

AUG-27-1996 09:25

DESMOS INC (619)435-3962

P.02

NASA-CR-202187

Final Report: NASA NAG 2-659
4/13/93

Final
IN-52-CR
EIT 50375

a. **Justification:** This project has investigated mechanisms that influence alterations in compartmental fluid and electrolyte balance in microgravity and evaluates countermeasures to control renal fluid and electrolyte losses. Determining the alterations in fluid compartments and renal function to spaceflight is an important component in understanding long term adaptation to spaceflight and the contribution to post-flight orthostatic intolerance.

b. **Accomplishments:** Four definition phase studies and two studies examining neuro-humoral and vascular mechanisms have been completed. 1) Renal function alterations and extracellular fluid and blood volume changes were examined in awake rats during 14 days head-down tilt (HDT) and for 7 days following HDT. 2) Determined the impact of 24 hr HDT on renal function, glomerular hemodynamics, and segmental tubular fluid reabsorption as well as glomerular hemodynamic changes after acute restoration (1 hr) to orthostatic position. 3) Examined the alterations in fluid spaces and renal function during prolonged (30 day) HDT and 7 days post-tilt. and 4) Determine if glomerular and tubular responses readapts after 14 day HDT and the renal response to acute return to orthostasis. 5) Systemic transcapillary albumin escape rate is increased in prolonged HDT shifting fluid balance from plasma to interstitium. 6) Renal function is more responsive to baroreceptor (adrenergic and AII) control mechanisms than to alterations in plasma volume during HDT such that acute saline loading alone prior to return to 1g fields may not restore plasma volume sufficiently to act as an orthostatic intolerance countermeasure.

c. **Plans:** Submit remaining studies to peer reviewed publications. Loss of funds prevents any further plans.

d. **Publications:**

1. Tucker, B.J. Fluid compartment and renal function alterations in the rat during 7 and 14 day head-down tilt. NASA Conference Pub., 10068:97-108, 1991.
2. Provost, S.B. and B.J. Tucker. Effect of 14 day head-down tilt on renal function and vascular and extracellular fluid volumes in the conscious rat. The Physiologist, 35:S105-S106, 1992.
3. Tucker, B.J. and M.M. Mendonca. Alterations in glomerular hemodynamics and tubular reabsorption after 24 hours head-down tilt and following acute return to orthostasis. The Physiologist, 35:S103-S104, 1992.

AUG 27 1996
CASI

Tucker BJ, UCSD # 93-5393

Progress Report and Preliminary Studies

Progress Report, 5/1/90 to 8/1/92:

Note: This progress report only covers a little over 2 years of research effort due to a delayed start of funding (5/1/90). We have successfully completed five studies and have obtained preliminary data on the sixth during this time period. This was accomplished despite the marked reduction in awarded direct costs (approximately \$28,500 in the first eight months and \$43,600 in the following years) and only a 15% effort committed by the PI. We have made substantial progress in the definition phase of the first grant period in spite of these severe restraints and have begun investigation into the effects of atrial natriuretic factor on renal function changes during simulated microgravity which are scheduled to be completed during the current grant year.

1. Changes in blood and plasma volumes during prolonged head-down tilt in the rat.

In rats, chronic cannulation of the femoral artery and vein was performed and the animals were allowed one week to recover. The rats were separated into four subgroups (n=6 in each group) and either tail suspended for 1, 7, or 14 days or left in the normal orthostatic position. Blood volume was measured in each of the four subgroups of awake rats utilizing ^{51}Cr labeled endogenous erythrocytes and the changes in volume with duration of HDT were compared to non-tilted controls (Figure 1). Although not significant, blood volume tended to increase from 5.4 ± 0.1 in Control to $5.6 \pm 0.1\%$ of body weight after 24 hrs HDT. By day 7, blood volume significantly decreased to $5.0 \pm 1\%$ of body weight and decreased further to $4.8 \pm 0.1\%$ by day 14 of HDT similar to observations made in humans. There were no differences in body weight, systemic hematocrit, or plasma protein concentrations among the four subgroups indicating that the observed blood volume changes were independent of these factors. The results of these studies have been published. In addition, we have recently measured blood volume in 30 day HDT (n=3) and have included those values in Figure 1.

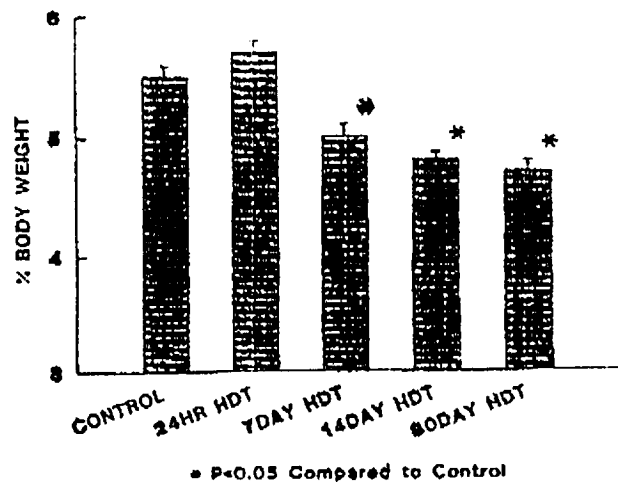


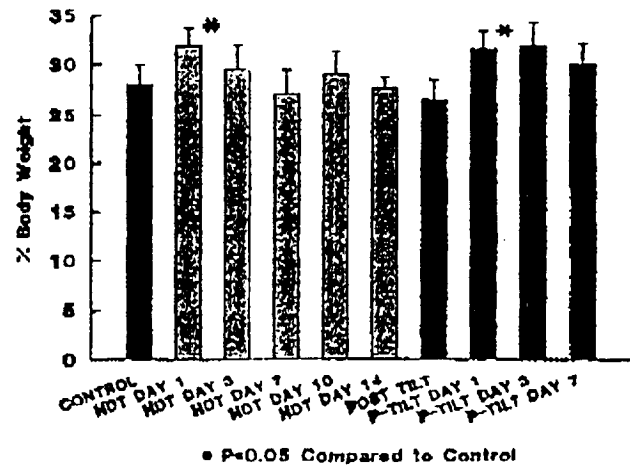
Figure 1. Alterations in blood volume during prolonged head-down tilt (HDT). * P<0.05 Compared to Control

2. Fluid compartment and renal function alterations in the rat during 14 day head-down tilt.

In this study, rats were cannulated in the femoral artery and vein and bladder as in prior studies for chronic monitoring of extracellular volume, systemic electrolytes and plasma protein concentration as well as renal function (GFR, renal plasma flow, urine

Tucker BJ, UCSD # 93-5393

flow rate, urinary sodium and potassium excretion). Serial measurements of these parameters were performed twice prior to HDT and then at 24 hrs, 3, 7, 10, and 14 days during HDT. After the 14 day HDT period was completed, all rats were returned to normal orthostatic position and, after a 45 min waiting period, the measurements were repeated. Measurements were also performed at 24 hrs, 3, and 7 days post-HDT. All values were compared to pre-tilt control measurements in the same rat on a paired basis. Similar to our previous findings, extracellular fluid volume increased from 28.2 ± 3.1 to 31.4 ± 3.5 % of



* P<0.05 Compared to Control
Figure 2. Effects of HDT and post-tilt recovery on extracellular fluid volume.

body weight after 24 hrs of HDT and then steadily decreased to 24 ± 2.1 % of body weight by day 7 (Figure 2). By day 14, ECF returned to values not different from control (27.3 ± 1.2 % of body weight). ECF increased significantly at day 1 post-tilt recovery compared to both control values and measurements performed 1 hour after return to an orthostatic position. ECF did return to near normal values by day 7 post-tilt recovery. GFR increased during HDT from 2.1 ± 0.1 in control to 2.3 ± 0.2 after 24 hrs HDT and to 2.8 ± 0.2 ml/min after 3 days HDT. By day 7, GFR was not different from control (2.2 ± 0.1 ml/min) and GFR at day 14 HDT was 2.3 ± 0.2 ml/min, also not different from pre-tilt values. It was surprising that GFR remained at values not different from control despite the decrease in blood volume at 7 and 14 days HDT. Post-tilt GFR values were not different from pre-tilt values measured in this group of rats. Renal plasma flow increased by day 3 of HDT but did not significantly deviate from control values at the other measurement time points. In early HDT, there seems to be a mild volume expansion with concomitant increase in GFR and renal plasma flow, but after the initial expansion phase, ECF and renal function returned to values not different compared to pre-tilt measurements. However, there was a decrease in blood volume during 7-14 days HDT (Figure 1), which would indicate an alteration in the normal blood/interstitium compartment volume ratio. None of the fluid shifts correlated well with changes in blood pressure, which increased significantly at day 3 HDT and remained elevated during the remainder of HDT, implying potential neurohormonal changes and altered volume homeostasis regulatory mechanisms.

3. Alterations in glomerular hemodynamics and tubular reabsorption after 24 hours of head-down tilt and following acute return to orthostasis.

We have previously demonstrated that glomerular filtration rate is increased in early phases of HDT (1-3 days) in the rat. Although most of the increase in filtration rate during HDT has been associated with increased renal plasma flow, the specific changes in the determinants of glomerular ultrafiltration have not been assessed. In addition, the effect of acute return to orthostatic position after HDT on glomerular hemodynamics have not been previously examined. After 24 hours HDT, single nephron glomerular filtration

Tucker R.I. UCSD # 93-5393

rate (SNGFR) increased more than 30% (Figure 3) due primarily to increased single nephron plasma flow with no change in either the glomerular hydrostatic pressure gradient or the glomerular ultrafiltration coefficient. After 24 hr HDT, both absolute proximal tubule fluid reabsorption and loop of Henle fluid reabsorption was significantly increased compared to control and was sufficient to maintain constant delivery of fluid to the distal tubule, and did not contribute to the observed increase in urine flow. One hour orthostasis after 24 hours simulated microgravity restored

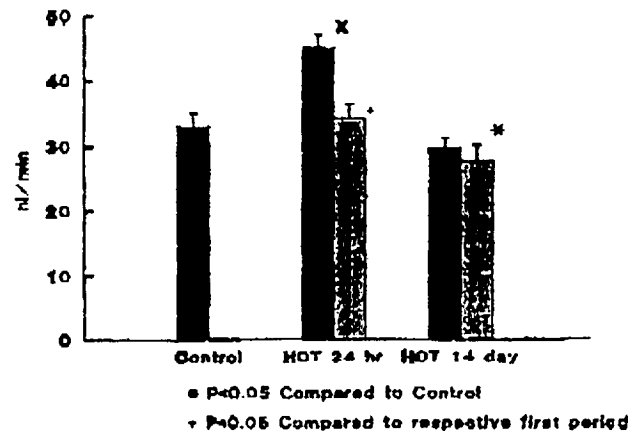


Figure 3. Changes in single nephron filtration rate during HDT and following one hour after return to orthostasis.

most renal function parameters to near normal values as well as returned the determinants of glomerular ultrafiltration and SNGFR to values not different from Control but urine flow remain increased compared to Control. The data indicate a dissociation in glomerular filtration rate, distal tubule flow rate and urine flow indicating a reduction in reabsorptive function in later portions of the distal tubule and/or the collecting duct.

4. Effect of 30 day head-down tilt and 7 day post tilt recovery on renal function and extracellular fluid volume.

This study has just been completed with comprehensive analysis of the 30 day HDT and 7 day post-HDT recovery results in the awake rat. We had a 60-70% success rate with rats in these studies, which is quite good considering that the implanted catheters are in use for almost 7 weeks. In addition, with the animal training protocol prior to the studies, body weight increased by 7% by day 28 and was $8 \pm 3\%$ at the end of post-tilt recovery period ($P < 0.05$ compared to initial control values, see Figure A on page 5). Continuing from the 14 day HDT study, at 21 days after onset of HDT, mean arterial pressure (MAP) had increased above pre-tilt values and remained increased at the 28 and 30 day HDT measurement period. Although blood pressure did not immediately decrease after one hour of orthostatic recovery, MAP returned to values not different from control at 24 hours post-tilt and during the 7 day recovery period. Despite the increase in MAP in this later period of prolonged HDT, there was no significant increase in either glomerular filtration rate or renal plasma flow compared to control, pre-tilt values. In a parallel group of rats ($n=6$), total body water was assessed during this time period, and no significant alteration in total body water was observed (control value = $75 \pm 2\%$ of body wt.). Extracellular fluid volume increased at both days 1 and 3 of HDT and returned to values not significantly different from Control for the remainder of HDT with a trend of increasing ECF (blood + interstitial volume) during the 7 day post-tilt period. As in both the 7 day HDT study previously published (33) and the 14 day HDT study in awake rats, both GFR and RPF in the 30 day HDT study increased significantly during the early phases of HDT. In conjunction with the blood volume study, there is a clear dissociation between plasma and interstitial volumes during prolonged head-down tilt, resulting in a net increase in interstitial volume (which would contribute to

AUG-27-1995 09:28

DESMOS INC (619)455-3962

P.05

Tucker BJ, UCSD # 93-5393

edema formation) with decreased plasma volume. Determination of fluid volume status based upon plasma volume measurements would significantly overestimate the fluid and electrolyte losses during conditions of microgravity.

5. Alterations in glomerular hemodynamics and tubular reabsorption after 14 days HDT and following acute return to orthostatic position.

This study has been completed and rigorously analyzed for publication in the near future. Single nephron filtration rate (SNGFR) after 14 days HDT, in the anesthetized surgically prepared rat, is not different compared to control values (Figure 3) and analysis indicates a linear relationship to single nephron plasma flow. These results parallel the findings of glomerular filtration rate and renal plasma flow observed at the 14 day HDT time point in the awake rat. Return to orthostasis did not decrease SNGFR despite a significant reduction in blood pressure, factors not observed after 24 hrs HDT (Figure 3). At 14 day HDT, absolute proximal tubule reabsorption (APR) was normal. However, upon return to orthostasis, APR decreased by 30%, without a decline in SNGFR. With the moderate increase in loop of Henle reabsorption after return to orthostasis, flow rate in the early distal tubule was not different compared to the 14 day HDT values. However, distal tubule flow rate was significantly less than that observed in non-tilt control rats. Since the distal tubule flow rate was only $\approx 60\%$ of Control values and urine flow was significantly increased, the data would indicate that (as observed in the 24 hr HDT group) there was a significant decrease in reabsorptive function in later portions of the distal tubule and/or the collecting duct. It is possible that if circulating levels of anti-diuretic hormone are normal at 14 day HDT, that the kidney is not responding to the anti-diuretic stimulus. Overall, the major alterations in glomerular hemodynamics observed after return to orthostasis from 24 hours HDT were not present at 14 day HDT. This is indicative of adequate renal compensatory and regulatory capacity to maintain most glomerular hemodynamic parameters at pre-return values after 14 day HDT but not response to volume conserving stimuli due to alterations in the neurohormonal milieu.

6. Evidence of increased systemic transcapillary albumin escape rate (TER_{sb}) after 30 days of head-down tilt.

We have recently hypothesized that the dissociation in volume ratio between the plasma and interstitium is due to the shift in Starling forces to produce positive fluid movement from the distal portions of the capillaries and venules outward to the extravascular space (see Significance) in the upper torso and cephalad regions of the body. This increased filtration in these regions will produce edema and may not be totally compensated by the reverse fluid movement in the lower portion of the body. This hypothesis is testable by examining the systemic

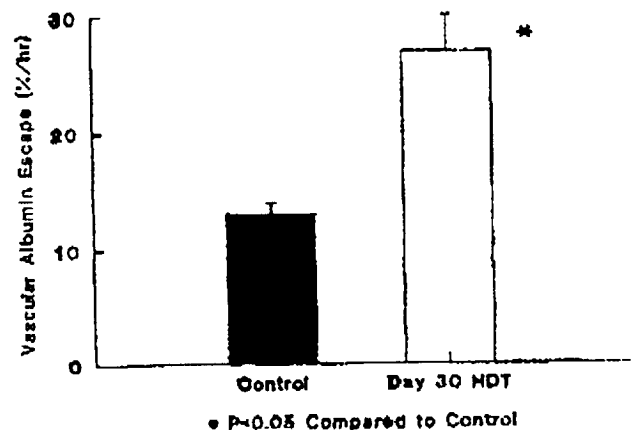


Figure 4. Systemic transcapillary albumin escape rate alterations after 30 days HDT.

Tucker BJ, UCSD # 93-5393

transcapillary escape rate of albumin (TER_{alb}) due to the differences in macromolecular permeability between normal filtering and reabsorbing sections of the peripheral vasculature. In some initial studies, we found that at 30 days HDT, TER_{alb} was significantly increased compared to non-tilt controls (Figure 4). In these rats, blood volume was less than non-tilt controls (Figure 1), whereas hematocrit and systemic plasma protein concentration was not significantly altered. Renal interstitial volumes were also obtained in 30 day HDT rats and were found not to be different from control (23 ± 1 vs $22 \pm 1\%$ of kidney volume, respectively). The increased systemic TER_{alb} may help define the mechanisms responsible for the significant and persistent decrease in plasma volume with little or no alteration of ECF during HDT.

7. Effects of angiotensin II infusion on glomerular hemodynamics and tubular reabsorption in 14 day head-down tilt rats.

We have recently completed studies examining the effects of angiotensin II infusion in both 14 day head-down tilt and in tail suspended non-tilt controls. Studies have previously demonstrated (Progress Report) that at 14 days HDT plasma volume is significantly reduced and extracellular fluid volume is not different from pre-tilt values. In humans, plasma renin activity is not different from normal after extended HDT, bed-rest, or spaceflight (3,19). Long term negative fluid and electrolyte balance should result in conditions, similar to chronic salt depletion, where there is either downregulation or no change in glomerular angiotensin II receptors (23,38). Therefore, infusion of exogenous angiotensin II should produce the same or reduced renal vasoconstriction in HDT rats compared to that of non-tilt controls. However, when angiotensin II was infused at the same dose in both HDT and non-tilt controls, producing a similar increase in systemic blood pressure, there was a greater vasoconstrictive effect in the HDT down-tilt rats compared to controls. This resulted in a significant decrease in both glomerular filtration rate and single nephron glomerular filtration rate in HDT rats compared to non-tilt controls (Figure 5). The reduction in nephron filtration rate in HDT rats was due to reductions in both nephron plasma flow (from 128 ± 10 to 67 ± 4 nl/min in HDT after All, $P < 0.05$) and the glomerular ultrafiltration

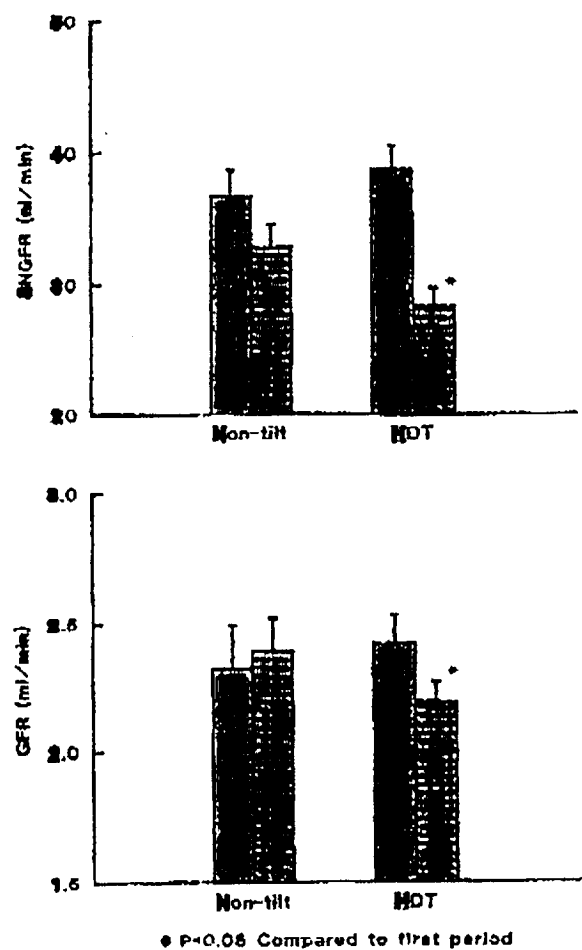


Figure 5. Effect of angiotensin II infusion on GFR and SNGFR in Non-tilt and after 14 day HDT. The paired bars are before and after All infusion respectively.

Tucker BJ, UCSD # 93-5393

coefficient (from 0.060 ± 0.011 to 0.027 ± 0.004 nl/sec mm Hg in HDT after AI) that was insufficiently opposed by the increase in the glomerular hydrostatic pressure gradient. These unexpected renal responses to AI infusion in HDT are similar to a response observed in plasma volume expanded rats (24), and not in euvoletic or volume depleted conditions. These data indicate that glomerular angiotensin II receptors may be increased in 14 day HDT compared to non-tilt controls. This results in a disproportionate response to angiotensin II considering the volume status of the rat. Measurement of glomerular AI receptors in 14d HDT and non-tilt controls is proposed in the current application. One potential stimulus for upregulation of angiotensin II receptors in the glomerulus is a reduction in renal sympathetic activity (24). These data clearly demonstrate the need for further understanding of the dynamics of the neurohormonal axis in HDT and potentially spaceflight and that extrapolation of data from circulating hormone levels and volume status would, at best, only provide partial information. This dissociation between angiotensin II response and volume status has not been previously observed. If countermeasures for orthostatic intolerance and negative fluid balance alter angiotensin II activity, the enhanced renal response to angiotensin II and the resulting effect on renal function must be considered.

Another observation resulting from this study is that despite a greater reduction in single nephron filtration rate after angiotensin II infusion in HDT rats compared to non-tilt controls, both early distal tubule flow rate (7.6 ± 0.7 vs 7.5 ± 0.7 nl/min, NS) and the resulting urine flow (3.8 ± 0.5 in HDT vs 3.4 ± 0.7 μ l/min) were not altered indicating a resetting of glomerular-tubular balance.

These findings are critical to the further understanding of the physiologic response to simulated microgravity and indicate resetting of the mechanisms responsible for renal function control, responses that would not have been predicted from volume status alteration studies.

Publications During Project Period:

Supported by NAG 2-659:

Abstracts:

1. Tucker, B.J., and M.M. Mendonca. Alterations in glomerular hemodynamics and tubular reabsorption after 24 hours head-down tilt and following acute return to orthostasis. Presented at the International Meeting of the IUPS Commission on Gravitational Physiology, 1991. *The Physiologist*. 34:235, 1991.
2. Provost, S.B., and B.J. Tucker. Effect of 14 day head-down tilt on renal function and vascular and extracellular fluid volumes in the conscious rat. Presented at the International Meeting of the IUPS Commission on Gravitational Physiology, 1991. *The Physiologist*. 34:235, 1991.
3. Tucker, B.J., and M.M. Mendonca. Alterations in glomerular and tubular dynamics in the rat after 1 and 14 days head-down tilt and following acute return to orthostasis. Presented at the 8th annual meeting of the American Society for

AUG-27-1995 09:30

DESMOS INC (619)455-3962

P.09

Tucker BJ, UCSD # 93-5393

Gravitational and Space Biology.

4. **Tucker, B.J., and M.M. Mendonca.** *Effects of 30 day head-down tilt and post-tilt recovery on fluid spaces and renal function in the awake rat. Presented at the 8th annual meeting of the American Society for Gravitational and Space Biology.*

Papers:

1. **Tucker, B.J., C.A. Mundy, M.G. Ziegler, C. Baylis, and R.C. Blantz.** *Head-down tilt and restraint on renal function and restraint on renal function and glomerular dynamics in the rat. J. Appl. Physiol. 63:505-513, 1987. (Prior to funded period)*
2. **Tucker, B.J.** *Fluid compartment and renal function alterations in the rat during 7 and 14 day head down tilt. NASA Conference Publication, 10068:97-108, 1991.*
3. **Provost, S.B. and B.J. Tucker.** *Effect of 14 day head-down tilt on renal function and vascular and extracellular fluid volumes in the conscious rat. The Physiologist, 35:S105-S106, 1992.*
4. **Tucker, B.J. and M.M. Mendonca.** *Alterations in glomerular hemodynamics and tubular reabsorption after 24 hours head-down tilt and following acute return to orthostasis. The Physiologist, 35:S103-S104, 1992.*

Papers in preparation:

5. **Tucker, B.J. and M.M. Mendonca.** *Alterations in glomerular and tubular dynamics in 24 hour and 14 day simulated microgravity and during acute orthostatic recovery. In preparation for J. Appl. Physiol., 1992.*
6. **Tucker, B.J. and S.B. Provost.** *Effect of 14 and 30 day simulated microgravity and orthostatic recovery on volume homeostasis and renal function in the awake rat. In preparation for J. Appl. Physiol., 1992.*

NASA related papers authored or co-authored by Principal Investigator:

7. **Parazynski, S.E., A.R. Hargens, B.J. Tucker, M. Aratow, J. Styf, and A. Crenshaw.** *Transcapillary fluid shifts in head and neck tissues during and after simulated microgravity. NASA Technical Memorandum. 103847:1-16, 1991.*
8. **Parazynski, S.E., A.R. Hargens, B.J. Tucker, M. Aratow, J. Styf, and A. Crenshaw.** *Transcapillary fluid shifts in tissues of the head and neck during and after simulated microgravity. J. Appl. Physiol., 71:2569-2475, 1991.*
9. **Parazynski, S.E., B.J. Tucker, M. Aratow, A. Crenshaw, and A.R. Hargens.** *Direct measurement of capillary blood pressure in the human lip. J. Appl. Physiol., Submitted, 1992.*

AUG-27-1995 09:30

DESMOS INC (619)455-3962

P. 10

Tucker BJ, UCSD # 93-5393

Other publications with the Principal Investigator during the project period:

10. Tucker, B.J., O.W. Peterson, K.A. Munger, J.E. Bird, M. Mitchell, J.C. Pelayo, and R.C. Blantz. Glomerular hemodynamic alterations during renal nerve stimulation in high and low salt diets. *Am. J. Physiol.*, 258:F133-F143, 1990.
11. Thomson, S.C., B.J. Tucker, F.B. Gabbai, and R.C. Blantz. Glomerular hemodynamic and alpha-2 adrenoreceptor stimulation: The role of renal nerves. *Am. J. Physiol.*, 258:F21-F27, 1990.
12. Tucker, B.J. and R.C. Blantz. Acute and subacute prostaglandin and angiotensin inhibition on glomerulotubular dynamics in rats. *Am. J. Physiol.*, 258:F1026-F1035, 1990.
13. Blantz, R.C., F.B. Gabbai, S.C. Thomson, and B.J. Tucker. Adrenergic influences and interactions with Angiotensin II. *Kidney Int.*, 38(Suppl.30):S84-S86, 1990.
14. Tucker, B.J. Early onset of increased transcapillary albumin escape in awake diabetic rats. *Diabetes*, 39:919-923, 1990.
15. Tucker, B.J., R.C. Collins, M.G. Ziegler, and R.C. Blantz. Disassociation between glomerular hyperfiltration and extracellular volume alterations in diabetic rats. *Kidney Int.* 39:1176-1183, 1991.
16. Thomson, S.C., B.J. Tucker and R.C. Blantz. Effects of beta-adrenergic blockade on the glomerular and tubular response to acute renal denervation. *J. Cardiovas. Pharmacol.* 17:397-402, 1991.
17. Hirschberg, R., J.D. Kopple, R.C. Blantz, and B.J. Tucker. Effects of recombinant human insulin-like growth factor I on glomerular dynamics in the rat. *J. Clin. Invest.* 87:1200-1206, 1991.
18. Tucker, B.J., F.B. Gabbai, and R.C. Blantz. Pathophysiology and renal hemodynamic changes in diabetic nephropathy. In Diabetic Nephropathy. (In Japanese), Shigeta S. and Kalzu K, Editors. Igaku-Shoin Ltd Publishers, Tokyo, Japan, 1992, pp. 69-86.
19. Thomson, S.C., F.B. Gabbai, B.J. Tucker, and R.C. Blantz. Interaction between α_2 -adrenergic and angiotensin II systems in the control of glomerular hemodynamics as assessed by renal micropuncture in the rat. *J. Clin. Invest.* 90:604-611, 1992.
20. Tucker, B.J., C.M. Anderson, R.S. Theis, R.C. Collins, and R.C. Blantz. Glomerular hemodynamics during acute hyperinsulinemia in normal and diabetic rats. *Kidney Int.* 42(5):, 1992.

AUG-27-1996 09:31

DESMOS INC (619)455-3962

P.11

ALTERATIONS IN GLOMERULAR AND TUBULAR DYNAMICS AT 1 AND 14 DAYS SIMULATED MICROGRAVITY AND AFTER ACUTE RETURN TO ORTHOSTASIS

Bryan J. Tucker and Margarida M. Mendonca

*Physiology/Pharmacology Consulting
San Diego, CA 92129*

*and
Department of Medicine,
University of California, San Diego
La Jolla, CA 92093*

INTRODUCTION

Head-down tilt (HDT) is utilized to simulate microgravity and produces a cephalad fluid shift, which results in alterations in fluid and electrolyte balance. These changes in volume homeostasis are due, in part, to alterations in multiple volume control mechanisms in which renal function is a major participant. We have previously demonstrated that glomerular filtration rate increases early in HDT and eventually returns to values not different from non-tilt measurements (1). This early increase in glomerular filtration rate was also demonstrated during days 2 and 8 of the SLS-1 mission. However, urine flow and electrolyte excretion does not parallel the alterations in glomerular filtration rate and the site of this change in nephron fluid reabsorption pattern has not been previously examined. Through determination of the location of alterations in tubular fluid reabsorption within the nephron, a more detailed hypothesis can be forwarded as to which specific neuro-humoral agents participating in control of renal function in microgravity conditions. The importance of this type of examination is that measurements in circulating neuro-humoral agents and urinary excretion patterns alone are not accurate predictors of how renal functional response may alter to head-down tilt or other models of simulated weightlessness.

To examine this issue, renal micropuncture techniques were utilized in Munich-Wistar rats submitted 24 hour and 14 day head-down tilt, measuring all the determinants of glomerular ultrafiltration and obtaining data regarding segmental tubular fluid reabsorption. Following these measurements, the rats were returned to an orthostatic position and after 60 min, the measurements were repeated.

METHODS

Munich-Wistar rats (males, Simonsen) were utilized in the present study. The rats were divided, at random, into three groups; 1) controls with no tail suspension (Control, n=8), 2) rats placed in 25° head-down tilt (HDT 24 hr, n=7) using tail suspension techniques developed by Morey-Holton and Wronski (2) and modified by Sweeny et al. (3), and 3) rats placed in tail suspension for 14 days (HDT 14 day, n=6). All animals were anesthetized with Inactin, a long acting anesthetic, (Research Biochemicals, Inc.) and prepared for renal micropuncture techniques (4). Following preparation for micropuncture and a 60 min period for equilibration of

H multi infusion and volume reabsorption (1% BW donor plasma in 60 min followed by 0.15% BW donor plasma/hr) glomerular hemodynamics and proximal tubule and loop of Henle fluid reabsorption were measured. In the head-down tilt groups, after completion of the first period micropuncture measurements, the rats were re-positioned to 0° tilt. Following return to orthostasis the rats were allowed one hour for re-equilibration and then the micropuncture measurements were repeated. Paired proximal and distal tubule collections were obtained in 4-6 separate nephrons on the surface of the kidney in each rat-period for assessment of single nephron filtration rate and segmental tubular fluid reabsorption. Glomerular capillary, Bowman's Space, and proximal tubule hydrostatic pressures were obtained utilizing a Servo-null pressure measuring system (IPM, San Diego). Protein concentration from efferent arteriolar plasma, obtained from the vascular stars on the surface of the kidney, was compared to systemic plasma protein concentration to determine single nephron filtration fraction. Whole kidney glomerular filtration rate and urine flows were also obtained.

RESULTS

Mean arterial pressure (MAP) was not different from control in either 1 or 14 day HDT (figure 1). However, upon acute return to orthostasis, MAP decreased significantly at both HDT durations (figure 1).

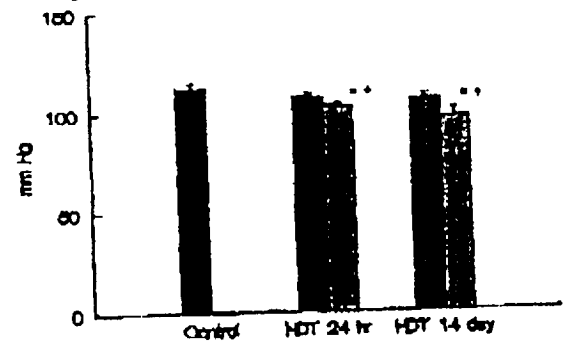


Figure 1. The effect of 1 and 14 day head-down tilt (HDT) and acute return to orthostasis (hatched bars) on mean arterial pressure (MAP). MAP was not increased in the anesthetized rat after 1 and 14 days HDT compared to Control. However, acute return to orthostasis resulted in a decrease of MAP in both durations of HDT. * $P < 0.05$ compared to control. + $P < 0.05$ compared to respective first period.

Two kidney glomerular filtration rate (GFR) was increased at 14 day HDT and a similar but non-significant trend at 1 day HDT (figure 2). After return to orthostasis, GFR returned to values not different from Control at both 1 and 14 day HDT duration with a significant decrease in GFR in the 1 day HDT group (figure 2).

Micropuncture data at the single nephron level yielded similar results with an increase in single nephron glomerular filtration rate (SNGFR) increased at 1 day HDT and returned to Control values in acute post-HDT (Table 1). However, at 14 day HDT, SNGFR was not significantly different from Control as opposed to the whole animal GFR. Post-HDT values were not different from control (Table 1). The only glomerular hemodynamic factor contributing to the increase in SNGFR was single nephron plasma flow (SNPF). The increase in SNPF was due to decreases in both afferent (AR, Table 1) and efferent arteriolar resistance. There

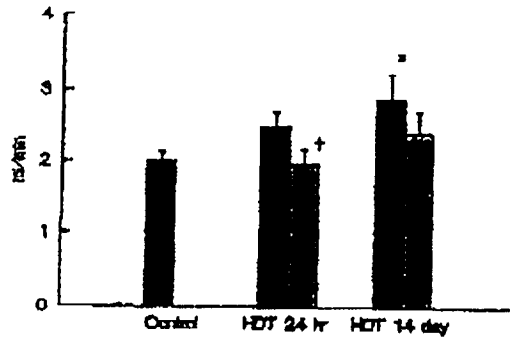


Figure 2. Effect of 1 and 14 day HDT on glomerular filtration rate (GFR, two kidney values) and the effect of acute return to orthostasis (hatched bars). GFR was increased at 14 day HDT compared to Control. Acute return to orthostasis significantly decreased GFR to values not different from Control at 1 day HDT but there was no significant decrease in GFR post-HDT in the HDT 14 day rats. * P<0.05 compared to Control. + P<0.05 compared to respective first period.

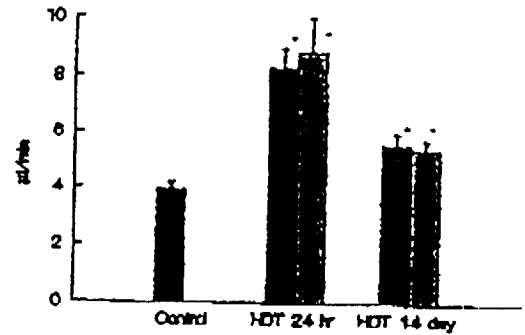


Figure 3. Effect of 1 and 14 day HDT on urine flow (UV) and the effect of return to orthostatic position (hatched bars). UV was increased 1 day HDT compared to Control with no increase in DFR. Urine flow was also increased in the HDT 14 day group despite a significant decrease in DFR. After acute return to orthostasis urine flow remained elevated compared to Control. * P<0.05 compared to control.

was no significant contribution to increases in SNGFR by changes in the glomerular hydrostatic pressure gradient (ΔP), glomerular ultrafiltration coefficient (LpA), or systemic protein concentration.

Table 1. Effect of HDT on single nephron hemodynamics.

| | SNGFR | SNPF | ΔP | LpA | AR |
|-----------|-------|---------|------------|---------|-------|
| CON | 33±2 | 93±8 | 34±1 | .05±.01 | 30±3 |
| 1d HDT | 45±2* | 166±11* | 34±1 | .08±.02 | 13±1* |
| P-1d HDT | 34±2* | 122±10* | 35±1 | .05±.01 | 18±2* |
| 14d HDT | 30±1 | 101±9 | 31±1 | .05±.01 | 24±3 |
| P-14d HDT | 28±3 | 83±8 | 31±1 | .05±.01 | 32±5 |

* P<0.05 compared to Control. + P<0.05 compared to respective first period.

As depicted in Table 2, segmental reabsorption of fluid in the proximal segment and loop of Henle portions of the nephron were significantly affected by HDT and acute post-HDT recovery. Absolute proximal tubule fluid reabsorption (APR) was increased by 1 day HDT and remained at values greater than control at 14 days HDT (Table 2). In contrast, loop of Henle reabsorption (LHR) was increased only at 1 day HDT (Table 2). During acute return to orthostasis, LHR decreased after 1 day HDT and increased after 14 days HDT. These alterations in nephron fluid reabsorption retained distal tubule flow rate at values similar or significantly less than control.

Table 2. Effect of HDT on nephron reabsorption.

| | SNGFR | APR | LHR | DFR |
|-----------|-------|-------|-------|----------|
| CON | 33±2 | 13±1 | 12±1 | 8.0±0.8 |
| 1d HDT | 45±2* | 19±1* | 16±1* | 10.1±1.0 |
| P-1d HDT | 34±2* | 13±1* | 13±1* | 9.0±0.8 |
| 14d HDT | 30±1 | 16±1* | 13±1 | 4.9±0.8* |
| P-14d HDT | 28±3 | 12±1* | 15±1* | 4.8±0.8* |

Since distal tubule flow rate was preserved or significantly decreased, the observed increase in urine flow both at 1 and 14 days HDT and during 1 hr post-HDT must be due to decreased fluid in latter portions of the nephron or the collecting duct (figure 3).

DISCUSSION

The only determinant of GFR that increased at HDT 24 hr was SNPF as a result of decreases in both afferent and efferent arteriolar resistance, resulting in the increase in SNGFR. There were no changes in the ΔP or the LpA. Upon acute return to orthostasis, SNPF was returned to values not different from Control and returning SNGFR to normal values. This pattern was paralleled by the measurements of whole animal GFR.

Absolute proximal tubule reabsorption (APR) was increased in HDT 24 hr and returned to control values after return to orthostasis. APR in 14 day HDT was not different from control and significantly decreased following return to orthostasis. However, alterations in LHR post-HDT resulted in no change in distal tubule flow rate in either HDT group.

In conclusion, the changes in glomerular filtration rate during head-down tilt are most likely due to alterations in renal plasma flow and not the glomerular hydrostatic pressure gradient or the glomerular ultrafiltration coefficient. The pattern of increased fluid reabsorption within the nephron indicates during head-down tilt is that the observed diuresis is the result of decreased reabsorption of fluid in either the later portion of the distal tubule or the collecting duct. Despite return to orthostasis and the resulting fluid shift, decreased glomerular filtration rate, and reduction in MAP, diuresis persists for at least an hour after the return from head-down tilt. This is most likely due to a lack of response to acute increased levels of anti-diuretic hormone and potentially increased renal sympathetic activity.

ACKNOWLEDGEMENTS

These studies were supported by NASA grants NAG 2-659 and NCC 2-556.

REFERENCES

1. Tucker, B.J., C.A. Mundy, M.G. Ziegler, C. Baylis, R.C. Blantz. Head-down tilt and restraint on renal function and glomerular dynamics in the rat. *J. Appl. Physiol.*, 63:505-513, 1987.
2. Morcy-Holton, E., T.J. Wronski. Animal models for simulating weightlessness. *Physiologist* 24, Suppl.:S45-S48, 1981.
3. Sweeney, J.R., G.J. Marshall, H. Gruber, M.E. Kirchen. Effects of non-weight bearing on fracture healing. *Physiologist*, 27:S35-S36, 1984.
4. Blantz R.C., B.J. Tucker. Measurements of glomerular dynamics. In: *Methods of Pharmacology, Renal Pharmacology*. M. Martinez-Maldonado ed., New York: Plenum, 1978, Vol. 4B, p. 141-163.

AUG-27-1995 09:33

DESMOS INC (619)455-3962

P. 13

EFFECTS OF 30 DAY SIMULATED MICROGRAVITY AND RECOVERY ON FLUID HOMEOSTASIS AND RENAL FUNCTION IN THE RAT

Bryan J. Tucker and Margarida M. Mendonca

*Physiology/Pharmacology Consulting
San Diego, CA 92129
and
Department of Medicine,
University of California, San Diego
La Jolla, CA 92093*

INTRODUCTION

Transition from a normal gravitational environment to that of microgravity eventually results in decreased plasma and blood volumes, increasing with duration of exposure to microgravity. This loss of vascular fluid is presumably due to negative fluid and electrolyte balance and most likely contributes to the orthostatic intolerance associated with the return to gravity. The decrease in plasma volume is presumed to be a reflection of a concurrent decrease in extracellular fluid volume with maintenance of normal plasma-interstitial fluid balance. In addition, the specific alterations in renal function contributing to these changes in fluid and electrolyte homeostasis are potentially responding to neuro-humoral signals that are not consistent with systemic fluid volume status.

We have previously demonstrated an early increase in both glomerular filtration rate and extracellular fluid volume and that this decreases towards control values by 7 days of simulated microgravity (1). However, longer duration studies relating these changes to plasma volume alterations and the response to return to orthostasis have not been fully addressed. Male Wistar rats were chronically cannulated, submitted to 30 days head-down tilt (HDT) and followed for 7 days after return to orthostasis from HDT. Measurements of renal function and extracellular and blood volumes were performed in the awake rat.

METHODS

Male Wistar rats (Simonsen, n=7) were utilized in the present study. One week prior to the initial measurements, the animals were anesthetized with a short duration anesthetic (Brevital, 65 mg/kg i.p.) and sterile tygon catheters were placed in the left femoral artery and vein. The vascular catheters were threaded under the skin and exteriorized at the back of the neck. A 14 gauge steel cannula, enclosed in silastic tubing, was implanted in the bladder and plugged with a stainless steel pin. This is a modification of the preparation described in detail by Gellai and Valtia (2). The rats received at least six hours of training by sitting quietly in a restraining cage of not more than three hours at any one time in preparation for the conscious studies. Two control, pre-tilt measurement periods were performed at least two days apart in each rat, while awake to ascertain extracellular fluid volume, glomerular filtration rate, and fluid and electrolyte excretion. After the control measurements were completed, the rats were tail suspended in a 25° head-down position (3) and the extracellular fluid volume and

10, 14, 21, 28, and 30 days head-down tilt (HDT). On day 30 of HDT, the rats were reverted to a 0° tilt position and the measurements repeated after 1 hour in the horizontal position. Post-HDT measurements were also performed a 1, 3, and 7 days after HDT. In a second group of chronically catheterized awake rats (n=25), plasma and blood volumes were measured utilizing ¹²⁵I labeled albumin and ⁵¹Cr labeled red blood cell distribution spaces in non-tilt, and 1, 7, 14, and 30 days 25° HDT (n=5 at each time point).

RESULTS

During the 30 days of HDT in the rat, there was no decrease in body weight. However, the increase in body weight was less than would be predicted in the normal growing rat. During the seven day recovery, growth rate returned to values not different from normal (~3 g/day). Mean arterial pressure increased during the 30 day HDT period as depicted in figure 1.

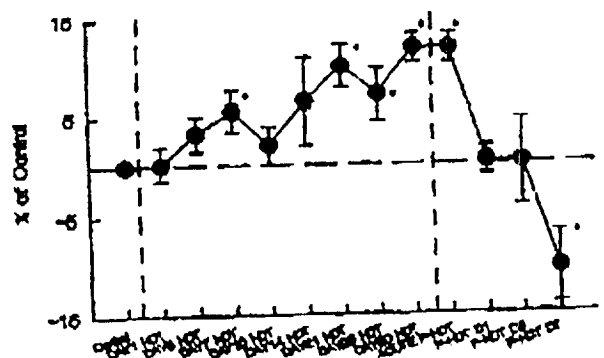


Figure 1. Alterations in mean arterial pressure (MAP) during 30 day HDT and post-HDT recovery. MAP increased at day 3 of HDT and was elevated for most of the remainder of HDT. In post-HDT, MAP decreased by day 7 to values less than control. * P<0.05 Compared to Control

Mean control value for MAP was 134±5 mm Hg and increased to 147±1 mm Hg by day 28 HDT.

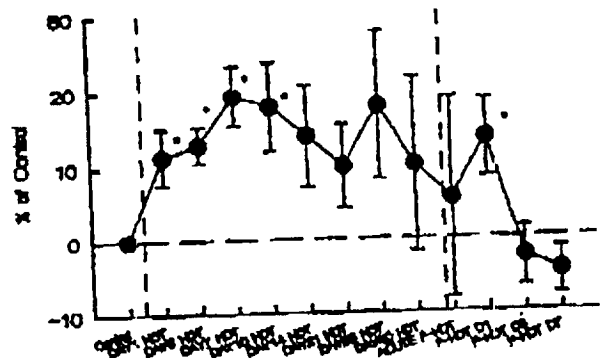


Figure 2. Effect of HDT and post-HDT recovery on glomerular filtration rate (GFR). GFR increased early in HDT and returned to values not different from control by day 21 HDT. Post-HDT was only different from control at day 3. * P<0.05 compared to Control.

Glomerular filtration rate (GFR) was 0.8 ± 0.1 ml/min/100g BW in Control and increased to a maximum of 1.0 ± 0.2 ml/min/100g BW during HDT. The specific changes in GFR as a percent of Control values are depicted in figure 2. GFR changes became more variable as the duration of HDT increased. In post-HDT, GFR did not consistently return to control values until 3 days post-HDT (figure 2).

The increase in GFR was most likely due to the increase in renal plasma flow (RPF) as depicted in figure 3. One hour after return to orthostasis, RPF decreased to values numerically less than Control but was not significantly different from Control during the 7 day post-HDT period (figure 3).

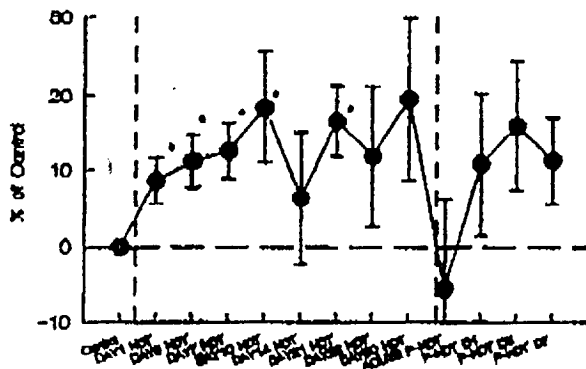


Figure 3. Effect of HDT and post-HDT on renal plasma flow (RPF). RPF increased by day 3 of HDT and remained increased to day 14 of HDT with an overall increase during the 30 day HDT. Post-HDT immediately returned RPF to values not different from Control. * $P < 0.05$ compared to Control.

As observed in previous studies (1,4) extracellular fluid volume (ECF) increased early in HDT and returned to Control values by day 10 HDT as depicted in figure 4.

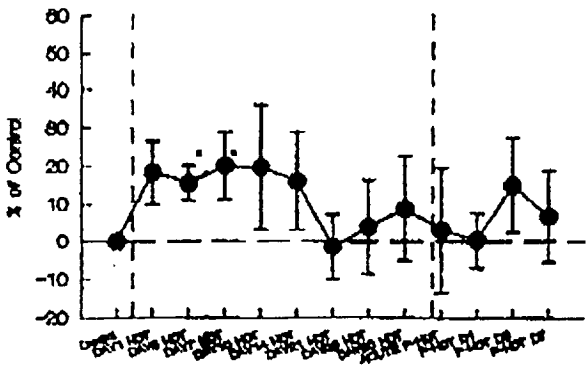


Figure 4. Alterations in extracellular fluid volume (ECF) during 30 day HDT and post-HDT recovery. There was a significant and early increase in ECF at 1 and 3 days HDT that decreased to values not different from Control by day 10 HDT. * $P < 0.05$ compared to Control.

The changes in blood volume did not parallel the alterations in ECF which did not increase and was significantly decreased by day 7 HDT (figure 5). These data indicate a significant dissociation in the renal response to alterations in blood volume and a change in the normal blood:interstitial volume ratio.

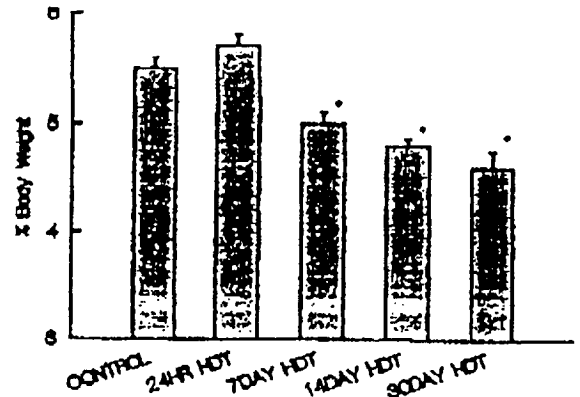


Figure 5. Alterations in blood volume (BV) in prolonged HDT compared to non-tilt Control. At 24 hr HDT there was no significant change in BV compared to Control. By day 7, BV decreased and continued a downward trend at days 14 and 30 HDT. * $P < 0.05$ compared to Control.

DISCUSSION

Glomerular filtration rate increased early in HDT, through increases in renal plasma flow, most likely responding to extracellular fluid volume expansion. However, over time, glomerular filtration rate, renal plasma flow, and extracellular fluid volume decreased to values not different from pre-tilt controls demonstrating a close functional correlation in these parameters. However, plasma and blood volume changes were dissociated from alterations in extracellular fluid volume resulting in decrease in the normal plasma:interstitial volume ratio. This change in the plasma:interstitial volume ratio is indicative of either alteration in Starling forces at the peripheral capillary beds and/or changes in the neuro-humoral axis controlling fluid volume homeostasis.

After acute return to orthostasis, there were significant decreases in both renal plasma flow and mean arterial pressure indicative of a degree of orthostatic intolerance in the rat model of simulated microgravity.

ACKNOWLEDGEMENTS

These studies were funded by NASA grants NAG 2-659 and NCC 2-556.

REFERENCES

1. Tucker, B.J., C.A. Mundy, M.G. Ziegler, C. Baylis, and R.C. Blantz. Head-down tilt and restraint on renal function and glomerular dynamics in the rat. *J. Appl. Physiol.*, 63:505-513, 1987.
2. Gellai, M., H. Valtin. Chronic vascular constrictions and measurements of renal function in conscious rats. *Kidney Int.*, 15:419-427, 1979.
3. Morey-Holton, E., T. J. Wronski. Animal models for simulating weightlessness. *Physiologist* 24, Suppl.:S45-S48, 1981.
4. Provost, S.B. and B.J. Tucker. Effect of 14 day head-down tilt on renal function and vascular and extracellular fluid volumes in the conscious rat. *Physiologist* 35, Suppl.:S105-S106, 1992.

Direct measurement of capillary blood pressure in the human lip

S. E. PARAZYNSKI, B. J. TUCKER, M. ARATOW, A. CRENSHAW, AND A. R. HARGENS
Life Science Division, National Aeronautics and Space Administration-Ames Research Center,
Moffett Field, California 94035-1000

PARAZYNSKI, S. E., B. J. TUCKER, M. ARATOW, A. CRENSHAW, AND A. R. HARGENS. *Direct measurement of capillary blood pressure in the human lip*. *J. Appl. Physiol.* 74(2): 946-950, 1993. —In this study, we developed and tested a new procedure for measuring microcirculatory blood pressures above heart level in humans. Capillary and postcapillary venule blood pressures were measured directly in 13 human subjects by use of the servo-nulling micropressure technique adapted for micropuncture of lip capillaries. Pressure waveforms were recorded in 40 separate capillary vessels and 14 separate postcapillary venules over periods ranging from 5 to 64 s. Localization and determination of capillary and postcapillary vessels were ascertained anatomically before pressure measurements. Capillary pressure was 33.2 ± 1.5 (SE) mmHg in lips of subjects seated upright. Repeated micropunctures of the same vessel gave an average coefficient of variation of 0.072. Postcapillary venule pressure was 18.9 ± 1.6 mmHg. This procedure produces a direct and reproducible means of measuring microvascular blood pressures in a vascular bed above heart level in humans.

postcapillary venule pressure; servo-nulling technique; micropressures; micropipette; microcirculation

CAPILLARY BLOOD PRESSURE is of central importance for maintaining tissue nutrition and transcapillary fluid balance, yet its direct measurement is difficult because of the relative inaccessibility of superficial capillary beds and technical difficulties relating to cannulation of such small vessels. Pulsatile pressure in capillaries was first observed by Landis (6), who used a microinjection technique within frog mesentery. Rappaport et al. (11) were able to record dynamic pressures by use of a pressure transducer in vessels as small as 30 μ m in diameter. The pioneering work of Wiederhielm et al. (15) developed a servo-controlled dynamic micropressure recording system to measure capillary blood pressure accurately in frog mesentery for prolonged periods of time. This technique makes use of a low electrical resistance fluid (1-2 M NaCl) within the micropipette. Displacement of the interface of plasma and the salt solution within the micropipette, due to changes in microvascular blood pressure in the vessel lumen, alters electrical resistance, which is then counteracted and sensed by a pressure transducer. This system allows for a rapid-frequency response time of 20 Hz.

Other investigators have applied the servo-nulling technique to direct measurements of capillary pressure in human fingernailfolds (3, 7, 8) and in tissues of other

mammals (4, 14, 17). Indirect methods for measuring capillary pressure, such as the venous occlusion technique described in humans by Perry et al. (10), have been criticized because of inaccuracies due to venous backflow (2). Moreover, none of the previously reported techniques obtained measurements of capillary pressure above heart level in humans.

Obtaining capillary pressure measurements in tissues above heart level is important for understanding the responses of tissues to changes in hydrostatic pressure due to both alterations in systemic blood pressure, such as hypertension, and local pressure changes due to posture. The regulatory mechanisms controlling capillary pressure and transcapillary fluid exchange have not been explored in capillary beds located above heart level and may differ from those of tissues located at or below heart level. The concept of a linear contribution of the hydrostatic blood column due to gravity to the capillary bed may not pertain to microvasculature above heart level, where adequate perfusion pressure must be maintained (9). In fact, Levick and Michel (7) observed that capillary pressure in the foot is less than that predicted by the hydrostatic blood column from the heart to the feet.

The purpose of this study was to develop and validate a technique to measure dynamic capillary and postcapillary venule blood pressures in the human lower lip by application of the servo-nulling technique described by Wiederhielm et al. (15). Such a technique could have important application in understanding transcapillary regulation in a wide variety of experimental studies of humans, such as orthostatic adaptation, orthostatic intolerance, dehydration, and local edema.

METHODS

Subjects. Ten male and three female volunteers, aged 24-52 yr (mean 34 ± 9 yr), were studied in the Human Research Facility, NASA-Ames Research Center, Moffett Field, CA. Protocols for this investigation were previously approved by the Human Research Experiments Review Board at NASA-Ames Research Center and the Human Subjects Committee of Stanford Medical Center, Stanford, CA, and informed written consent was obtained. As determined by prior physical examinations, all subjects were in excellent health and not currently taking any medication. Systolic blood pressure ranged from 87 to 138 mmHg (mean 116.5 ± 14.6 mmHg); diastolic blood

TABLE 1. Human subject and environment parameters

| | Mean \pm SD | Range |
|-------------------------|-----------------|-------------|
| Age, yr | 33.7 \pm 9.1 | 24.0-52.0 |
| Height, cm | 173.4 \pm 9.5 | 157.0-188.0 |
| Weight, kg | 69.3 \pm 11.8 | 44.1-86.1 |
| SBP, mmHg | 116 \pm 15 | 87-139 |
| DBP, mmHg | 79 \pm 9 | 62-96 |
| Heart rate, beats/min | 63 \pm 9 | 48-82 |
| Lip temperature, °C | 33.6 \pm 0.7 | 31.7-34.8 |
| Ambient temperature, °C | 23.9 \pm 0.9 | 21.9-24.9 |

SBP and DBP, systolic and diastolic blood pressures, respectively.

pressure ranged from 62 to 96 mmHg (mean 78.6 \pm 9.4 mmHg). Table 1 gives subject and environmental parameters.

Techniques. The subjects were seated upright in a dentist's chair with the head extended backward. The inner aspect of the lower lip was exposed with two padded surgical soft tissue clamps (Fig. 1). This configuration did not compromise vascular blood flow as visualized under $\times 40$ magnification with a Zeiss surgical microscope. The subject's head rested on a custom-designed platform to minimize vibration and to keep the micromanipulator/micropipette assembly in line with the subject's lip. The lower lip was illuminated with a quartz fiber-optic bundle and a light source (Ehrenreich Photo Optical Industries, Garden City, NY). A thermistor was applied to the lip, and mucosal temperature ranged between 31.7 and 34.8°C (mean 33.6 \pm 0.7°C). Ambient room temperature for all measurements ranged between 21.9 and 24.9°C (mean 23.9 \pm 0.9°C).

Pressure measurements were obtained with a servo-nulling pressure recorder (Instrumentation for Physiology and Medicine, San Diego, CA) by use of specially prepared glass micropipettes, which were inserted under direct visualization into the vessel lumen with a Zeiss surgical microscope ($\times 40$) and a Leitz micromanipulator device. No anesthetics were used, and micropuncture was essentially painless. The only reported sensation was slight pressure, which occurred infrequently. Three to five pressure measurements of each vessel type were attempted in each individual. Uniform micropipettes were prepared from custom 0.9-mm borosilicate capillary tubing with wall thickness of 0.2 mm (Drummond Glass) by use of a commercial pipette puller (Kopf) to obtain a sharply tapered tip. Pipettes were ground on a Crysalan grinding wheel to a 25- to 30°-beveled tip and a 1- to 3- μ m OD. Micropipettes were filled with 1.2 M NaCl solution containing FD&C Green no. 3 to improve contrast of the micropipette tip. Breakage of the micropipette tip occurred occasionally, but the fragments were easily removed and produced no untoward complications.

Micropuncture was performed in the lower lip under a drop of isotonic saline from an angle of $\sim 45^\circ$. Before micropuncture, null pressure was established in this drop of saline. The vessel was identified by location with respect to arterioles and venules before the pressure recording. In addition, only capillaries with single-file flow of erythrocytes were selected for pressure measurements. The micropipette was advanced through the superficial lip mucosa toward the capillary or postcapillary venule

chosen for cannulation. The position of the micropipette was adjusted such that blood flow was not impeded and a reliable waveform was obtained. Pressure measurements were obtained in a single blind fashion with the micropuncturist unaware of the recorded pressure at the time of measurement. Simultaneous electrocardiogram monitoring allowed correlation of microvascular pressure wave patterns to the cardiac cycle. Vessel diameters were not quantified in this study.

Data and statistical analyses. Microvascular pressures were determined by two procedures. If a tracing provided clear systolic peaks and diastolic troughs and was insensitive to increases in gain from the servo-null system, the peaks and troughs were averaged and mean capillary pressure (or postcapillary venular pressure) was calculated. If the signals had superimposed electrical noise on the pulse pressure wave, values were measured at regular time intervals (during the recording) and averaged to obtain a mean microvascular pressure value.

Linear regression analysis was performed to determine significant relationships between microvascular pressures, arterial blood pressure, and temperature. Multiple regression analyses were performed to determine whether any correlation existed between microvascular pressures in the lip and such variables as systolic blood pressure, mean arterial blood pressure, diastolic blood pressure, and lip surface temperature. None of the curves yielded statistical significance. The null hypothesis was rejected when $P < 0.05$. Coefficients of variation (SD/mean $\times 100\%$) were calculated to document the reproducibility of measuring capillary blood pressure (13).

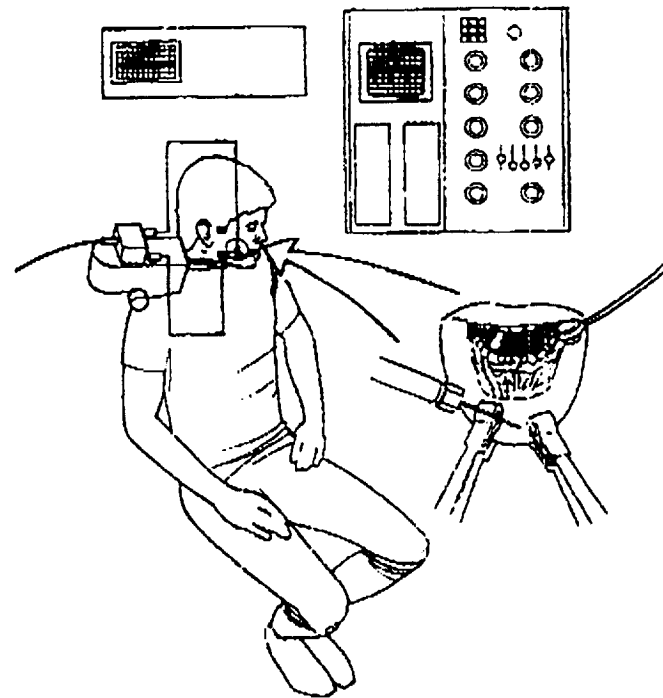


FIG. 1. Microvascular pressure recording system. Subjects were stabilized in a dentist's chair with a specially designed headrest for micropuncture of lower lip capillaries and venules. Enlargement (right) details positioning of padded surgical soft tissue clamps on lip.

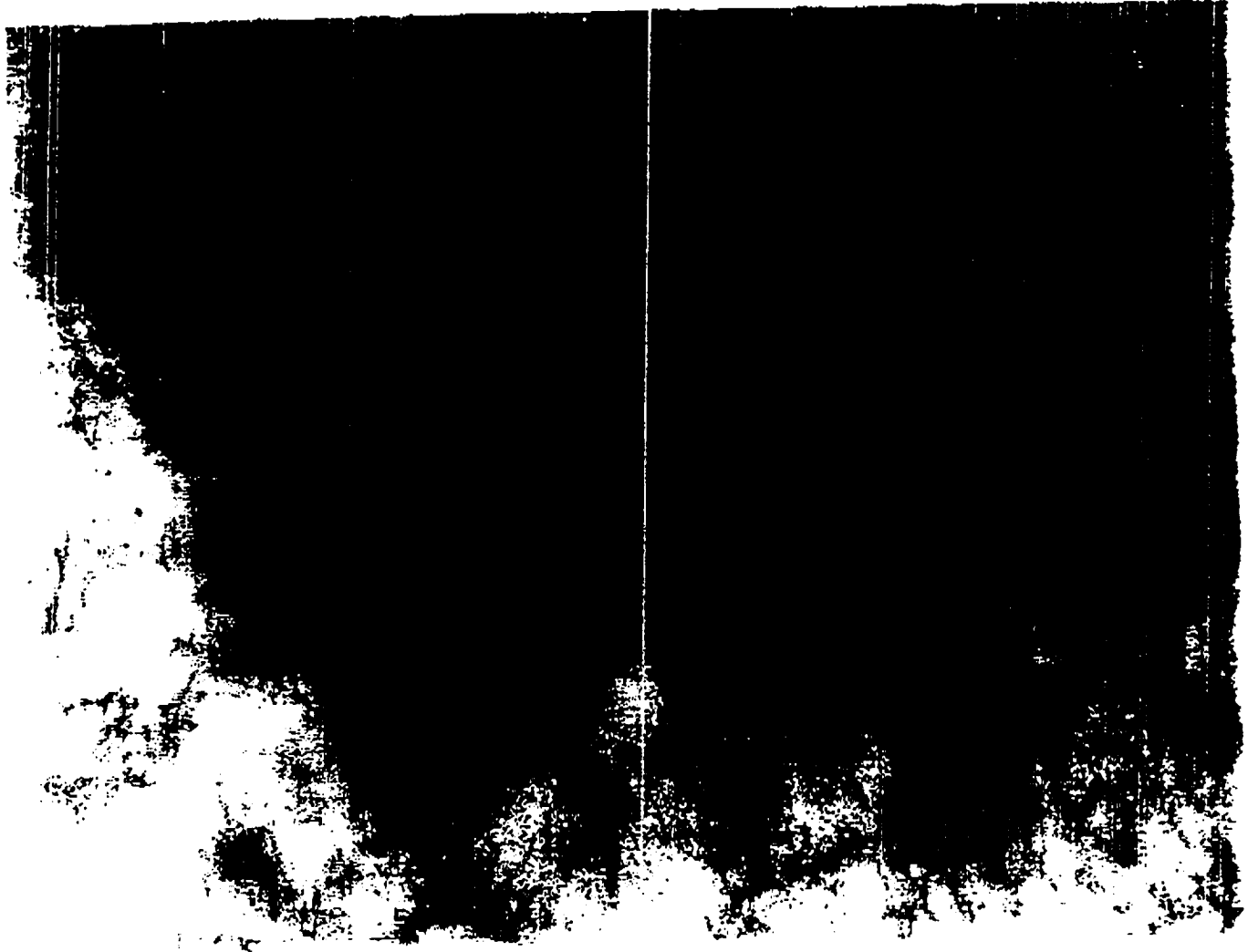
AUG-27-1995 09:36

DESMOS INC (619)495-3962

P. 17

CAPILLARY BLOOD PRESSURE IN THE LIP

948

FIG. 2. Micrograph of lower lip capillary network ($\times 40$).

RESULTS

Success of micropuncture and period of capillary pressure monitoring were limited by subject fatigue and a subsequent increase in involuntary movements. This procedure was performed without anesthesia and without pain in our 13 subjects. Pipette breakage occurred occasionally. Nevertheless, despite the technical difficulty of the procedure, micropressure recordings were obtained in 40 separate capillaries and 14 postcapillary venules (≥ 5 recordings of each vessel type in a single individual). We found that the capillary network within superficial tissues of the lower lip consisted of a branching capillary arcade (Fig. 2).

Capillary pressure in the lip was 33.2 ± 1.5 (SE) mmHg during the upright seated position, whereas mean lip venular pressure was 18.9 ± 1.6 mmHg. In the four subjects who had repeated cannulation of the same capillary, the coefficient of variation averaged 7.2% (Table 2). In all measurements, characteristic pulse-pressure tracings showing pulsatile flow corresponding with the cardiac cycle were obtained (Fig. 3A). Respiration had no effect

on the wave pattern. The resolution and magnitude of the pressure tracings did not allow for visualization of a dicrotic notch. In general, pulsatile pressure tracings from postcapillary venules were more attenuated than those obtained in capillary vessels (Fig. 3B). After considerable practice, each recording took ~ 5 –10 min, including set-up time, for a given subject.

Neither capillary pressure nor postcapillary venule pressure correlated significantly with systolic blood pressure, mean arterial blood pressure, diastolic blood pressure, or lip surface temperature.

TABLE 2. Reproducibility of blood pressure measurements within a given capillary vessel

| Subject No. | Capillary Pressure (mmHg) | Duration of Measurement (min) | Mean Capillary Pressure (mmHg) | Number of Measurements | Mean \pm SE | Coefficient of Variation |
|-------------|---------------------------|-------------------------------|--------------------------------|------------------------|-----------------|--------------------------|
| 1 | 31.6 | 26 | 33.6 | 27 | 33.6 \pm 1.41 | 4.0 |
| 2 | 18.1 | 64 | 48.5 | 22 | 26.5 \pm 1.4 | 6.8 |
| 3 | 52.7 | 16 | 43.1 | 11 | 27.2 \pm 1.7 | 14.2 |
| 4 | 33.0 | 25 | 31.8 | 17 | 32.6 \pm 1.67 | 5.3 |

Pc, capillary pressure.

AUG-27-1996 09:37

DESMOS INC (619)455-3962

P.18

CAPILLARY BLOOD PRESSURE IN THE LIP

949

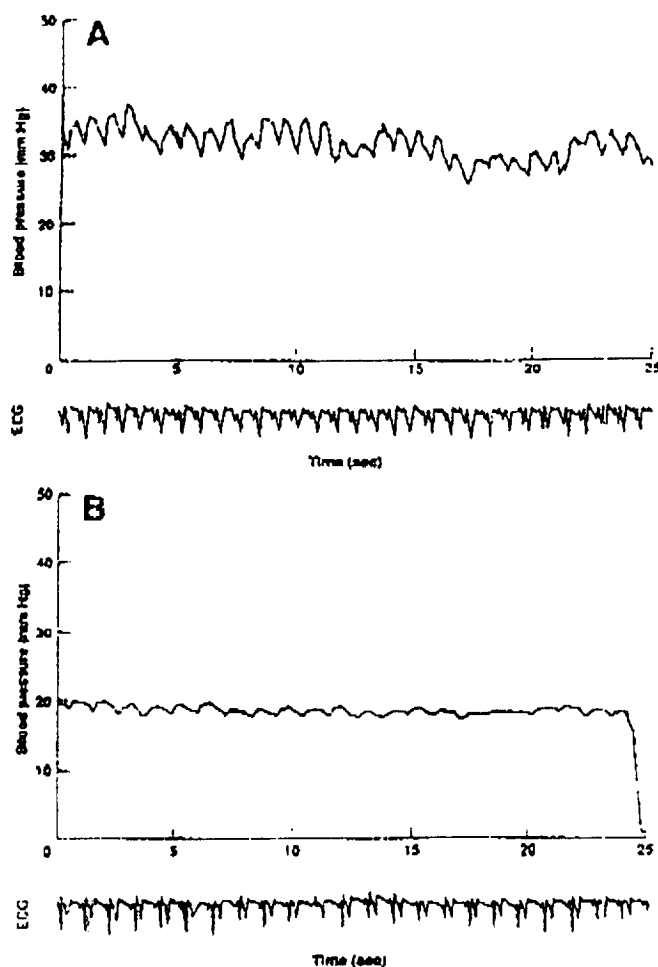


FIG. 3. Variation of microcirculatory pressures in lip with time. A: capillary pressure tracing with corresponding electrocardiographic (ECG) recording. B: postcapillary venule pressure tracing with corresponding ECG recording.

DISCUSSION

We have applied the servo-nulling micropressure system to measure dynamic microvascular pressures above heart level for the first time in human subjects. Continuous tracings were obtained with pulsatile pressures corresponding to the cardiac cycle. Although the technique is technically challenging, it provides painless, direct, and reproducible measurements in the lip capillary arcade. In a previous study (9), lip capillary pressure increased significantly during 6° head-down-tilt posture, a recognized model for microgravity.

The mean value of capillary pressure in the lip during upright posture was ~10 mmHg less than that measured by Levick and Michel (7) and Mahler et al. (8) in the fingernailfold placed at heart level. We observed that the vertical distance between the heart and lower lip, when our subjects were seated in pressure-recording position, was 10–15 cm, corresponding to an 8- to 12-mmHg blood pressure gradient. This hydrostatic pressure gradient explains the relatively lower pressure values we obtained compared with those in the finger. Furthermore, the basic structures of the fingernailfold capillary bed and the

lip capillary bed are substantially different. Whereas the lip mucosa is supported by a branching capillary arcade, the less vascular fingernailfold capillary bed consists of relatively large-caliber single loops.

Microvascular pressure was not related to body or room temperature over the temperature ranges involved in this study. Although arterial pressure provides the input force for propelling blood through downstream capillaries, capillary pressure did not correlate with blood pressure over the limited ranges of pressure in this study. Several possibilities exist to explain why there was no significant correlation between the measured microvascular pressures and systemic arterial pressures (systolic, mean, and diastolic blood pressures). Therefore, other factors are probably more important in modulating microvascular blood pressures in the lip. For example, regional nutritional and thermoregulatory requirements vary from individual to individual at any given time, resulting in variable capillary recruitment and regional perfusion (12). Also, precapillary sphincter and vasomotor activities are important regulators of microvascular pressure. Nonetheless, repeated punctures of the same vessel yielded fairly reproducible blood pressures.

Our pressure values are generally higher than those reported by Joyner and Davis (6) using vascular beds of small animals. However, the microvasculature of these small animals does not have to contend with great variations of local blood pressure due to height and posture. Other investigators who measured pressures in fingernailfold capillary beds at heart level have calculated capillary pressure in the head to be 25 mmHg (6). This calculation is based on the hydrostatic pressure difference between the heart and the head. Higher than expected capillary pressure may be necessary to maintain capillary perfusion and filtration in the head. Our results in this study agree with previous findings that reduction of capillary blood flow due to precapillary vasoconstriction may be less in tissues of the head than in tissues of the feet (1). With less precapillary vasoconstriction, peripheral vascular resistance in the head may shift toward the venules, resulting in increased capillary and postcapillary pressures, as observed in this study. Because these tissues are located 10–25 cm above heart level, a shift in vascular resistance from pre- to postcapillary vessels would benefit the metabolic demands of the surrounding tissue by providing adequate perfusion during episodes of decreased blood pressure.

In summary, we have developed a rapid, reliable, and reproducible means for measuring capillary and postcapillary venule pressures continuously for short periods of time in the human lip. Furthermore, to our knowledge, this is the first time that capillary pressures have been recorded above heart level. Compared with capillary pressures in the fingernailfold at heart level, the lower capillary pressures found in the lip are likely due to a hydrostatic pressure gradient between the heart and lip. This technique may find application in studies of transcapillary fluid regulation and microvascular disease.

The authors thank Dee O'Hara and Rick Ballard for their help with this project. We also thank Karen Hutchinson for assistance in manuscript preparation and Dr. Ralph Fellgr for subject monitoring.

This research was supported by National Aeronautics and Space Administration (NASA) Grant 199-14-12-04. S. E. Parazyński was supported by a NASA Graduate Student Fellowship and the Stanford Medical Scholars Program.

Present addresses: S. E. Parazyński, Astronaut Office (code CB), NASA-Johnson Space Center, Houston, TX 77068; B. J. Tucker, Dept. of Medicine, University of California, San Diego, La Jolla, CA 92093-6151.

Address reprint requests to S. E. Parazyński.

Received 20 March 1992; accepted in final form 29 July 1992.

REFERENCES

- ARATOW, M., A. R. HARGENS, J.-U. MEYER, AND S. B. ARNAUD. Postural responses of head and foot cutaneous microvascular flow and their sensitivity to bed rest. *Aviat. Space Environ. Med.* 62: 246-251, 1991.
- BRAAM, B., H. A. KOOMANS, T. J. RABELINK, AND R. BERCKMANS. Pitfalls of venous occlusion for determination of capillary pressure in humans. *J. Appl. Physiol.* 66: 997-1002, 1989.
- HARGENS, A. R., J. B. COLOGNE, F. J. MENNINGER, J. S. HOGAN, B. J. TUCKER, AND R. M. PETERS. Normal transcapillary pressures in human skeletal muscle and subcutaneous tissues. *Microvasc. Res.* 22: 177-189, 1981.
- INTAGLIETTA, M., R. F. PAWULA, AND W. R. TOMPKINS. Pressure measurements in the mammalian microvasculature. *Microvasc. Res.* 2: 212-220, 1970.
- JOYNER, W. L., AND M. J. DAVIS. Pressure profile along the microvascular network and its control. *Federation Proc.* 46: 266-289, 1987.
- LANDIS, E. M. The capillary pressure in frog mesentery as determined by micro-injection methods. *Am. J. Physiol.* 75: 648-670, 1926.
- LEVICK, J. R., AND C. C. MICHEL. Capillary pressures in the fingers and toes. *J. Physiol. Lond.* 274: 97-109, 1978.
- MAHLER, F., M. H. MUNDIM, M. INTAGLIETTA, A. BOLLINGER, AND M. ANLIKER. Blood pressure fluctuations in human nailfold capillaries. *Am. J. Physiol.* 236 (Heart Circ. Physiol. 5): H888-H893, 1979.
- PARAZYNSKI, S. E., A. R. HARGENS, B. TUCKER, M. ARATOW, J. STYF, AND A. CRENSHAW. Transcapillary fluid shifts in tissues of the head and neck during and after simulated microgravity. *J. Appl. Physiol.* 71: 2469-2476, 1991.
- PERRY, M. A., J. O. COLEBATCH, W. E. GLOVER, AND I. C. RODDIE. Measurement of capillary pressure in humans using a venous occlusion method. *J. Appl. Physiol.* 60: 2114-2117, 1986.
- RAPPAPORT, M. B., E. H. BLOCH, AND J. W. IRWIN. A manometer for measuring dynamic pressures in the microvascular system. *J. Appl. Physiol.* 14: 651-656, 1959.
- SALERUD, E. G., T. TENLAND, G. E. NILSSON, AND P. A. OBERG. Rhythmical variations in human skin blood flow. *Int. J. Microcirc. Clin. Exp.* 2: 91-102, 1983.
- SOKAL, R. R., AND F. J. ROHLF. *Biometry: The Principles and Practice of Statistics in Biological Research*. San Francisco, CA: Freeman, p. 82-83, 1969.
- TUCKER, B. J., AND R. C. BLANTZ. Factors determining superficial nephron filtration in the mature, growing rat. *Am. J. Physiol.* 232 (Renal Fluid Electrolyte Physiol. 1): F97-F104, 1977.
- WIEDERHIELM, C. A., J. W. WOODBURY, S. KIEH, AND R. F. RUSHMER. Pulsatile pressure in the microcirculation of the frog's mesentery. *Am. J. Physiol.* 207: 173-176, 1964.
- WILLIAMS, S. A., S. WASSERMAN, D. W. RAWHINSON, R. I. KITNEY, L. A. SMAJE, AND J. E. TOOKE. Dynamic measurement of human capillary blood pressure. *Clin. Sci. Lond.* 74: 507-512, 1988.
- ZWEIFACH, B. W. Quantitative studies of microcirculatory structure and function. I. Analysis of pressure distribution in the terminal vascular bed. II. Direct measurement of capillary pressure in splanchnic mesenterics. *Circ. Res.* 34: 843-868, 1974.