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Intestinal glucose uptake in normal, untreated and insulin-treated diabetic dogs

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Summary
The study was carried out on fasted, anaesthetized diabetic and non-diabetic dogs. Diabetes was induced by i.v injection of alloxan (60mg/kg). A vein draining a segment of the upper jejunum was cannulated for blood flow measurements, and blood samples were obtained for determination of glucose content of arterial and venous blood. Glucose uptake was calculated as the product of jejunal blood flow and arterio-venous glucose difference ((A-V) glucose. The results showed that following induction of diabetes, there were significant increases in jejunal blood flow, (A-V) glucose and jejunal glucose uptake when compared with non-diabetic dogs. For instance, the glucose uptake increased from 23.10± 2.34 to 178.40± 6.93 mg/min. When the diabetic dog was challenged with different doses of insulin (2.5, 5.0, 7.5, 10.0 iu/kg), the blood glucose levels and the intestinal glucose uptake decreased in a dose-dependent manner. In normal dogs, insulin administration caused negative glucose uptake at the lower dose (5.0 iu/kg) while at the higher dose, 8.0 iu/kg, insulin caused just a transient negative glucose uptake. From the results, it was concluded that the small intestine increased its glucose uptake in response to the hyperglycemia in alloxan-induced diabetes and when the blood glucose was reduced with insulin the intestine also reduced its glucose uptake accordingly. The result of insulin administration in normal dogs suggests that glucose uptake by the gut cannot be explained on the basis of blood glucose concentration alone.

Keywords: *Glucose uptake, intestine, diabetes, dog.*

Résumé
Cette étude était faite sur des chiens a jeune anesthésie et diabétique. Le diabéte était induit par injection intraveneuse de l'olloxan (60 mg/kg). Le segment entérique du jéjunum était cannulé pour mesurer la coulée du sang, la composition du glucose arteriel et veneux. L'absorption du glucose était calculé comme produit de la coulée et la difference entre le taux du glucose artériel et veneux. Aprés le diabete induit, les résultats montraient une augmentation significative de la coulée du sang jugénal, du glucose A-V et l'absorption du glucose jugénale comparés aux chiens non-diabétiques.Lorsqu'on administrait difference doses d'insuline(2.5,5.0,7.5,10 iu/kg), le taux du glucose et son absorption intestinal réduisaient a dose dependante. Aux chiens normal, l'administration d'insuline causait une absorption lente. Nous avons conbclu que l'intestin grele augmente l'absorption du glucose en response à l'hyperglycémie aux alloxan-induit de diabéte et ce taux de glucose absorbé réduisait avec la réduction d'insuline. Le résultat de l'administration de l'insuline aux chiens normaux sugére que l'absorption du glucose ne peut pas etre expliqué à base de la concentration du glucose **seulement.**

Introduction

Diabetic mellitus is a condition which results from deficiency of or insensitivity of the tissues to insulin leading to significant hyperglycemia [1]. It is a common disease of man [2,3] and animals, especially dogs and cats [4] which has been extensively studied. Of particular interest to researchers is the role of various tissues in glucose homeostasis in normal and diabetic animals and man. In this regard, the roles of the muscles, liver, intestines and kidneys in glucose homeostasis have received a lot of attention of researchers. Previous studies in normal dogs have shown that the canine intestine takes up substantial amount of glucose at rest and the glucose uptake increased further when blood glucose was increased [5-11]. Thus, when hyperglycemia was induced by adrenaline, glucagon and glucose [6, 8, 9, and 10], nicotine [7] and cow's urine concoction [5] the gastro-intestinal tract increased its glucose uptake by as much as 400-700%. On the other hand, when hypoglycemia was induced by insulin injection [9], the gastro-intestinal tract pushes out glucose into the circulation. It was therefore concluded that the gastro-intestinal tract plays a modulatory role in glucose homeostasis. Also, since there was increased glucose uptake in all cases of hyperglycemia, it was concluded that the canine gut increased its glucose uptake whenever there was hyperglycemia irrespective of the cause of hyperglycemia [9].

All the studies cited above were in fasting normal dogs. An extensive search of the literature showed that there have been no studies of glucose uptake by the gut in the post-absorptive state (i.e., when no glucose is coming from the gut) in diabetic animals or man. That is, there has been no study of glucose uptake by the intestine of diabetic animals in the post-absorptive state. This is in spite of the fact that hundreds of studies have been carried out on post-ingestion uptake of glucose in the gut, liver and muscles in normal and diabetic animals and man. [12-15]. Yet, diabetes mellitus is a very common disease in animals [4] and man [2, 3]. Since the gut of normal animals has been shown in previous studies from our laboratory to play a modulatory role in glucose homeostasis, a logical question that came to us is: what is the role of the gut in

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glucose handling in diabetes mellitus? For instance, does our earlier conclusion that the gut of normal dogs will increase their glucose uptake during hyperglycemia irrespective of its cause apply to hyperglycemia of diabetes mellitus? That is, how does the intestine of a diabetic animal respond to hyperglycemia due to diabetes mellitus with respect to glucose uptake? Administration of insulin injection is one of the main forms of treating diabetes mellitus, especially Type I or insulin-dependent diabetes. Our study on the effect of insulin on glucose uptake in the gut of normal dogs showed that insulin-induced hypoglycemia caused a negative glucose uptake. This leads us to the second question. What is the effect of insulin on gut glucose uptake in diabetic animals and man? That is, what is the effect of insulin administered to diabetics on the post-absorptive glucose uptake by their gut?

The need to provide answers to the above questions led us to undertake the present study. We have chosen to study the gut glucose uptake in the post-absorptive state because, as in our earlier studies in normal dogs, it will eliminate the problem of deciding where the glucose we are measuring in the blood is coming from. For instance, after carbohydrate ingestion, glucose enters the blood circulation from the gut [15, 16] and this makes the job of deciding where a particular glucose in the arterial or venous blood is coming from more complex. Besides, not all ingested glucose reaches the systemic circulation because a portion of it is metabolized by the gut and/or stored in the liver as glycogen [13, 14, and 17].

Materials and methods

The surgical preparation of the animals in this study is as described earlier [6, 9]. Male mongrel dogs weighing 8-14 Kg were used for the study. Each animal was fasted for 18-24 hours before the start of an experiment; but was allowed free access to drinking water. Anesthesia was induced by intravenous sodium pentobarbitone, 30mg/Kg. Light anesthesia was maintained with supplementary doses of intravenous sodium pentobarbitone. The trachea was intubated using a Y-piece cannula and the animal was allowed to breathe room air (temp. 25°C) spontaneously. Respiration was assisted by a respiratory pump (Palmer, England) whenever necessary. Cannulae were placed in the right femoral vein and right femoral artery.

Through a midline laparatomy, the jejunum was identified, secured and a vein draining the proximal segment of the jejunum was cannulated using 1.9mm (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 iu/Kg was administered intravenously to prevent blood clotting. The abdomen was closed in two layers with interrupted sutures.

Following surgery, a period of 60 minutes was allowed for each animal to stabilize. Diabetes mellitus was induced by intravenous injection of freshly prepared alloxan monohydrates solution, 60mg/Kg body weight through the tibia vein [18, 19]

Experimental procedure:

The study was carried out on four groups of dogs:

Group I (Non-diabetic and untreated): Four dogs were used. In each animal, arterial and venous blood samples for the basal (zero minute) glucose levels were collected through the femoral artery and jejunal vein respectively. Each animal was then injected with 0.6ml/Kg body weight freshly prepared normal saline (0.9% NaCl). Arterial and venous glucose concentrations were again measured at 5, 10, 15, 30, 45, 60, 75, and 90 minutes post-injection observation period. Jejunal blood flow was measured during each sampling period by timed collection as previously described [8, 9].

Group II (Diabetic and untreated): Four dogs were also used. Each dog was injected with alloxan solution, 60mg/Kg a week before the experiment to induce diabetes mellitus [20]. Blood and urine samples after a week confirmed that the dogs were diabetic (Blood glucose above 200mg/dl [4]. The experiment was then carried out on the diabetic dogs. After surgery and stabilization as described above, blood samples for basal arterial and venous glucose were collected. The animal was thereafter injected with normal saline (0.6ml/kg body weight) through the cannula in the femoral vein. Arterial and venous samples were again obtained at 5, 10, 15, 30, 45, 60, 75, and 90 minutes post- injection observation period. Jejunal blood flow was measured intermittently as described earlier.

Group III (Diabetic with Insulin treatment): Sixteen dogs, divided into four sub-groups of four dogs per sub-group were used. Each dog was given i.v injection of alloxan solution, 60mg/Kg a week before the start of the experiment to induce diabetes mellitus. Development of diabetes was confirmed through blood and urine glucose tests as in group II. Arterial and venous blood samples for basal blood glucose levels were first collected. After the basal collections, the animal was given a bolus injection of soluble insulin 2.5iu/Kg through the cannula in the femoral vein; thereafter, arterial and venous blood samples were obtained at 5, 10, 15, 30, 45, 60, 75,and 90 minutes post-injection period. Jejunal blood flow was again measured as in groups I and II. The experiment was repeated in dogs in the other three sub-groups using 5.0iu/Kg, 7.5iu/Kg, and 10.0iu/Kg of insulin respectively and sampling carried out as previously described.

Group IV (Non-diabetic with Insulin): Four normal, non-diabetic dogs were also used. Following surgery and stabilization of the animal, arterial and venous blood samples for basal blood glucose levels were collected. The animals were then given insulin (5 iu/Kg) through the cannula in the femoral vein. Arterial and venous blood sampling were carried out for glucose measurement as previ-

ously described. Jejunal blood flow was also measured as described earlier. After full recovery from the effect of first insulin injection, a repeat injection of insulin, 8 iu/kg was given and all measurements were repeated.

Blood glucose was determined with ONE TOUCH BASIC-plus glucometer (Life Scan data file, 2000). The meter was checked against the standard glucose solution at regular intervals to ensure accuracy. Values obtained correlate excellently with those from the use of standard biochemical methods (21, 22). Glucose uptake was computed as the product of the (A-V) glucose and blood flow.

All values given are the mean ± S.E of the variables measured. Significance was assessed by the student's t-test for two means of independent variables. P values of 0.05 or less were taken as statistically significant.

Effect of normal saline on glucose uptake in normal dogs. This is shown in Table 1. Normal saline had no effect on arterial and venous blood glucose levels, (A-V) glucose, jejunal blood flow and intestinal glucose uptake. The mean fasting arterial blood glucose level was 109.00± 1.76 mg/dl while the resting jejunal blood flow was 10.26± 0.09 ml/min. The mean resting intestinal glucose uptake was 23.10± 2.34 mg/min.

Effects of normal saline on glucose uptake in diabetic dogs

This is shown in Table 2. The fasting arterial blood glucose level in the diabetic dog was 247.75± 4.75mg/dl. Fasting venous glucose was correspondingly elevated while resting blood flow (10.35± 0.08) was essentially the same as that of normal dog. Diabetes mellitus resulted in huge

Table 1: Effect of normal saline on arterial and Venous glucose, (A-V) glucose, jejunal blood flow and intestinal glucose uptake in normal dogs. - (n - 4)

	0 min	5 min	10mm	15min	30min`	45 min	60 min	75min	90 min
Arterial glucose	109.00	109.25	107.25	108.25	109.25	109.00	108.50	109.00	109.25
(mg/dl)	± 1.76	± 1.71	± 1.82	± 2.36	±2.30	± 2.09	± 1.08	±2.37	± 1.82
Venous glucose	106.75	107.50	105.75	106.50	107.00	107.00	106.50	107.50	107.00
(mg/dl)	± 1.85	± 2.02	±2.13	± 2.19	±2.00	± 2.00	± 1.60	± 2.56	±2.09
(A-V) glucose	2.25	1.75	2.00	1.75	2.25	2.00	2.25	1.50	2.00
(mg/dl)	±0.22	± 0.42	±0.35	± 0.42	±0.22	± 0.20	±0.22	± 0.25	±0.35
Blood flow	10.27	10.20	10.20	10.20	10.25	10.35	10.25	10.35	10.20
(ml/min)	±0.09	± 0.07	±0.07	± 0.10	±0.11	± 0.04	±0.08	± 0.04	±0.16
Glucose uptake	23.10	17.75	20.35	17.80	23.05	20.70	23.05	15.55	20.30
(mg/min)	±2.34	± 4.09	±3.57	± 4.13	±2.19	± 2.09	±2.19	± 2.63	±3.48

Table 2: Effect of normal saline on arterial and venous glucose, (A-V), glucose, jujunal blood flow and intestinal glucose uptake in diabetic dogs. - (n - 4)

	0 min	5 min	10mm	15min	30min`	45 min	60 min	75min	90 min
Arterial glucose	247.75	247.50	248.50	248.75	246.75	249.25	248.50	248.25	245.25
(mg/dl)	±4.42	± 3.65	±4.48	± 3.65	±5.33	± 5.75	±5.25	± 4.48	±4.90
Venous glucose	230.50	230.75	230.75	229.75	229.75	232.50	231.50	232.25	232.50
(mg/dl)	±3.85	± 3.34	±3.58	± 4.26	±4.32	± 4.42	±4.82	± 4.22	±2.25
(A-V) glucose	17.25	16.75	17.25	17.25	17.00	16.75	17.00	16.50	16.25
(mg/dl)	±0.74	± 0.74	±0.89	± 0.42	±1.28	± 0.96	±1.46	± 0.25	±0.74
Blood flow	10.35	10.40	10.30	10.40	10.35	10.30	10.35	10.40	10.40
(ml/min)	±0.08	± 0.07	±0.09	± 0.10	±0.10	± 0.11	±0.04	± 0.12	±0.12
Glucose uptake	178.40	174.25	177.60	179.30	176.00	172.10	175.90	171.55	168.70
(mg/min)	±6.93	± 8.05	±9.11	± 4.15	±13.64	± 8.10	±15.03	± 2.50	±6.88

Results

The results are shown in tables 1 to 9 and in the figure. The asterisks in the tables indicate the levels of significance of the variables measured compared with their basal (zero minute) values.

increases in the glucose extraction and glucose uptake. Glucose extraction in the diabetic dog was almost eight times that of normal dogs. Also, the resting glucose uptake of 178.40± 6.93 mg/min in the diabetic dog was almost eight fold the glucose uptake in normal dogs.

Following the injection of 0.9% saline into the diabetic dogs, there was no change in blood glucose levels, A-V glucose, blood flow and glucose uptake above basal values throughout the 90 min. post-injection observation period (Table 2). Although normal saline had no effect of its own, a comparison of the effect of normal saline on glucose uptake in normal and diabetic dogs shows that glucose uptake in diabetic dogs was between 672 to 1003% higher than in normal dogs from the basal levels to the peak of uptake and these differences are highly significant at the 1% level (Tables 1 and 2).

shows the effect of the different doses of insulin on arterial blood glucose level. 2.5 iu/kg of insulin caused significant reduction in arterial glucose from 10 to 30 minutes post-injection, while 5.0 iu/kg insulin caused significant fall in arterial glucose from 10 to 60 minutes post-injection. For the higher doses, 7.5 and 10.0 iu/kg insulin, significant reduction in arterial glucose started 10 minutes post-injection and lasted the entire 90 minutes post-injection period. The levels of significance of the effects of different doses of insulin on glucose uptake are indicated in the tables.

There were significant changes in venous glu-

Table 3: Arterial glucose (mg/dl) after 2.5, 5.0, 7.5 and 10.0 iu/kg of insulin in diabetic dogs.

	0 min	5 min	10mm	15min	30min`	45 min	60 min	75min	90 min
2.5 iu/kg	245.75 ±4.08	246.00 ± 2.82	218.75 ±5.06 **	221.25 ± 4.64 **	230.75 ±4.44 *	233.75 ± 2.70	239.75 ±3.56	240.25 ± 1.29	243.00 ±3.62
5.0 iu/kg	243.00 ±3.76	242.00 ± 4.06	196.50 ±3.05 **	187.25 ± 1.63 **	212.25 ± 1.67 **	222.25 ± 1.67 **	231.00 ±2.67 *	234.00 ± 3.01	234.75 ±3.30
7.5 iu/kg	235.25 ±4.25	236.25 ± 3.27	172.50 ±3.27 **	141.50 ± 4.71 ***	151.50 ± 6.63 **	170.00 ± 5.18 **	180.50 ±2.28 **	188.00 ± 2.55 **	192.25 ±4.94 **
10.0 iu/kg	235.50 ±4.85	235.75 ± 5.26	174.25 ±2.06 **	146.75 ±34.86 ***	154.25 ±2.53 **	178.50 ±3.63 **	183.75 ±4.22 **	190.25 ± 5.58 **	191.75 ±5.80 **

*- *P<0.05*; **- *P<0.01*; ***- *P<0.001*; *N = 4*

Table 4: Effect of 5.0 iu/kg insulin on arterial and venous blood glucose, (A-V) glucose, jejunal blood flow and intestinal glucose uptake in diabetic dogs.

	0 min	5 min	10mm	15min	30min`	45 min	60 min	75min	90 min
Arterial glucose (mg/dl)	243.00 ±3.76	242.00 ± 4.06	196.50 ±3.05 **	187.25 ± 1.63 **	212.25 ± 1.67 **	222.25 ± 2.56 **	231.00 ±2.67 *	234.00 ± 3.01	234.75 ±3.30
Venous glucose (mg/dl)	225.50 ±3.05	225.25 ± 3.73	185.00 ±2.53 **	176.25 ± 1.60 **	199.25 ± 1.67 **	207.75 ± 2.43 **	215.75 ±2.41 *	218.25 ± 2.60	218.75 ±3.00
(A-V) glucose (mg/dl)	17.50 ±0.56	16.75 ± 0.42	11.50 ±0.56 **	11.00 ± 0.35 **	13.00 ±0.35 **	14.50 ± 0.25 **	15.25 ±0.42 **	15.75 ± 0.56 *	16.00 ±0.35
Blood flow (ml/min)	10.40 ±0.09	10.45 ± 0.07	10.80 ±0.07	11.00 ± 0.12	10.50 ±0.05	10.50 ± 0.04	10.45 ±0.06	10.45 ± 0.05	10.45 ±0.04
Glucose uptake (mg/min)	182.10 ±6.77	174.25 ± 4.92	124.30 ±6.54 ***	121.15 ± 5.06 ***	136.55 ±4.20 **	144.43 ± 5.47 **	159.45 ±5.13 *	163.88 ± 6.22	168.85 ±4.25

*- *P<0.05*; **- *P<0.01*; ***- *P<0.001*: *N = 4*

Effects of insulin on glucose uptake in diabetic dog. The results of insulin administration in doses of 2.5, 5.0, 7.5 and 10.0 iu/kg are shown in Tables 3, 4, 5 and 6. Table 3

cose levels in all the groups that correspond with changes in arterial glucose level, but in all cases, venous glucose levels were less than arterial glucose levels (Tables 4 and 5).

Table 5: Effect of 7.5 iu/kg insulin on arterial and venous blood glucose, (A-V) glucose, jejunal blood flo and intestinal glucose uptake in diabetic dogs.

	0 min	5 min	10mm	15min	30min`	45 min	60 min	75min	90 min
Arterial glucose (mg/dl)	235.25 ±4.25	236.25 ± 3.27	172.50 ± 3.27 **	141.50 ± 4.71 ***	151.50 ± 6.63 **	170.00 ± 2.28 **	180.50 ± 2.28 **	188.00 ± 2.55 **	192.25 ±4.94 **
Venous glucose (mg/dl)	219.00 ± 3.48	220.50 ± 3.33	164.00 ± 2.72 **	136.25 ± 4.52 ***	145.25 ± 6.11 **	161.50 ± 4.62 **	170.50 ± 1.58 **	176.00 ± 2.03 **	180.00 ± 3.86 **
(A-V) glucose (mg/dl)	16.25 ± 0.22	15.75 ± 0.65	8.50 ± 0.56 **	5.25 ± 0.22 ***	6.25 ± 0.55 ***	8.50 ± 0.43 **	10.00 ± 0.61 **	12.00 ± 0.61 *	12.25 ± 0.82 *
Blood flow (ml/min)	10.40 ± 0.06	10.50 ± 0.07	11.10 ± 0.11	11.15 ± 0.08	10.80 ± 0.10	10.60 ± 0.06	10.50 ± 0.05	10.50 ± 0.06	10.50 ± 0.06
Glucose uptake (mg/min)	169.85 ± 3.04	164.00 ± 7.76	91.95 ± 5.06 ***	58.60 ± 2.84 ***	67.66 ± 6.36 ***	90.20 ± 5.14 ***	105.05 ± 6.69 *	126.02 ± 6.64 *	128.73 ± 9.04 *

*- $P<0.05$; **- $P<0.01$; ***- $P<0.001$; $N = 4$*

Table 6: Effects of different doses of insulin on intestinal glucose uptake in diabetic dogs.

Dose of insulin	0 min	5 min	10mm	15min	30min`	45 min	60 min	75min	90 min
2.5 iu/kg (mg/min)	173.35 ±4.17	174.30 ± 8.28	165.15 ± 5.33 ***	172.28 ± 2.80 ***	174.25 ±2.75 **	175.75 ± 4.95 **	175.28 ± 0.62 *	178.76 ± 2.45	179.40 ± 6.78
5.0 iu/kg (mg/min)	182.10 ± 6.77	174.25 ± 4.92	124.30 ± 6.54 ***	121.15 ± 5.06 ***	136.55 ± 4.20 **	144.43 ± 5.47 **	159.45 ± 5.13 *	163.88 ± 6.22	168.85 ± 4.25
7.5 iu/kg (mg/min)	169.85 ± 3.04	164.00 ± 7.76	91.95 ± 5.06 ***	58.60 ± 2.84 ***	67.66 ± 6.36 ***	90.20 ± 5.14 ***	105.05 ± 6.69 **	126.02 ± 6.64 *	128.73 ± 9.04 *
10.0 iu/kg (mg/min)	166.40 ± 4.78	168.00 ± 6.65	90.75 ± 4.94 ***	69.00 ± 4.68 ***	70.20 ± 6.94 ***	95.40 ± 4.97 **	108.14 ± 5.92 **	126.60 ± 6,64 *	133.88 13.10 *

*- $P<0.05$; **- $P<0.01$; ***- $P<0.001$; $N = 4$*

Tables 4 and 5 show the effects of 5.0 and 7.5 iu/kg of insulin on blood glucose levels, A-V glucose and glucose uptake. First, it should be noted that insulin had no effect on blood flow at all the dose-levels studied. Apart from the changes in blood glucose reported above, insulin caused significant decreases in glucose extraction and glucose uptake (Tables 4 and 5). A comparison of Tables 4 and 5 showed that the higher dose of insulin (7.5 iu/kg) caused more profound decreases in glucose extraction and glucose uptake than the lower dose, 5.0 iu/kg. Table 6 shows the glucose uptake at the four dose levels of insulin used in this study. An important point to note here is that further increase in insulin dosage to 10.0 iu/kg did not result in further decrease in glucose uptake when compared with the glucose uptake at the 7.5 iu/kg dose level.

Table 7 shows the percentage changes in arterial glucose levels and in glucose uptake at three insulin dose levels. It is to be noted that the percentage changes in glucose uptake in all cases is far higher than the corresponding percentage changes in arterial glucose levels. The figure compares the glucose uptake in the untreated diabetic dog given normal saline and in the four groups of diabetic dogs given different doses of insulin. The figure shows that insulin decreased intestinal glucose uptake in a dose-dependent manner. The figure also shows that increase in dosage of insulin above 7.5 iu/kg did not result in a more significant decrease in glucose uptake.

Table 7: Percentage (%) change in arterial glucose and glucose uptake in insulin treated dog*.

Insulin	% Change	0min	5min	10min	15min	30min	45min	60min	75min	90min
5.0iu/kg	Arterial glucose	243.00 mg/dl	0.0	19.0	22.9	12.6	8.3	4.9	3.7	3.3
	glucose uptake	182.10 mg/dl	4.3	31.7	33.5	25.0	20.7	12.4	10.0	7.3
7.5 iu/kg	Arterial glucose	235.25 mg/dl	0.0	26.7	39.8	35.6	26.4	23.2	20.0	18.2
	glucose uptake	169.85 mg/dl	3.4	45.8	65.5	60.1	46.9	38.1	25.8	24.2
10 iu/kg	Arterial glucose	235.50 mg/dl	0.0	26.0	37.7	30.2	24.2	21.9	19.2	18.6
	glucose uptake	166.40 mg/dl	0.0	45.5	85.6	57.8	42.7	35.0	23.9	19.5

**All values from 5min to 90min are in percent (%) (N = 4)*

Table 8: Effects of low dose (5.0 iu/kg) and high dose (8 iu/kg) of insulin on arterial and venous glucose, (A-V) glucose, jejunal blood and intestinal glucose uptake in normal dog.

		0min	5min	10min	15min	30min	45min	60min	75min	90min
Arterial glucose level (mg/dl)	Low Dose	107.45 ±3.82	108.00 ±4.39	106.31 ±2.62	104.81 ± 1.57	107.33 ±4.63	107.70 ± 4.15	106.75 ±2.57	106.56 ± 4.81	105.61 ± 2.03
	High Dose	119.20 ±2.91	119.10 ± 2.86	120.30 ±4.15	92.15 ± 3.09*	75.40 ± 2.32**	99.10 ± 2.32*	109.32 ±5.16	123.10 ± 2.19	120.00 ±2.21
Venous glucose level (mg/dl)	Low Dose	105.69 ±2.76	106.21 ± 4.66	104.90 ±3.30	105.34 ± 6.69	110.55 ±5.28	108.78 ±6.01	106.72 ±4.69	106.01 ± 1.88	105.60 ±1.68
	High Dose	116.00 ± 1.87	116.20 ± 1.92	115.10 ±3.58	87.21 ± 4.10*	80.00 ± 6.63**	95.24 ± 4.81*	106.17 ±4.02	118.25 ± 3.13	115.74 ±3.22
(A-V) glucose (mg/dl)	Low dose	1.76 ±0.81	1.79 ± 0.31	1.41 ±0.28	-0.53 ± 0.46	-3.22 ±0.22	-1.08 ± 0.63	0.03 ±0.52	0.55 ± 0.50	0.01 ±0.23
	High dose	1.89 ±0.53	1.94 ± 0.45	2.16* ±0.51	1.06 ± 0.56	-4.60 ±0.40	3.86 ± 0.51***	3.15 ± 0.52***	4.85 ± 0.44***	4.27 ±0.34 ***
Blood flow (ml/min)	Low dose	9.30 ±0.14	9.30 ± 0.13	9.20 ±0.21	9.50 ± 0.22	9.20 ±0.20	9.00 ± 0.16	9.00 ±0.23	9.00 ± 0.17	9.00 ±0.19
	High dose	9.40 ±0.10	9.40 ± 0.09	9.30 ±0.18	9.70 ± 0.17	9.40 ±0.21	9.40 ± 0.19	9.20 ±0.17	9.20 ± 0.15	9.20 ±0.20
Glucose uptake (mg/min)	Low dose	16.37 ±2.03	16.65 ± 2.28	12.97 ±3.15	-5.05 ± 4.87	-29.62 ±3.23	-9.72 ± 1.20	0.27 ±1.71	4.95 ± 1.17	0.09 ±0.02
	High dose	17.77 ± 1.56	18.24 ± 3.12	24.08 ±4.31	10.28 ± 7.42	43.24 ±4.40 **	36.28 ± 3.18 ***	29.98 ±5.68 ***	44.62 ± 3.72 ***	39.28 ±3.69 ***

**P<0.05; **P<0.01; ***P<0.001; N = 4*

Table 9: Comparison of the effects of low and high doses of insulin on intestinal glucose uptake in normal and diabetic dogs.

		0min	5min	10min	15min	30min	45min	60min	75min	90min
Low dose insulin (mg/min)	Normal	16.37 ± 2.03	16.65 ± 2.28	12.97 ± 3.15 *	- 5.05 ± 4.87 **	-29.62 ± 3.23 ***	-9.72 ± 1.20 **	0.27 ± 1.71 **	4.95 ± 1.17 **	0.09 ± 0.02 **
	Diabetic	182.10 ± 6.77	174.25 ± 4.92	124.30 ± 6.54 ***	121.15 ± 5.06 ***	136.55 ± 4.20 **	144.43 ± 5.47 **	159.45 ± 5.13 *	163.88 ± 6.22	168.85 ± 4.25
High dose insulin (mg/min)	Normal	17.77 ±1.56	18.24 ±3.12	24.08 ±4.31 *	10.28 ± 7.42 **	-43.24 ± 4.40 ***	36.28 ± 3.18 **	29.98 ± 5.68 **	44.62 ± 3.72 ***	39.28 ± 3.69 **
	Diabetic	169.85 ± 3.04	164.00 ± 7.76	91.95 ± 5.06 ***	58.60 ± 2.84 ***	67.66 ± 6.36 ***	90.20 ± 5.14 ***	105.05 ± 6.69 **	126.02 ± 6.64 *	128.73 ± 9.04 *

(- P<0.05; **- P<0.01; ***- P< 0.001; N = 4)*

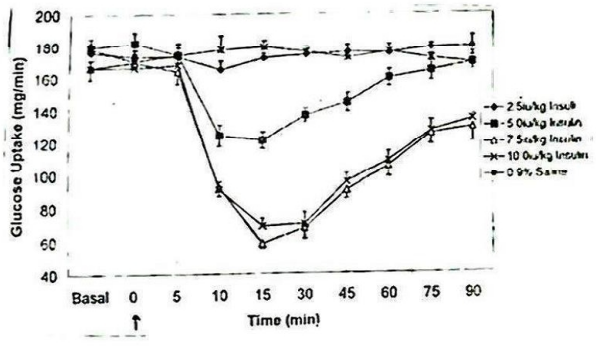

Fig. 1: Effect of normal saline and different doses of insulin on intestinal glucose uptake. (Arrow shows point of injection of saline or insulin)

Effect of insulin on glucose uptake in normal dogs. The effect of two doses of insulin on arterial blood glucose, venous blood glucose, (A-V) glucose difference, jejunal blood flow and intestinal glucose uptake in normal dogs are shown in Table 8. Insulin, 5 iu/Kg caused significant decreases in the arterial and venous blood glucose levels. However, the venous blood glucose levels were slightly but significantly higher than the arterial blood glucose levels during most of the post-injection observation period. Insulin 5 iu/Kg caused significant but negative difference in (A-V) glucose during the post-injection observation period. There was no significant change in the jejunal blood flow following administration of insulin. When the glucose uptake was calculated, there was a significant negative intestinal glucose uptake (P<0.01) which lasted about 30 minutes (between 15-45 min.) post-injection. At 60 min post-injection, glucose uptake was as low as 0.27 ± 1.71mg/min, which represents an upward climb from negative to positive glucose uptake. Even at 90 min post-injection, glucose uptake was very close to zero value of 0.09 ± 0.02mg/min.

The higher dose of insulin (8 iu/Kg) produced more significant effect on the arterial and venous glucose levels. The intestinal glucose uptake first increased significantly at 10 min post-injection, and then uptake decreased between 15 min and 30 min. Thus glucose uptake decreased significantly from a basal level of 17.77 ± 1.56 mg/min. to 43.24± 4.40 mg/min, at 30 min post-injection, that is, a decrease of about 250%. However, the negative glucose uptake at this dose did not persist for as long as the 5.0 iu/kg dose. Also, after this transient negative glucose uptake at 5.0 iu/kg dose, glucose uptake increased significantly from 45 min to the end of the post-injection observation period.

Table 9 compares the effects of insulin at two dose levels on glucose uptake in normal and diabetic dogs. The

point to note in Table 9 is that while insulin decreased glucose uptake in diabetic dogs, it caused a negative glucose uptake in normal dogs, that is to say, it resulted in the gut pushing out glucose into the arterial blood so that the venous glucose level became higher than the arterial level. In the normal dogs, the higher dose of insulin caused an increase in glucose uptake after a transient negative uptake.

Discussion

The substantial glucose uptake by normal canine intestine reported in this study is consistent with findings in previous experiments in dogs [6, 8, and 9]. Failure of normal saline to produce an effect on all the parameters measured also concurs with previous reports [9].

The present study is the first report as far as we are aware, of glucose uptake in the gut of a diabetic animal *in the fasting (post-absorptive) state*. There are of course, hundreds of studies of gut glucose uptake in both normal and diabetic animals and man following ingestion of different meals [17]. An interesting finding in the present study is that the hyperglycemia of diabetes mellitus resulted in an enormous increase in glucose uptake by the canine intestine. This result is similar to our earlier findings in normal dogs where glucose uptake by the gut was increased following hyperglycemia induced by adrenaline [6], glucose infusion, adrenaline injection and glucagon injection respectively [9], nicotine [7] and cow's urine concoction [5]. The present result provides further support for our earlier conclusion that the gut of a normal or a diabetic dog will increase its glucose uptake in response to hyperglycemia, irrespective of the cause of hyperglycemia [9].

It has been reported by earlier workers that in hyperglycemia of diabetes mellitus, glucose uptake in the liver and muscle is impaired [23]. In the present study, the reverse occurred in the gut, that is to say, glucose uptake in the gut is tremendously increased in untreated diabetes mellitus. We did not measure liver and muscle glucose uptake in the present study, but if the findings of Butler and Rizza [23] about a decrease in glucose uptake in the liver and muscle in diabetes mellitus apply to our animals, this suggests but does not prove, that a reciprocal relationship exists between the liver and the intestine in their response to diabetic hyperglycemia with respect to glucose uptake. Whether such reciprocity actually exists can only be proven by simultaneous measurements in the same animal of hepatic and gut glucose uptakes in the post-absorptive state. This possibility deserves further investigation. However, the enormous quantity of glucose taken up by the gut in diabetic hyperglycemia suggests that the post-absorptive gut in the diabetic state may well be involved in reducing the degree of diabetic hyperglycemia by mopping up a lot of glucose from the circulation. Since there was no increase in blood flow in the present study, the huge glucose uptake observed in the diabetic state is due mainly to increased glucose extraction. This contrasts with the effects of adrenaline, glucagon and glucose infusion on glucose uptake [9] where significant increases in gut blood flow contributed substantially to the increased glucose uptake.

An interesting observation in this study is the huge difference in the arterial glucose levels in the normal and diabetic dogs. While arterial glucose level in normal dogs was 109.00± 1.76 mg/dl, a value that is similar to values reported for normal dogs in our earlier study [9], the resting arterial glucose level in the diabetic dogs was 247.75± 4.42mg/dl (Table 2). At no time was resting arterial glucose less than 235.25 mg/dl in any of the diabetic dogs (Table 4). It should be noted that these fasting glucose levels in the diabetic dogs are far higher than the peak of hyperglycemic response to adrenaline, glucagon and glucose administrations in our earlier study [9]. In other words, the hyperglycemia of diabetes mellitus is far more profound than those induced by adrenaline, glucagon and glucose injections. This difference is most probably due to the fact that the pancreases were intact in the latter group of animals and were able to produce insulin to counter the effects of the hyperglycemic agents injected.

Since the present and previous studies have established that the canine intestine will increase its glucose uptake in response to hyperglycemia irrespective of its cause, it is tempting to expect that the amount of glucose taken up should be proportional to the degree of hyperglycemia. The possibility of such a relationship was examined in this study. The results presented in Table 7 however showed that it is unlikely that such a direct relationship exists at least in insulin-treated dogs. The percentage changes in glucose uptake were far in excess to the corresponding percentage changes in arterial blood glucose. These suggest that insulin (and other unknown factor(s)) influence glucose uptake apart from the glycemic level. There is need for further investigation of this aspect.

Insulin caused a reduction in gut glucose uptake in a dose-dependent manner in the diabetic dogs. Since insulin had no effect on blood flow at all the doses studied, the decrease in glucose uptake was due mainly to reduction in glucose extraction. However, at no instance was a negative glucose uptake observed as recorded in normal dogs given insulin in this study and in our earlier study [9]. The reason for this is not clear. The diabetic state may be responsible for the difference in some yet to be identified ways. The glycemic level may be involved in this. For instance, a close look at arterial glucose levels in all animals given insulin (Table 3) shows that at no instance did arterial glucose fall below 141 mg/dl in any of the animals. This value is still well above the resting arterial blood glucose level of 109.00 ± 1.76 mg/dl in normal dogs.

The finding of negative glucose uptake following insulin administration in normal dogs in this study concurs with our earlier report [9]. The result of the lower

dose of insulin in normal dogs suggests that the reduced uptake produced by insulin as well as the negative glucose uptake is most probably not due to the blood glucose lowering effect of insulin. Other mechanism(s) not related to the glycemic are probably involved. This deserves further study.

It is surprising that the higher dose of insulin (8.0 iu/kg) in normal dogs caused just a transient negative glucose uptake and that for most of the post-injection period, there was in fact significant increase in glucose uptake. This was in spite of the fact that this dose caused significant reductions in both arterial and venous glucose levels. This result shows that it is not the degree of hyperglycemia alone (and therefore the amount of glucose transported to the gut tissue in a given time) that determines the glucose uptake in the gut. Other factors that are yet to be identified are involved. For now it is reasonable to suggest that the reduced blood glucose level produced by insulin triggered the secretion of counter-regulatory hormones, especially adrenaline, glucagon and growth hormone and these hormones may be responsible for the increased glucose uptake. Production of counter-regulatory hormones in response to lowering of blood glucose is well-documented [3, 24, and 25]. This may well be a similar phenomenon to the post-hypoglycemic hyperglycemia or the Somogyi effect which has long been known to occur in humans given insulin therapy [26]. Further studies, including measurement of these hormones, will provide more insight as to the mechanism of increased glucose uptake during insulin-induced lowering of blood glucose.

Type I diabetes is normally treated with insulin. Insulin administration is known to increase tremendously glucose uptake into the liver and muscles in diabetes mellitus [4]. Reduced uptake of glucose by the gut following insulin injection may well be a reverse play of the earlier suggested reciprocity between the liver and the gut as far as glucose uptake is concerned.

In the present study, although no glucose is coming from the gut after an overnight fast, it is well-documented that the liver and kidney are primary sites of glucose production during fasting [27, 28, and 29]. We have not measured the quantity of glucose produced by the liver and kidney in the present study. Whatever its magnitude, it will at best only influence the level of hyperglycemia in the systemic blood.

In summary, we have shown in this study that diabetes mellitus resulted in an eight-fold increase in canine intestinal glucose uptake when compared with normal dogs. Normal saline had no effect on gut glucose uptake in both normal and diabetic dogs. Insulin however, caused significant reduction in gut glucose uptake in both normal and diabetic dogs.

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