JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Buckwheat Concentrate Reduces Serum Glucose in Streptozotocin-Diabetic Rats

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The antihyperglycemic effects of chemically synthesized D-*chiro*-inositol (D-CI), a component of an insulin mediator, have been demonstrated in rats. Buckwheat contains relatively high levels of D-CI: thus, it has been proposed as a source of D-CI for reducing serum glucose concentrations in diabetics. The present study evaluates the effects of a buckwheat concentrate, containing D-CI, on hyperglycemia and glucose tolerance in streptozotocin (STZ) rats. In fed STZ rats, both doses of the buckwheat concentrate (containing 10 and 20 mg of D-CI/kg of body weight) were effective for lowering serum glucose concentrations by 12−19% at 90 and 120 min after administration. Findings from this study demonstrate that a buckwheat concentrate is an effective source of D-CI for lowering serum glucose concentrations in rats and therefore may be useful in the treatment of diabetes.

KEYWORDS: D-*chiro*-Inositol; buckwheat concentrate; diabetes; streptozotocin rats

INTRODUCTION

Of nine inositol isomers, *myo*-inositol is the most commonly occurring isomer in nature, whereas D-*chiro*-inositol (D-CI) is relatively rare. Buckwheat seeds contain relatively high amounts of free D-CI and galactosyl derivatives of D-CI known as fagopyritols (*1*). Obendorf and Horbowicz (*1*) detected free D-CI in lupine, pigeon pea, soybean, chickpea, mungbean, and buckwheat. Among all of the seeds analyzed, only mungbean seeds contained higher levels of free D-CI than buckwheat. According to Horbowicz et al. (*2*), one of the fagopyritols has been identified in soybean, lupine, lentil, and chickpea seeds, whereas buckwheat contains five different fagopyritols.

Besides their existence in plant sources, D-CI and *myo*-inositol have been identified as components of two different inositol-phosphoglycan (IPG) molecules in mammalian systems (*3*, *4*). The role of IPG molecules as putative insulin secondary messengers has been demonstrated in numerous studies (*5*−*8*). IPGs are released from glycosylphosphatidylinositols (GPIs) in cell membranes in response to insulin. Following GPI hydrolysis by phospholipases, IPGs are incorporated into the cell, where they can affect enzymes implicated in insulin action. The insulin-mimetic effects of IPGs isolated from various mammalian tissues and their analogues have been widely documented (*8*).

The use of buckwheat in the management of diabetes mellitus has been previously reported. Consumption of buckwheat as flour or biscuits made from buckwheat flour has been demonstrated to have hypoglycemic effects in patients with diabetes (*9*, *10*); however, the active components were not identified.

The effectiveness of acute administration of chemically synthesized D-CI on lowering plasma glucose has been evaluated in rats. A single dose of intragastric D-CI (2−15 mg/kg) administered to normal rats 2 h before intraperitoneal glucose produced a 30−50% decrease in plasma glucose concentrations (*11*). A single dose of intragastric D-CI (10 mg/kg) administered to fed streptozotocin-treated rats produced a 30−40% decrease in plasma glucose concentrations (*11*). The acute effects of D-CI on plasma glucose were also demonstrated in STZ rats when a single dose of 15 mg/kg attenuated elevated plasma glucose concentrations by 21% in 120 min (*12*). Ortmeyer et al. (*11*) administered acute doses of 1−30 mg of D-CI/kg of body weight in STZ rats and found maximal effects for plasma glucose lowering at 10 mg of D-CI/kg of body weight. Fonteles et al. (*12*) reported significant plasma glucose lowering in STZ rats with a 15 mg of D-CI/kg of body weight dose but not with a 5 mg of D-CI/kg dose. These studies used chemically synthesized D-CI. However, buckwheat contains sufficient amounts of D-CI to be evaluated as a natural source of D-CI for reducing serum glucose concentrations in diabetes.

The purpose of the present study was to evaluate the effect of an acute dose of a buckwheat concentrate on elevated serum glucose and glucose tolerance in rats. To determine the efficacy of buckwheat as a natural source of D-CI, we administered the buckwheat concentrate to normal and fed STZ rats under conditions similar to those used in the studies mentioned above. We also administered the buckwheat concentrate to fasted rats, prior to an oral glucose tolerance test (OGTT). Performing an OGTT is beneficial because it provides a functional measurement of whole body glucose tolerance. We hypothesized that a buckwheat concentrate equivalent to 10 or 20 mg of D-CI/kg of body weight would improve glucose tolerance in normal and STZ rats and decrease hyperglycemia in fed STZ rats. Thus, the specific objectives of this study were (1) to determine the effectiveness of a buckwheat concentrate as a natural source of

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10.1021/jf0302153 CCC: $25.00
Published on Web 11/01/2003

D-CI on glucose tolerance in normal rats and on hyperglycemia in fed STZ rats and (2) to determine the effect of an acute dose of a buckwheat concentrate given to fasted rats 1 h before an OGTT on serum glucose concentrations.

MATERIALS AND METHODS

Standards and Chemical Reagents. The *myo*-inositol standard, phenyl-α-D-glucoside (internal standard), trimethylsilylimidazole, pyridine, and streptozotocin (STZ) were purchased from Sigma Chemical Co. (St. Louis, MO). Reagent alcohol was purchased from Fisher Scientific (Ontario, Canada). D-*chiro*-Inositol standard was a gift from Dr. S. G. Angyal (University of New South Wales, Australia).

Buckwheat Concentrate. Buckwheat variety Koto (*Fagopyrum esculentum* Moench) was provided by Kade Research Ltd. (Morden, MB, Canada). Concentrates were produced from buckwheat according to a modified method of Horbowicz and Obendorf (*1*). Briefly, dehulled buckwheat groats were fractionated by milling on a Buhler mill (MLU-202). The bran and shorts fractions were combined, ground, and thoroughly homogenized in ethanol/water (1:1, v/v) for 10 min. Five volumes of extraction solvent were used for every 1 volume of ground seed fractions. The homogenate was vacuum filtered and the remaining residue re-extracted with the same volume of solvent. The combined filtrates were evaporated using a rotary evaporator (Yamato RE200, Orangeburg, NY) under vacuum until a 40-fold reduction in solvent volume was achieved. This form was used for acute administration to rats in the present study.

Analysis of Inositols and Soluble Carbohydrates in Buckwheat Concentrate. Aliquots (100 μL) of the concentrate solution were transferred to silylation vials (Pierce) and evaporated to dryness under nitrogen at 40 °C. The dry residues were derivatized with 1.6 mL of silylation reagent (trimethylsilylimidazole/pyridine, 1:1, v/v, containing 200 μg of phenyl-α-D-glucoside) at 75−80 °C for 1 h (*1*).

Two microliters of derivatized carbohydrates was injected into a Shimadzu gas chromatograph GC-17A (Columbia, MD) equipped with a flame ionization detector and split injector. Carbohydrates were separated on an RTX-5MS capillary column (25 m length, 0.25 mm i.d., and 0.25 μm film thickness; Restek, Bellefonte, PA). Column temperature was programmed from 150 to 200 °C at the rate of 3 °C/min and then to 325 °C at the rate of 7 °C/min. Initial and final temperatures were held for 5 and 20 min, respectively. The injector and detector temperatures were held at 270 and 350 °C, respectively. The carrier gas was hydrogen at a flow rate of 1.5 mL/min, whereas the split ratio used was 1:40. Soluble carbohydrates including inositols were quantified using phenyl-α-D-glucoside as the internal standard.

Protein and Mineral Composition of Buckwheat Concentrates. Concentrates were analyzed for content of protein using AOAC method 955.04 (*13*) and minerals by atomic absorption spectrometry.

Animals and Treatment Groups. Fifty-two male Sprague−Dawley rats (Central Animal Holding, Winnipeg, MB, Canada) weighing 150−180 g were acclimatized for a period of 7 days. Throughout the acclimatization and subsequent study period, rats were maintained in a controlled environment of 21−23 °C, 55% humidity, and a 14-h light/10-h dark cycle. Rats were housed in groups of two in plastic hanging cages and fed standard laboratory chow (Prolab RMH 3000, Purina Mills, Richmond, IN) ad libitum; fresh water was available in polypropylene bottles with stainless steel sipper tubes. Rats were familiarized with subsequent testing procedures during this adaptation period. A protocol for animal care procedures was approved by the University of Manitoba Protocol Management and Review Committee.

Treatments were a low-dose buckwheat concentrate, a high-dose buckwheat concentrate, a low-dose placebo, or a high-dose placebo. The low-dose and high-dose buckwheat groups received a single dose of buckwheat concentrate containing 10 or 20 mg of D-CI/kg of body weight, respectively. The low-dose and high-dose placebo groups received a sucrose/deionized water solution (3 and 6% sucrose, respectively) that was administered at a dose level equivalent to the amount of sucrose provided in the buckwheat concentrate. For rats assigned the lower dose (10 mg of D-CI/kg of body weight), the buckwheat concentrate was diluted 2-fold so that equivalent volumes of the concentrate were administered to rats in both treatment groups.

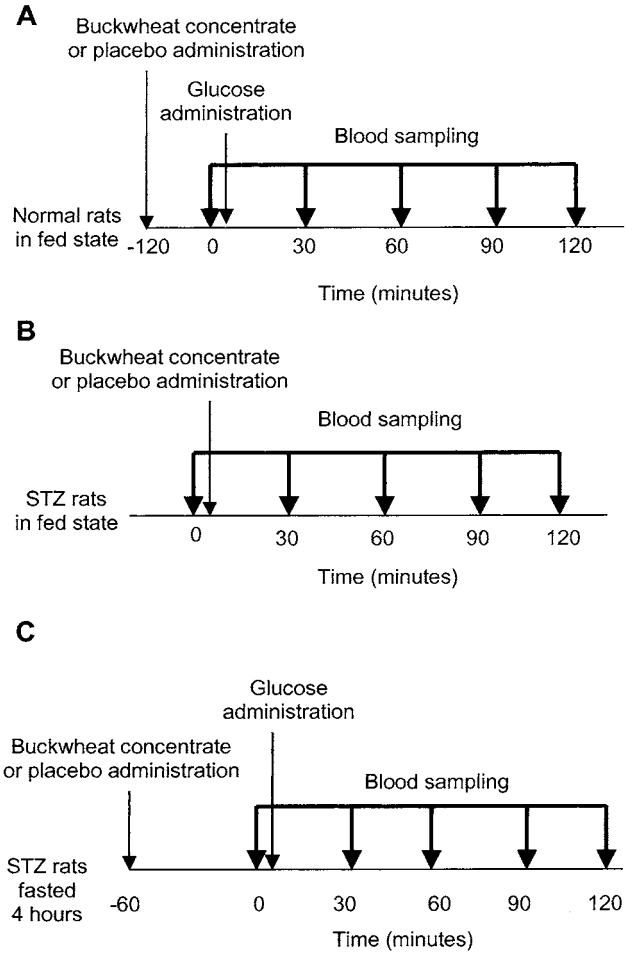

Figure 1. Experimental design for (**A**) IPGTT 2 h following an acute dose of a buckwheat concentrate or placebo given to normal rats, (**B**) fed state response to an acute dose of a buckwheat concentrate or placebo in STZ rats, and (**C**) OGTT 1 h following an acute dose of a buckwheat concentrate or placebo given to fasted STZ rats.

Normal Rats Given a Glucose Load. Following the acclimatization period, 12 male Sprague−Dawley rats were randomly assigned to either the low-dose buckwheat group or the low-dose placebo group. Following treatment administration, rats were fasted for 2 h and given an intraperitoneal glucose tolerance test (IPGTT). For the IPGTT, blood was collected from the saphenous vein for the 0 min time point and, immediately following, a 70% glucose solution was injected intraperitoneally (4 g of glucose/kg of body weight). Blood was collected from the saphenous vein at 30, 60, 90, and 120 min from the time of the initial glucose administration. Blood samples were held on ice until centrifuged to obtain serum. Serum samples were stored at −20 °C until analysis. The protocol for the test procedure in normal rats is shown in **Figure 1A**.

Streptozotocin Rats. Following the acclimatization period, 40 rats received intraperitoneal injections of 60 mg of STZ/kg of body weight/day on days 1 and 2 of the experiment. STZ was freshly dissolved in 0.9% NaCl, pH 5.5, at a concentration of 15 mg/mL. Three days after the second injection, a blood sample to measure blood glucose levels was taken via the saphenous vein. Diabetes was defined when a blood glucose concentration of 13 mmol/L or greater was achieved. Rats with a positive response to STZ administration were randomly assigned to either the low-dose buckwheat, high-dose buckwheat, low-dose placebo, or high-dose placebo group. Rats were individually housed for the remainder of the experiment. Two different test procedures were performed on STZ rats. The protocols for test procedures in STZ rats are shown in **Figure 1B**,**C**.

Effect of Buckwheat Concentrate on Serum Glucose of Fed STZ Rats. For STZ rats, the first test procedure was performed on day 7 of

Table 1. Composition of Buckwheat Concentrates[a]

component	contribution
carbohydrates (%)	
D-*chiro*-inositol	0.2
myo-inositol	0.1
sucrose	6.0
fagopyritols	5.7
protein (%)	5.0
minerals (ppm)	
calcium	130
iron	2
magnesium	900
manganese	2
phosphorus	2800
selenium	26
zinc	60

[a] Amount in high-dose buckwheat concentrate. For the low-dose buckwheat concentrate, the high-dose buckwheat concentrate was diluted 2-fold so that equal volumes of the concentrate were administered to rats for the two different dose amounts (10 or 20 mg of D-CI/kg of body weight).

the experiment. Blood was collected via the saphenous vein for the 0 time point and, immediately following, either a buckwheat concentrate or a placebo was administered intragastrically to fed rats (**Figure 1A**). Blood samples were also collected at 30, 60, 90, and 120 min following treatment administration.

Effect of Buckwheat Concentrate on Glucose Tolerance in Fasted STZ Rats. For the second test procedure (on day 14 of the experiment), treatments were administered to fasted STZ rats prior to an OGTT (**Figure 1B**). After 4 h of fasting, rats were given intragastrically either a buckwheat concentrate or placebo. One hour following treatment, blood was collected via the saphenous vein for the 0 time point. Immediately following, rats received 1 g of glucose/kg of body weight (70% glucose solution) intragastrically. Blood was collected at 30, 60, 90, and 120 min after administration of glucose. For both tests, blood samples were held on ice until centrifuged to obtain serum. Serum samples were stored at −20 °C until analysis.

Analysis of Serum Glucose. Glucose in the serum was assessed in triplicate using an enzymatic colorimetric kit (procedure 315, Sigma Chemical Co., St. Louis, MO).

Statistical Analysis. Statistical significance between buckwheat and placebo groups was determined by Student's *t* test using SAS Statistical software (v. 8.2, SAS Institute Inc., Cary, NC). For effects over time within each treatment group, repeated-measures ANOVA was used. Data are expressed as the mean ± standard error.

RESULTS

Composition of Buckwheat Concentrate. The protein, mineral, and carbohydrate composition of the buckwheat concentrate is shown in **Table 1**. Soluble carbohydrates comprised 12% of the buckwheat concentrate and included D-CI, *myo*-inositol, sucrose, and fagopyritols. The majority of carbohydrates found in the concentrate were from sucrose and fagopyritols. Protein content of the buckwheat concentrate was 5%. The various minerals found in the buckwheat concentrate were present at a concentration of 0.4%.

Effect of Buckwheat Concentrate on Glucose Tolerance in Normal Rats. Administration of the low-dose buckwheat concentrate to normal rats 2 h prior to an IPGTT resulted in serum glucose concentrations that were 17% lower 30 min after glucose administration compared to rats given the low-dose placebo (**Figure 2**).

Effect of Buckwheat Concentrate on Serum Glucose of Fed STZ Rats. The low-dose buckwheat concentrate group had a 14−16% decrease in serum glucose concentrations 60−120 min after treatment administration (**Figure 3**). In contrast, rats given the low-dose placebo had 4−10% increased serum glucose

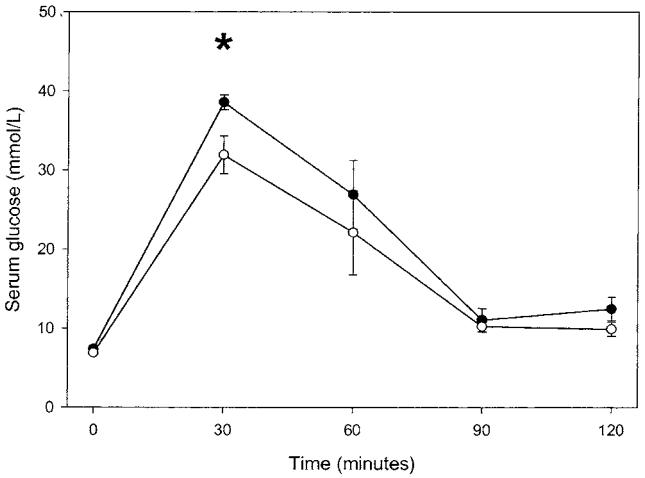

Figure 2. Serum glucose concentrations during an IPGTT given 2 h following administration of a low-dose buckwheat concentrate (10 mg of D-*chiro*-inositol/kg of body weight) or placebo in normal rats: (●) placebo low-dose ($n = 6$) group; (○) low-dose buckwheat concentrate ($n = 6$) group. The asterisk (*) indicates difference ($p < 0.05$) between placebo-treated and buckwheat-treated rats.

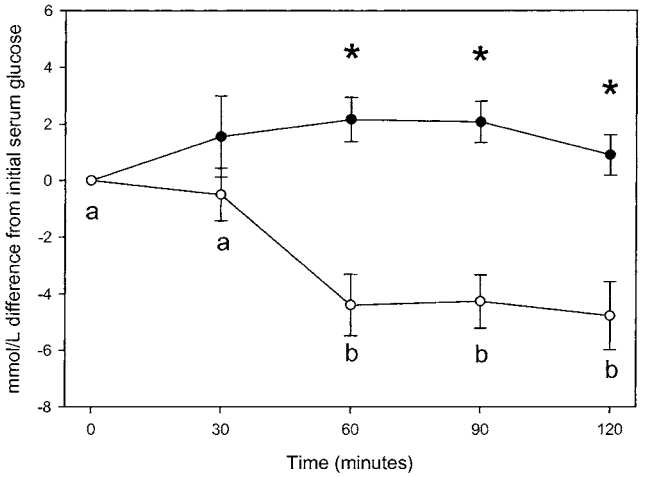

Figure 3. Effect of a low-dose buckwheat concentrate (10 mg of D-*chiro*-inositol/kg of body weight) or placebo given to STZ rats in the fed state on serum glucose concentrations. Data are expressed as the mmol/L difference from initial serum glucose concentrations (28.4 ± 0.95 mmol/L) for the placebo low-dose (●, $n = 9$) and the low-dose buckwheat concentrate (○, $n = 8$) groups. Asterisks (*) indicate differences ($p < 0.001$) between placebo-treated and buckwheat-treated rats. Data points with different letters indicate differences ($p < 0.05$) within a group as determined by Duncan's multiple-range test.

concentrations after 60−120 min (**Figure 3**). The high-dose buckwheat concentrate also reduced serum glucose concentrations in fed STZ rats by 12% after 90 min and by 19% after 120 min (**Figure 4**). In rats given the high-dose placebo, serum glucose concentrations were similar to baseline values after 60−120 min (**Figure 4**).

Effect of Buckwheat Concentrate on Glucose Tolerance in Fasted STZ Rats. Administration of the buckwheat concentrates to fasted rats prior to an OGTT did not affect serum glucose concentrations. The mean serum glucose concentrations of both the low-dose (**Figure 5**) and high-dose (**Figure 6**) buckwheat concentrate-treated rats were lower than those of the placebo-treated rats, but the difference was not statistically significant.

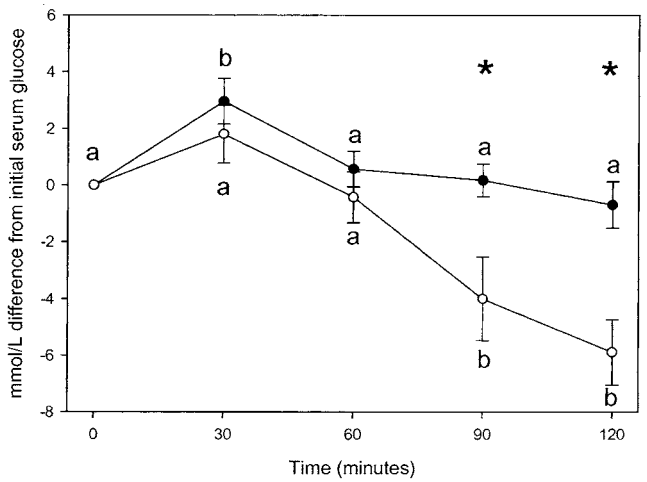

Figure 4. Effect of a high-dose buckwheat concentrate (20 mg of D-*chiro*-inositol/kg of body weight) or placebo given to STZ rats in the fed state on serum glucose concentrations. Data are expressed as the mmol/L difference from initial serum glucose concentrations (29.6 ± 0.7 mmol/L) for the placebo high-dose (●, $n = 10$) and the high-dose buckwheat concentrate (○, $n = 9$) groups. Asterisks (*) indicate differences ($p < 0.05$) between placebo-treated and buckwheat-treated rats. Data points with different letters indicate differences ($p < 0.05$) within a group as determined by Duncan's multiple-range test.

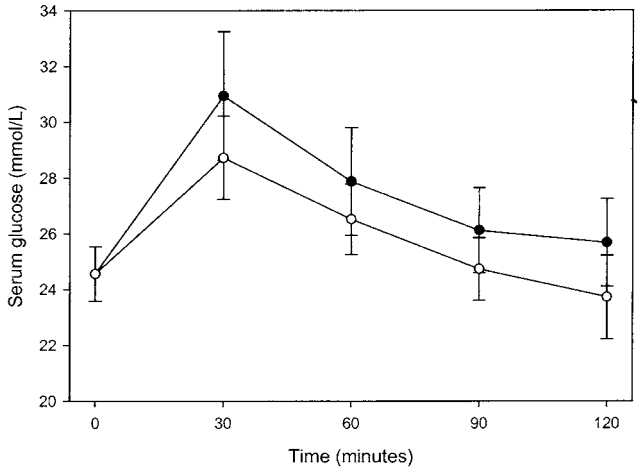

Figure 5. Serum glucose concentrations during an OGTT given 1 h following administration of a low-dose buckwheat concentrate (10 mg of D-*chiro*-inositol/kg of body weight) or placebo in fasted STZ rats: (●) placebo low-dose ($n = 9$) group; (○) low-dose buckwheat concentrate ($n = 8$) group.

DISCUSSION

The major finding of the present study was that a single oral dose of a buckwheat concentrate was effective in lowering elevated serum glucose concentrations in fed STZ rats. In fed STZ rats, both doses (10 and 20 mg of D-CI/kg of body weight) of the buckwheat concentrate were effective in lowering serum glucose concentrations by 12−19% at 90 and 120 min after treatment administration. Similar results were demonstrated when chemically synthesized D-CI was administered to STZ rats. Fonteles et al. (*12*) reported that a single dose of D-CI (15 mg/kg) injected into the jugular vein promoted a 21% decrease in plasma glucose of STZ rats, which was different from the control rats at 80, 100, and 120 min after administration. The glucose lowering effect of the buckwheat concentrate demonstrated in the present study is of a similar magnitude to that of

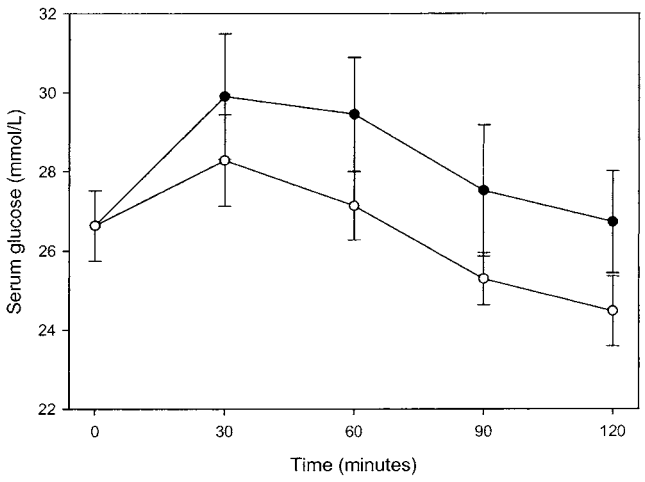

Figure 6. Serum glucose concentrations during an OGTT given 1 h following administration of a high-dose buckwheat concentrate (20 mg of D-*chiro*-inositol/kg of body weight) or placebo in fasted STZ rats: (●) placebo high-dose ($n = 10$) group; (○) high-dose buckwheat concentrate ($n = 9$) group.

synthesized D-CI, suggesting that D-CI in the buckwheat concentrate is primarily responsible for the observed effects.

In addition to D-CI, the buckwheat concentrate contained *myo*-inositol, also identified as a component of an IPG with insulin-mimetic effects. However, previous work suggests that administration of *myo*-inositol has no effects on plasma glucose concentrations. For example, dietary supplementation with 1.0% *myo*-inositol for 14 days had no effects on hyperglycemia of STZ rats (*14*). Besides free D-CI, the buckwheat concentrate used in the present study contained fagopyritols. The role of these D-CI derivatives is not known; however, the contribution of fagopyritols to the glucose-lowering effects of the buckwheat concentrate observed in the present study appear to be minimal. Future research may elucidate a beneficial role for fagopyritols and phytochemicals present in the buckwheat concentrate in other aspects of health and disease.

The mechanism by which administration of D-CI acts to lower plasma glucose is unknown. Ortmeyer et al. (*11*) and Fonteles et al. (*12*) suggested that acute administration of D-CI may act to lower plasma glucose by being incorporated into a mediator precursor. Sanchez-Arias et al. (*15*) demonstrated that STZ rats have impaired GPI-dependent insulin signaling. Isolated hepatocytes from STZ rats had lower amounts of GPI compared to control rats. STZ-induced diabetes also blocked the hydrolysis of GPI in response to insulin and markedly reduced the uptake of IPG. We have reported that urinary *chiro*-inositol excretion is elevated 336-fold in STZ rats compared to normal rats (*16*). This pattern of inositol excretion may be related to altered GPI−IPG signaling system. It is possible that administration of D-CI corrects the GPI-dependent signaling defect of STZ rats. STZ rats are a model of type 1 diabetes mellitus, characterized by hyperglycemia and hypoinsulinemia. Insulin deficiency in type 1 diabetes leads to a decrease in glucose utilization by the liver, muscle, and adipose tissue and an increase in hepatic glucose production (*17*). The antihyperglycemic effect of D-CI may result from inhibition of hepatic glucose output or enhanced glucose transport, glucose utilization, glucose disposal, or glycogen synthesis. The mechanism by which administration of D-CI lowers serum glucose concentrations still needs to be established.

The results from this study suggest that an acute dose of D-CI is effective only under specific conditions. Administration of D-CI promoted a decrease in serum glucose concentrations when

STZ rats were in the fed state but did not improve glucose tolerance of fasted STZ rats. To date, this is the first study to report the acute effects of D-CI given to fasted STZ rats prior to an OGTT. In the present study, D-CI can be effective despite subsequent glucose administration in normal rats. Similarly, Ortmeyer et al. (*11*) demonstrated that in normal rats, synthetic D-CI administered intragastrically 2 h before an intraperitoneal glucose load (4 g/kg) produced a 30−50% decrease in plasma glucose. It is possible that administration of glucose to a diabetic animal may compromise the effects of D-CI. Shaskin et al. (*18*) reported that the activity of the IPG containing D-CI increased following glucose ingestion in healthy men, whereas no difference in IPG activity was observed in men with type 2 diabetes. Determining the mechanism by which D-CI lowers serum glucose in the fed state may also elucidate why D-CI is ineffective in fasted rats given a glucose load.

Findings from this study demonstrate that a buckwheat concentrate is an effective source of D-CI for lowering serum glucose concentrations in rats. Buckwheat can provide a concentrated source of D-CI in the food supply and therefore may be useful in the treatment of diabetes.

ABBREVIATIONS USED

IPG, inositolphosphoglycan; GPI, glycosylphosphatidylinositols; D-CI, D-*chiro*-inositol; OGTT, oral glucose tolerance test; STZ, streptozotocin; IPGTT, intraperitoneal glucose tolerance test.

ACKNOWLEDGMENT

We acknowledge the following for their contributions to this research: Dr. S. G. Angyal for providing the D-*chiro*-inositol standard; Kade Research Ltd. for providing buckwheat; Lisa Maximiuk, Jennifer Zahradka, Amy Noto, Melissa Zirk, Heather Hosea, Heather Simpson, and the staff of the Animal Holding Facility at the University of Manitoba for their assistance with animal test procedures; and the Canadian International Grains Institute for their guidance and use of their milling facility to produce the milled buckwheat fraction.

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Received for review March 25, 2003. Revised manuscript received September 24, 2003. Accepted September 24, 2003. We acknowledge Agri-Food Research and Development Initiative and Kade Research Ltd. for funding.

JF0302153