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Metabolic Fate of the Glucose Taken up by the Intestine During Induced Hyperglycaemia in Dogs

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Summary: Available data showed that the intestine increases it glucose uptake in response to hyperglycemia induced by any cause. However, what the intestine does with the glucose is not known. This study investigated the metabolic fate of the glucose taken up by the intestine during hyperglycaemia in dogs. Experiments were carried out on fasted, male, anaesthetized mongrel dogs divided into 4 groups. The control (group 1, n=5) received normal saline (0.2 ml/kg) while groups 2-4 (subdivided into two as low or high dose, n=5 each) received adrenaline (1 µg/kg or 5 µg/kg), glucagon (3 ng/kg or 8 ng/kg) and glucose (10 mg/kg/min or 20 mg/kg/min). Through a midline laparatomy, the upper jejunum was cannulated for Intestinal Blood Flow (IBF) measurement. Blood glucose and lactate levels were determined using glucose oxidase and lactate dehydrogenase methods, respectively. Intestinal Glucose/Lactate Uptake (IGU/ILU) was calculated as the product of IBF and arterio-venous glucose /lactate difference [(A-V) $_{glucose/lactate}$]. Jejunal tissue samples were obtained for the determination of Glycogen Content (GC) and activities of Glycogen Synthase (GS), Glycogen Phosphorylase 'a' (GPa), hexokinase and glucose-6-phosphatase. Anthrone method was used to determine GC while activities of GS, GPa, hexokinase and glucose-6-phosphatase were determined spectrophotometrically. Data were subjected to descriptive statistics and analyzed using student's t-test and ANOVA at $\alpha_{0.05}$. Arterial and venous blood glucose and lactate were increased by adrenaline, glucagon and glucose. Venous lactate was higher than arterial lactate in all groups. Intestinal blood flow, (A-V) glucose and (A-V) lactate were increased in all the experimental groups. Intestinal glucose uptake increased by 624% (adrenaline), 705% (glucagon) and 589% (glucose) while intestinal lactate release increased by 422%, 459% and 272% respectively. Intestinal GC increased from 138.72 ± 4.58 mg/100 g to 167.17 ± 4.20 mg/100 g (adrenaline), 229.21 ± 6.25 mg/100 g (glucagon) and 165.17 ± 4.20 mg/100 g (glucose). Adrenaline and glucose had no effect on GS activity but it was increased by glucagon; GPa was decreased while hexokinase activity was increased by adrenaline, glucagon, and glucose. Glucose-6-phosphatase activity was not affected by adrenaline and glucagon but decreased by glucose. The intestine modulates blood glucose levels through lactate formation, glycogen formation and most probably conversion of lactate to glucose through gluconeogenesis.

Keywords*:* Glucose uptake, Lactate uptake, Glycogen, Dogs.

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INTRODUCTION

Available evidence in the last three decades have shown conclusively that the gastrointestinal tract increases its glucose uptake during hyperglycaemia irrespective of the cause of the increase in blood glucose. Thus, the intestinal glucose uptake increased by 250 – 1000% following hyperglycaemia induced by adrenaline (Grayson and Oyebola, 1983, Alada and Oyebola, 1995, Alada et al; 2000, Oyebola et al; 2007), nicotine (Grayson and Oyebola, 1985, Oyebola et al; 2008), glucagon (Alada and Oyebola, 1995), glucose infusion (Alada and Oyebola, 1995, Salman and Alada, 2014) and diabetes mellitus (Alada et al; 2000) in dogs or rabbits.

When Grayson and Oyebola (1983, 1985) measured both glucose uptake and oxygen consumption in the upper jejunum of dogs in response to adrenaline or nicotine induced hyperglycaemia, they showed that the intestine has a capacity for huge glucose and oxygen consumption but the increase in glucose uptake was far more than the increase in oxygen consumption. Also, the increase in glucose uptake and oxygen consumption did not occur at corresponding times. It was therefore difficult to conclude that the huge amount of glucose taken up by the gut was involved in glucose metabolism. However, available evidence suggests that the glucose taken up by the gastrointestinal tract may not be utilised for oxidative metabolism. Indeed, Windmueller and Spaeth (1978) showed that isolated, vascularly perfused preparations of rat intestine took up from the circulation nearly as much glutamine as glucose, and glutamine accounted for more than 30% of the carbon dioxide produced.

These results and those of earlier workers suggest that the gastrointestinal tract is more involved in glucose homeostasis than in glucose metabolism. The nature of this involvement is however not clear. The

question therefore is, will the gastrointestinal tract take up glucose and convert it to glycogen or oxidise it to lactate/pyruvate as it is done in the liver? The present study was therefore designed to investigate the metabolic fate of the huge amount of glucose taken up by the intestine following concomitant measurements of the plasma lactate and tissue glycogen during hyperglycaemia induced by adrenaline, glucagon or glucose infusion.

MATERIALS AND METHODS

Experiments were carried out on anaesthetized (Sodium Pentobarbitone, 30 mg/kg) adult male mongrel dogs weighing 9 – 11 kg. Each dog was fasted for 18-24h before the start of the experiment. Light anaesthesia was maintained with supplementary doses of i.v. sodium pentobarbitone as necessary. The trachea was intubated using a Y-piece cannula and the dog was allowed to breathe room air (temp. 25ºC) spontaneously. Cannulae were placed in the left femoral artery and vein. The cannula in femoral artery was advanced to the level of the superior mesenteric artery. Through a midline laparotomy, the jejunum was identified and a vein draining the proximal segment of the jejunum was cannulated using a 1.8 mm (i.d) polyethylene tubing (P.E. 260). The jejunal vein cannula was moved into an extra-corporeal position and a non-crushing clamp was applied to its free end. Sodium heparin, 300 unit per kg was administered i.v to prevent intravascular blood clotting. The abdomen was then closed in two layers with interrupted sutures. Each dog was then allowed to stabilize for 60-90 minutes prior to commencement of experimental procedure.

Experimental procedure

After stabilization, basal measurements were done for jejunal blood flow, arterial and venous glucose and lactate levels. Jejunal blood flow was determined by timed collection as described by Alada and Oyebola (1996). Arterial samples for glucose and lactate estimation were collected from the cannula at the femoral artery while venous samplings were collected from the cannula at the jejunal vein. After the basal measurements, a bolus injection of adrenaline (1 µg/kg or 5 µg/kg), glucagon (3 ng/kg or 8 ng/kg) or 10 minutes infusion of glucose (10 mg/kg/min or 20 mg/kg/min) was given intravenously. Five dogs each were used for the low or high doses of adrenaline, glucagon or glucose. Glucose infusion was carried out using an infusion pump (Palmer, England). Measurement of jejunal blood flow and arterial and venous blood sampling for glucose and lactate were carried out at 0, 5, 10, 15, 20, 30, 45, 60, 75 and 90 minutes post- injection of adrenaline or glucagon or during infusion and post-infusion of glucose. Arterial and Venous glucose and lactate levels were determined using glucose oxidase and lactate dehydrogenase methods, respectively; arterio-venous difference (A-V) was determined 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 done between 10-15 minutes post injection or post infusion for determination of jejunal glycogen content and homogenised in phosphate buffer saline (PBS, pH 7.4) for activities of glycogen synthase, glycogen phosphorylase, hexokinase and glucose-6-phosphatase enzymes. The protein content of tissue supernatant was determined to allow the expression of the enzyme activities per milligram protein.

Another group of 5 dogs was studied for the effect of normal saline (0.2 ml/kg) on intestinal blood flow, arterial and venous glucose and lactate, intestinal glycogen content and enzyme activities as in the other groups. This group served as the control

Determination of jejunal glycogen content

The glycogen content of the jejunum was determined using the method of Seifter *et al.* (1950) as modified by Jermyn (1975). Briefly, 1 g of jejunum was digested in 10ml of 30% KOH over heat. An aliquot of 4 ml of the digested tissue was washed by adding 5 ml of 95% ethanol, centrifuged for 5 minutes and drained. The precipitate was reconstituted with 0.5ml of distilled water and rewashed to obtain a white precipitate which was reconstituted with 2 ml of distilled water. The reconstituted precipitate or distilled water (0.5 ml) was pipetted into a test tube followed by stepwise addition of 0.5 ml concentrated HCl, 0.5 ml 88% formic acid and 4 ml of anthrone reagent (added slowly to minimize frothing). The solution was mixed thoroughly, incubated at 100^0C for 10 minutes and cooled. A standard curve was obtained by treating several double dilutions of 0.2 mg/ml of standard glycogen with HCl, formic acid and anthrone reagent as it was done for the test/blank. Absorbance of the blue colored solution formed was read at 630 nm against a reagent blank. Glycogen concentration (mg/ml) was obtained from the standard curve while glycogen content/100 g tissue was calculated as follows:

Glycogen content/100 g wet tissue

$$= glycogen\ concentration\ X\ \frac{10}{4}\ X\ \frac{2}{0.5}\ X\ \frac{100}{total\ tissue\ weight}$$

Determination of Liver glycogen synthase activity

Jejunal glycogen synthase activity was assayed using the spectrophotometric stop rate (Kinetic) method of Danforth (1965). Briefly, 100 µl of hepatic 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° C. The reaction was stopped by heating both the test or blank for 5 minutes at 100^0C then cool over running tap water. The solutions were transferred into eppendorf tubes and centrifuged. Supernatant obtained (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 Liver 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 hepatic 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 38^0C in a buffer solution containing ATP, Magnessium, KCl and Fluoride. Briefly, 2 ml of Glucose buffer [0.0025 M glucose, 0.0025 M $MgCl_2$, 0.01 M K_2HP0_4, 0.077 M KCl, and 0.03 M Tris (Hydroxy-methyl) aminomethane, (Trizma base) pH 8] was pippetted 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 38^0C, 1 ml of liver homogenate was added and 100µl of the homogenate-buffer substrate mixture was taken immediately for initial glucose analysis. The mixture was then incubated at 38^0C 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 hepatic homogenate were mixed and incubated at 37^0C 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

Effects of normal saline

Normal saline had no effect on blood flow, (A-V)glucose, (A-V)lactate, intestinal glucose uptake and intestinal lactate level. The resting intestinal blood flow was 10.4 ± 1.88 ml/min, (A-V) glucose was 2.51± 0.52 mg/dl, (A-V)lactate was -3.11± 0.83 mg/dl, intestinal glucose uptake was 25.96 ± 6.25 mg/min while intestinal lactate uptake was -30.99 ± 6.4 mg/min. The resting value of intestinal glycogen content was 138.72 ± 4.58 mg/100g tissue, glycogen synthase activity was 1.29 ± 0.13 activity/mg.pr, glycogen phosphorylase activity was $1.74 \pm 0.21 \times 10^{-3}$ activity/mg.pr, hexokinase activity was 1.28 ± 0.20 activity/mg.pr and glucose-6-phosphatase activity was 30.71 ± 1.56 activity/mg.pr.

Effects of adrenaline

Intestinal blood flow (IBF) increased significantly in both the low dose and high dose reaching its peak at

15 minutes post-injection. There was no difference in the increased IBF produced by the two doses of adrenaline. The effects of adrenaline on blood glucose levels and arterio-venous (A-V) glucose difference are shown in table 1. The arterial glucose level increased significantly from a basal value of 102.6 ± 6.05 mg/dl to 162.8 ± 5.94 mg/dl at 15 min post-injection, for low dose adrenaline, while the venous glucose also increased from a basal value of 100.4 ± 5.78 mg/dl to 157.0 ± 5.24 mg/dl at the same period. The (A-V) glucose also increased significantly. High dose adrenaline produced a greater increase in arterial and venous glucose and the (A-V) glucose. As shown in figure 1a, low dose adrenaline produced 546% increased intestinal glucose uptake while high dose of adrenaline produced 624% increased uptake. The effect of the high dose of adrenaline on intestinal glucose uptake is significantly higher than the low dose of adrenaline.

The effect of adrenaline on blood lactate levels and arterio-venous (A-V) lactate difference are shown in table 2. The two doses of adrenaline significantly increased arterial and venous blood lactate levels throughout the post-injection observation period. At each dose of adrenaline, the venous blood lactate level was higher than the arterial blood lactate level. The arterio-venous lactate level was negative at rest which indicates that the intestine releases lactate into circulation at rest. Adrenaline at the two doses administered increased the amount of lactate that is released into circulation during the 90 minutes observation period. The maximum lactate release occurred within the first 15 minutes post-injection.

There was however no difference in the increased blood lactate level or arterio-venous lactate difference produced by the two doses of adrenaline. As shown in figure 2a, intestinal lactate uptake was negative throughout the experiments with the two doses of adrenaline. This is indicative of intestinal lactate release. At the peak of the intestinal response to adrenaline, low dose produced 438% while high dose produced 422% increase in intestinal lactate release at 15 minutes post injection There was however no significant difference in the peak of intestinal lactate production in response to low dose adrenaline and high dose adrenaline.

Table 1: Effect of intravenous injection of low (1µg/kg) and high (5µg/kg) doses of adrenaline on arterial glucose level, venous glucose level, and arterio-venous glucose difference [(A-V) glucose in dogs (n=5). (*P<0.05, **P<0.01, ***P<0.001)

	Dose	0min	5min	10min	15min	20min	30min	45min	60min	75min	90min
Arterial Glucose level (mg/dl)	Low dose	102.6 ±6.05	118.4 ±7.57	151.4 ±9.42**	162.2 ±7.56***	162.8 ±5.94***	153.8 ±5.98***	148.2 ±5.26***	133.8 ±5.66**	125.2 ±6.87*	119.8 ±7.12
	High dose	104.9 ±4.50	124 ±5.79	161.6 ±9.47***	171.2 ±6.91***	171 ±4.50***	158.8 ±5.43***	148.8 ±5.26***	135.6 ±6.19**	128.6 ±6.50*	124.2 ±5.70
Venous Glucose level (mg/dl)	Low dose	100.4 ±5.78	115.2 ±7.18	145.4 ±9.21**	154.2 ±7.60***	157 ±5.24***	150.8 ±5.98***	146 ±5.27***	131.8 ±5.53**	123 ±6.82*	117.4 ±6.83
	High Dose	102.4 ±4.24	119.8 ±5.46	153.6 ±8.70***	161.6 ±6.69***	164.8 ±4.96***	154.6 ±4.11***	146 ±5.27***	133.2 ±5.24**	126.2 ±5.84*	122 ±5.42*
A-V glucose (mg/dl)	Low dose	2.2 ±0.33	3.2 ±0.44	6.0 ±0.63**	8.0 ±0.49***	5.8 ±0.71**	3 ±0.63	2.2 ±0.33	2 ±0.28	2.2 ±0.44	2.4 ±0.36
	High Dose	2.29 ±0.58	4.2 ±0.80	8.0 ±1.30**	9.6 ±0.51***	6.2 ±0.73**	4.2 ±1.65	2.8 ±0.20	2.4 ±1.29	2.4 ±1.32	2.2 ±0.58

Table 2: Effect of intravenous injection of low (1µg/kg) and high (5µg/kg) doses of adrenaline on arterial lactate level, venous lactate level and arteriovenous lactate difference [(A-V) lactate] in dogs (n=5). (*P<0.05, **P<0.01, ***P<0.001

	Dose	0min	5min	10min	15min	20min	30min	45min	60min	75min	90min
Arterial lactate level (mg/dl)	Low Dose	17.48 ±1.69	20.18 ±2.81	16.09 ±2.90	19.63 ±2.02	23.36 ±2.84	24.01 ±2.11*	27.97 ±1.87**	27.24 ±1.23**	27.51 ±1.01**	27.96 ±1.66**
	High Dose	17.71 ±0.75	20.75 ±2.83	21.32 ±1.05**	20.47 ±2.03	22.126 ±1.96*	25.646 ±4.11	26.742 ±2.69**	28.468 ±3.58**	27.74 ±3.83*	22.71 ±3.40
Venous lactate level (mg/dl)	Low Dose	20.98 ±1.67	24.92 ±3.87	24.27 ±3.52	24.80 ±1.90	27.75 ±2.52*	30.69 ±2.48**	31.50 ±1.84**	32.30 ±2.02**	29.43 ±1.56**	31.16 ±0.57**
	High Dose	21.37 ±0.96	26.798 ±2.76*	26.774 ±1.56**	28.846 ±2.43**	29.692 ±2.21**	30.312 ±4.45*	32.34 ±4.10*	33.5 ±4.07**	30.942 ±2.62**	29.55 ±3.02*
A-V Lactate level (mg/dl)	Low Dose	-2.89 ±0.65	-4.74 ±1.08	-8.19 ±1.55**	-5.17 ±0.94*	-4.39 ±1.33	-6.68 ±1.98	-3.53 ±1.13	-5.06 ±1.56	-1.92 ±0.88	-3.20 ±1.53
	High Dose	-2.97 ±0.54	-6.05 ±2.10	-5.44 ±1.05*	-8.38 ±1.47**	-7.57 ±1.08**	-4.67 ±1.90	-5.59 ±2.33	-5.03 ±1.75	-3.20 ±2.92	-6.84 ±1.84
Blood Flow (ml/min)	Low dose	10.2 ±0.86	12.2 ±0.97	15 ±0.71**	15.5 ±0.74***	13.1 ±1.25*	11.8 ±1.11	10.4 ±0.81	10.2 ±0.66	10.2 ±1.16	10.4 ±1.36
	High dose	10.3 ±0.87	11.7 ±0.54	14.8 ±0.80**	15.6 ±1.03**	13.6 ±0.51**	12.2 ±0.37	10.2 ±0.37	10.1 ±0.40	9.6 ±0.75	9.2 ±0.86

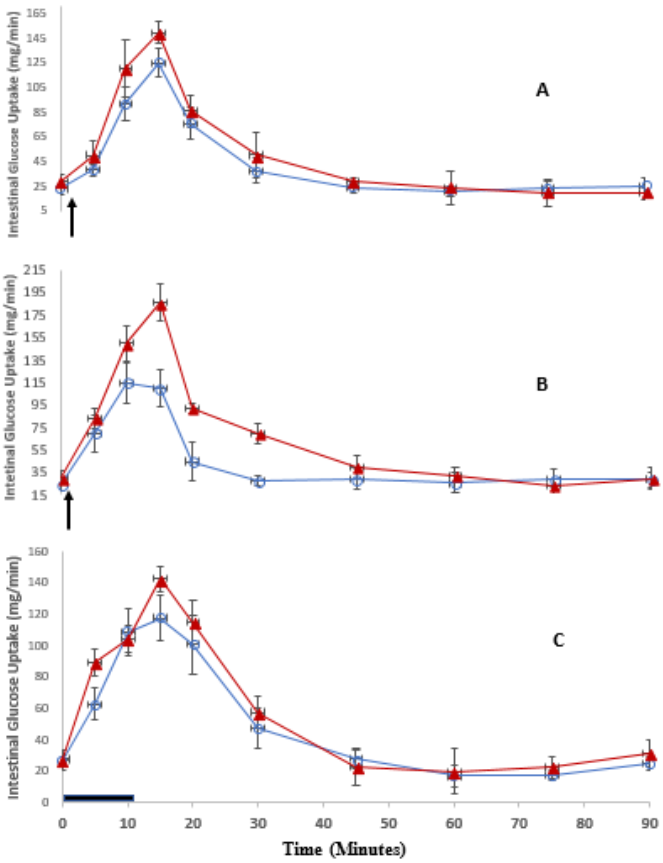

Figure 1. Effect of low(blue) and high (red) doses of adrenaline injection (A), glucagon injection (B) and glucose infusion (C) on intestinal glucose uptake in dogs (n=5). Black arrow indicates point of drug injection. Black bar indicates period of infusion.

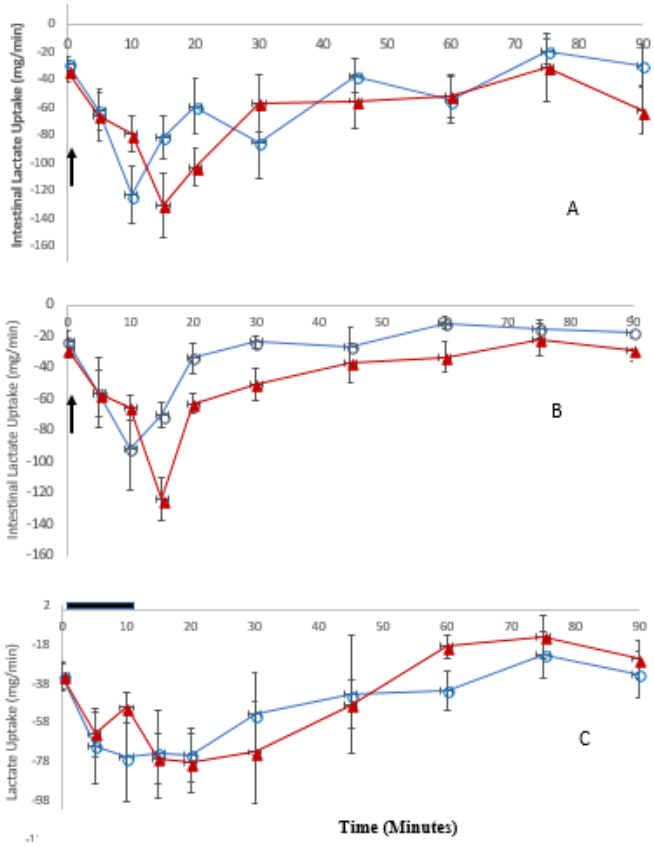

Figure 2. Effect of low(blue) and high (red) doses of adrenaline injection (A), glucagon injection (B) and glucose infusion (C) on intestinal lactate uptake in dogs (n=5). Black arrow indicates point of drug injection. Black bar indicates period of infusion.

Table 3. Effect of intravenous injection of low (1µg/kg) and high (5µg/kg) doses of adrenaline on glycogen contents and activities of glycogen synthase, glycogen phosphorylase, hexokinase and glucose-6-phosphatase enzymes in jejunum of dogs (n=5). (*P<0.05, **P<0.01, #P<0.0.05 low vs high dose)

	Control	Low Dose	High Dose
Intestinal Glycogen Content (mg/100g tissue)	138.72 ± 4.58	141.84 ± 4.13	167.17 ± 4.20**
Glycogen Synthase (Activity/mg.pr)	1.29 ± 0.13	1.35 ± 0.11	1.17 ± 0.03
Glycogen Phosphorylase a ($x\ 10^{-3}$ Activity/mg.pr)	1.74 ± 0.21	1.02 ± 0.12**	1.05 ± 0.11**
Hexokinase (Activity/mg.pr)	1.28 ± 0.20	4.26 ± 0.25**	3.21 ± 0.17** #
Glucose 6-Phosphatase (Activity/mg.pr)	30.71 ± 1.56	28.52 ± 1.18	31.41 ± 1.00

Table 3 shows the effects of adrenaline on intestinal glycogen content and activities of glycogen synthase, glycogen phosphorylase, hexokinase and glucose-6-phosphatase. Adrenaline at low dose had no effect on glycogen content when compared with the control. However, high dose adrenaline significantly increased intestinal glycogen contents. The two doses of adrenaline significantly decreased activity of glycogen phosphorylase activity and increased hexokinase activity while they had no effect on glycogen synthase and glucose-6-phosphatase activities.

Effects of glucagon

Glucagon injection produced a transient but significant increase in blood flow which occurred within the first twenty minutes post-injection of the two doses and declined towards basal value in the remaining part of the 90 minutes observation period. Low dose glucagon caused intestinal blood flow to increase from a basal value of 10.6 ± 0.86 ml/min to peak value of 12.5 ± 0.40 ml/min at 10 minutes while high dose of glucagon increased intestinal blood flow from 10.4 ± 0.51 ml/min to a peak value of 12.4 ± 0.51 ml/min at 15 minutes. There was no difference in the increased intestinal blood flow produced by the two doses of glucagon. glucagon on blood glucose and arterio-venous (A-V) glucose difference is shown in table 4.

Table 4. Effect of intravenous injection of low (3ng/kg) and high (8ng/kg) doses of glucagon on arterial glucose level, venous glucose level, and arterio-venous glucose difference [(A-V) glucose in dogs (n=5). (*P<0.05, **P<0.01, ***P<0.001)

	Dose	0min	5min	10min	15min	20min	30min	45min	60min	75min	90min
Arterial Glucose level (mg/dl)	Low dose	104.6 ±8.22	126.2 ±10.37	141.0 ±8.73*	146.8 ±7.19**	143 ±6.71**	133.6 ±8.06*	131.8 ±10.16	121 ±6.43	115.2 ±6.41	110.6 ±5.77
	High dose	106.1 ±5.63	128.6 ±5.51*	150.8 ±6.32**	159.4 ±5.37***	158 ±5.40***	144.6 ±5.45**	134.8 ±7.69*	125.6 ±5.25	119.2 ±5.03	115.4 ±3.77
Venous Glucose level (mg/dl)	Low dose	102.2 ±8.11	120.4 ±9.54	132.2 ±7.90*	138 ±8.11*	139 ±7.62**	131 ±7.93*	129 ±9.59	118.6 ±6.71	112.4 ±6.57	107.8 ±5.50
	High Dose	103.4 ±5.06	120.8 ±5.24	138.4 ±4.88**	144.4 ±5.89**	149.8 ±5.69**	138.2 ±5.97**	130.8 ±6.85*	122.4 ±5.36	117 ±5.25	112.4 ±3.50
A-V glucose (mg/dl)	Low dose	2.2 ±0.33	5.8 ±0.99**	8.8 ±0.87***	8.8 ±1.07***	4.0 ±1.26	2.6 ±0.22	2.8 ±0.66	2.4 ±0.61	2.8 ±0.59	2.8 ±0.82
	High Dose	2.6 ±0.66	7.8 ±1.02**	12.4 ±1.53***	15.0 ±1.18***	8.2 ±0.37***	6.4 ±0.68**	4.0 ±1.05	3.2 ±0.58	2.2 ±0.58	3.0 ±0.63

Table 5. Effect of intravenous injection of low (3ng/kg) and high (8ng/kg) doses of glucagon on arterial lactate level, venous lactate level and arteriovenous lactate difference [(A-V) lactate] in dogs (n=5). (*P<0.05, **P<0.01, ***P<0.001)

	Dose	0min	5min	10min	15min	20min	30min	45min	60min	75min	90min
Arterial lactate level (mg/dl)	Low dose	19.87 ±2.18	23.78 ±2.82	19.98 ±2.34	21.10 ±2.92	24.73 ±2.82	24.30 ±1.67*	26.74 ±2.94*	29.14 ±3.28*	25.86 ±3.55*	26.98 ±2.35*
	High dose	18.85 ±1.08	23.15 ±2.78	21.15 ±1.23**	22.31 ±2.70*	24.70 ±2.06**	25.38 ±3.85*	30.09 ±2.16**	30.72 ±2.59**	27.17 ±3.10**	22.79 ±2.82**
Venous lactate level (mg/dl)	Low dose	21.64 ±1.23	27.86 ±2.15*	28.25 ±2.12*	27.14 ±3.04	28.11 ±3.02*	26.68 ±1.88*	29.25 ±3.22*	30.32 ±3.59*	28.07 ±3.47	28.49 ±2.74*
	High Dose	21.73 ±0.96	27.97 ±3.19*	26.48 ±1.75**	32.26 ±2.38**	30.34 ±2.34**	30.15 ±3.32**	33.90 ±3.65**	34.05 ±2.51**	29.38 ±2.47**	26.16 ±3.33*
A-V lactate (mg/dl)	Low dose	-2.33 ±0.80	-4.28 ±1.76	-6.87 ±1.90*	-5.65 ±0.73**	-2.98 ±0.74	-2.38 ±0.56	-2.52 ±1.11	-1.19 ±0.45	-1.61 ±0.64	-1.50 ±0.98
	High dose	-2.64 ±0.51	-5.36 ±1.67	-5.34 ±0.70	-9.95 ±1.13**	-5.63 ±0.64*	-4.77 ±1.15	-3.81 ±1.51	-3.33 ±1.04	-2.21 ±1.27	-3.37 ±1.29
Blood Flow (ml/min)	Low dose	10.4 ±0.35	11.5 ±0.29	11.8 ±0.37*	11.6 ±0.28	11.4 ±0.47	10.3 ±0.51	10.6 ±0.50	10.4 ±0.39	10.2 ±0.48	10.2 ±0.19
	High dose	10.6 ±0.24	11.2 ±0.24	12.5 ±0.43**	12.1 ±0.19**	11.5 ±0.71	10.4 ±0.68	10.6 ±0.39	10.2 ±0.19	10.1 ±0.32	10.1 ±0.37

Table 6. Effect of intravenous injection of low (3 ng/kg) and high (8 ng/kg) doses of glucagon on glycogen contents and activities of glycogen synthase, glycogen phosphorylase, hexokinase and glucose-6-phosphatase enzymes in jejunum of dogs (n=5). (*P<0.05, **P<0.01, #P<0.0.05 low vs high dose)

	Control	**Low Dose**	**High Dose**
Intestinal Glycogen Content (mg/100g tissue)	138.72 ± 4.58	138.30 ± 4.14	229.21 ± 6.25**
Glycogen Synthase (Activity/mg.pr)	1.29 ± 0.13	2.67 ± 0.13**	2.72 ± 0.19**
Glycogen Phosphorylase a (x 10^{-3}Activity/mg.pr)	1.74 ± 0.21	2.17 ± 0.11*	1.01 ± 0.11** #
Hexokinase (Activity/mg.pr)	1.28 ± 0.20	3.39 ± 0.28**	3.22 ± 0.22**
Glucose 6-Phosphatase (Activity/mg.pr)	30.71 ± 1.56	34.56 ± 1.99	32.31 ± 1.47

Glucagon injection caused significant increase in arterial and venous blood glucose level. The maximum increase in arterial and venous blood glucose level was achieved at 15 minutes post-injection of glucagon and the glucose levels declined thereafter throughout the remaining observation period. Arterio-venous glucose difference was significantly increased by the two doses of adrenaline with the peak values attained at 15 minutes post-injection. High dose glucagon produced a more significant effect on arterial and venous blood glucose. As shown in figure 1b, intestinal glucose uptake in response to glucagon is 458% and 705% increases for low and high doses of glucagon respectively. The latter dose had a significantly higher effect than the former dose.

Blood lactate level and arterio-venous lactate difference in response to glucagon injection are shown in table 5. Glucagon produced an immediate rise in both arterial and venous lactate level. The increased in arterial and venous lactate levels were sustained throughout the 90 minutes post-injection observation period. The venous lactate levels produced by the two doses of glucagon were higher than their corresponding arterial lactate level throughout the experiment. At the peak, arterio-venous lactate difference increased from -2.33 ± 0.51 mg/dl to -6.87

±1.33 mg/dl and -2.64 ± 0.51 mg/dl to -9.95 ±1.13 mg/dl in response to low dose and high dose of glucagon respectively. Figure 2b shows the effect of glucagon on intestinal lactate uptake. Intestinal lactate uptake was negative throughout the experiments with the two doses of glucagon. This is indicative of intestinal lactate release. Intestinal lactate release increased from basal value of 24.35 ± 6.44 mg/min to a peak value of 108.22 ± 20.14 mg/min at 10 minutes post injection of low dose glucagon. Following high dose injection, intestinal lactate release increased from 27.92 ± 5.51 mg/min to 124.08 ± 13.58 mg/min at 15 minutes post injection. There was no difference in the peak response produced by the two doses of glucagon on intestinal lactate release.

The effects of glucagon on intestinal glycogen content and activities of glycogen synthase, glycogen phosphorylase, hexokinase and glucose-6-phosphatase are shown in table 6. While low dose of glucagon had no effect on intestinal glycogen content, high dose of glucagon produced a 65% increase intestinal glycogen content. Glycogen synthase activity was increased by the two doses of glucagon while glycogen phosphorylase activity was increased by low dose of glucagon but decreased by high dose of glucagon. The two doses of glucagon increased hexokinase activity with no effect on glucose-6-phosphatase activity. There was no difference in the increases in glycogen synthase and hexokinase activity produced by the high dose of glucagon compared with the low dose.

Effects of glucose

Intestinal blood flow increased from basal value of 10.2±0.37 ml/min to a peak value of 11.8±0.37 ml/min and 10.2±0.58 ml/min to 12.4±0.68 ml/min in response to low dose and high dose of glucose respectively. As shown in table 7, arterial blood glucose increased from 103.4 ± 4.90 mg/dl to 170.4 ± 12.81 mg/dl and venous blood glucose increased from 101.1 ± 5.26 mg/dl to 160.0 ± 12.49 mg/dl for the low dose at 5 minutes post-infusion while; arterial blood glucose increased from 104.8 ± 6.54 mg/dl to 205.6 ± 6.67 mg/dl and the venous blood glucose increased from 102.2 ± 6.33 mg/dl to 194.8 ± 5.66 mg/dl for the high dose at 10 minutes post-infusion. Following infusion of glucose, arterio- venous glucose difference

Table 7. Effect of intravenous infusion of low (10 mg/kg/min) and high (20 mg/kg/min) doses of glucose on arterial glucose level, venous glucose level, and arterio-venous glucose difference [(A-V) glucose in dogs (n=5). (*P<0.05, **P<0.01, ***P<0.001)

	Dose	0min	5min	10min	15min	20min	30min	45min	60min	75min	90min
Arterial Glucose level (mg/dl)	Low Dose	103.40 ±4.90	138.00 ±10.09*	153.40 ±11.38**	170.40 ±12.81**	163.00 ±8.15***	141.60 ±8.54**	130.80 ±6.57**	121.40 ±4.38*	117.40 ±6.39*	113.60 ±5.81
	High Dose	104.8 ±6.54	142.8 ±4.76**	169.2 ±7.56***	185.4 ±5.62***	205.6 ±6.67***	172.2 ±5.80***	150.6 ±6.14**	135.8 ±6.10*	125 ±5.08*	118 ±3.22*
Venous Glucose level (mg/dl)	Low Dose	101.10 ±5.26	132.20 ±10.25*	143.20 ±10.57**	160.00 ±12.49**	153.20 ±6.50***	137.20 ±8.40**	128.20 ±6.29**	119.80 ±4.07*	115.60 ±6.25	111.00 ±6.12
	High Dose	102.2 ±6.33	134.6 ±4.16**	159.6 ±7.87***	171.8 ±5.06***	194.8 ±5.66***	166.4 ±5.33***	148.2 ±4.97***	133.8 ±5.73**	122.8 ±5.53	114.8 ±3.42
A-V Glucose (mg/dl)	Low dose	2.40 ±0.51	5.80 ±0.73**	10.20 ±1.49***	10.40 ±0.93***	9.80 ±1.71**	4.40 ±1.03	2.60 ±0.51	1.60 ±0.68	1.80 ±0.37	2.60 ±0.51
	High Dose	2.8 ±0.37	8.2 ±0.97***	9.6 ±0.60***	13.6 ±1.16***	10.8 ±1.24***	5.8 ±0.97*	2.4 ±1.29	2.0 ±1.45	2.2 ±0.58	3.2 ±0.86

Table 8. Effect of intravenous infusion of low (10 mg/kg/min) and high (20 mg/kg/min) doses of glucose on arterial lactate level, venous lactate level and arteriovenous lactate difference [(A-V) lactate] in dogs (n=5). (*P<0.05, **P<0.01, ***P<0.001)

	Dose	0min	5min	10min	15min	20min	30min	45min	60min	75min	90min
Arterial Lactate level (mg/dl)	Low Dose	20.49 ±2.17	24.05 ±1.49	22.49 ±2.40	22.36 ±2.16	21.02 ±2.09	20.84 ±1.69	25.39 ±2.95	24.98 ±3.57	26.27 ±4.24	24.33 ±2.34
	High Dose	19.40 ±1.44	23.20 ±1.62*	25.86 ±1.93*	24.14 ±2.36*	25.94 ±2.03**	26.53 ±1.81**	31.60 ±2.64**	30.59 ±2.79**	26.08 ±2.80**	24.89 ±4.59
Venous lactate level (mg/dl)	Low Dose	21.778 ±2.04	30.67 ±2.06*	29.256 ±1.78*	28.784 ±3.378	28.29 ±2.43*	26.12 ±2.40*	29.354 ±4.17*	29.248 ±3.42*	28.588 ±4.24*	27.8 ±3.64
	High Dose	22.74 ±1.19	29.15 ±2.36*	29.43 ±1.56**	31.396 ±2.31***	33.28 ±1.99***	33.91 ±2.00***	36.38 ±3.31***	32.60 ±2.98**	27.75 ±2.66*	27.50 ±5.33
A-V lactate (mg/dl)	Low Dose	-2.88 ±0.95	-6.62 ±2.08	-6.76 ±2.04	-6.43 ±2.03	-7.27 ±1.57*	-5.28 ±2.38	-3.97 ±3.15	-4.27 ±1.29	-2.32 ±1.39	-3.47 ±1.49
	High Dose	-2.79 ±0.70	-5.95 ±1.44	-4.57 ±0.72	-7.25 ±1.52*	-7.34 ±1.53*	-7.38 ±2.91*	-4.78 ±1.32	-2.01 ±0.65	-1.67 ±1.36	-2.61 ±1.08
Blood flow (ml/min)	Low dose	10.2 ±0.37	10.6 ±0.51	11 ±0.45	11.8 ±0.37*	11 ±0.77	10.6 ±0.60	10.4 ±0.51	10 ±0.32	10 ±0.32	10.2 ±0.37
	High dose	10.2 ±0.58	11.2 ±0.37	11.8 ±0.20*	12.4 ±0.68*	10.6 ±0.24	10.4 ±0.51	10.2 ±0.49	9.6 ±0.24	9.8 ±0.37	10.2 ±0.37

Table 9. Effect of intravenous infusion of low (10 mg/kg/min) and high (20 mg/kg/min) doses of glucose on glycogen contents and activities of glycogen synthase, glycogen phosphorylase, hexokinase and glucose-6-phosphatase enzymes in jejunum of dogs (n=5). (*P<0.05, **P<0.01, #P<0.0.05 low vs high dose)

	Control	Low Dose	High Dose
Intestinal Glycogen Content (mg/100g tissue)	138.72 ± 4.58	155.26 ± 7.17**	165.45 ± 10.59**
Glycogen Synthase (Activity/mg.pr)	1.29 ± 0.13	1.40 ± 0.12	1.32 ± 0.11
Glycogen Phosphorylase a ($x 10^{-3}$ Activity/mg.pr)	1.74 ± 0.21	1.29 ± 0.17*	1.33 ± 0.08*
Hexokinase (Activity/mg.pr)	1.28 ± 0.20	2.13 ± 0.31*	3.01 ± 0.26**#
Glucose 6-Phosphatase (Activity/mg.pr)	30.71 ± 1.56	16.93 ± 1.21**	16.52 ± 0.68**

immediately increased reaching its peak 20 minutes post-infusion and thereafter returned gradually to the basal level. The low dose of glucose increased arterio-venous glucose difference from a basal value of 2.40 ± 0.51 mg/dl to a peak value of 10.4 ± 0.93 mg/dl while at high dose of glucose, arterio-venous glucose difference increased from 2.8 ± 0.37 mg/dl to 13.6 ± 1.16 mg/dl. High dose of glucose produced a more profound effect on blood glucose and arterio-venous glucose difference. Intestinal glucose uptake response to glucose infusion also followed the same pattern as that of arterio-venous glucose difference. While low dose of glucose produced 456% increase in intestinal glucose uptake, high dose of glucose increased intestinal glucose uptake by 589% (figure 1c).

The effects of glucose infusion on blood lactate levels and arterio-venous lactate difference are shown in table 8. Low dose of glucose has no effect on the arterial lactate level throughout the observation period while it caused an immediate increase in venous lactate level that was sustained throughout the post-infusion observation period. High dose of glucose on the other hand caused significant increase in both the arterial and venous lactate within 5 minutes of infusion and these increases were sustained throughout the post-infusion observation period. The venous lactate level was consistently higher than arterial lactate level produced by infusion of the two doses of glucose. Arterio-venous lactate difference increased from -2.88 ± 0.95 mg/dl to -7.27 ±1.57 mg/dl and -2.79 ± 0.70 mg/dl to -7.34 ±1.13 mg/dl in response to low dose and high dose of glucose respectively. As shown in figure 2c, intestinal lactate uptake was negative at basal and this was increased by glucose infusion up to the 45th minutes of post-infusion observation period. In other words, within the first 45 minutes post-infusion observation period, glucose significantly increase lactate release into the circulation. Intestinal lactate release increased from basal value of 29.53 ± 8.88 mg/min to a peak value of 79.22 ± 15.14 mg/min in response to infusion of low dose of glucose while, it increased from 28.64 ± 3.56 mg/min to 86.51 ± 13.58 mg/min in response to infusion of high dose of glucose. There was however, no difference in response produced by the two doses of glucose on intestinal lactate release.

The two doses of glucose infused significantly increased intestinal glycogen content. While low dose of glucose caused a 12% increase in intestinal glycogen content, high dose of glucose caused a 19% increased glycogen content. Glycogen synthase activity was unchanged while glycogen phosphorylase activity was significantly reduced during infusion of the two doses of glucose. Hexokinase activity increased in a dose-dependent manner in response to glucose infusion. Low dose of glucose increased intestinal hexokinase activity from 1.28 ± 0.20 activity/mg.pr to 2.13 ± 0.31 activity/mg.pr while at high dose, hexokinase activity increased from 1.28 ± 0.20 activity/mg.pr to 3.04 ± 0.22 activity/mg.pr. Glucose-6-phosphatase activity was significantly reduced by the two doses of glucose (table 9).

DISCUSSION

The resting blood flow of 10.4 ± 1.88 ml/min observed in this study is similar to the resting jejunal blood flow observed in previous studies on dogs (Alada and Oyebola, 1996; Alada et al., 2005 and Salman et al., 2014). The blood flow results obtained in this study suggest that the jejunal territory drained in this study is approximately equal to the territory of the upper jejunum drained in previous studies using dogs (Alada and Oyebola, 1996; Alada et al., 2005 and Salman et al., 2014).

The significant increase in the intestinal glucose uptake following intravenous injection of adrenaline is consistent with earlier findings in previous experiments in dogs (Grayson and Oyebola, 1983; Alada and Oyebola, 1996) and rabbits (Oyebola et al., 2009). Following administration of adrenaline, both arterial and venous blood glucose levels significantly increased. The increase in arterial blood glucose was however greater than the venous increase. The hyperglycemic response to adrenaline is well-known and has been extensively studied. The response is highly complex and incompletely understood (Oyebola and Alada, 1991). Basically, adrenaline has been reported to induce hyperglycemia by increasing glucose production, decreasing glucose clearance and increase glycogenolysis in muscle producing lactic

acid which is subsequently converted in the liver to glucose (Rizza et al., 1979;1980; Steiner et al., 1991; Stevenson, 1991).

Administration of adrenaline also significantly increase both the arterial and venous blood lactate levels. However, the venous lactate level was greater than the arterial level. This observation shows that the intestinal tissue was releasing lactate into the blood circulation. The increase in the quantity of lactate that is released into circulation following adrenaline is also consistent with the hyperlactatemic effect of adrenaline as reported in many animals (Issekutz, 1985; Laurent et al., 1998; Gjedsted et al., 2011). Adrenaline caused significant increase in plasma lactate levels in dogs during rest and exercise (Issekutz, 1985). Pronounced hyperlactatemia is associated with conditions characterized by elevated plasma adrenaline level such as pheochromocytoma (Bornemann et al., 1986) or patient treated with adrenaline after cardiopulmonary bypass (Totaro and Raper, 1997). *In vitro* studies using intestinal smooth muscles (Hanson and Parson, 1976; Ishida and Takagi-Ohta, 1996), vascular smooth muscles (Paul et al., 1979) and urinary bladder smooth muscles (Haugaard et al., 1987; Waring and Wendt, 2000) showed significant increases in the utilization of glucose and production of lactate following administration of adrenaline. The utilization of glucose and increase production of lactate in response to adrenaline was irrespective of the level of oxygenation in the intestinal smooth muscle (Paul, 1980).

The increase of about 400% in (A-V) glucose at the peak of response to adrenaline injection in this study shows that adrenaline caused a marked increase in glucose extraction. Interestingly, (A-V) lactate at the peak of response to adrenaline injection was about 300%. Studies using rabbit intestinal smooth muscle have reported that adrenaline relaxant effect on smooth muscle is through production of lactate (Mohme-Lundholm, 1953; 1957). Lundholm and Mohme-Lundholm (1956) also demonstrated that in the presence of high concentration of glucose, adrenaline stimulates huge glucose utilization and lactate production by intestinal smooth muscle of rats.

When the intestinal glucose uptake and lactate uptake were calculated from the product of (A-V) glucose and blood flow and (A-V) lactate and blood flow respectively, it was found that the large increases in intestinal glucose uptake after adrenaline injection corresponded in timing with the increases in intestinal lactate production. The increase in intestinal lactate production is most probably a metabolic response by this segment of the gut to the huge amount of glucose taken up by the small intestine. The response is similar to that reported by Hanson and Parson (1976) in *in vitro* preparations of rat small intestine whereby vascular perfusion of small intestinal tissue resulted in increased utilization of glucose and production of lactate. Also, the uptake of glucose and appearance of lactate were linearly related to time.

However, in the present study, the magnitude of glucose taken up by the small intestine is not the same as the amount of lactate that is released. While intestinal glucose uptake is about 600%, the intestinal lactate production is about 400%. Haugaard et al (1987) had earlier reported on this observation. From an *in vitro* incubation of rabbit bladder, the latter (Haugaard et al., 1987) reported that 11% of the glucose is oxidized to CO_2, 81% is utilized for lactate production while 4.7% was incorporated into glycogen. This partitioning of glucose utilization by the smooth muscle is in contrast to the 52.1% used for lactate production while the remaining was incorporated into glycogen in rat's diaphragm, an example of skeletal muscle (Haugaard et al.,1976).

The observed increase in intestinal glycogen in this study is in agreement with the earlier observation of Haugaard et al (1987) whereby adrenaline injection resulted in increased glycogen level in rabbit urinary bladder. In actual fact, the results of the present study showed significant reduction in the activity of intestinal phosphorylase and a significant increase in the activity of glycogen synthase. In other words, adrenaline injection resulted in significant increases in intestinal glucose uptake which is most probably the effect of induced hyperglycemia. Also, the adrenaline-induced increase in intestinal glucose uptake resulted in significant increase in the production lactate into the blood stream and a significant increase in the intestinal glycogen content. Although some studies (Axelsson et al., 1961; Andersson and Mohme, 1969, 1970; Andersson, 1971) have reported that the relaxing effect of adrenaline on smooth muscle is a result of activation of phosphorylase, glycogenolysis and production of lactic acids, Kolnes et al (2015) have recently shown that the effect of adrenaline on glycogen breakdown depends on the initial level of glycogen in a skeletal muscle. Such that the muscle with high glycogen content resulted in activation of phosporylase enzyme and glycogenolysis while a tissue with low glycogen content resulted in stimulation of glycogen synthase and glycogenesis. This observation led the authors (Kolnes et al., 2015) to the conclusion that glycogen content autoregulates glycogen synthase.

Based on the results of the present study, it could be reasonably concluded that adrenaline administration produced a huge increase in intestinal glucose uptake which is most probably converted largely to produce lactate (through oxidation) and to a little extent form glycogen in the intestinal tissue.

Following administration of glucagon, both the arterial and venous blood glucose levels significantly increased. The increase in arterial blood glucose was however greater than the venous increase. The hyperglycaemia induced by glucagon is consistent

with earlier observations on the effect of glucagon in animals (Myers et al., 1991; Young et al., 1993; Alada and Oyebola, 1996; Oyebola et al., 1998) and humans (Lins et al., 1983; Freychet et al., 1988; Hvidberg et al., 1994). Glucagon stimulates hepatic output of glucose (Beuers and Jungermann, 1990; Doi et al., 2001) by binding its G-coupled receptor which stimulates adenylate cyclase via the Gsα sub-unit to increase intracellular cAMP levels and activates protein kinase A (PKA) while it also stimulates phospholipase C via Gq sub-unit to increase production of inositol 1,4,5-triphosphate and mobilize intracellular calcium (Burcelin et al., 1994; Christophe, 1995). The net effect of glucagon on glucose homeostasis is to increase hepatic glucose production by potentiating glycogenolysis and gluconeogenesis via the activation of Gsα while inhibiting glycolysis and glycogenesis via activation of Gq signal transduction (Jiang and Zhang, 2003).

The direct effect of glucagon on tissue lactate production is obscure. However, the immediate rise in arterial and venous blood lactate level following glucagon administration in this study is most probably a secondary response by the intestine to the glucagon-induced hyperglycaemia. Since both the increase in blood glucose and lactate occurred at corresponding times. Previous studies have shown that during hyperglycaemia, there is active increase in tissue lactate release (Hangström et al., 1990; Henry et al., 1996). It has also been reported that glucose at high concentration can stimulate hexokinase activity hitherto inhibited by glucose-6-phophate in human erythrocyte (Fujii and Beutler, 1985). The increase in hexokinase activity following glucagon induced hyperglycaemia observed in this study is most probably a consequence of the increased glucose availability. Thus, the increase in lactate release is a consequence of the increase in blood glucose, since a linear relationship exist between hexokinase activity and lactate release (Crabtree and Newsholme, 1972). Also, studies showed that glucagon has a relaxant effect on gastrointestinal smooth muscle which is mediated through lactic acid. It has been shown that glucagon inhibits intestinal smooth muscle in the jejunum of man (Dotevall and Koch, 1963) as well as the stomach and duodenum of dog (Necheles et al., 1966). The inhibition of intestinal smooth muscle by glucagon could occur through its stimulation of the adrenal medulla to produce catecholamine which inhibits the activity of the intestinal smooth muscles (Fasth and Hultén, 1971). The relaxant effect of adrenaline is associated with lactate production (Mohme-Lundholm, 1953; 1957). It could therefore be suggested that the increased intestinal lactate production following glucagon injection in this study is most probably a secondary effect of glucagon on adrenal medulla to release catecholamines.

The huge increase in intestinal glucose uptake following glucagon injection in this study agrees with an earlier report in dog experiment (Alada and Oyebola, 1996). The observed increase of about 580% in (A-V) glucose at the peak of response to glucagon injection in this study also, is consistent with previous study of Alada and Oyebola (1996). It also shows that glucagon caused a marked increase in glucose extraction as evident from the observed increase in the activity of hexokinase in the present study. The lactate production from the intestine following glucagon injection was also huge. The increase in intestinal glucose uptake is most probably a metabolic response by the intestine to the hyperglycemia induced by glucagon. In other words, as a result of a significant rise in blood glucose following glucagon injection, the intestine also increased its glucose uptake from bloods circulation. A similar observation has been reported in previous studies (Alada and Oyebola, 1996) and in the present study following adrenaline-induced hyperglycemia. In the present study, administration of glucagon did not just increase intestinal glucose uptake but also produced a significant increase in the amount of lactate released into circulation. While the intestinal glucose uptake increased by about 700%, intestinal lactate release increased by about 400% in response to glucagon-induced hyperglycaemia. Again, the intestinal glucose uptake and lactate release are linearly related with time. Based on the above observation, it would therefore not be unreasonable to conclude that the increase in lactate production is metabolically related to the glucagon-induced hyperglycemia.

The increase in intestinal glycogen in this study is of great interest. Glucagon is a well-known glycogenolytic hormone. However, the observed increased activity of glycogen synthase and reduction in the activity of glycogen phosphorylase in this study seems to show that during glucagon-induced hyperglycemia, the intestinal tissue is actually synthesizing glycogen; thus, the observed increase in intestinal glycogen content following glucagon administration. This observation is consistent with the earlier postulate of Kolnes et al (2015) which shows that glycogen content autoregulates glycogen synthesis.

The observed increases in arterial and venous blood glucose following glucose infusion in this study is consistent in magnitude and pattern to those reported in previous studies (Grayson and Oyebola, 1983; Alada and Oyebola, 1996; Salman et al., 2014). Also, the glucose extraction by the intestine is dependent on the level of blood glucose. In other words, the increase in (A-V) glucose is a result of the increase in blood glucose levels following increase in the dose of glucose infused into the animal. Similar observations have been reported by Alada and Oyebola (1996) and Salman et al (2014). The observed increase in

intestinal glucose uptake in response to glucose-induced hyperglycaemia is similar to the result of previous studies (Alada and Oyebola 1996; Salman et al., 2014) where different doses of glucose infusions produced different magnitude of intestinal glucose uptake. These findings were further strengthened by the observed increase in the activity of hexokinase in the present study. This observation therefore provides further evidence to show that the increase in the intestinal glucose uptake is a metabolic response to the increase in blood glucose.

The observed increases in both arterial and venous blood lactate levels in response to glucose infusion in this study is consistent with the report that intravenous infusion or oral load of glucose increases plasma lactate concentration in dog (Cianciaruso et al., 1991; Youn and Bergman, 1991) and rat (Vine et al., 1995). Furthermore, the present results show that the increases in blood lactate and glucose are linearly related with time. Generally, lactate formation in any tissue is a by-product of anaerobic glycolysis whereby there is insufficient amount of oxygen for the tissue. Ordinarily, glucose is converted to pyruvate which is incorporated in the formation of ATP. However, in the absence of sufficient oxygen, the tissue converts pyruvate to lactate with the help of lactate dehydrogenase. Although lactate dehydrogenase activity has not been determined in this study, the presence of large amount of lactate in blood provided evidence of strong anaerobic glycolysis, the observed increase of about 600% in intestinal glucose uptake followed by an increase of about 300% in intestinal lactate release in the present study provides evidence to show that the intestinal lactate release is most possibly a metabolic response to the remarkable increase in intestinal glucose uptake. Again, both increases in intestinal glucose uptake and lactate release occur at corresponding times. The significant difference in the magnitude of glucose taken up by the gut and the amount of lactate being released shows that the lactate output from the intestine may not be the only by-product of the intestinal glucose taken up.

Indeed, the results of the present study showed a significant increase in intestinal glycogen following infusion of glucose. The observed increases in the activity of intestinal glycogen synthase and inhibition of intestinal glycogen phosphorylase 'a' provides strong evidence to show that glycogenesis probably occurred in response to glucose-induced hyperglycaemia. Similar observations have been reported in this study following hyperglycaemia induced by adrenaline and glucagon.

In conclusion, the present results demonstrated that the fate of the large amount of glucose that is taken up by the intestine following a rise in blood glucose induced by adrenaline, glucagon or glucose is through formation and release of lactate and synthesis of glycogen.

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