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Exogenous administration of adenosine enhanced glucose uptake in canine hind limb at rest and during contraction

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Abstract

Background: Glucose metabolism increases during contraction of skeletal muscle and can be influenced by the endogenous adenosine. However, the role of exogenous adenosine in regulating glucose uptake at rest or during contraction has not been elucidated in dogs. We studied the effects of exogenous adenosine on glucose uptake in canine hind limb at rest and during contraction.

Methods: The study was carried out using thirty (30) fasted and anaesthetized male dogs divided into six groups (5dogs/group). Groups I (control) and II received normal saline (0.1ml/kg) at rest and during contraction of hind limb respectively. Group III received adenosine (0.1, 0.5 and 1mg/kg) at rest. Group IV were treated with adenosine (1mg/kg) during contraction. Groups V and IV were pretreated with caffeine (6mg/kg) and infused with adenosine (1mg/kg) for thirty minutes at rest and during contraction of the hind limb respectively. Blood glucose was measured by glucose oxidase method. Arterio-venous (A-V) glucose and venous blood flow (VBF) were measured; hind limb glucose uptake (HGU) was calculated as the product of A-V glucose and VBF.

Results: The results showed that exogenously administered adenosine significantly ($P<0.05$) increased A-V glucose, VBF and HGU in a dose dependent manner at rest. During contraction adenosine increased A-V glucose significantly from14.2±0.5mg/dl to 45.4±1.8ml/min. VBF also significantly increased from 4.7±0.6ml/min to 16.3±1.2 and HGU from 34.8±2.4 to 450.8±8.2mg/ /min. Pretreatment with caffeine significantly reduced adenosine-induced hyperglycemia at rest and during contraction.

Conclusion: Exogenous adenosine at rest and during contraction increases the skeletal muscle glucose uptake and the increase appears to be mediated by inhibition of adenosine receptors.

Keywords: *Adenosine, Caffeine, Dog, glucose uptake, hind limb*

Résumé - 3710

Contexte: Le métabolisme du glucose augmente pendant la contraction du muscle squelettique et peut être influencé par l'adénosine endogène. Cependant, le rôle de l'adénosine exogène dans la régulation de l'absorption de glucose au repos ou pendant la contraction n'a pas été élucidée chez les chiens. Nous avons étudié les effets de l'adénosine exogène sur l'absorption de glucose dans le membre postérieur canin au repos et pendant la contraction.

Méthodes: L'étude a été menée à l'aide de trente (30) chiens mâles en jeûnés et anesthésiés répartis en six groupes (5 chiens / groupe). Les groupes I (témoin) et II ont reçu une solution saline normale (0,1 ml / kg) au repos et pendant la contraction du membre postérieur respectivement. Le groupe III a reçu de l'adénosine (0,1 ; 0,5 et 1 mg / kg) au repos. Le groupe IV a été traité avec de l'adénosine (1 mg / kg) pendant la contraction. Les groupes V et IV ont été prétraités avec de la caféine (6 mg / kg) et infusés avec de l'adénosine (1 mg / kg) pendant 30 minutes au repos et pendant la contraction du membre postérieur respectivement. La glycémie a été mesurée par la méthode de l'oxydase du glucose. Le glucose sanguin artério-veineux (A-V) et le flux sanguin veineux (VBF) ont été mesurés; l'absorption de glucose des membres postérieurs (HGU) a été calculée comme le produit du glucose A-V et du VBF.

Résultats: Les résultats ont montré que l'adénosine administrée exogène de manière significative (P <0,05) a augmenté le glucose A-V, VBF et HGU de manière dépendante de la dose au repos. Pendant la contraction, l'adénosine a augmenté le glucose A-V significativement de 14,2 ± 0,5 mg / dl à 45,4 ± 1,8 ml / min. VBF a également augmentée de 4,7 ± 0,6 ml / min à 16,3 ± 1,2 et HGU de 34,8 ± 2,4 à 450,8 ± 8,2 mg / min. Le prétraitement avec la caféine a considérablement réduit l'hyperglycémie induite par l'adénosine au repos et pendant la contraction.

Conclusion: L'adénosine exogène au repos et pendant la contraction augmente l'absorption du glucose dans le muscle squelettique et l'augmentation semble être par la médiation de l'inhibition des récepteurs de l'adénosine.

Mots-clés: *Adénosine, Caféine. Chien, absorption de glucose, membre postérieur*

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Introduction

Skeletal muscle comprises about 40% of total body mass in mammals and accounts for 30% of the resting metabolic rate in adult humans [1]. Skeletal muscle has a critical role in glycaemic control, metabolic homeostasis, and is the predominant site of glucose disposal under insulin stimulated conditions [2]. It is the largest glycogen storage organ having 4-fold the capacity of the liver. During exercise, the increase in glucose uptake from the circulation provides fuel to meet energy demand of contracting muscles [3,4]. Therefore, the regulation of glucose transport, metabolism or storage of glycogen by insulin and exercise is of critical importance in maintaining glucose homeostasis with significant implications for patients with insulin resistance [5]. Decrease in response to insulin but not to exercise leading to decreased glucose transport in skeletal muscle is a major factor responsible for insulin resistance associated with diabetes mellitus [6].

Reports indicate that one of the locally produced compounds in muscles, adenosine, has potent glucose metabolism and uptake activity [7]. Adenosine is a naturally occurring compound that is elaborated in the myocardium in response to hypoxia and under conditions in which there is increased demand for oxygen. Adenosine is principally formed on degradation of intracellular ATP when high-energy phosphate use exceeds its formation [8,9]. ATP is hydrolyzed to ADP and then to AMP when high energy phosphate reserves are compromised through the action of 58-nucleotidase. AMP is hydrolyzed to adenosine, which then diffuses into the interstitial space [8]. Report indicate that biological functions of extracellular adenosine are mediated by four different G-protein coupled receptors that are classified as adenylyl cyclase inhibiting (A_1 and A_3) or adenylyl cyclase activating (A_{2a} and A_{2b}) receptors [10,11]. Previous pharmacological studies on the effect of adenosine on glucose uptake have shown that it increases or stimulates glucose uptake in adipose tissues in human [12,13], dogs [14] and rats [15]. Studies have also established adenosine's ability to activate myocardial glucose uptake [16,17].

Reports on the role of adenosine on glucose uptake by the skeletal muscle are inconsistent. For example; a study indicates that adenosine deaminase (ADA), which converts adenosine to inactive metabolite inosine, and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) can decrease both insulin and contraction stimulated glucose uptake [18]. It was also shown that N^6-cyclopentyladenosine (CPA) a selective adenosine A_1 receptor agonist increases the glucose uptake in steptozotocin-induced diabetic rats, an effect that was blocked by adenosine antagonists [19]. Furthermore during a euglycemic-hyperinsulinemic clamp, it was also observed that caffeine an adenosine antagonist impaired the glucose uptake at rest and in exercising human skeletal muscle [20].

Contrary to this, several reports have consistently shown that while adenosine impairs on one hand, on the other hand adenosine deaminase and adenosine antagonists improve insulin sensitivity in skeletal muscle [21-23]. Furthermore, one study showed that adenosine, via A_1 affects insulin-mediated glucose uptake in rat skeletal muscles only in the presence of a submaximal concentration of insulin [24]. This supports the finding of a stimulatory action of adenosine on insulin-stimulated glucose uptake in striated muscle during contraction. Skeletal muscle contains several interstitial metabolites including adenosine. There are reports that suggest that interstitial concentration of adenosine was very low and the rate of its release in well- oxygenated muscle is very slow at the resting state. This rate may be insufficient to cause significant adenosine receptors activation in the resting skeletal muscle preparation when it is compared to during contraction.

The question to ask now is: What is the effect of exogenous infusion of adenosine on glucose uptake at rest and during electrical stimulation?

The present study was therefore designed to study the effect of infusion of adenosine on the glucose uptake by the canine hind limb at rest and during contraction. In addition, we also investigate the effect of caffeine a non-selected antagonist on effect of adenosine.

Materials and methods

Experimental design and treatment

Male mongrel dogs weighing 11-13 kg were used for the study. The animals were divided into six groups with 5 dogs per group.

Group I served as control and received normal saline (0.1 ml/kg) at rest, group II received normal saline with hind limb muscle contraction, Group III were infused with adenosine at doses of 0.1, 0.5 or 1 mg/kg for thirty minutes at rest, group IV was infused with adenosine (1 mg/kg) with hind limb muscle contraction, group V animals were pretreated with caffeine (6 mg/kg) before infusion of adenosine (1 mg/kg) at rest and lastly group VI were pretreated with caffeine (6 mg/kg) adenosine (1 mg/kg) with hind limb contraction.

Experimental procedure

The protocols and procedures used in this study were approved by the Animal Ethics Committee of the Lagos State University College of Medicine and conform to the 1985 guidelines for laboratory animal care of the National Institute of Health (NIH).

Each animal was fasted for 18-24 hr before the start of experiment. Anaesthesia was induced by i.v injection of sodium pentobarbitone, 30 mg/kg. Light anaesthesia was maintained with supplemental doses of i.v. sodium pentobarbitone as necessary during dissection. The trachea was intubated using endotrachea tube and the animal was allowed to breathe room air (temp. 25 °C) spontaneously.

The right femoral vein and artery were cannulated. The cannula in the right femoral vein was moved into an extracorporeal position and a non-crushing clamp was applied to its free end. The left femoral vein was cannulated for the administration of drug and left femoral artery was also cannulated and connected to a two-Channel physiographic recorder through pressure transducer model 7070 Gemini (Ugo Basil) to monitor blood pressure and heart rate. The right femoral nerve was surgically isolated and stimulated by student electrical stimulator (Brooks Instruments, UK) to induce muscular contraction. The output voltage was limited to 5Hz for non-painful muscle contraction for thirty minutes [25]. At the end of the dissection, sodium heparin 300unit per kg-body weight was administered intravenously to prevent blood clotting. After all surgical procedures were completed, a 60-90 minutes stabilization period was observed. The blood flow to the hind limb was measured by timed collection of the blood from the right femoral vein as previously described [26]. Arterial and venous blood samples for glucose estimation were obtained from the cannula placed in the right femoral artery and vein respectively.

Blood pressure was recorded continuously throughout the duration of the experiment. After stabilization, basal measurements of femoral venous blood flow, arterial and venous glucose levels were recorded. Then, these measurements were repeated at 0, 5, 15, 20, 25, 30, 45, 60, 75, and 90 minutes post-injection of drugs. The arterial and venous samples (0.05ml per sample) for glucose determination were obtained simultaneously via three-ways tap cocks placed on the right femoral venous outflow and in the femoral artery cannula. After the basal samples have been taken, the effects of intravenous injection of normal saline, and adenosine under resting (basal) and muscles contractions on the hind limb glucose uptake were studied.

Measurement of blood flow

The technique requires arterial cannulation with an extra corporal circuit with or without a pump. A free flow of blood from the distal end of the right femoral vein cannula into a clear, graduated cylinder was allowed for 30 seconds. The volume of blood thus collected multiplied by two gave flow per minute.

Blood glucose measurement

Blood glucose was determined by modified glucose oxidase method [27]. Glucose uptake was computed as the product of the A-V glucose and blood flow.

Statistical analysis

Data was analyzed using GraphPad Prism version 5.0 statistical software. All values given were expressed as mean ± S.E of the variables measured. Significance was assessed by the student's t-test of two means of independent variables. P values of 0.05 or lesser were taken as statistically significant.

Results

Effects of adenosine (0.1, 0.5 and 1 mg/kg) on blood glucose, arterial-venous (A-V) glucose difference, and hind limb glucose uptake (HGU) in dogs at rest and during contraction

Adenosine produced varying effects on blood glucose, arterial-venous glucose difference and hind limb glucose uptake (HGU). At low doses, (0.1 and 0.5 mg/kg) adenosine has no significant effect on the arterial blood glucose levels when compared with normal saline ($p>0.05$). However, at a high dose of 1.0 mg/kg/min, adenosine produced significant increases in arterial blood glucose levels ($p<0.05$). The effect did not occur until 25min post-injection and was sustained for the rest of the observation period. There was however no significant change in the venous blood glucose levels (table 1).

As shown in Figure 2a, there were dose-dependent increases in A-V glucose following administration of different doses of adenosine when compared to control. Doses of 0.1 and 1 mg/kg/30min of adenosine produced a maximum A-V glucose of about 10.1 mg/dl and 16.3 mg/dl respectively (Fig. 2a) while control was 4.2 ± 0.2 mg/dl (Fig. 1a) [$p<0.05$]. Infusion of adenosine (0.1, 0.5 and 1 mg/kg) significantly increased blood flow to 8.5 ± 0.2, 12.4 ± 0.3 and 18.5 ± 0.6 respectively from resting blood flow of 4.5 ± 0.5 ml/min (Fig. 1b) [$p<0.05$]. It is to be noted that blood flow to the hind limb during adenosine infusion remains high and sustained throughout the post-infusion observation period (Fig. 2b).

Table 1: Effects of normal saline and intravenous infusion of adenosine (0.1, 0.5, 1 mg/kg) on arterial and venous glucose levels (mg/dl) in dogs at rest.

		Time										
	Treatment	0	5	10	15	20	25	30	45	60	75	90
Arterial	Control	110±1.4	107±0.9	108±0.8	106±1.9	105±1.2	99±1.0	97±1.4	96±0.9	96±0.8	96±1.0	96±0.4
	Adenosine 0.1mg/kg	99±0.7	94±2.9	88±2.2	89±3.6	93±4.4	92±4.3	97±6.3	98±6.4	96±5.2	96±7.5	97±5.3
	Adenosine 0.5mg/kg	99±1.4	103±6.2	96±8.4	93 ±3.3	89±2.7	89±3.9	92±2.5	92±1.5	92±2.6	92±2.0	91±2.3
	Adenosine 1mg/kg	99±1.2	104±4.0	103±3.0	107±4.4	109±3.3	114±3.7*	113±1.8*	108±3.1	110±3.2	110±1.2	110±2.7
Venous	Control	104±1.5	99±1.7	98±1.1	97±1.2	96±1.5	92±1.4	91±1.2	90±1.6	90±1.4	90±1.6	90±1.7
	Adenosine 0.1mg/kg	95±1.5	77±3.3	74±3.3	77±3.3	79±4.4	80±4.5	82±5.0	80±5.3	77±6.2	76±7.8	79±6.2
	Adenosine 0.5mg/kg	95±1.5	83±2.9	81±5.9	78±2.1	75±3.9	75±2.8	77±1.9	79±1.7	78±1.5	76±2.4	79±6.2
	Adenosine 1mg/kg	95±1.5	89±3.9	90±2.0	89±3.7	91±2.1	92±3.4	97±2.5	96±4.0	91±3.6	95±2.9	65±2.9

*Values are expressed as Mean ± SEM. (N=5) (*p<0.05; **p <0.01 when compared with control)*

Table 2: Effects of normal saline and intravenous infusion of adenosine (1mg/kg) on arterial and venous blood glucose levels (mg/dl) during hind limb muscles contraction in dogs.

		Time										
	Treatment	0	5	10	15	20	25	30	45	60	75	90
Arterial	Control	99±0.7	118±5.4	115±5.7	128±6.5	110±6.5	109±6.6	107±6.1	104±7.1	105±5.6	106±4.2	105±5.5
	Adenosine	99±2.1	131±2.4*	154±3.2*	160±3.4**	156±4.1**	151±2.5**	156±3.4**	148±5.2**	149±4.6**	149±3.2**	148±2.5**
Venous	Control	95±0.5	94±1.8	94±6.2	96±2.1	98±2.1	98±3.3	92±4.2	94±4.7	93±5.1	93±5.6	93±5.6
	Adenosine	93.2±3.1	115±3.2*	130±3.5**	123±4.3*	123±2.3*	120±3.1*	112±2.1*	108±4.2	109±5.3	109±4.3	109±5.2

Values are expressed as Mean ± SEM. (N=5) (*p<0.05; **p <0.01 when compared with control)

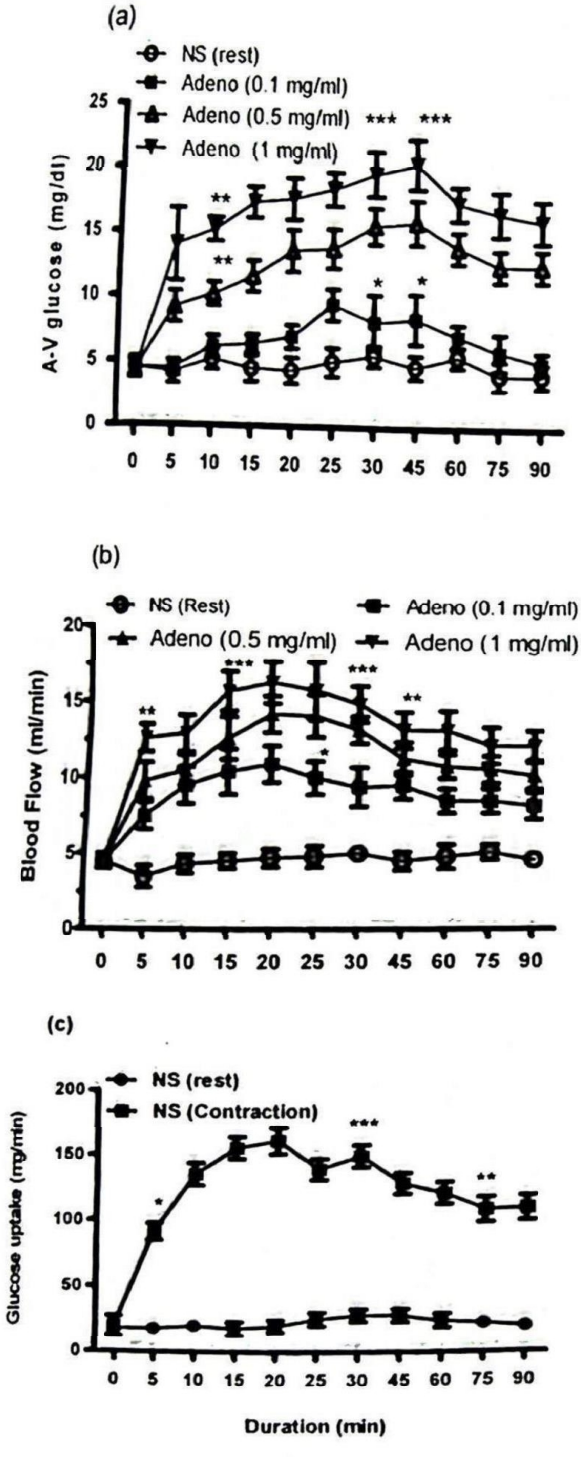


Fig. 1: Effects of intravenous injection of normal saline on (A) arterio-venous glucose (B) blood flow (C) glucose uptake at rest and during hind limb muscle contractions in dogs (n=5). Values are expressed as mean ± SE (**P*< 0.05; ***P*<0.01; ****P*<0.001).

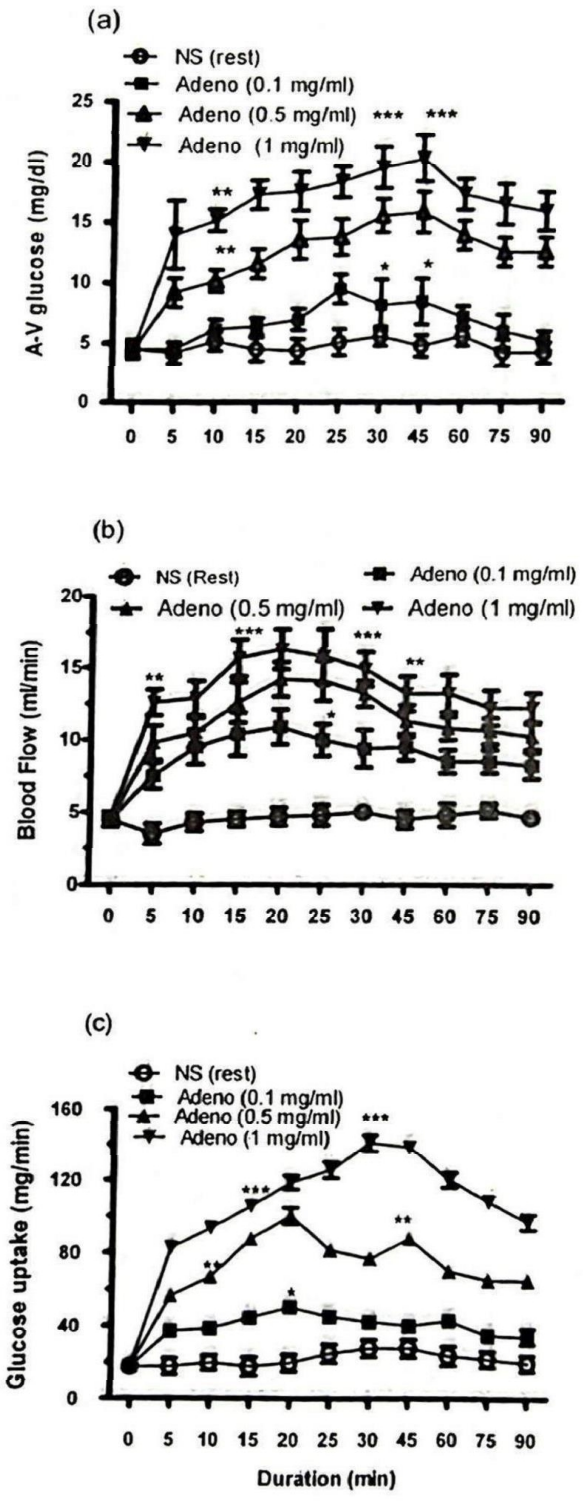


Fig. 2 : Effects of intravenous injection of normal saline and intravenous infusion of adenosine (0.1; 0.5; and 1mg/ml) on (A) arterio-venous glucose (B) blood flow (C) glucose uptake at rest in dogs (n=5). Values are expressed as mean ± SE (**P*< 0.05; ***P*<0.01; ****P*<0.001).

At rest the hind limb glucose uptake (HGU) in the dogs was 18.0 ± 1.5 mg/min and was sustained following administration of normal saline (Fig. 1c). However, adenosine produced dose-dependent increases in HGU. The HGU increased from 18.0.0 ± 0.5mg/min to 50.3 ± 2.5 mg/min, 100.1 ± 4.5 mg/min and 140.7 ± 4.4 mg/min for 0.1, 0.5 and 1.0 mg/kg/30min respectively [p<0.05] (Fig. 2c).

Table 2 shows the effect of contraction of the hind limb on arterial and venous blood glucose level. Contraction and infusion of adenosine (1 mg/kg/min) caused significant ($p<0.05$) increase in both arterial and venous glucose levels of the hind limb. (Table 2).

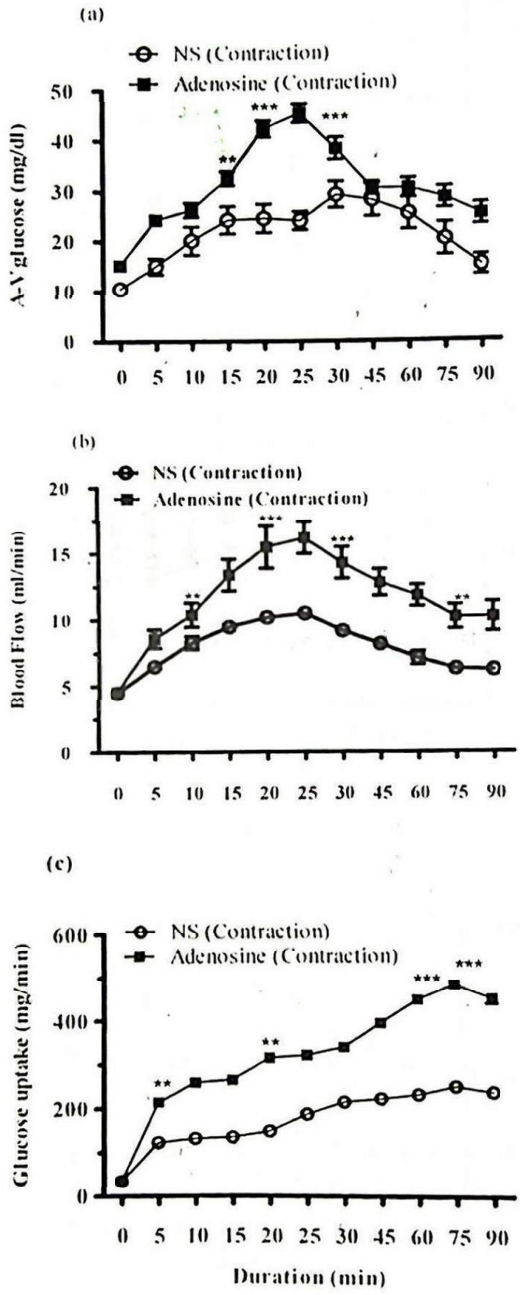


Fig 3: : Effects of intravenous infusion of adenosine (1mg/ml) on (A) arterio-venous glucose (B) blood flow (C) glucose uptake at hind limb during contraction in dogs (n=5). Values are expressed as mean ± SE (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

Figure 1a shows the effect of hind limb contraction on A-V glucose difference. There was a steady rise in A-V glucose, from 4.5 ± 0.7 mg/dl reaching its peak at 29.1 ± 2.6 mg/dl about 30 mins into the contraction period. Administration of adenosine significantly increased A-V glucose higher than normal saline in contracting hind limb [$p<0.05$] (figure 3a).

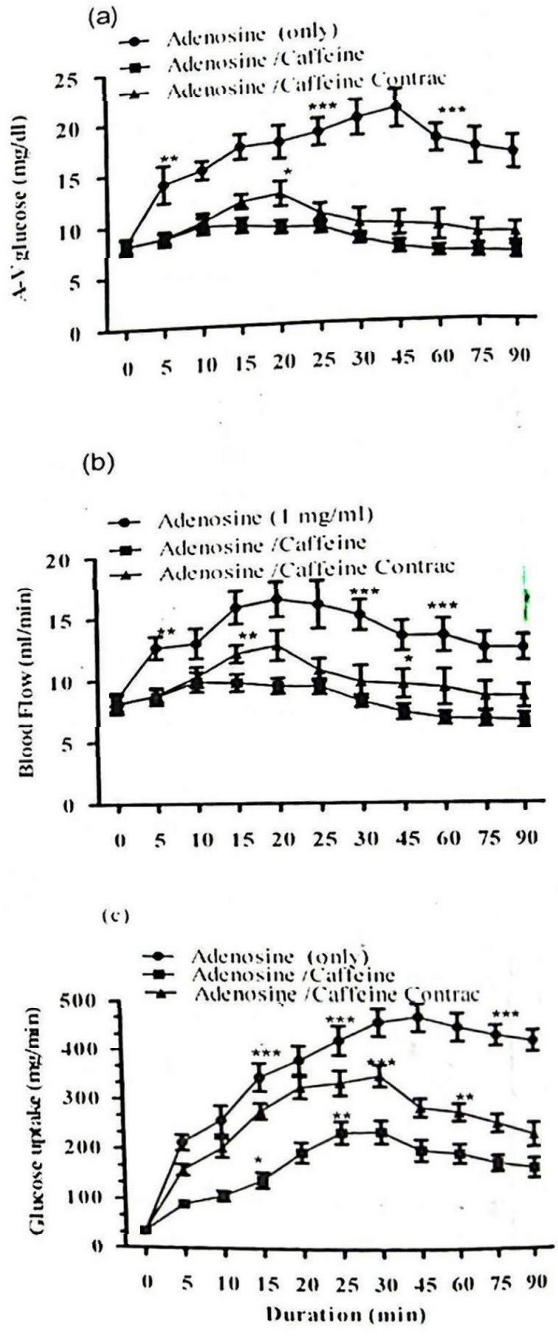


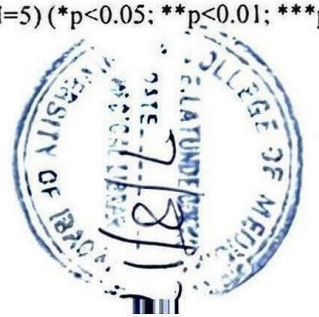
Fig. 4: : Effects of intravenous infusion of adenosine (1mg/ml) on (A) arterio-venous glucose (B) blood flow (C) glucose uptake in hind limb pre-treated with caffeine (6mg/ml) at rest and during hind limb contraction in dogs (n=5). Values are expressed as mean ± SE (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

Following contraction, the blood flow to the hind limb significantly increased from 4.5 ± 0.5 ml/mins to 13.2 ± 0.6 ml/mins. This was sustained throughout the contraction and post contraction period (Fig. 1b).

Table 3: Effects of intravenous infusion of adenosine (1mg/kg) on arterial and venous glucose levels (mg/dl) at rest and during hind limb contraction in non-caffeine and caffeine (6mg/kg) pre-treated dogs.

		Time										
	Treatment	0	5	10	15	20	25	30	45	60	75	90
Arterial	Adenosine only (Rest)	95±1.2	115±4.0	126±3.0	132±4.4	123±3.3	121±3.7	121±1.8	120±3.1	126±3.2	120±1.2	118±1.2
	Adenosine+Caffeine (Rest)	99.3±1.2	104.4±4.0	102.6±3.0	107.4±4.4	108.6±3.3*	113.8±3.7	113.4±1.8	108±3.1	109.6±3.2	110.2±1.2	110.2±2.7
	Adenosine only (Contraction)	99±2.1	131±2.4*	154±3.2*	160±3.4***	156±4.1***	151±2.5**	156±3.4**	148±5.2**	149±4.6**	149±3.2**	148±2.5**
	Adenosine+Caffeine (Contraction)	98±0.5	128±1.8*	130±2.0*	133±1.5**	134±2.1**	134±1.2**	132±1.2**	131±2.2**	126±2.4**	118±2.1**	118±1.2*
Venous	Adenosine only (Rest)	94±2.3	109±3.1	115±4.3	118±5.2	120±4.7	116±5.2	107±3.2	104±3.2	105±3.2	106±4.2	105±3.2
	Adenosine+Caffeine (Rest)	95.6±6.0	89.4±3.3	89.6±2.0	88.6±3.7	91.4±2.1	92.2±3.4	96.6±2.5	95.8±3.7	91.2±4.0	95.4±3.6	95.4±2.9
	Adenosine only (Contraction)	93.2±3.1	115±3.2	130±3.5	123±4.3	123±2.3	120±3.1	112±2.1	108±4.2	109±5.3	109±4.3	109±5.2
	Adenosine+Caffeine (Contraction)	95±0.6	102±1.2*	100±1.1*	100±1.2*	97.4±2.5	99.6±1.2	105±±2.5	105±1.2	103±0.5	100±1.2	100±1.7

Values are expressed as Mean ± SEM. (N=5) (*p<0.05; **p<0.01; ***p<0.001 when compared with control)



During contraction adenosine significantly increased the blood flow to the hind limb compared to control (Fig. 3b) [$p<0.05$].

The effect of contraction of the hind limb on glucose uptake is shown in Figure 1c. In normal saline-treated animals, HGU significantly increased during contraction ($p<0.05$) but remained steady at rest. It is to be noted that in the post-contraction observation period HGU did not return to the basal level. During contraction treatment with adenosine also significantly increased HGU higher than that of normal saline ($p<0.05$). (Fig. 3c).

Effect of adenosine on blood glucose, A-V glucose, and hind limb glucose uptake (HGU) in caffeine-treated dogs at rest and during contraction

Pre-treatment with caffeine significantly reduced hyperglycemia induced by adenosine infusion at rest and during contraction compared to non- caffeine treated group (table 3) and also significantly inhibit A-V glucose differences (Fig. 4a).

Figure 4b shows the effect of adenosine on blood flow in dogs pretreated with caffeine. Caffeine significantly reduced adenosine induced increase in blood flow to the hind limb. As shown in figure 4c, pretreatment with caffeine significantly reduced the effect of adenosine on glucose uptake.

During pre-treatment with caffeine, there was also significant increase in A-V glucose during contraction compared to control [$p<0.05$] (Fig 4a). Figure 4b shows the effect of adenosine on blood flow in caffeine pre-treated dogs during contraction. Pre-treatment with caffeine reduced significantly the effect of adenosine on blood flow during contraction of the hind limb ($p<0.05$). Pre-treatment of the animal with caffeine also reduced significantly the glucose uptake. For instance, caffeine pretreatment decreased hind limb glucose uptake from 420.7 ± 2.3 mg/min to 231.8 ± 3.7 mg/min (a decrease of about 44.9%) during contraction of the hind limb ($p<0.05$).

Discussion

The present study examined the effects of exogenously administered adenosine on arterial-venous (A-V) glucose, venous blood flow and hind limb glucose uptake (HGU) during resting and contraction states in experimental dogs. The observed increase in blood flow following the contraction of the hind limb in this study agrees with the report of Hespel et al. [28]. It is also consistent with previous reports [3,4], whereby exercise-induced contraction was reported to increase blood flow probably through recruitment of capillaries with increase surface area for glucose delivery and exchange in rats and humans [29,30]. Hind limb contraction induced increase in muscle A-V glucose difference and glucose uptake. This is consistent with the reports of many other studies [28, 31]. The increase in A-V glucose observed in the present study showed that muscular contraction increased glucose extraction by the canine hind limb. This may be partially due to the increase in blood flow or probably due to the increase in blood glucose levels, since the arterial glucose level is the other important determinant of muscle glucose uptake during contraction.

The molecular signaling mechanisms by which contraction/exercise induced glucose uptake are not fully understood. However, it is proposed that the rise in intracellular Ca^{2+} is a mediator of increased glucose transport during skeletal muscle contraction and hypoxia. This was based on the evidence that hypoxia, verapamil, a calcium inhibitor or dantrolene which lower Ca^{2+} efflux from sarcoplasmic reticulum inhibit glucose transporter during skeletal muscle contraction. In addition, agents that increase the cytoplasmic Ca^{2+} such as caffeine and Ca^{2+} ionophores may activate the glucose transporter [32,33]. Therefore, the increase in the intracellular calcium may facilitate the activation of key intracellular signaling molecules that increased muscle transporter. Ca^{2+} are also known to activate conventional protein kinase (cPKC) and Phorbol 12-myristate 13-acetate (PMA) an activator of cPKC are reported to increase glucose disposal by distinct mechanism from insulin [32-34]. In this study, the significant increase in blood flow and glucose uptake to the hind limb observed with exogenous infusion of adenosine at rest is similar to the observation in similar study [35]. Heinomen et al. [36] reported that adenosine infusion at rest increased glucose uptake by several-fold in healthy young men [36], and in patients with essential hypertension [37]. Reports also observed that the increase in forearm glucose uptake by adenosine infusion was not insulin-mediated [38] since the observed glucose uptake was inhibited in the present of adenosine receptor antagonist [39]. Also reports indicate that adenosine action via the A_1 adenosine receptor activates and regulates both insulin- and contraction-induced glucose uptake [39,40]. In contrast, absence of the receptor decreases glucose transport in both situations [41]. The presence of adenosine receptors in skeletal muscle have been well documented [11,20]. Earlier report indicates that the resting and contraction adenosine concentrations in dog model are similar to those of humans [42]. Reports indicate that exogenous administration of

adenosine stimulate the formation of nitric oxide and prostacyclin in both the intravascular and interstitial compartments of skeletal muscle [43,44]. Nitric oxide and prostacyclin have been shown to contribute to the exercise hyperaemia response [44,45] which suggest that about two thirds of the vasodilator response to adenosine during muscle contraction may be mediated through the formation of nitric oxide and prostacyclin. Therefore, the observed increase in blood flow and glucose uptake in this study may be due in part to the effect of adenosine on NO production since NO has been shown to increase glucose uptake [45].

Furthermore, the significant inhibiting effect of caffeine on the exogenous adenosine at rest and during contraction on blood flow and glucose uptake is of interest. After extensive search of literature, there was no reported work on the inhibiting effects of caffeine on exogenous adenosine actions on blood glucose in dog. One study in humans showed that caffeine impaired insulin sensitivity and glucose uptake [46]. This effect was attributed to increased plasma epinephrine and free fatty acid levels rather than peripheral adenosine receptor antagonism. In contrast other studies suggesting that endogenous adenosine may modulate muscle glucose uptake during muscle contraction have been conducted in rats [41,47]. Although we did not measure plasma levels of cathecholamines in this study, previous study have shown that adenosine reduces catecholamine mediated activation of phosphorylase by inhibiting beta receptor adenylate cyclase coupling [48]. In animal study, caffeine stimulates the release of cathecholamines to enhance contraction-induced glycogenolysis [40] by inhibition of phosphodiesterase, and increasing cAMP, [13]. There are also studies that showed that caffeine decreases insulin sensitivity through the blockage of adenosine receptors [13,20]. Therefore, the significant reduction in hind limb blood flow and glucose uptake in caffeine pretreated group observed in this study is also consistent with the reported vasoconstriction effect of caffeine [49]. The possible explanation for the vasoconstriction effect of caffeine is the blockage of vasodilatory actions of adenosine receptors [50].

In conclusion, there were several important findings in the present study. Firstly, we observed that moderate electrical stimulation led to increase in blood flow and glucose uptake in contracting canine hind limb. Secondly, exogenous infusion of adenosine increased blood flow and glucose uptake at rest and during contraction of canine hind limb. Thirdly, inhibition of adenosine action by caffeine significantly affect glucose uptake in canine hind limb at rest and during contraction. The mechanism regulating skeletal muscle glucose uptake during contraction is complex and could be mediated directly via adenosine receptors.

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