triglycerides, along with increased HDL-cholesterol levels, are desirable in the effective management of type 2 diabetes mellitus. Hence, treatment with the combined IP6 and inositol supplement may be effective in controlling dyslipidaemia which is a common feature of type 2 diabetes mellitus.

Conclusion

This study showed that consumption of an IP6 and inositol combination by diabetic animals resulted in reduced serum

amylase, increased insulin sensitivity and increased serum leptin activity. These may be possible mechanisms for the significant reduction in blood glucose concentration observed in these animals. Further studies regarding the molecular mechanisms underlying these observations are needed. Total cholesterol, triglycerides and food and fluid intake were also significantly reduced in diabetic rats treated with the combination. These are factors proven to be beneficial in the management of type 2 diabetes mellitus. Data from this study indicate that treatment of type 2 diabetic rats with the combination may be more effective in curtailing some indices of diabetes including polyphagia, polydipsia and dyslipidaemia, compared with the glibenclamide treatment. Further studies are needed on the effects of the combination on leptin and ghrelin regulation as well as its effects on adipogenesis and dyslipidaemia in the diabetic state.

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Conflict of Interest

The authors report no declarations of interest.

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