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Research Article

Intestinal Glucose Release Following Insulin-Induced Hypoglycemia in Dogs: Implication of Gluconeogenesis and Glycogenolysis

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Summary: This study was designed to investigate the source of the glucose released by the small intestine during insulin-induced hypoglycaemia in dogs. Experiments were carried out on fasted, male, anaesthetized mongrel dogs divided into 3 groups (n = 5 each). Group 1 received normal saline (0.2 ml/kg) and served as the control while groups 2-3 were injected with low (5 i.u/kg) and high (8 i.u/kg) doses of insulin. The left femoral artery and vein were cannulated for arterial blood sampling and intravenous administration of normal saline or insulin, respectively. Through a midline laparatomy, a vein draining the upper jejunum was cannulated for Intestinal Blood Flow (IBF) measurement and jejunal venous blood sampling. In stabilized animals, basal measurement of IBF and levels of glucose and lactate in blood were obtained prior to the injections and monitored for 90 minutes post injection. Intestinal Glucose/Lactate Uptake was calculated as the product of IBF and arterio-venous glucose /lactate difference. Jejunal tissue samples were obtained for the determination of Glycogen Content and activities of glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase. Data are presented as Mean ± SEM and compared by student's t-test and ANOVA. Intestinal blood flow was significantly increased by insulin. Within 20 minutes post injection of insulin, glucose uptake was negative while lactate uptake increased. Glycogen content, glycogen synthase activity and hexokinase activity were significantly reduced in the insulin groups compared with the control while glycogen phosphorylase 'a' and glucose-6-phosphatase activities were increased significantly. In conclusion, the glucose released during insulin-induced hypoglycemia may receive inputs from the breaking down of glycogen and synthesis of glucose within the small intestine.

Keywords*: Glucose release, lactate uptake, Glycogen metabolism, Jejunum, Dogs*

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INTRODUCTION

Previous studies have shown conclusively that the gastro-intestinal tract plays a modulatory role in glucose homeostasis in dogs (Alada and Oyebola, 1996; Alada *et al*., 2005; Salman *et al*., 2014; Shittu *et al*., 2018). Thus, when the blood glucose was increased following hyperglycaemia induced by adrenaline (Grayson and Oyebola, 1983; Alada and Oyebola, 1996, Oyebola *et al*., 2011), glucagon (Alada and Oyebola 1996; 1997), nicotine (Grayson and Oyebola, 1985; Oyebola *et al*., 2009), infusion of glucose and other sugars (Alada and Oyebola 1996; Salman *et al*., 2014) or by hyperglycaemia that occurs in diabetes mellitus (Alada *et al*; 2005), the intestine significantly increased its glucose uptake by as much as 700 to 1000 %. Also, when the blood glucose was reduced, for instance, by administration of insulin to hypoglycaemic level, the intestine pushes out glucose into blood circulation (Alada and Oyebola, 1996; Alada *et al*., 2005). Interestingly, a reduction in blood glucose above the hypoglycaemic levels as reported by Alada *et al*. (2005) in dogs did not cause a release of glucose from the intestine. Thus, the intestinal glucose release is dependent on the presence of hypoglycemia.

Recently, Shittu *et al*. (2018) provided strong evidence to suggest that the huge amount of glucose taken up by the intestine in response to the induced hyperglycaemia was mostly converted to glycogen and to some extent oxidized by glycolysis, judging by the increased levels of lactate in circulation. However, the metabolic source of the glucose that is released into circulation in response to insulin-induced hypoglycaemia (Alada and Oyebola, 1996; 1997) is still not known. For example, in the liver, which is the major blood glucose regulating organ in the body, glucose is released into circulation through the processes of gluconeogenesis and glycogenolysis because the liver possesses the enzyme glucose-6-phosphatase which is a key enzyme involved in the last biochemical step leading to the production of glucose in gluconeogenesis and glycogenolysis. Mitheux *et al*. (2004) reported the expression of glucose-6-phosphatase gene in the intestine of rat and man, thereby suggesting that the intestine of both rat and man could also be involved in endogenous glucose production. With the use of molecular techniques and monitoring gene expression of glucose-6-phosphatase, Penhoat *et al*. (2014) were able to show that during fasting, the small intestine of mice also contributes significantly to endogenous glucose production. It was therefore, concluded that under certain conditions such as fasting and hypoglycaemia, the small intestine of rat, mouse and man behaves essentially like the liver and kidney in regulation of glucose homeostasis. For instance, the endogenous glucose production of the small intestine could increase from 5 – 10 % in post-absorptive rat to 20 – 25 % after 24hr of fasting (Croset *et al*., 2001; Mithieux *et al*., 2006). It must however,

be noted that the metabolic pathways for endogenous glucose production in the liver is not the same as in the small intestine (Penhoat *et al*., 2014).

The present study was therefore designed to investigate the possible source of glucose that is released into circulation following insulin-induced hypoglycaemia in dogs.

MATERIALS AND METHODS

The experimental procedure was as earlier described (Shittu *et al*., 2018). Adult male mongrel dogs (9 – 11 kg) were anaesthetized (Sodium Pentobarbitone, 30 mg/kg) after an overnight fast prior to the commencement of the experiments. They were allowed to breathe at room temperature via a Y-piece cannula inserted into the trachea. The left femoral artery and vein were cannulated with the arterial canula advanced to the level of the superior mesenteric artery for arterial sampling while the venous canula was for drug delivery. A midline laparotomy was carried out to isolate the jejunum, then a vein draining the proximal segment of the jejunum was cannulated for jejunal venous blood sampling. The jejunal vein cannula was moved into an extra-corporeal position and a non-crushing clamp was applied to its free end. Intravascular blood clotting was prevented by administration of Sodium heparin (300 I.U/kg, i.v) and supplemental doses of the anaesthetic agent were given as required. By two layered interrupted sutures, the abdomen was closed and the dogs were allowed to stabilize for 60 minutes.

Experimental procedure: The experiments were carried out on three groups (control and insulin groups) with 5 dogs per group. Basal measurements of jejunal blood flow, arterial and venous glucose and lactate levels were done in the stabilized animals. Low (5 i.u/kg) or high doses (8 i.u/kg) of insulin (Actrapid®, Norvo Nordisk) was then injected intravenously and the variables were monitored for 90 minutes at 5 minutes interval post- injection of insulin. Timed collection was used to determine jejunal blood flow (Alada and Oyebola, 1996). Arterial and Venous glucose and lactate levels were determined using glucose oxidase and lactate dehydrogenase methods, respectively; arterio-venous differences (A-V) were calculated for glucose [$(A\text{-}V)_{glucose}$] and lactate [$(A\text{-}V)_{lactate}$]. Intestinal Glucose Uptake (IGU) and lactate uptake were calculated as the product of intestinal blood flow and [$(A\text{-}V)_{glucose}$] or [$(A\text{-}V)_{lactate}$]. Jejunal tissue biopsy was taken 10-15 minutes post injection for glycogen content determination and for the assays of glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase enzymes activities. The effect of normal saline (0.2 ml/kg) on the measured variables was studied in another 5 dogs and this served as the control group.

Determination of jejunal glycogen content: The method of Seifter *et al.* (1950) as modified by Jermyn (1975) was used to quantify jejunal glycogen content. The tissue was digested in potassium hydroxide, washed with ethanol, centrifuged and drained. The obtained white precipitate was reconstituted in water and step-wisely reacted with hydrochloric acid, formic acid and anthrone reagent. It was incubated at 100 ^{0}C for 10 minutes, cooled and the absorbance of the blue colored solution was taken at 630 nm. The glycogen concentration (mg/ml) was read from a glycogen standard curve. Glycogen content/100 g tissue was calculated as detailed earlier (Shittu *et al*., 2018).

Tissue homogenization for enzyme activities: The tissues were first rinsed in ice cold 1.15% KCl, freed of intestinal contents and dabbed on filter paper. Samples for glycogen synthase, glycogen phosphorylase and hexokinase activities were homogenized in 0.05 M phosphate buffer saline (pH 7.4) while samples for glucose-6-phosphatase activity were homogenized in sucrose buffer containing 0.25 M sucrose, 4 mM EDTA and 1 mM NaF. Protein contents of the supernatants were determined by Biuret method.

Determination of jejunal glycogen synthase activity: Jejunal glycogen synthase activity was assayed using the spectrophotometric stop rate (Kinetic) method of Danforth (1965). Briefly, 100 µl of tissue supernatant was added to a reaction cocktail containing 0.5M Tris HCl Buffer (pH 8.2), $MgCl_2$, EDTA-tetrasodium, β-Mercaptoethanol, UDPG, glycogen and deionized water. It was mixed by inversion and incubate for 5 minutes at 30 ^{0}C. The reaction was stopped by heating the test or blank for 5 minutes at 100 ^{0}C then cool over running tap water. The solutions were transferred into eppendorf tubes and centrifuged. The obtained supernatant (100 µl) was added into another reaction cocktail containing 0.2M Tris HCl Buffer (pH 7.5), KCl, $MgSO_4$, Phosphoenol pyruvate, EDTA-tetrasodium, β-NADH, deionized water and PK/LDH enzyme suspension. It was mixed immediately by inversion and the decrease in absorbance was recorded for five minutes. The final absorbance was obtained for both test and blank supernatant.

Glycogen synthase (Units/mg. protein)

$$= \frac{\Delta \text{ AbsorbanceTest} - \Delta \text{ Absorbance Blank } (2.91)}{(5)(6.22)(0.1)(protein\ concentration)}$$

Determination of jejunal glycogen phosphorylase 'a' activity: Glycogen phosphorylase 'a' activity was assayed using the spectrophotometric stop rate (Kinetic) method of Fischer *et al*. (1962) and Bergmeyer *et al.* (1974). Briefly, in the absence of 5´-AMP, 100 µl of jejunal tissue supernatant was added to a reaction cocktail containing 0.5M Potassium Phosphate Buffer, Glycogen, $MgCl_2$, EDTA, phosphoglucomutase and NADPH, pH 6.8 at 30 ^{0}C. It was mixed by inversion and increase in absorbance was monitored at 340nm for approximately 10 minutes and the ΔA_{340}/minute was obtained for both the Test and Blank. The Phosphorylase a units/ml enzyme was calculated as:

$$\frac{\left(\Delta\frac{\text{Absorbance}}{\text{min}}\text{Test without 5´AMP} - \Delta\frac{\text{Absorbance}}{\text{min}}\text{Blank without 5´AMP}\right)(2.95)(df)}{(6.22)(0.1)}$$

Where df is the diluting factor

The Phosphorylase a units/mg protein

$$= \frac{\text{Phosphorylase a units/ml enzyme}}{protein\ concentration}$$

Determination of Hexokinase activity: Hexokinase activity in the jejunum was determined by the method described by Branstrup *et al.* (1957) wherein the rate of disappearance of glucose was determined at 38 ^{0}C in a buffer solution containing ATP, Magnesium, KCl and

Fluoride. Briefly, 2 ml of Glucose buffer [0.0025 M glucose, 0.0025 M $MgCl_2$, 0.025 M NaF, 0.01 M K_2HP0_4, 0.077 M KCl, and 0.03 M Tris (Hydroxy-methyl) aminomethane, (Trizma base) pH 8] was pipetted into a test tube followed by 0.1 ml of 0.18 M ATP solution and 0.9 ml of distilled water. The mixture was preheated in water for 5 minutes at 38 ^{0}C, 1 ml of jejunal homogenate was added and 100 µl of the homogenate-buffer substrate mixture was taken immediately for initial glucose analysis. The mixture was then incubated at 38 ^{0}C for 30 minutes and another 100 µl was taken for final glucose analysis. The difference in the level of glucose was calculated and hexokinase activity was expressed as glucose metabolised/mg. pr/30min. All assays were carried out in duplicates. In this assay, glucose was assayed using a commercially available Glucose GOD-PAP kit (Fortress Diagnostic®, United Kingdom).

Determination of Glucose-6-Phosphatase activity: Glucose-6-phosphatase activity was assayed according to the method of Koide and Oda (1959) based on the principle that the enzyme acts as phosphohydrolase and phosphotransferase. The reaction involves the formation of covalently bound enzyme-inorganic phosphate intermediate that can liberate inorganic phosphate in the presence of an acceptor. The liberated inorganic phosphate can then be quantified using a suitable method. Briefly, into a test tube, 0.3 ml of 0.1 M citrate buffer (pH 6.5), 0.5 ml of 150 mM glucose-6-phosphate solution and 0.2 ml of sucrose buffer extracted jejunal homogenate were mixed and incubated at 37 ^{0}C for 1 hour. At the end of the incubation period, 1.0 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction and placed on ice. After 10 minutes on ice, the mixtures were centrifuged. Aliquot (1 ml) of the supernatant was then used for the determination of liberated phosphate by the method of Fiske and Subbarow (1925).

Statistical Analysis

Data were presented as Mean± SEM of the variables measured. Differences in mean values were compared using student' t-test and ANOVA. P values of 0.05 or less were taken as statistically significant.

RESULTS

Effect of insulin on arterial and venous blood glucose level: The effects of insulin on arterial and venous blood glucose levels are shown in Table 1. The two doses of insulin administered to the dog produced immediate reduction in blood glucose level which was sustained throughout the post-injection observation period. At low dose of insulin, the arterial blood glucose decreased from a basal value of 106.4 ± 4.69 mg/dl to a minimum value of 91.8 ± 1.59 mg/dl. When high dose of insulin was administered, the arterial blood glucose level further decreased from 106.2 ± 4.48 mg/dl to 52.20 ± 7.28 mg/dl.

Insulin administration also reduced significantly the venous blood glucose level. However, between 5 to 20 minutes post-injection, the venous blood glucose was higher than the arterial blood glucose level.

Effect of insulin on intestinal blood flow, arterio-venous glucose difference and intestinal glucose uptake: The effects of insulin on intestinal blood flow is shown in figure 1(A). Insulin produced a significant increase in intestinal blood flow within the first 15 minutes post-injection which declined gradually to basal value in the remaining part of the post-injection observation period. Intestinal blood flow increased from basal value of 10.4 ± 0.35 ml/min to a maximum value of 11. 8 ± 0.37 ml/min in response to low dose of insulin and further increased from 10.6 ± 0.24 ml/min to 12.5 ± 0.43 ml/min in response to high dose of insulin. There was however no difference in the increased intestinal blood flow produced by the two doses of insulin. The effect of insulin on arterio-venous glucose difference is shown in figure 1(B). Insulin caused negative arterio-venous glucose difference in the first twenty minutes post-injection of insulin and thereafter increased towards basal value in the remaining part of the post-injection observation period. Low dose decreases arterio-venous glucose difference from a basal value of 2.8 ± 0.73 mg/dl to a minimum value of -4.40 ± 1.94 mg/dl. High dose of insulin had a more profound effect on arterio-venous glucose difference, for instance, arterio-venous glucose difference decreased from the basal value of 3.00 ± 0.55 mg/dl to the minimum value of -6.80 ± 1.28 mg/dl in response to high dose of insulin.

The effect of insulin on intestinal glucose uptake is shown in figure 1(C). Insulin significantly produced negative intestinal glucose uptake between 5 minutes to 20 minutes post injection and returned to values that is not different from the basal in the remaining part of the observation period. In other words, insulin pushes out glucose in to circulation between 5-20 minutes post-injection. Low dose of insulin produced decreased intestinal glucose uptake from a basal value of 29.2 ± 8.17 mg/min to -49.0 ± 10.86 mg/min. while high dose of insulin decreased intestinal glucose uptake from a basal value of 29.8 ± 5.76 mg/min to -82.0 ± 14.02 mg/min. The percentage decrease in intestinal glucose uptake is about 275%.

Table 1.

Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on arterial and venous glucose level in dogs (n=5). *P<0.05, **P<0.01, ***P<0.001.

	Dose	0 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	75 min	90 min
Arterial Glucose level (mg/dl)	Low Dose	106.4 ±4.69	102.6 ±2.73	97.4 ±2.52	91.8 ±1.59**	94.8 ±3.74*	93.4 ±2.80*	96.4 ±2.94*	94 ±1.87*	94.6 ±4.65	95.4 ±2.50
	High Dose	106.20 ±4.48	82.60 ±4.29***	52.20 ±7.28***	55.40 ±8.13***	70.60 ±5.03***	90.80 ±3.82**	100.00 ±3.33*	102.80 ±3.32	98.00 ±2.07	103.00 ±5.18
Venous Glucose level (mg/dl)	Low Dose	103.6 ±5.32	103.4 ±3.12	99 ±2.84	96.2 ±1.85	95.2 ±3.74	91.2 ±2.55*	93.6 ±3.00	90.8 ±1.88*	92.1 ±4.48	92.6 ±2.67
	High Dose	103.30 ±4.13	94.60 ±6.24**	59.00 ±7.46**	57.80 ±8.66**	72.20 ±5.75**	88.20 ±4.20**	97.40 ±3.36*	99.40 ±3.21	93.80 ±2.13	100.40 ±5.45

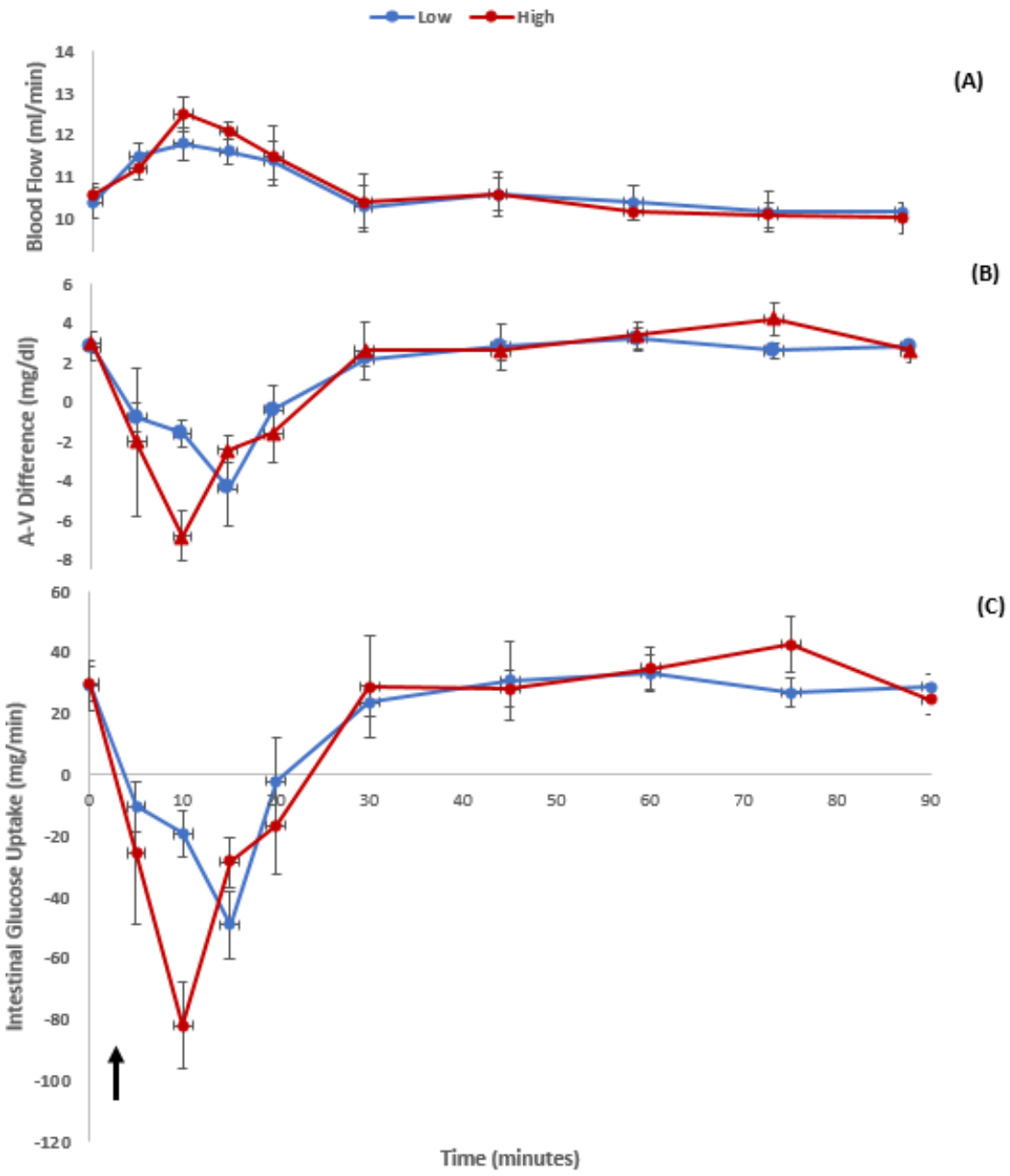

Figure 1.
Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on (A) jejunal blood flow, (B) Arterio-venous glucose difference and (C) Intestinal glucose uptake in dogs (n=5). Black arrow indicates point of injection.

Table 2.
Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on arterial and venous lactate level in dogs (n=5). *P<0.05.

	Dose	0 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	75 min	90 min
Arterial Lactate level (mg/dl)	Low dose	20.60 ±1.90	21.03 ±1.93	22.97 ±1.00	25.62 ±1.64*	26.42 ±2.64*	27.648 ±2.12*	28.02 ±3.37*	26.93 ±2.78*	29.05 ±2.92*	31.09 ±3.22*
	High dose	22.22 ±2.67	25.71 ±2.65	27.38 ±2.05*	26.43 ±2.98	25.91 ±2.82	25.16 ±3.26	27.36 ±2.19*	25.54 ±3.08	29.52 ±2.51*	27.78 ±3.28
Venous lactate level (mg/dl)	Low dose	24.05 ±2.25	19.62 ±1.70*	21.62 ±2.40	22.09 ±1.37	25.09 ±2.36	26.71 ±2.42	28.98 ±3.24	28.47 ±3.55	31.92 ±3.10	33.71 ±2.08
	High	26.36 ±1.64	22.92 ±1.32*	21.86 ±1.71*	25.68 ±1.84	26.52 ±2.27	27.25 ±1.81	28.85 ±1.45	28.18 ±2.05	31.04 ±2.40*	31.03 ±2.87*

Effect of insulin on arterial and venous blood lactate level: The effects of insulin on arterial and venous blood lactate levels are shown in Table 2. Insulin caused an immediate increase in arterial blood lactate level which was sustained throughout the post-injection observation period. Venous lactate level was transiently decreased between 5 to 15 minutes post-injection of insulin and thereafter increased throughout the remaining part of the post-injection observation period.

Hypoglycemia induces intestinal glycogenolysis and gluconeogenesis

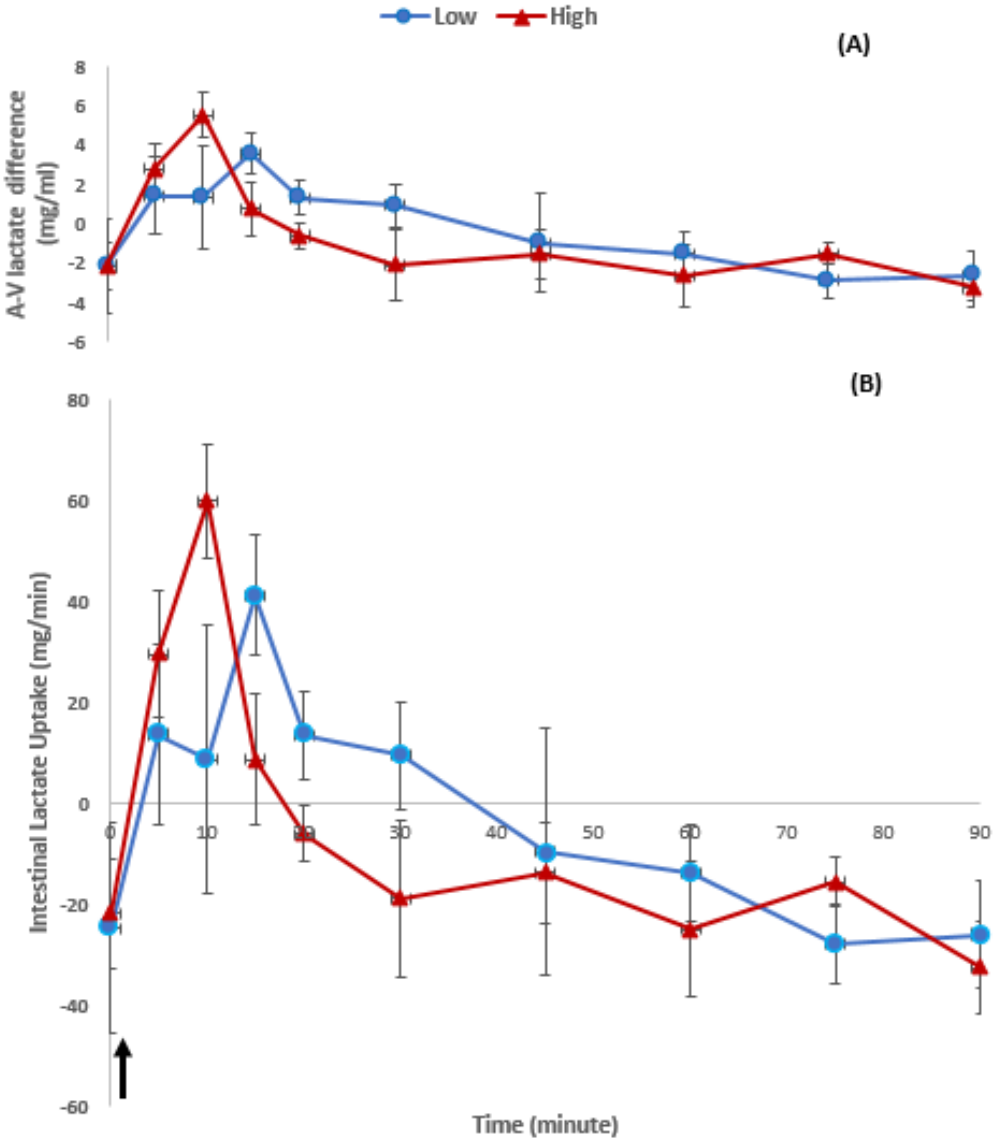

Figure 2.
Effect of intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on (A) Arterio-venous (A-V) lactate difference and (B) intestinal lactate uptake in dogs (n=5). Black arrow indicates point of injection.

Effect of insulin on arterio-venous lactate difference and intestinal lactate uptake: The effect of insulin on arterio-venous lactate difference is shown in figure 2(A). Insulin injection produced a positive arterio-venous lactate difference between 5 minutes to 15 minutes post-injection. In other words, arterio-venous lactate difference which hitherto was negative was reversed by insulin injection within 5-15 minutes post-injection and gradually became negative till the end of the observation period. For instance, following low dose, arterio-venous lactate difference increased from a basal value of -2.14 ± 2.41 mg/dl to a peak value of 3.53 ± 1.04 mg/dl at 15minutes post injection. High dose insulin produced a more profound effect on arterio-venous lactate difference which increased from a basal value of -2.14 ± 1.23 mg/dl to a peak value of 5.54 ± 1.14 mg/dl at 10 minutes post-injection.

The effect of insulin on intestinal lactate uptake is shown in figure 2(B). Insulin reversed the negative intestinal lactate uptake between 5 minutes to 15 minutes post injection which became negative again at 20 minutes till the end of the observation period. That is to say that insulin produced a positive intestinal lactate uptake which indicates that the intestine actually extracted lactate from the blood stream between 5 to 15 minutes following insulin injection. Low dose increased intestinal lactate uptake from basal value of -24.64 ± 20.87 mg/min to a peak significant value of 41.35 ± 11.85 mg/min at 15 minutes post-injection while high dose increased intestinal lactate uptake from -21.76 ± 10.96 mg/min to 60.09 ± 11.36 mg/min at 10 minutes post-injection. The percentage increase in intestinal lactate uptake for low dose and high dose are 168% and 276%, respectively.

Effect of insulin on intestinal glycogen content
The effect of insulin on intestinal glycogen content is shown in Figure 3. Intestinal glycogen content decreased from 138.72 ± 4.58 mg/100g to 106.90 ± 2.29 mg/100g and 97.39 ± 4.33 mg/100g following low and high dose insulin

injection these represented a 23% and 30% depletion following injection of low and high dose of insulin, respectively. There was no significant difference in the depletion of intestinal glycogen content caused by the two doses of insulin.

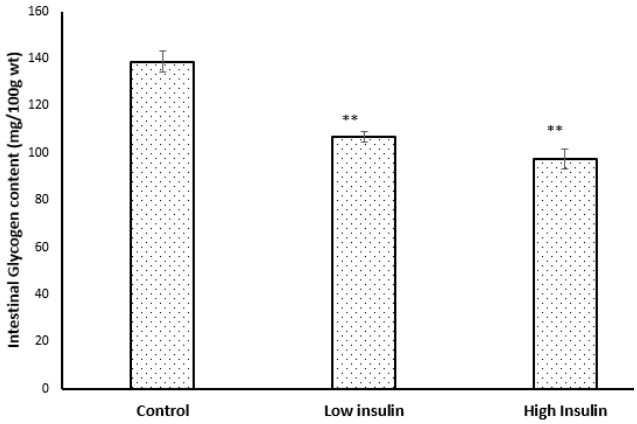

Figure 3:
Effect intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on intestinal glycogen content (n=5). **P<0.01.

Table 3.
Effect intravenous injection of low (5 i.u/kg) and high (8 i.u/kg) doses of insulin on intestinal glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase activities

Enzyme activity	Control	Low Dose	High Dose
Glycogen Synthase (Activity/mg.pr)	1.29 ± 0.13	0.79 ± 0.13**	0.41 ± 0.11**#
Glycogen Phosphorylase a (x 10^{-3}Activity/mg.pr)	1.74 ± 0.21	5.14 ± 0.45**	6.58 ± 0.18**#
Hexokinase (Activity/mg.pr)	1.28 ± 0.20	0.34 ± 0.02*	0.26 ± 0.03**#
Glucose 6-Phosphatase (Activity/mg.pr)	30.71 ± 1.56	45.73 ± 3.10**	41.87 ± 2.72**

Effect of insulin on intestinal glycogen synthase, glycogen phosphorylase 'a', hexokinase and glucose-6-phosphatase activities

The effect of insulin on the intestinal enzyme activities are shown in Table 3. Insulin decreased intestinal glycogen synthase activity in a dose-dependent manner. In other words, high dose of insulin produced a more profound effect on the activities of glycogen synthase. For example, intestinal glycogen synthase activity decreased from 1.29 ± 0.13 activity/mg.pr to 0.79 ± 0.13 activity/mg.pr for low dose of insulin and 0.41 ± 0.11 activity/mg.pr for high dose of insulin.

The two doses of insulin significantly increase glycogen phosphorylase 'a' activity in a dose-dependent manner. Glycogen phosphorylase 'a' activity increased from 1.74 ± 0.21 activity/mg.pr 5.14 ± 0.45 activity/mg.pr and 6.58 ± 0.18 activity/mg.pr for low dose and high dose of insulin respectively.

Hexokinase activity significantly decreased from 1.28 ± 0.20 activity/mg.pr to 0.34 ± 0.02 activity/mg.pr and 0.26 ± 0.03 activity/mg.pr for the low dose and high dose of insulin respectively. On the other hand, glucose-6-phosphatase activity increases from 30.71 ± 1.56 activity/mg.pr to 45.73 ± 3.01 activity/mg.pr and 41.87 ± 1.56 activity/mg.pr. There was however, no difference in the increase in glucose-6-phosphatase activity induced by the two doses of insulin.

DISCUSSION

The observed increase in the blood flow through the intestinal mesenteric veins is consistent with reports in previous studies in rats (Grayson and Kinnear, 1958) and dogs (Grayson and Mendel, 1965; Alada and Oyebola, 1996). This increase occurs irrespective of blood pressure change (Alada and Oyebola, 1996). There are however reports that the rise in blood flow is not a direct effect of insulin *per se* on intestinal vessels but rather secondary to stimulation of sympathetic system and release of catecholamines (Alada and Oyebola, 1997).

The hypoglycaemic action of insulin is well- known. Insulin acts through activation of tyrosine kinase in the beta subunit and phosphorylating several proteins to increase glucose uptake essentially in the liver, skeletal muscles and adipose tissue (Kasuga *et al*., 1983, Yamauchi *et al*., 1996)

The most relevant results in this study is the negative intestinal glucose uptake observed within the first twenty minutes post-injection of insulin when the arterio-venous glucose balance across the intestinal segment was negative. In other words, the intestine was actually involved in endogenous glucose production in dogs during the insulin-induced hypoglycaemia. Similar observations have been reported in rat and humans (Mitheux *et al*., 2004, Rajas *et al*., 2009) and mouse (Penhoat *et al*., 2014) in studies employing tracer techniques and gas chromatography in determining arterio-venous glucose balance. Previous workers (Mitheux *et al*., 2001; Rajas *et al*., 2001) have also provided strong evidence to show that the small intestine is involved in endogenous glucose production through gluconeogenesis. Indeed, gene expression of major gluconeogenic enzymes, including glucose-6-phosphatase, pyruvate carboxylase and phosphoenol carboxykinase have been reported in the small intestine of rat and human (Croset *et al*., 2001; Mithieux *et al*., 2004). Mithieux *et al*. (2004) reported that the small intestine produces glucose using glutamine and glycerol as the main precursors. Abundance of the enzyme glutaminase was also reported in the small intestine of rat and human (Mithieux *et al*., 2005).

Interestingly, intestinal gluconeogenesis was reported to occur only during 24 to 48 hours fasting and under diabetic condition and this could account for as high as 30% of endogenous glucose production in the body (Penhoat *et al*., 2014). There have been other reports of intestinal gluconeogenesis following hepatectomy-induced hypoglycaemia (Battezatti *et al*., 2004, Penhoat *et al*., 2014). In the present study, hypoglycaemia was induced by a single intravenous injection of insulin at both low and high doses. A similar observation of intestinal glucose release was reported in an earlier study (Alada *et al*., 2005) with diabetic dogs following significant reduction of diabetic hyperglycaemia with insulin injections.

The observed increase in intestinal lactate uptake following a rise in arterial blood lactate level in this study is noteworthy. The increase in intestinal lactate uptake also corresponds in timing with the negative arterio-venous glucose balance of the intestine. In other words, while there was an increase in the uptake of lactate by the intestine, glucose was also being released into circulation by the intestine. Although Mitheux and his co-workers (2004) have reported that in rat and human, glutamine and glycerol are the main metabolic precursors for the formation of glucose in the intestinal gluconeogenesis, while lactate is the major precursor in the liver and the kidney. To the best of our knowledge, there has been no report of intestinal gluconeogenesis in the dog. However, even in those animals where there was evidence of intestinal gluconeogenesis, there are still doubts on the metabolic pathways involved in the endogenous glucose production. In this study, an observation of the presence of and a significant increase in the activity of glucose-6-phosphatase in the intestine of dog give support to the hypothesis that the intestine of dog is most probably also involved in gluconeogenesis as earlier reported for rat (Croset *et al*., 2001), mouse (Penhoat *et al*., 2014) and human (Mithieux *et al.*, 1999). Further studies using molecular techniques and knockout model may throw more light on this observation.

Another possible source of the glucose released from the intestine following insulin-induced hypoglycaemia in this study is through glycogenolysis. Some workers (Rajas *et al*., 1999; Mithieux *et al*., 2004) had earlier reported on intestinal glycogenolysis during hypoglycaemia in rat and human. The observed decrease in intestinal glycogen content and activity of intestinal glycogen synthase with concomitant increase in activity of intestinal phosphorylase 'a' following insulin-induced hypoglycaemia in this study is consistent with the possibility of intestinal glycogenolysis in dogs. The observed increase in the activity of glucose-6-phosphatase in this study also supports the possibility of intestinal glycogenolysis since the enzyme is important in breaking down glucose-6-phosphate into glucose and phosphate ion.

In conclusion, this study provided evidence to show that the intestine of dog could be involved in gluconeogenesis and glycogenolysis, thereby accounting for the source of the glucose released into circulation following insulin-induced hypoglycaemia. However, more studies employing tracer and molecular techniques with knockout animal models may be able to provide more information on our observations.

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