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Title: The Role of Blood Viscosity in the Regulation of Hematocrit in Tail-Suspended Rats (*Rattus norvegicus*) Treated with Pentoxifylline

Abstract approved: David K. Saunders

Previous studies have shown a significant increase in hematocrit with initial exposure to microgravity condition, with a corresponding return to normal hematocrit values with continued exposure to microgravity. Tail suspension studies have been used to mimic microgravity on earth and have shown similar changes in hematocrit. High hematocrit is correlated with increased blood viscosity. As such, increased blood viscosity during the initial exposure to microgravity may be a factor signaling the reduction of the hematocrit with continued exposure to microgravity. The purpose of this study was to use tail-suspension to investigate the role of blood viscosity might play in the regulation of hematocrit.

Seventy Sprague-Dawley (male) rats were randomly assigned to control and tail suspended groups. All rats were given the drug pentoxifylline which should cause a

reduction in blood viscosity. Suspended and control rats were monitored for hematological changes including blood viscosity over 4h, 24h, 72h, and 168h of tail suspension as well as during recovery periods from tail suspension of 24h, 72h, and 168h.

Hematocrit, red blood cell count, MCV, MCH, and MCHC were not significantly different between control and experimental groups at any time. There was no significant difference in blood viscosity and plasma viscosity between control and experimental groups. Further, it appeared that pentoxifylline had little effect on blood viscosity. The lack of a significant difference in hematocrit between control and tail suspended animals makes it difficult to determine if blood viscosity acts as a regulator of hematocrit.

THE ROLE OF BLOOD VISCOSITY IN THE REGULATION OF HEMATOCRIT IN TAIL-SUSPENDED RATS (*Rattus norvegicus*) TREATED WITH PENTOXIFYLLINE

A Thesis
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PREFACE

My thesis was written in the style according to the instruction for submission to Aviation, Space, and Environmental Medicine.

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INTRODUCTION

A consistent change in hematology has been reported with exposure to microgravity as occurs with space flight (19, 30). On earth, gravity causes blood to pool in peripheral blood vessels below the heart due to the hydrostatic force generated by gravity (1, 7, 33). With exposure to microgravity a cephalic fluid shift rapidly occurs within three to six hours (7, 21, 31). Further evidences for the occurrence of fluid shifts include decreases in leg volume, engorgement of neck veins and tissues, and a feeling of head fullness (7). Along with the cephalic shift, plasma volume, red cell mass (RBCM), and hemoglobin (Hb) concentration are also changed by exposure to the microgravity environment. The initial cephalic fluid shift results in a plasma volume decrease because of an increase in central blood volume and pressure within the thoracic cavity (21). A 15 to 25% decrease in plasma volume has been shown to occur during the beginning of microgravity exposure (2, 32). As a result, hematocrit (Hct) and Hb concentration temporarily increase within 24 hours of space flight (22).

During continued exposure to the microgravity, plasma volume remains decreased, but Hct and Hb concentration decrease to normal values (20, 22, 31). This is likely due to a destruction of circulating red blood cells in response to the plasma volume decrease (2, 19). Previous studies have shown that as much as a 10 to 20% reduction in the RBCM during space flight (18, 30, 31). This reduction in RBC numbers is likely not due solely to the suppression of erythropoiesis. If the production of new RBCs from the bone marrow were completely blocked, the expected decrease in red blood cells would be approximately 1% per day (2, 18). However, this decrease is not as great as the loss of RBCM that occurs with exposure to microgravity. Previous work has shown that released

RBCs from bone marrow are selectively destroyed during the first four to five days of space flight, a process called neocytolysis (1, 2). In addition, exposure to microgravity causes an increase in the rate of destruction of circulating RBCs and a decrease in red cell size, further decreasing Hct (1).

Upon return to earth, there is a rapid increase in plasma volume. The increase in plasma volume causes a decreased Hct and Hb concentration due to dilution (1, 20, 22). Since the recovery of plasma volume is more rapid than that of the RBCs, many astronauts suffer from a temporary condition, called space anemia (30).

Because space flight opportunities are rare, and the collecting and handling of blood is more difficult in space than on earth, analogous models for space flight have been developed on earth to mimic microgravity environments using experimental animals (16). Unlike space flight studies, using a ground-based model simulating spaceflight can be scheduled without concern for crew time, and modification can be made as necessary during the experiment with little impact on cost (23). Additionally, it is easier to handle samples under 1g conditions than it is in space (23). The most important benefit in using analogous models for space flight is repetition and extension of experiments on a routine basis (23). Ground-based models include antiorthostatic horizontal bed rest, hindlimb unloading in rodents using the tail suspension method, and wet immersion (16). Because both spaceflight and tail-suspension methods show similar atrophic changes and similar responses in heart, pulmonary, intestine, immune, and reproductive systems, the tail suspension method has been accepted by the scientific community as the model of choice for simulating spaceflight (23). Further, tail suspension studies on animals mimicking

microgravity have shown hematological responses which are similar to those of space flight. In the initial period of suspension, plasma volume is decreased, and Hct, Hb concentration (14, 25) and RBCM (14) are increased. With continued suspension time, Hct and Hb concentration return to within normal range (14, 25), while plasma volume is still decreased (14). During the recovery period, plasma volume is rapidly increased (14, 16, 25).

Blood viscosity, the resistance to bulk flow, is increased by low shear rates, high concentration of plasma proteins, decreased RBC deformability, and increased Hct, which has the greatest effect (5, 8). At the time of initial microgravity exposure, or tail suspension, the decrease in plasma volume and resulting increase in Hct (14, 25) lead to higher blood viscosities (25). During the tail suspension period, suppressed erythropoiesis as well as selective destruction of newly released RBCs results in return to normal Hcts (14, 25) which leads to the return of normal blood viscosity values (25).

The mechanism that stimulates the reduction in RBC numbers has yet to be elucidated. Soviet scientist have suggested that reduced erythropoiesis during microgravity exposure may be the result of decreased oxygen demand by muscles, because the oxygen requirement of the tissue in microgravity would be less than that on the earth (17, 20). However, initial decrease in RBCM may not be totally driven by the excess oxygen delivery to muscles. Another explanation for reduction in RBC numbers may be the temporary increase in Hct, due to the initial decrease in plasma volume, leading to an increase blood viscosity which may act as signal to decrease RBCM. So, the decrease in Hct with prolonged microgravity exposure may result from the body's response to an increased blood viscosity.

Oxygen delivery is maximized at some level of Hct, termed the optimal hematocrit (10). This can be seen using the Poiseuille-Hagen equation for laminar flow $Q= \Delta P\pi r^4/8\eta l$ where Q = blood flow, ΔP= change in pressure, r= radius, η= viscosity of fluid, and l= length of the tube. From the Poiseuille-Hagan equation, when blood viscosity is increased, blood flow (Q) is decreased, thus the relationship between blood viscosity and blood flow is inversely proportional. When this relationship is applied to the O_2 delivery equation; O_2 delivery = Q ($CaO_2 - CvO_2$), where Q = blood flow, CaO_2 = concentration O_2 in the arteries (O_2 carrying capacity), and CvO_2 = concentration of O_2 in the veins, the effect of blood viscosity on the maintenance of an optimal hematocrit can be seen. Increased Hct results in increased oxygen carrying capacity of blood, but when Hct increases above a theoretical optimal hematocrit, oxygen delivery decreases due to the effect of increased viscosity on decreasing blood flow. Conversely, low Hct leads to decreased blood viscosity and thereby increases blood flow, but also adversely affects the oxygen carrying capacity of blood. If the O_2 carrying capacity decreases more than Q increases, O_2 delivery could decrease. Hct is likely regulated at near optimal hematocrit values to maintain optimal delivery of oxygen to the tissues (5).

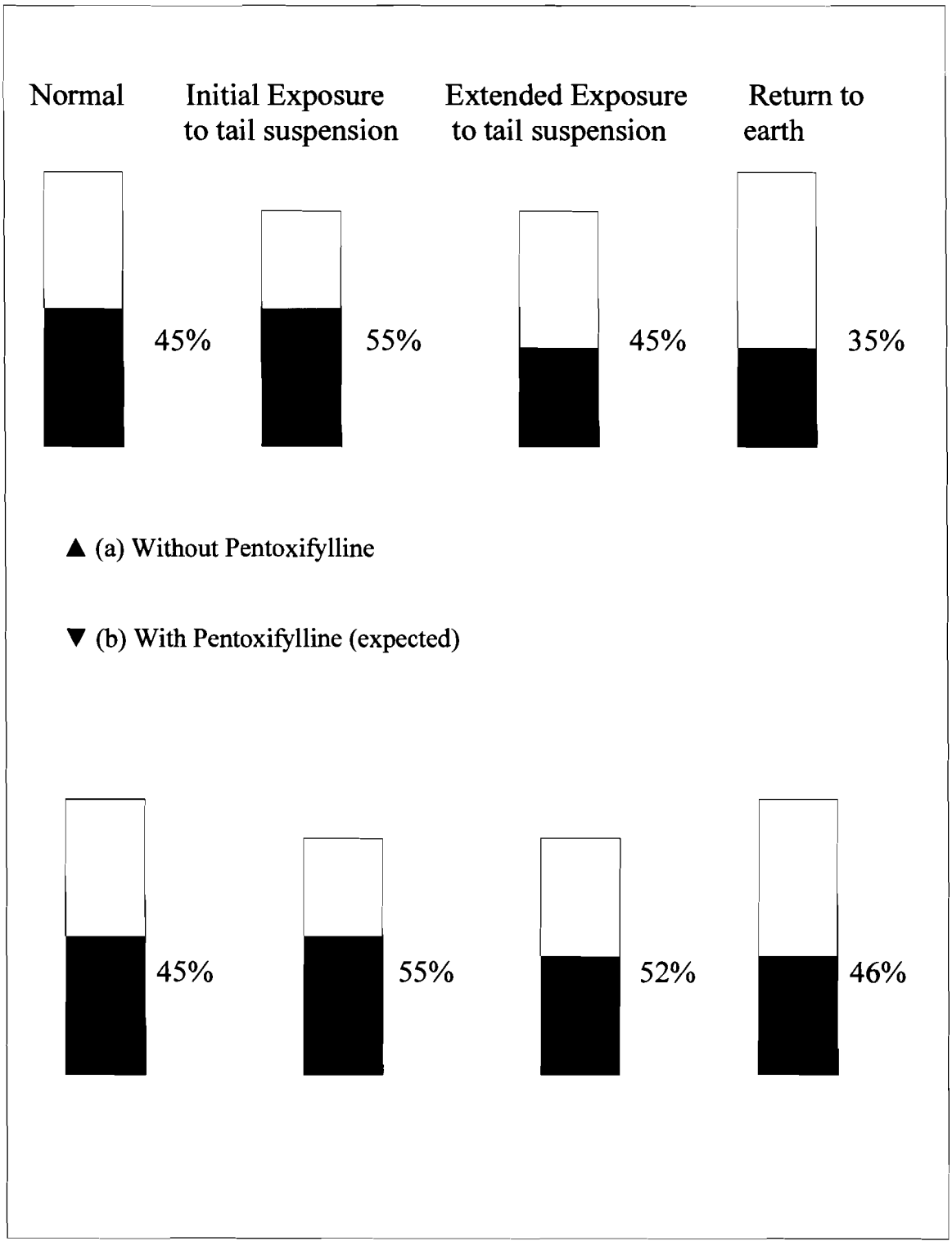
Pentoxifylline is a drug that has been suggested to reduces blood viscosity and improves microvascular blood flow by reducing erythrocyte aggregation and by increasing erythrocyte deformability, a major determinant of blood viscosity (3, 4). Thus, pentoxifylline permits RBC penetration into microcapillaries less than one-half the diameter of the RBCs (27). Pentoxifylline also inhibits calcium-dependent transglutaminases so that cross-linking of the membrane proteins becomes deficient and membrane fluidity increases (29). Pentoxifylline can increase blood flow without

elevating blood pressure (9). Other effects of pentoxifylline include inhibition of platelet aggregation, increase of white blood cell deformability, and decrease of leukocyte adhesiveness (3).

To determine if blood viscosity is a regulator for other hematological changes that occur with tail suspension, I used pentoxifylline in an attempt to lower the blood viscosity. Recall, Hct increases initially with exposure to microgravity then decreases toward normal values within days of continued microgravity exposure (Fig 1a). If Hct is regulated by blood viscosity, then lowering blood viscosity should delay and/or reduce the decrease in Hct toward normal values during continued exposure to microgravity leading to an Hct that is higher than normal Hct (Fig 1b).

Fig.1. (a) shows, in general, changes that occur in hematocrit during pre-tail suspension, initial exposure to tail suspension, extended exposure to tail suspension, and recovery (no longer tail suspended). (Black indicates red blood cells and white indicates plasma. Numbers to the right of each bar represent theoretical hematocrit value.)

(b) shows, in general, what I predict would occur to hematocrit if pentoxifylline were administered to rats during the same time periods as in (a). Such lack of changes in hematocrit as shown in (b) could suggest a role of viscosity in the regulation of hematocrit due to a decrease in blood viscosity with the treatment of pentoxifylline. (Black indicates red blood cells and white indicates plasma. Numbers to the right of each bar represent theoretical hematocrit value.)


Normal
Initial Exposure to tail suspension
Extended Exposure to tail suspension
Return to earth
45%
55%
45%
35%
▲ (a) Without Pentoxifylline
▼ (b) With Pentoxifylline (expected)
45%
55%
52%
46%

METHODS

Animals: Seventy male Sprague-Dawley rats maintained at the Emporia State University Animal Care facility were used for this study. Animals weighing between 330g and 400g were randomly assigned to either control or experimental groups. Rats were kept in Nalgene cages on 12 hour day-night cycle at a room temperature (25 ± 1°C). Experimental and control animals were given free access to a water solution containing 250 mg Pentoxifylline [(Trental), Sigma Chemical. Co. St.Louis, MO] per L of water. A previous study (25) observed 0.1 ml/g/day of water consumption in rats whose average body weight is 400g. The recommended dosage of pentoxifylline for humans is 0.017mg/g/day. I applied the human-dosage of pentoxifylline to the rats whose average body weight is about 400g. 170 mg of pentoxifylline per L of water is the proportional dosage of pentoxifylline for rats. However, since the metabolic rate of rat is higher than that of human, I gave the rats in this study 250 mg of pentoxifylline per L of water. This research was performed according to the guidelines of the Emporia State University Animal Care and Use committee.

Tail suspension: Test-rats were suspended using a similar method described by Chapes et al. (6) for mice. All tail suspended rats were skeletally unloaded from the floor of the suspension cage, such that approximately 50% of their body weight was placed on the front legs. Since the cables connected with tail were attached to the pulley system above the cages, rats were free to move in any direction within the cage. Control-rats were similarly treated within the suspension cages, but not tail- suspended.

Experimental design: The experimental tail-suspended groups consisted of: 4 h, 24 h, 72 h, and 168 h tail suspension. Each group consisted of five rats, and each experimental group had a corresponding control group, also containing five rats in each group. In order to investigate hematological factors and measure blood viscosity of recovery after tail suspension, three groups containing five rats each, with corresponding control groups, were created: 168 h tail suspension followed by a 24 h recovery period, 168 h tail suspension followed by a 72 h recovery period and 168 h tail suspension followed by a 168 h recovery period. Rats in all groups, experimental and control, received pentoxifylline.

Body mass was determined prior to and following tail suspension. For the tail-suspension recovery studies, body mass was determined before tail suspension, at the end of tail suspension, and at the end of recovery period. Water consumption was monitored daily to determine the amount of total fluid plus trental, which is a brand name of pentoxifylline, consumed. Final water consumption was determined for the duration of the tail suspension period for all groups and was also determined for the duration of the recovery period for those animals in the recovery groups.

Blood collection: At the end of each experimental period, blood was taken from each rat by heart puncture performed under anesthesia induced with halothane. Blood was drawn into a syringe containing the anti-coagulant heparin. A total of 6 - 8 ml of blood was collected from each rat and placed into a vacutainer containing 143 U.S.P. units of heparin. Once blood had been obtained from an animal, the animal was euthanized by cervical dislocation while still under anesthesia.

Hematological Measurements: Hct was determined for each rat using the microhematocrit method. RBC counts were determined by diluting the blood in a standard RBC pipette (1:200) with 0.9 % NaCl. Diluted blood was placed on a hemocytometer and cells were counted. Hb concentration was measured using the cyanomethemoglobin method (Sigma Chemical. Co. St. Louis, MO). Mean corpuscular volume (MCV) was calculated using the formula: MCV = (Hct/RBCC) x 10, mean cell hemoglobin (MCH) was calculated using equation: MCH = (Hb Concentration/RBCC) x 10, and mean cell hemoglobin concentration (MCHC) was calculated using the equation: MCHC = (Hb Concentration / Hct) x 100.

Blood Viscosity Measurements: Blood viscosity was determined using a Wells-Brookfield cone/plate viscometer (Model DV-II+, Brookfield Engineering Lab, Stoughton, MA, USA), with a CP-40 cone, using 0.5 ml of samples. The viscometer was calibrated with distilled water before the collection of blood viscosity data (cP: 0.678 at 38 °C). The temperature of the sample cup was kept at 38 °C with an external water bath. Viscosity determinations were made at a shear rate of 150 s^{-1}. To make a high and low hematocrit sample from each blood sample, blood was placed into three Eppendorf tubes. The tubes was centrifuged at 1200 g for approximately 30 s. Plasma was transferred from the first tube to the second tube to create packed cell volumes that were above and below that of the original sample. The blood viscosity was determined for each of the three Eppendorf tubes for a given rat, and a linear regression equation was generated for a shear rate of 150 s^{-1} on plots of log apparent viscosity versus Hct. From the regression

equation of each rat, apparent blood viscosity was predicted over a range of Hcts (from 10% to 70 %) for each animal. This allowed for the comparison of the apparent blood viscosity values at a similar Hct, temperature, and shear rate among the rats over a wide range of Hcts. Log apparent viscosity values were converted back to non-log apparent viscosity values. Plasma viscosity was also determined for each rat at a shear rate of 750 s^{-1} at 38 °C.

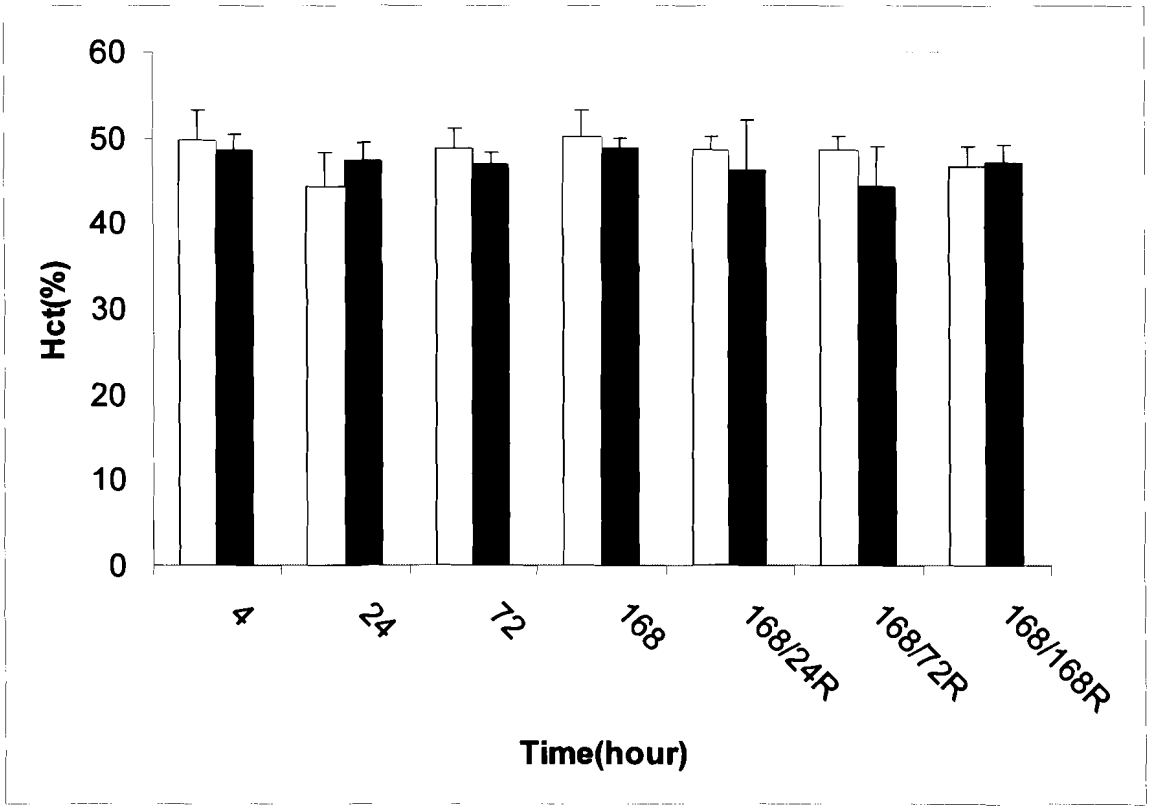
Statistical analysis: A student's t-test was used to compare the hematological parameters and viscosity between experimental groups and their corresponding control groups. Differences were considered significant at $P \leq 0.05$

RESULTS

Figures 2 through 7 show changes in Hct, RBCC, Hb concentration, MCV, MCH, and MCHC. Hct and RBCC were not significantly different between control and experimental groups for anytime period (Fig 2, Fig 3). However, Hb concentration was significantly higher in the 168/72 h (recovery) experimental group as compared with 168/72 h (recovery) control group and significantly lower in 168/168 h (recovery) experimental group relative to the corresponding control group (Fig 4). For MCV (Fig 5), MCH (Fig 6), and MCHC (Fig 7), there was no significant difference between the experimental groups and their corresponding control group.

Fig 8 shows the changes in body mass for all experimental and control groups immediately after the end of tail suspension. Fig 9 depicts changes in body mass after the end of the recovery period for the suspension/recovery group. There is no significant difference in the percentage change in body mass after 4 and 24 hours between the experimental groups and their corresponding control group. However, experimental rats in the 72 hours and 168 hours suspension groups show a significant decline in percentage body mass when compared to their corresponding control group. All experimental recovery groups showed a significant decline in body mass percentage after 168 h tail-suspension relative to their control groups. Experimental rats that experienced 24 hours and 72 hours of recovery show no significant difference in percentage change in body mass relative to their corresponding control groups during the recovery period.

Fig.2 Mean value (± SD) for hematocrit (Hct) at the end of suspension, and at the end of recovery period (R) for tail suspended rats (Experimental), and their corresponding control groups (Control). (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)



Control Experimental

Fig. 3. Mean value (± SD) for RBCC at the end of suspension, and at the end of the recovery period (R) for tail suspended rats (Experimental), and their corresponding control groups (Control). (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)

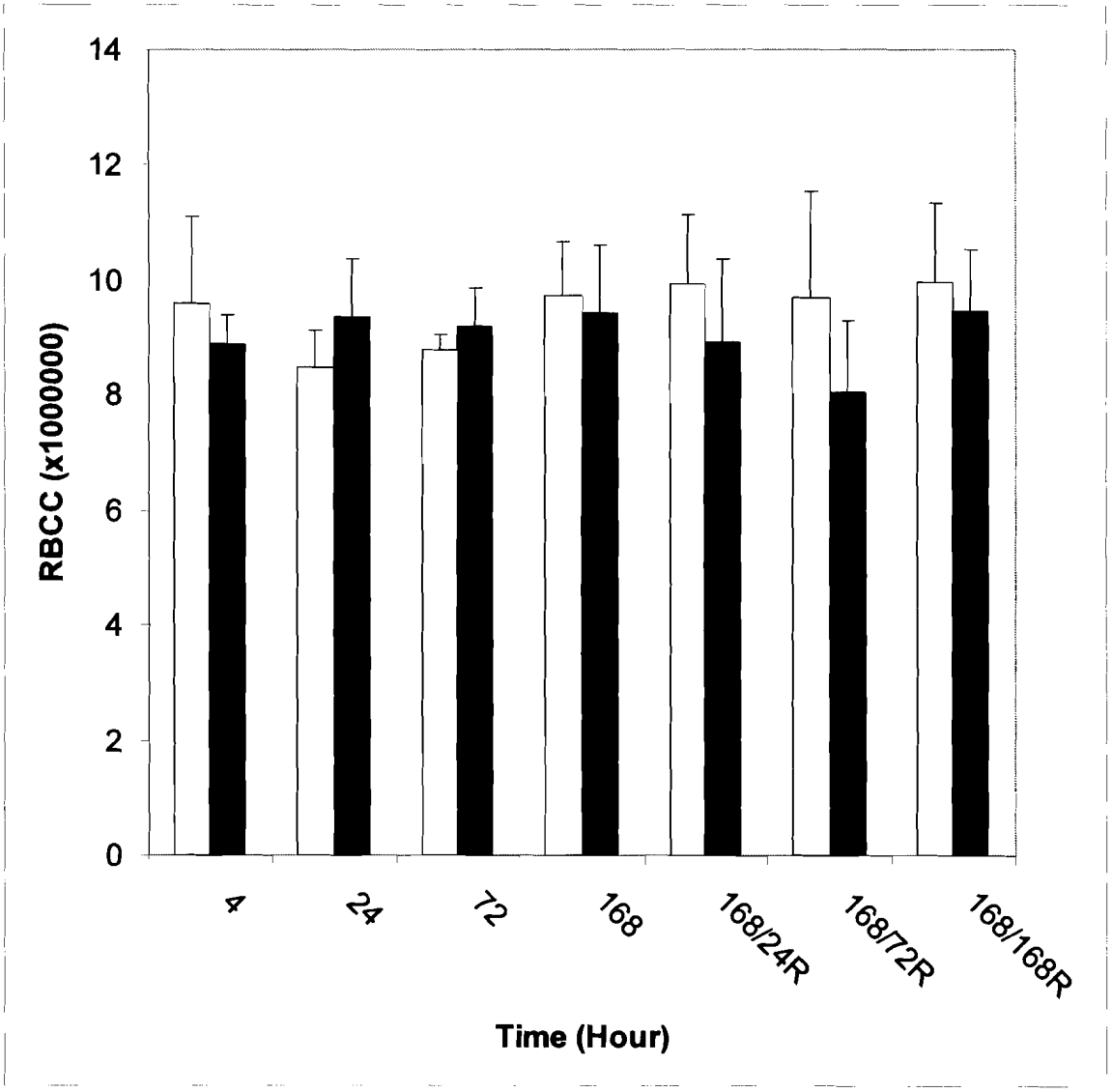

14
12
10
8
6
4
2
0
RBCC (x1000000)
4
24
72
168
168/24R
168/72R
168/168R
Time (Hour)
Control
Experimental

Fig. 4. Mean value (± SD) for Hb concentration at the end of suspension, and at the end of recovery period (R) for tail-suspended rats (Experimental), and their corresponding control groups (Control). (* indicates significant difference between experimental and its corresponding control at $P \leq 0.05$)

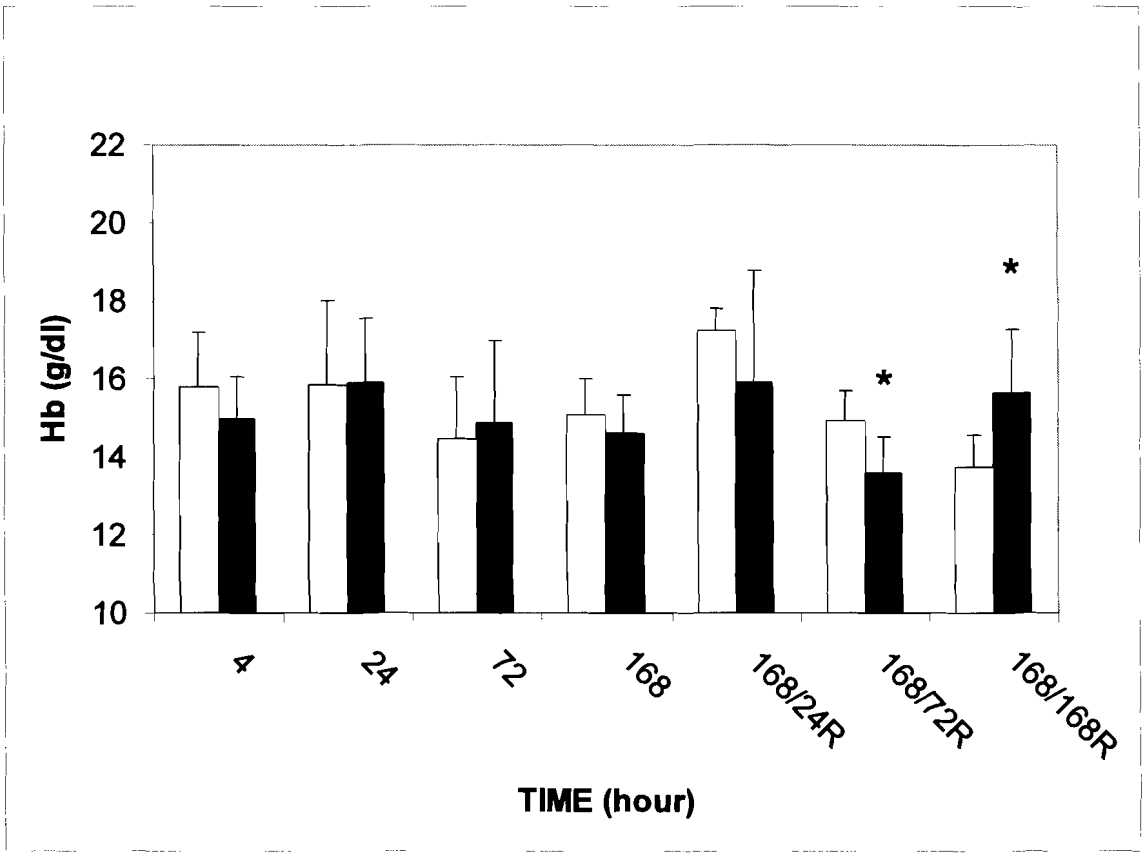

22
20
18
16
14
12
10
Hb (g/dl)
4
24
72
168
168/24R
168/72R
168/168R
*
*
TIME (hour)
Control
Experimental

Fig. 5. Mean cell volume (MCV) (± SD) for tail-suspended rats (Experimental) and their corresponding control groups (Control) during the tail-suspension and recovery period. (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)

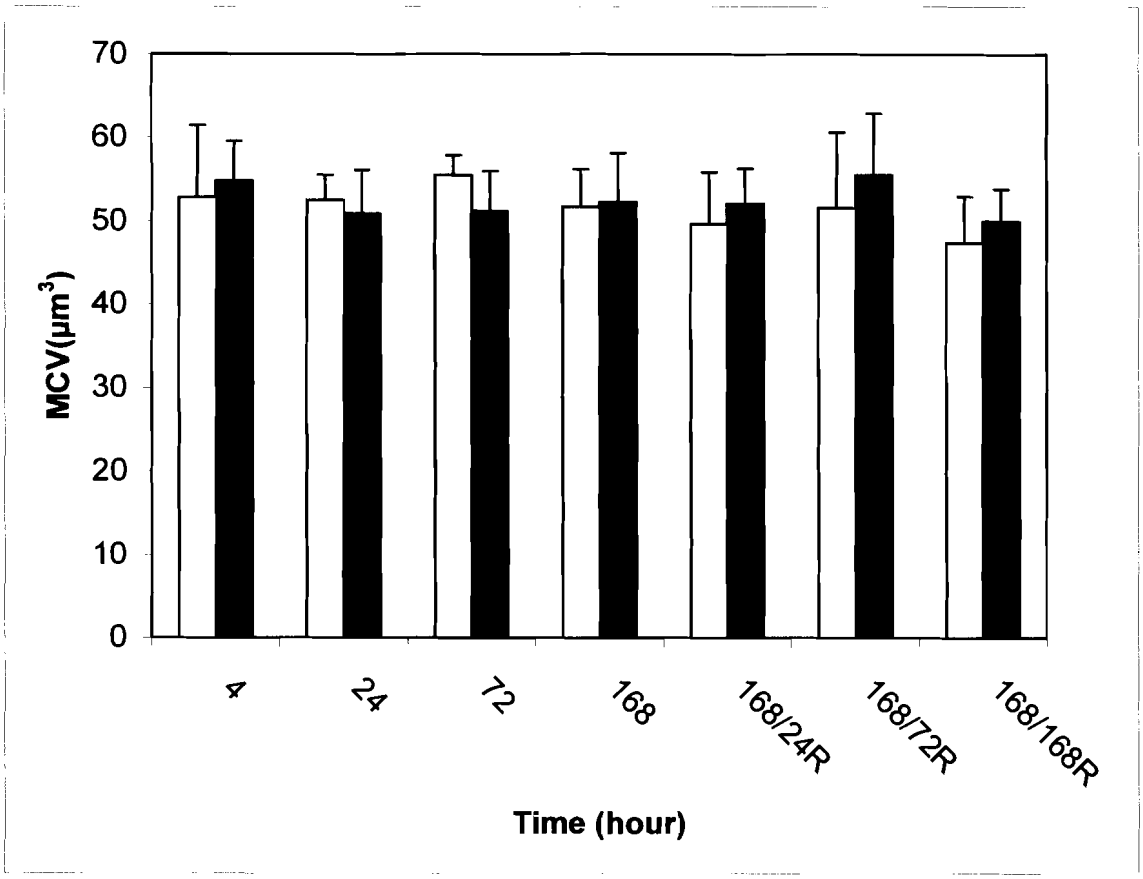
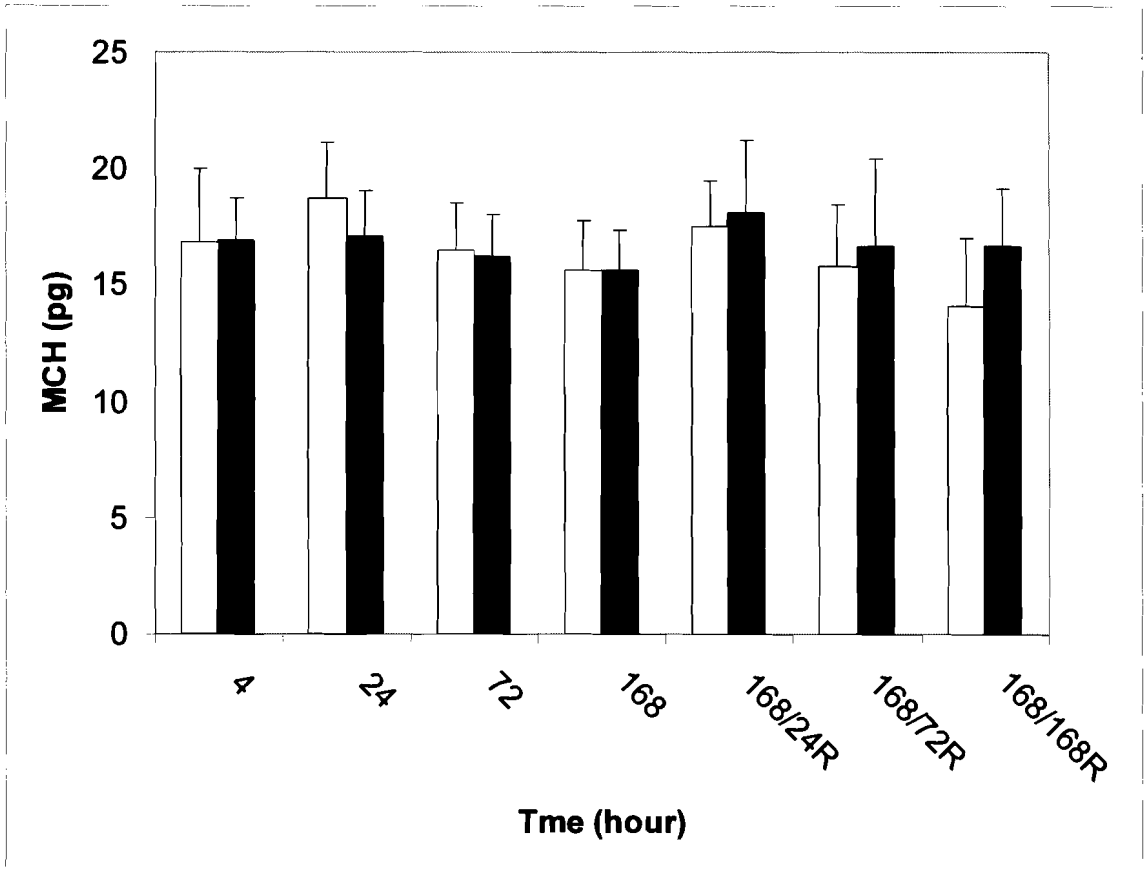

70
60
50
40
30
20
10
0
MCV(μm³)
4
24
72
168
168/24R
168/72R
168/168R
Time (hour)
Control
Experimental

Fig. 6. Mean cell hemoglobin (MCH) (± SD) for suspended rats (Experimental) and their corresponding control groups (Control) during the tail-suspension and recovery period. (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)



Control Experimental

Fig. 7. Mean cell hemoglobin concentration (MCHC) (± SD) for suspended rats (Experimental) and their corresponding control groups (Control) during the tail suspension and recovery period. (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)

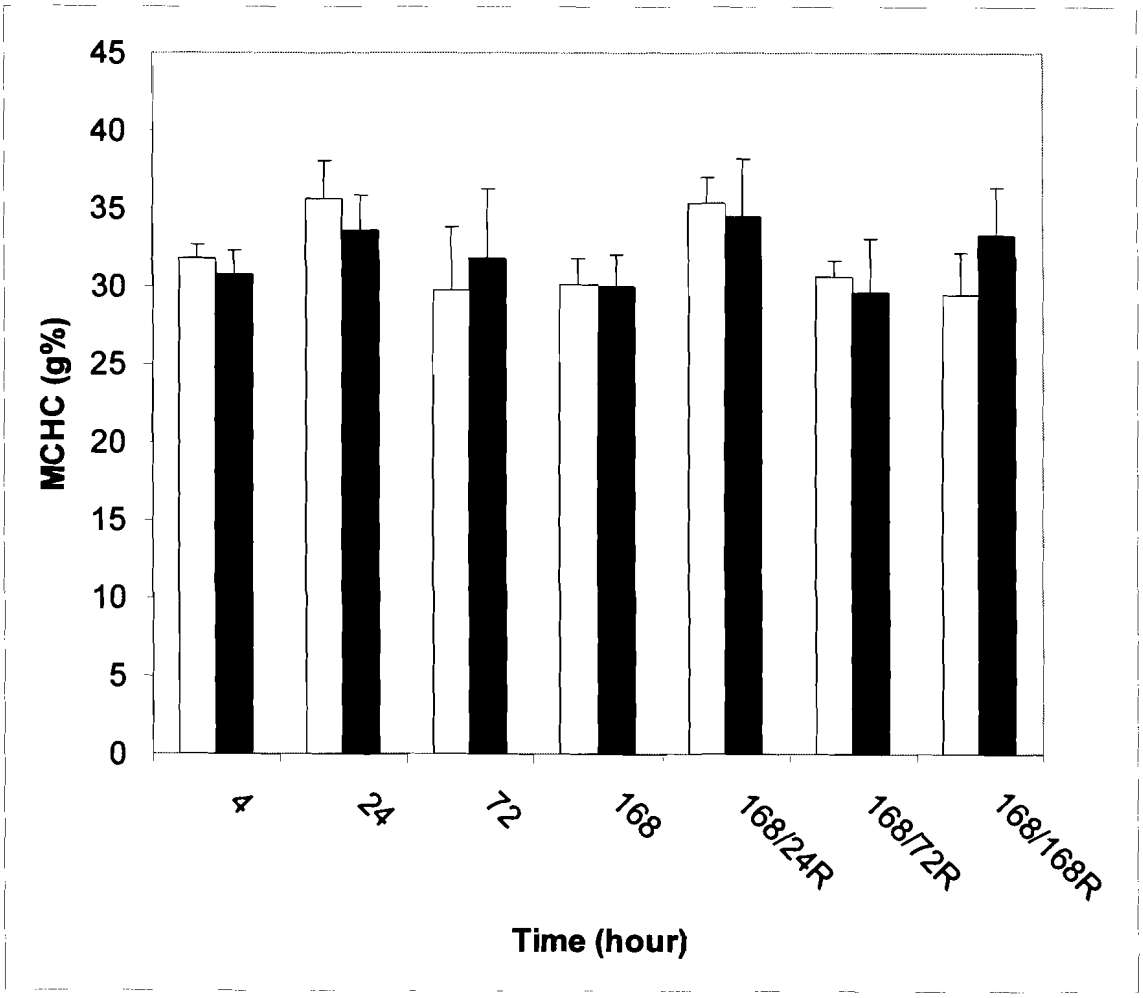

45
40
35
30
25
20
15
10
5
0
MCHC (g%)
4
24
72
168
168/24R
168/72R
168/168R
Time (hour)
Control
Experimental

Fig. 8. Changes in body mass (± SD) of suspended and suspension/ recovery rats and their corresponding control groups at the end of the suspension period. (* indicates significant difference between experimental and its corresponding control at $P \leq 0.05$)

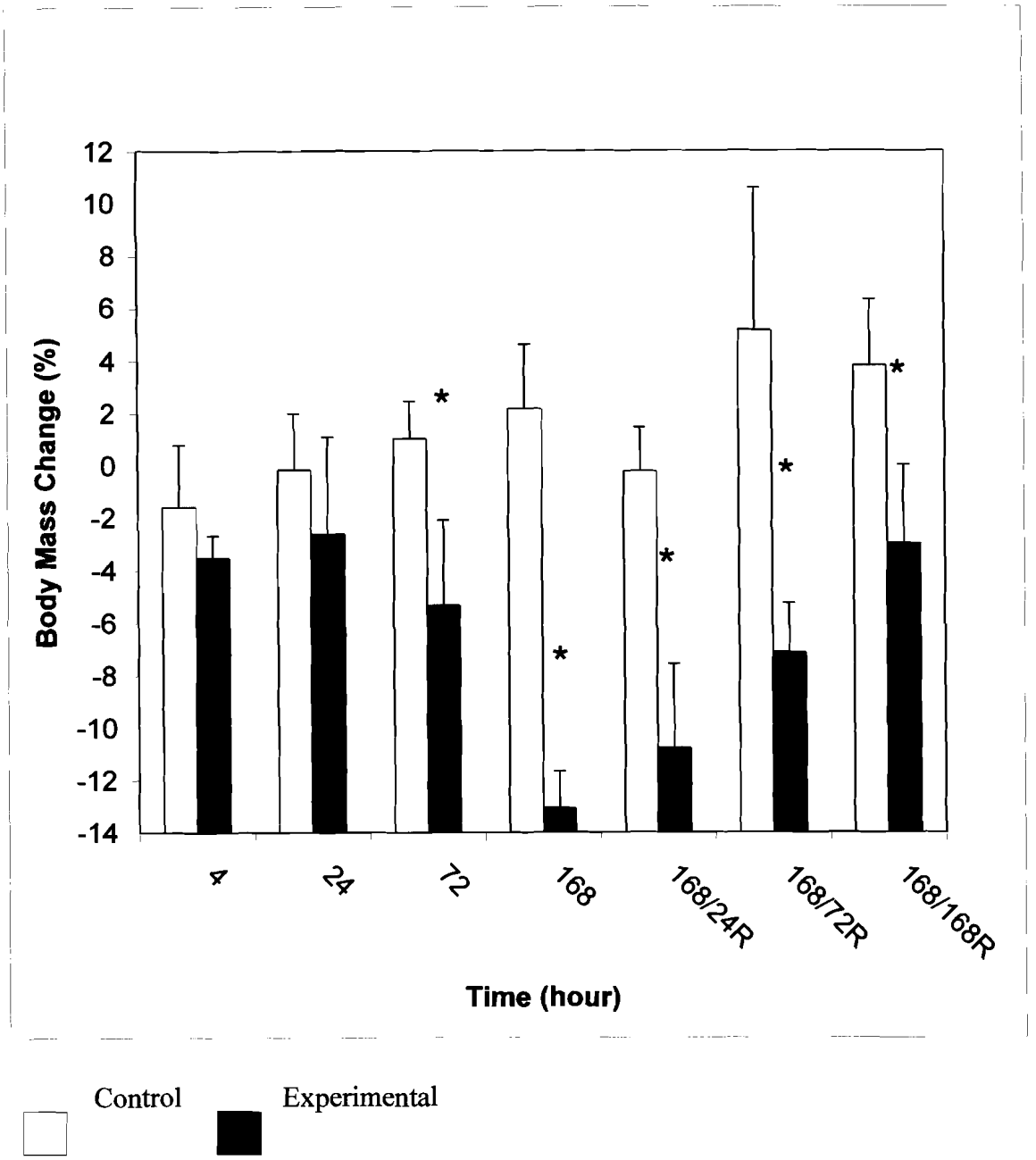
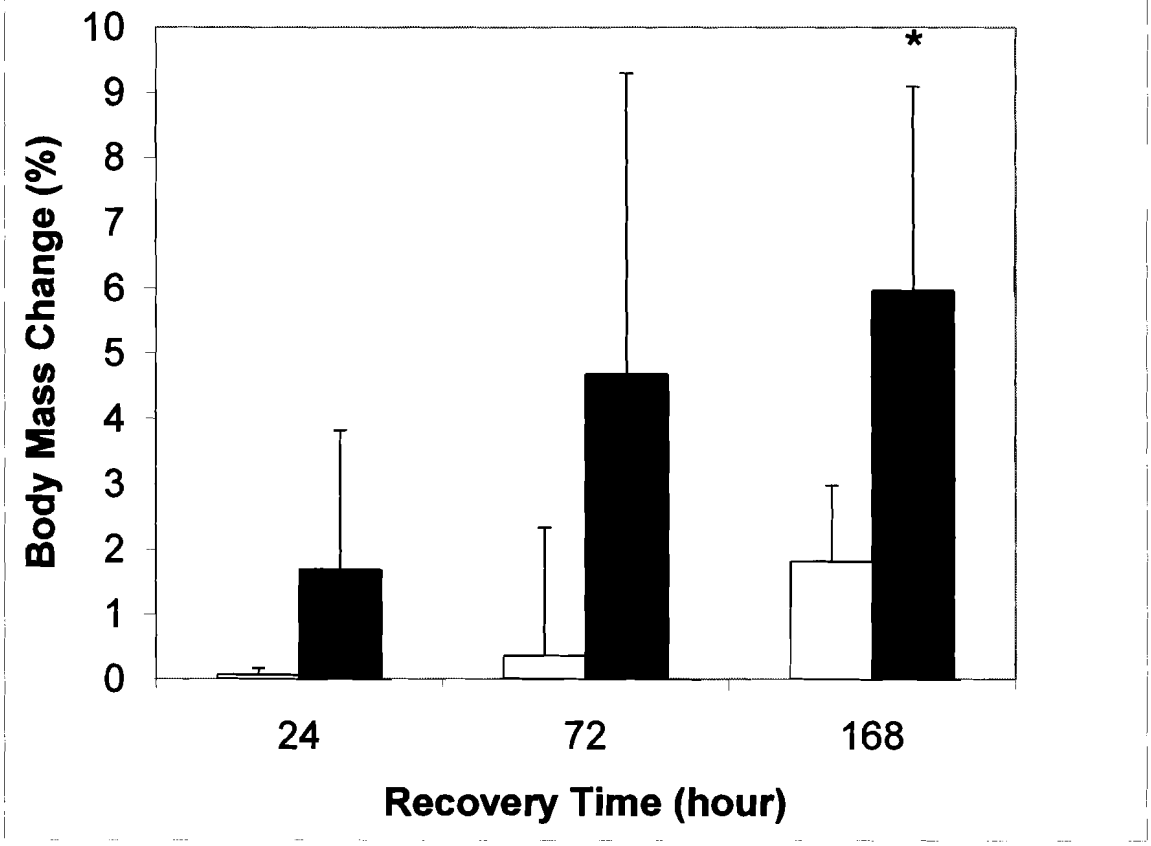

12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
Body Mass Change (%)
*
*
*
*
*
4
24
72
168
168/24R
168/72R
168/168R
Time (hour)
Control
Experimental

Fig. 9. Changes in body mass (± SD) after the recovery period for suspension/ recovery rats (Experimental) and their corresponding controls (Control). (* indicates significant difference between experimental and its corresponding control at $P \leq 0.05$)



Control Experimental

However, those rats that had been in the recovery period for 168 h after 168 h tail-suspension showed a significant increase in percentage body mass relative to their control group over the same post-suspension times.

Fig 10 shows water consumption (ml consumed/ g body mass/ hour) during the suspension period for all groups. No significant difference was found between control and experimental groups after 4 hours of suspension. However, at 24 hours, 72 hours, and 168 hours suspension, experimental groups consumed significantly less water than did their corresponding control group. Fig 11 shows water consumption during the recovery period for the suspension/ recovery groups for both experimental and control groups. There is no statistically significant difference in the water consumption between control and experimental groups during the recovery period (Fig 11). Water consumption by the rats in this study is very similar to the study of Saunders et al. in which pentoxifylline was not given (25).

Fig. 12 shows blood viscosity changes for the average hematocrit of each group in the suspended and recovery animals relative to their controls. Over all suspension and recovery times, no significant difference in viscosity between experimental groups and their corresponding control group was found. When I compare blood viscosity values at 150 s^{-1} in this research with those at 150 s^{-1} of Saunders (25)' research, blood viscosity in this study was not reduced (Fig 13)

No significant differences were found in plasma viscosity in experimental groups relative to their controls in 4 h, 24 h, 72 h, 168 h suspension groups, and 168/24 h, 168/ 72 h, 168/168 h suspension/recovery groups (Fig 14).

Fig. 10. Comparison of water consumption (± SD) during the tail suspension period for suspension, suspension/ recovery rats (Experimental), and their corresponding control groups (Control). (* indicates significant difference between experimental and its corresponding control at $P \leq 0.05$)

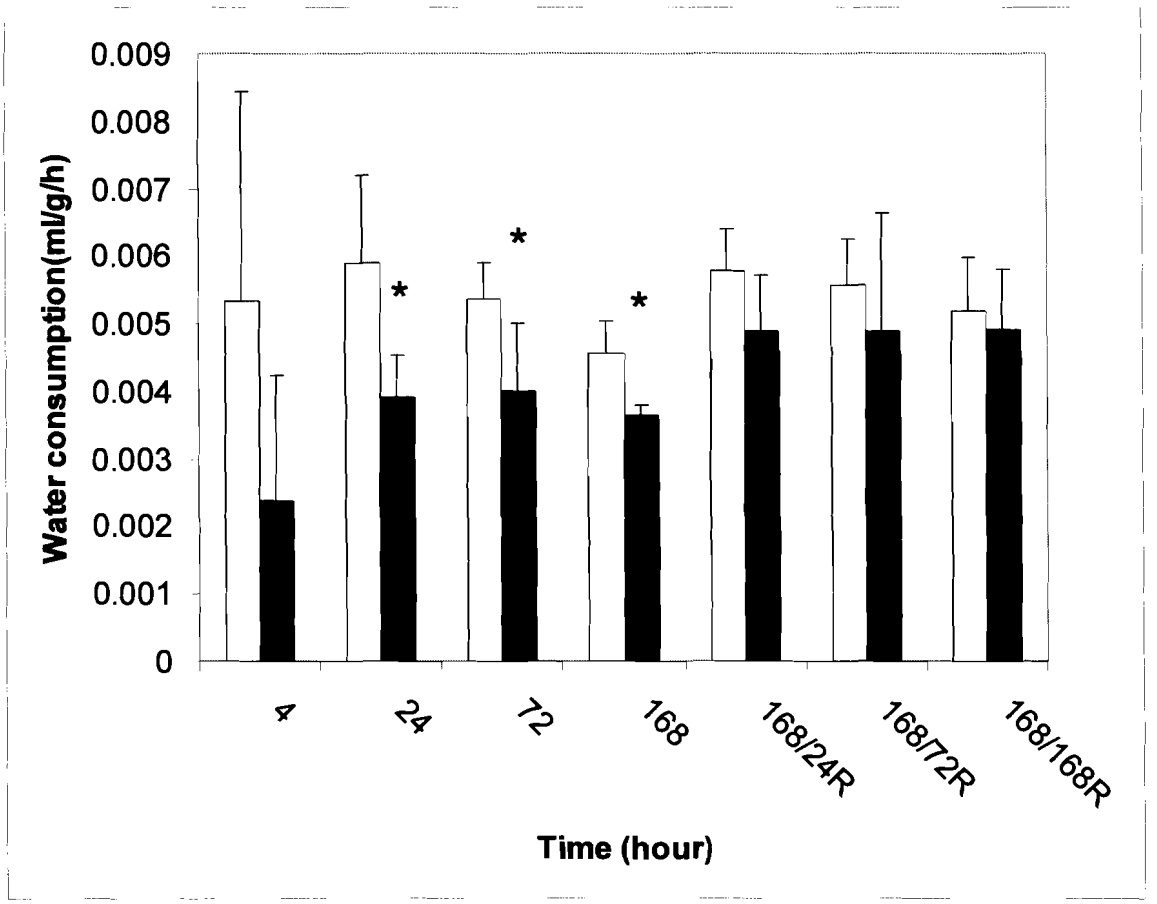

0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Water consumption(ml/g/h)
*
*
*
4
24
72
168
168/24R
168/72R
168/168R
Time (hour)
Control
Experimental

Fig.11. Comparison of water consumption (± SD) during the recovery period for suspension/recovery rats (Experimental) and their corresponding control groups (Control). (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)

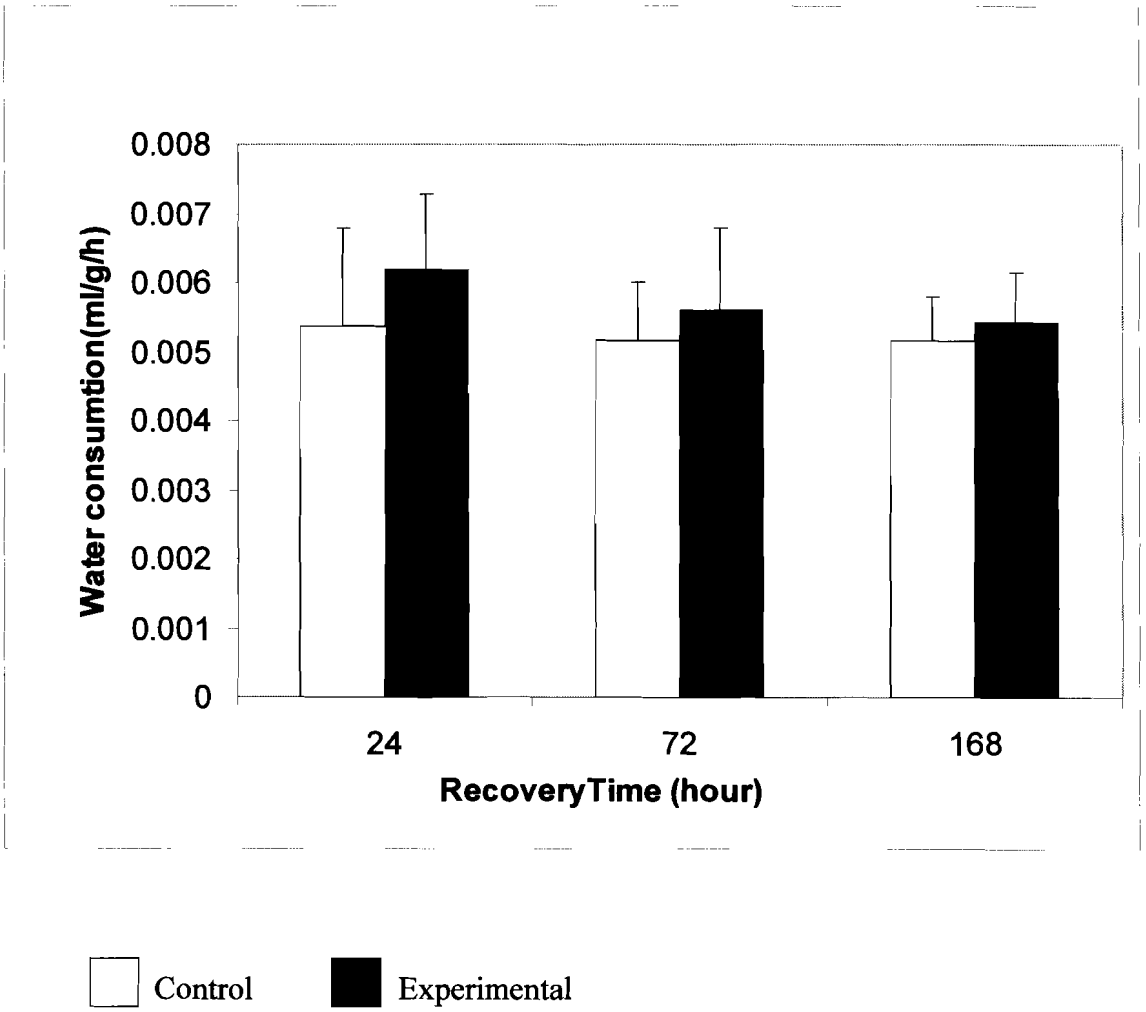

0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Water consumtion(ml/g/h)
24
72
168
RecoveryTime (hour)
Control
Experimental

Fig.12. Mean blood viscosity values (± SD) at a shear rate of 150 s^{-1} at the end of the suspension period for each suspension group and their corresponding control groups and at the end of recovery period for each suspension/recovery group and their corresponding control group. (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)

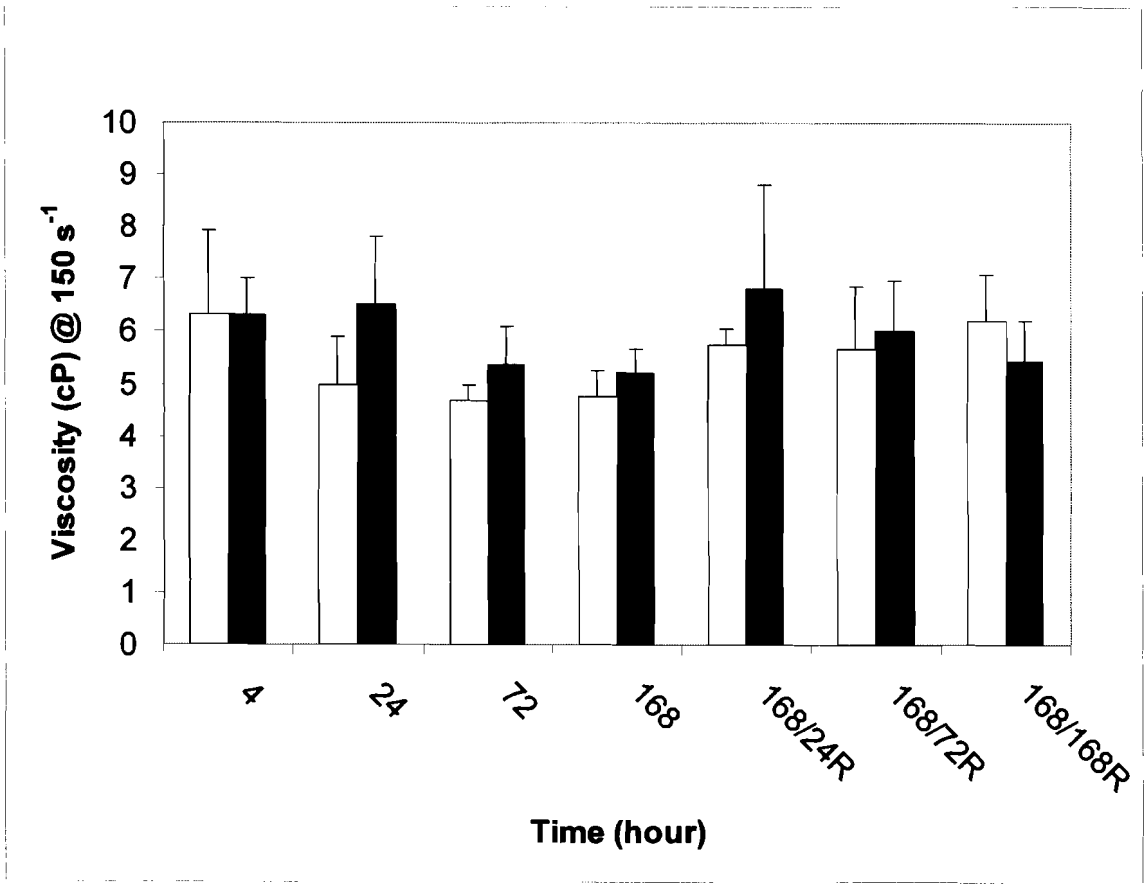
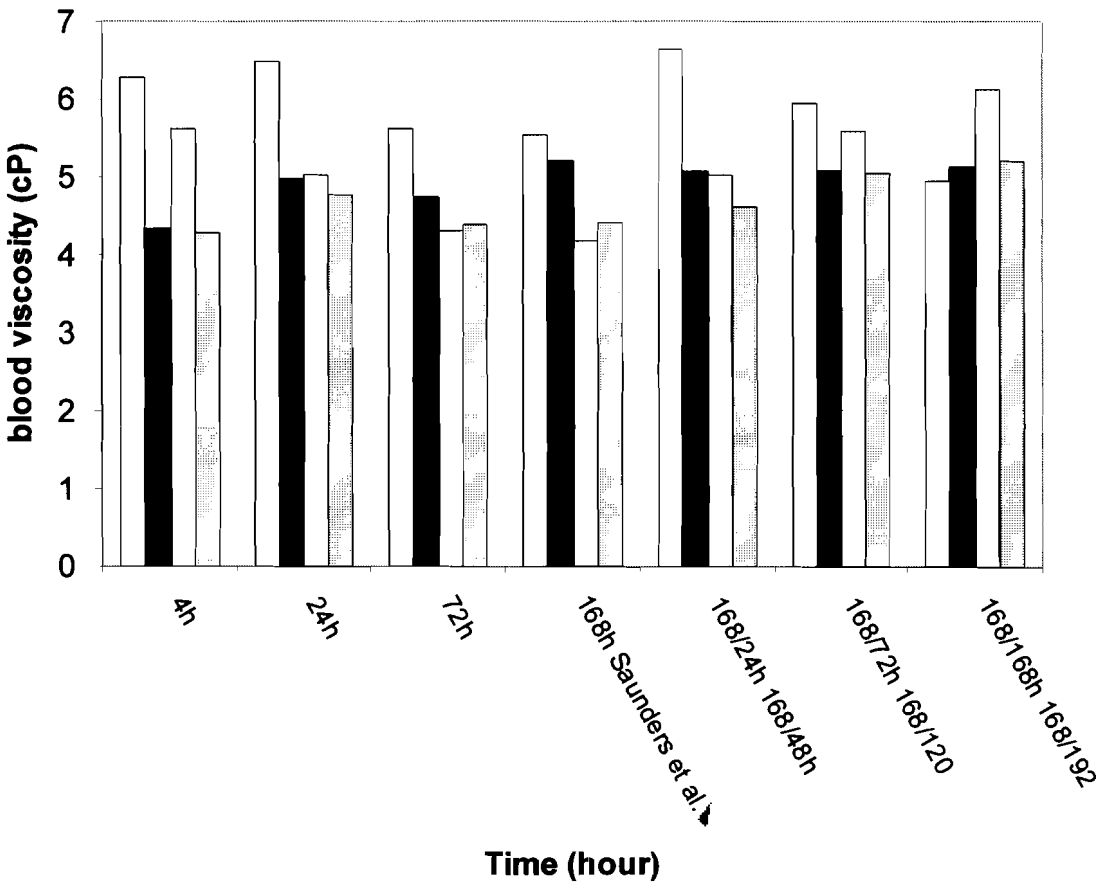

Viscosity (cP) @ 150 s-1
10
9
8
7
6
5
4
3
2
1
0
4
24
72
168
168/24R
168/72R
168/168R
Time (hour)
Control
Experimental

Fig.13. Comparison of blood viscosity values for experimental and control rats given pentoxifylline (current study) to experimental and control rats not receiving pentoxifylline (25).

Saunders et al. have different recovery period; 48h, 120h, and 192 h.



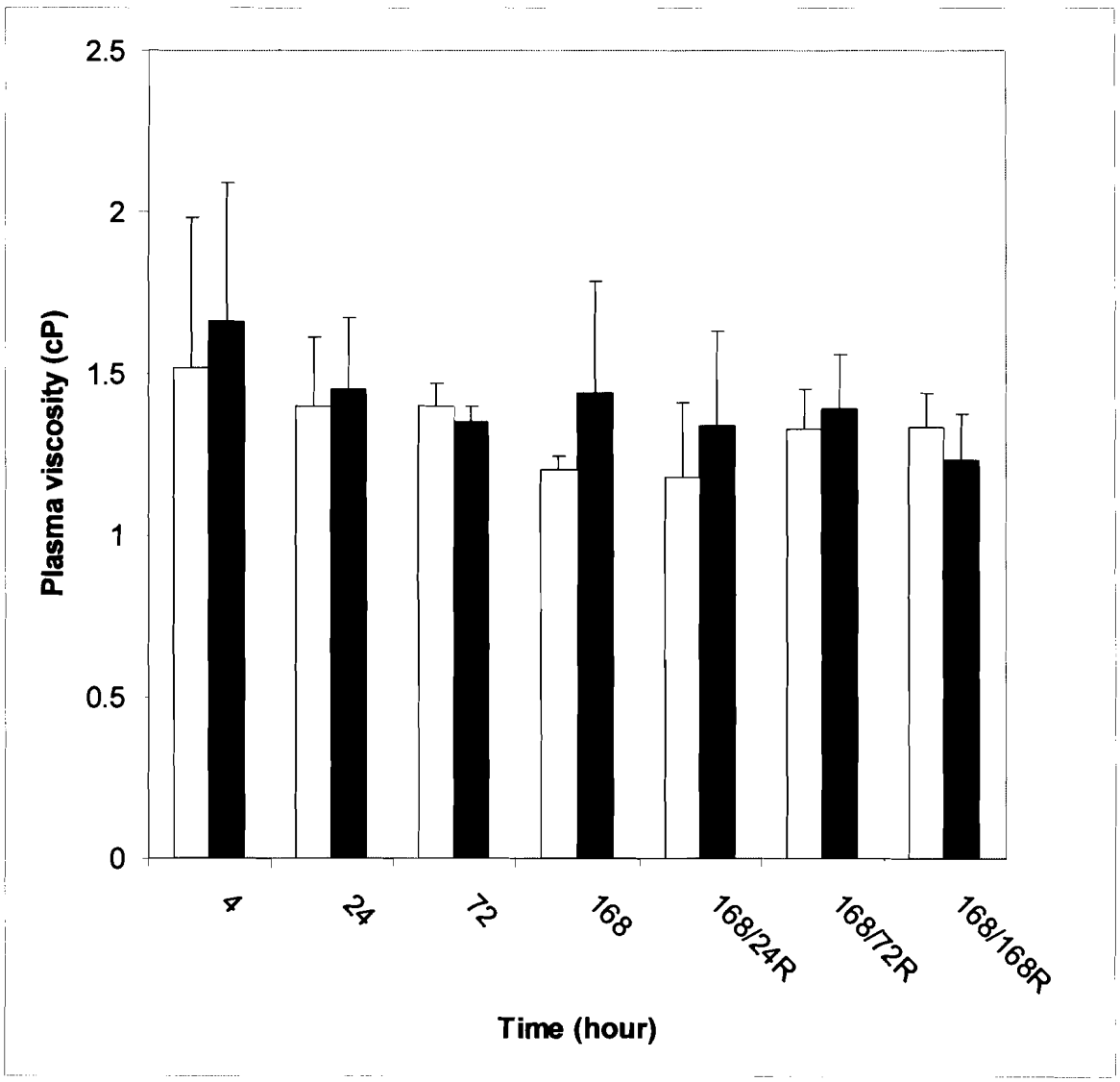
□ Experimental group of current study

■ Experimental group of previous pentoxifylline free study

⊡ Control group of current study

⊟ Control group of previous pentoxifylline free study

Fig.14. Mean plasma viscosity values (± SD) at the end of suspension period for each suspension group (Experimental) and their corresponding control groups (Control) and at the end of recovery period for each suspension/recovery (R) group (Experimental) and their corresponding control groups (Control). (No significant difference between experimental and corresponding control groups was found at $p \leq 0.05$)


2.5
2
1.5
1
0.5
0
Plasma viscosity (cP)
4
24
72
168
168/24R
168/72R
168/168R
Time (hour)
Control
Experimental

Discussion

Percent change in body mass in suspended and suspended/recovery rats in this study was similar to that previously reported for tail suspended and recovery rats which did not receive pentoxifylline (25). However, there is a slight difference in the time course of percent change in body mass of this study relative to that of Dunn (14). Dunn reported that suspended rats lost 6-10% of their body mass during the first 24 hours of suspension and either failed to gain body mass or gained it at a greatly reduced rate during the rest of the suspension period. On the other hand, Saunders et al. (25) found about a 3% and a 7% decrease in body mass by 24 and 72 hours of suspension, respectively, and continued decrease in body mass (10%) by 168 hours of suspension. I also found significant decrease in body mass of approximately 4% and 5% after 24 and 72 hours of suspension, respectively, and a continued decrease in body mass to about 13% in 168 hours of suspension. This weight loss may be related to food intake, fluid loss (urine output) and/or decreased water consumption and/or increased metabolic rate (31) during exposure to suspension. Dunn (14) reported that in suspended rats, food consumption was lower than that of control animals during the suspension phase. This was followed by 'rebound overindulgence' for the first 3-4 days of recovery. Water consumption was also lower in the control animals. However, there was no 'rebound overindulgence' in water consumption unlike that seen in the food loss (14). Dunn et al. (14) showed that the rate of body mass recovery of suspended rats rapidly increased during the recovery period. Saunders et al. (25) found by the fifth day of recovery, body mass increased by 6%. In my research, I did not measure food consumption or urine output, but I did notice

increased food consumption during the recovery phase of suspended rats. Body mass recovered greatly in experimental rats by 168 hours (the seventh day) of recovery, increasing by 6%. The amount of increase in body mass during the recovery period was not significant at 24 and 72 hours of recovery, a trend toward gradually increasing body mass throughout the recovery period was seen. This slow increase in body mass could be due to increased water and food consumption, and decreased stress levels during the recovery period. Because body mass changes were similar in my study, in which pentoxifylline was administered, to that of Saunders et al. (25), in which no pentoxifylline was given, it appears there is no effect of pentoxifylline on body mass.

Water consumption of experimental animals at 24, 72, and 168 hours of suspension was significantly less than that of the corresponding control groups. This could be a cause of body mass loss during exposure to microgravity and suspension. There is no statistical difference in water consumption during the recovery time between control and experimental groups. However, Saunders et al. (25) found water consumption did not differ between control and experimental animals at any time period, but that water consumption in the 120 and 192 hour recovery animals after 168 hours of suspension was significantly increased as compared with their water consumption during the suspension period. My study also found water consumption during the recovery period in experimental groups to be greater compared to their own water consumption during the suspension period. The increase in water consumption could result in a relatively rapid increase of the plasma volume. This greater increase in plasma volume relative to the increase in RBCM should result in a decreased Hct, Hb concentration, and RBC count during the recovery period.

In this study, Hct showed no significant change between suspended animals and their corresponding controls for any time period. Saunders et al. (25), in contrast, found significant increase in Hct after 4 hours of suspension and a significant decrease in Hct at the end of 120 hour and 192 hours of recovery period. RBC count also was not significantly different between control and experimental groups at any time period in my study. The effect of pentoxifylline could account for lack of significant difference in Hct values. It has been suggested that fluid shifts eventually increase central venous pressure, the primary sensory site for which the cardiovascular system realizes the increased fluid volume and thereby allowing the body to regulate fluid volume (7, 21). However, test results obtained from two Spacelab missions did not show increased central venous pressure during the space flight (7). Also Gerzer et al. (15) reported that central venous pressure did not clearly increase in simulation models for the effects of weightlessness, including the tail-suspension method. The lack of increased central venous pressure apparently then does not regulate fluid volume. Regulation of fluid volume may be regulated by increase levels of pulmonary edema. Previous work has shown that pretreatment with pentoxifylline can reduce pulmonary edema (26), which is observed after fluid shifts occur. Thus, pentoxifylline may reduce the build up of fluid in the lungs that normally occur with the cephalic fluid shifts. As such, pentoxifylline may have limited the quantity of the fluid shift, thereby limiting regulation of fluid volume. The resulting lack of fluid loss associated with initial tail suspension would cause little change in Hct.

Hb concentration in rats of the previous study by Saunders et al. (25) mirrored that of Hct. In contrast, in this study Hb concentration of the experimental group after 72 h

recovery period was significantly lower than those of corresponding controls, but after 168 h recovery period, the Hb concentration of the experimental group was significantly increased. Dunn et al. (14) showed that Hb concentration during the seven days of recovery period was less than that of corresponding control groups. At the eighth day of the recovery period, Hb concentration greatly increased (14). The significant increase in Hb concentration could be a result of small sample size. In both the previous study by Saunders et al. (25) and in this study, MCV was not significantly different between control and experimental groups at any time. However, unlike MCV of the previous study, which showed a trend toward decreasing MCV during the recovery periods, I found a trend toward increasing MCV. There were no significant differences in MCH and MCHC in this research. However, a previous study on humans found MCH was significantly increased upon landing and after 24 h recovery period, and MCHC was significantly increased during 168 h exposure to microgravity and on landing day (22). The only significant change in RBC hematology in the previous ground based experiment was an increase in MCHC on 13 days of recovery period (22).

My study and that of Saunders et al. show considerably different results in blood viscosity changes despite similar experimental conditions. Saunders et al. (25) found that blood viscosity significantly increased after the 72 and 168 hour suspension times, and after 48 hours of recovery from suspension, despite no difference in the Hct during those same time periods. However, in this study, no significant difference was found in the blood viscosity at any time. Blood viscosity is dependent on shear rate, RBC deformability, temperature, plasma protein concentration, and Hct, which has the greatest effect (5, 8).

Saunders et al. (25) reported that viscosity changes in their research could have resulted from decreased RBC deformability and/or alteration in plasma protein concentration associated with plasma volume change, with evidence of significant increase in plasma viscosity in the 72 and 168 hours of suspension groups relative to their controls and for the 168 /48 hour suspension/recovery group relative to its control. As such, the increased plasma protein concentration can lead to increased plasma viscosity, thereby leading to an increased blood viscosity. In this research, no significant differences were found in plasma viscosity in experimental groups relative their controls in 4, 24, 72, and 168 hours of suspension and 24, 72, and 168 hours of recovery after 168 hours of suspension. Since the Hct was not significantly different between control and suspended groups, potentially due to no fluid shifts occurring during the beginning of exposure to suspension, plasma dilution may not be influencing the concentration of plasma proteins, leading to stable plasma viscosity, or concentrations of plasma proteins, such as fibrinogen, could be decreased by pentoxifylline (4). Additionally, I used pentoxifylline which can lower blood viscosity by increasing red blood cell deformability (3, 4). The addition of pentoxifylline should counter the potential decrease cell deformability. This could explain the lack of significant differences in blood viscosity.

An increased Hct can give rise to both increased blood viscosity and oxygen carrying capacity. Oxygen delivery is maximized at some level of Hct, termed optimal Hct (10). This can be seen using the Poiseuille-Hagen equation for laminar flow ($Q= \Delta P\pi r^4/8\eta l$) where Q = blood flow, ΔP= change in pressure, r= radius, η= viscosity of fluid, l= length of the tube. Increased Hct results in an increased oxygen carrying capacity of the blood, but where Hct increases above a theoretical optimal Hct, oxygen delivery decreases due

to the effect of increased viscosity on decreasing blood flow. Conversely, low Hct leads to decreased blood viscosity and thereby increases blood flow, but also adversely affects the oxygen carrying capacity of blood. If O_2 carrying capacity decrease more than Q increase, O_2 delivery could decrease; O_2 delivery = Q ($CaO_2 - CvO_2$), where Q = blood flow, CaO_2 = concentration O_2 in artery (O_2 carrying capacity), and CvO_2 = concentration of O_2 in vein. Therefore, the graph of this relationship between Hct and oxygen delivery is parabolic in shape. Hct is likely regulated within optimal Hct values.

From the previous studies in which pentoxifylline was not used, the initial decrease in plasma volume resulted in an initial increase in Hct, RBC numbers, and Hb concentration. The removal of RBCs may be done to maintain optimal Hct thus optimal O_2 delivery in microgravity environments. I initially thought pentoxifylline would decrease blood viscosity even at the increased Hct. However, my study as well as other studies investigating hematological effects of pentoxifylline (11, 13, 24, 28), failed to corroborate earlier reports of essential changes in blood viscosity by using pentoxifylline. As such, pentoxifylline may have had little effect on blood viscosity resulting in no significant difference in hematocrit between control and suspended groups. As a result, it is difficult to determine the influence of blood viscosity in the regulation of hematocrit from this study.

Future studies need to determine pre-suspension hematocrit values of animals in which pentoxifylline is given to determine if overloading with excessive water occurs when pentoxifylline is absorbed. Additionally, the dosage of pentoxifylline should be regularly controlled. For example, a constant dose of 400 mg should be given three times a day. To switch from pentoxifylline to cilostazol, a new drug with antiplatelet and vasodilating

activity (12), could be beneficial in determining the role of blood viscosity in regulation of Hct. Dawson et al. (12) has shown that blood viscosity decreased with the use of cilostazol group, but was unaffected in the pentoxifylline treatment group. Also, Dawson et al. (12) showed that intermittent claudication patients treated with cilostazol improved more than those who treated with pentoxifylline.

In summary, pentoxifylline did not seem to affect blood viscosity or the direction and amplitude of body mass change during the tail-suspension periods. However, pentoxifylline may influence the body's response to fluid shifts at the beginning of exposure to space flight or tail-suspension. Because lack of fluid shifts resulted in no change in Hct, suggests the importance of pentoxifylline on influencing the body's response to fluid shifts. The lack of change in blood viscosity and Hct seen in tail suspended rats in this study make it difficult to determine the role of blood viscosity on the regulation of hematocrit.

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Date

The Role of Blood Viscosity in the Regulation of Hematocrit in Tail-Suspended Rats (*Rattus norvegicus*) Treated with Pentoxifylline

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