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Effects of hindlimb suspension and elevated CO2 on rat growth and renal function

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EFFECTS OF HINDLIMB SUSPENSION AND ELEVATED CO_2 ON RAT GROWTH AND RENAL FUNCTION

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ABSTRACT

EFFECTS OF HINDLIMB SUSPENSION AND ELEVATED CO_2 ON RAT GROWTH AND RENAL FUNCTION

Studies show that an ambient CO_2 concentration of 2.0% may interfere with animal experiments conducted in spaceflight, while 0.7% CO_2 exposure produces minimal effects. With additional spaceflight factors, such as microgravity, effects from the 0.7% CO_2 exposure may be amplified. To investigate the combined effects from microgravity and elevated CO_2 on growth and renal function, two groups of rats were hindlimb suspended (HLS) for 37 days, and a third group served as ambulatory vivarium controls. One suspension group was exposed to 0.7% CO_2 for 30 days, while the other groups breathed room air. HLS group showed effects consistent with past studies. When comparing experimental animals to suspended controls, exposure to CO_2 revealed lowered food consumption, increased urine output, urine NH_3 and CO_2 excretion. This study shows that chronic exposure to 0.7% ambient CO_2 and hindlimb suspension have little effect on rat growth and renal function.

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INTRODUCTION

Considerable research has been performed describing the effects of microgravity on mammals. Use of ground-based microgravity simulation models have facilitated our understanding of gravitational effects on mammals. Introduced by Morey-Holton and Wronski (21), hindlimb suspension (HLS) is a popular model used to simulate microgravity in rats by elevating the caudal end of the animal. It mimics microgravity in regard to fluid volume shifts (4,11,21,33,35,36), loss of bone mass (20,21,25,33,38,41) and muscle and tissue atrophy (10,16,21,22,33) and serves as a practical model to understand microgravity environments (such as spaceflight) and bedrest (4,19,21,33). HLS has also been shown to alter renal function (4,35,36,37).

In addition to microgravity, other factors play a role in the spacecraft environment that may significantly impact mammalian physiology. One such factor is elevated ambient carbon dioxide. Groups ranging from environmentalists to the military are concerned with the effects of elevated carbon dioxide on animals, and in particular, on humans (5,7,18,26,31,32). Elevated ambient carbon dioxide concentrations are of particular concern in confined areas such as airplanes, submarines, and manned spacecraft (6,23,30,40). The Space Shuttle and the Russian Space Station MIR have reported mean carbon dioxide concentrations of 0.3%, which is ten times that of normal ambient air (6,23). There have been extended periods with 0.7% carbon dioxide with peak concentrations at 2.0%. The International Space Station program has proposed that carbon dioxide concentrations be maintained, on average, at 0.7%, and not to exceed 1.0% (6,7,23,40). Concerns have been raised regarding the chronic impact of 0.7% carbon dioxide exposure on the physiological systems of rats during flight experiments on the

Space Station, since rats have been the primary experimental animal model for studies involved in the responses of living systems to spaceflight.

Many studies have been published describing the effects of elevated carbon dioxide on rats (1,13,14,15,29,31). It has been shown that an ambient carbon dioxide concentration of 2.0% can produce changes in acid-base balance and renal function in ambulatory rats (13). Urine pH values initially dropped, then increased throughout the remainder of the 30 day study, indicative of induced respiratory acidosis followed by compensatory metabolic alkalosis. Animals exhibited diuresis along with increased excretion of calcium, ammonia and carbon dioxide. Results from a 0.7% carbon dioxide exposure revealed attenuated effects, showing slight increases in urine volume and excretion of ammonia and carbon dioxide (13). Urine pH values did not drop initially, but showed an increase corresponding to the 2.0% carbon dioxide exposure. The rats were capable of renal compensation to maintain acid-base homeostasis in the presence of the elevated carbon dioxide and showed slight decreases in food consumption with no significant effects on weight gain.

Though the responses to elevated ambient carbon dioxide and microgravity have been extensively studied independently, it is important to consider both components simultaneously to gain a more accurate account of what occurs during spaceflight. The present study was designed to investigate the impact of elevated ambient carbon dioxide on growth and renal function in rats in a simulated microgravity environment (hindlimb suspension). The current recommended Space Station average exposure limit of carbon dioxide (0.7%) will be tested in conjunction with simulated microgravity. It is expected that with the introduction of hindlimb suspension, the ability to compensate for respiratory acidosis will be further compromised, greatly exaggerating the effects from 0.7% carbon dioxide alone.

MATERIALS AND METHODS

This experiment was approved by the NASA-Ames Animal Care and Use Committee (Protocol #95-017) and conducted at NASA-Ames Research Center in compliance with the National Research Council Guideline for the Care and Use of Laboratory Animals (24).

Animals: Thirty-six male Sprague Dawley albino rats (75 ± 5 g; mean±SEM, body weight) from Simonsen Laboratories (Gilroy, CA) were housed in pairs in standard vivarium cages, maintained on a 12:12 hour light-dark cycle (fluorescent lights on at 6 am), and provided Purina (St. Louis, MO) rat chow pellets (Diet #5012) and water *ad libitum* and were allowed to acclimate for four days prior to being placed in individual metabolic cages (12).

Hindlimb suspension: Allowing four days for shipping adaptation and another four days to adapt to the metabolic cages, twenty-four animals (136 ± 1.5 g; mean±SEM, body weight) were suspended by the procedure of Morey-Holton et al. (21), then acclimated to suspension for an additional seven days. The remaining twelve animals served as ambulatory controls in metabolic cages to confirm suspension effects. Animal weights were monitored daily and twenty animals were selected for the study from the pool of twenty-four suspended animals based on acclimation to suspension as indicated by a steady weight gain and durability of their harnesses. Ten animals were selected to serve as ambulatory control animals to asses the effect of HLS. During the study, Purina powdered rat chow (Diet #5012) and tap water were provided *ad libitum.*

Experimental protocol: Suspended animals were then divided into two suspension groups of ten rats each with paired weight distributions. The designated experimental animals (HLS, 0.7% CO_2), suspended in their metabolic cages, were placed in an environmental chamber, 4' W x 10' L x 4' H (Kent Scientific, Litchfield, Connecticut). The internal environment of the chamber was regulated for 0.7% CO_2 and 20.9% O_2 with gas sensors (Fuji Electronics, Orange, California and Teledyne Electronics, City of Industry, California, respectively) and data recorded with a data acquisition program (Strawberry Software, Sunnyvale, California) on a Macintosh Quadra 650 computer system (Apple Computer, Cupertino, California). Daily temperature (°C) and relative humidity (RH%) were also recorded for the chamber in 20 minute intervals by the environmental chamber system (Kent Scientific, Litchfield, CT), and also for room air by hand held monitors (Fisher Scientific, Pittsburgh, PA), recorded daily during animal maintenance. The suspension control animals (HLS) were kept outside of the environmental chamber and exposed to ambient room air (0.03% CO_2), along with the ambulatory control animals (AMB). Access to only one environmental chamber limited the ability to run a concurrent elevated CO_2 control group. A previous study detailing effects from elevated CO_2 concentrations alone will provide a comparison (13). Though a chamber control (doors opened to room air) versus ambient control experiment was never performed to assess potential effects from the chamber itself, data from a previous study indicated minimal chamber effects (13). Temperature readings within the chamber remained similar to the ambient room air; chamber 25.1 ± 0.01 °C (mean ± SEM), room air 25.2 ± 0.13 °C. However, humidity within the chamber (when doors were closed) was nearly twice that of room air; chamber 64.4 ± 0.24 %, room air 36.6 ± 1.16 %.

Both suspension groups were suspended for a total of 37 days. The experimental phase began immediately following seven days of HLS adaptation. Animals from the

experimental group were then exposed to 0.7% carbon dioxide for 30 days. Daily measurements were made for the last three days of suspension adaptation (for baseline data to adjust for any initial group differences) and the first ten days of the experimental phase. Thereafter, data were collected every 3-4 days. The chamber was exposed to room air for 5-10 minutes on these days to allow for measurements and collection. These measurements included food and water consumption, urine and fecal production, and body weights. In addition, urine pH, osmolality, electrolytes (sodium, potassium, calcium, phosphorus and creatinine), hormones (corticosterone and aldosterone) and gases (total urine carbon dioxide and ammonia) were measured for every collection day urine sample.

Tissue and plasma collection: Immediately following data collection on day 30 of the experimental phase, each rat was weighed and given a health check prior to being anesthetized with methoxyfluorane (Pittman Moore, Mundelein, Illinois). Once anesthetized, anaerobic blood samples were drawn from the descending aorta using a heparinized (lithium heparin) 6 cc syringe with a 19 g needle. Part of the sample was injected into two 5 cc lithium heparin vacutainers and placed in ice. Each rat was then decapitated and organs (heart, thymus, lung, kidneys, spleen, liver, adrenals, epididymal fat pads, testes, and right femur) were collected and weighed. Blood samples collected during the dissection were analyzed for pH, plasma osmolality, plasma electrolytes (sodium, potassium, calcium, phosphorous and creatinine), plasma hormones (corticosterone and aldosterone), bicarbonate and blood gases (ammonia and partial and total carbon dioxide).

Sample Analyses: Urine pH was measured using a Corning pH Meter 340 (Corning, New York). Blood gases and plasma pH were analyzed using AVL 995 Blood Gas Analyzer (Roswell, Georgia). Urine and plasma osmolality were measured using a

Fiske 2400 Osmometer (Norwood, Massachusetts). A Roche COBAS MIRA (Branchburg, New Jersey) was used to measure urine electrolytes and total urine gases and plasma electrolytes. Urine and plasma corticosterone and aldosterone were measured using a double antibody kit from ICN Biomedicals (Costa Mesa, California) and radioimmunoassay kit from Diagnostic Products Corporation (Los Angeles, California), respectively.

Statistical analyses: Body and fecal weights, food and water consumption, urine volume, pH, osmolality, creatinine, electrolytes, gases, and hormones were compared by two-way ANOVA for group (between subjects) and time (repeated) factors using Statistica (Tulsa, OK). Tissues, organs, and plasma and blood chemistries were compared by unpaired t-tests. A p-value of ≤ 0.05 was considered statistically significant. All values are presented as means $\pm$ SEM.

RESULTS

Hindlimb suspended animals showed similar results to previous studies, revealing significant differences in many of the measured parameters compared to non-suspended controls (Table 1). Since the AMB group was used primarily to confirm suspension effects, this group will not be discussed further. The following results compare the experimental group (HLS, 0.7% CO_2) with the suspension controls (HLS) to examine the effects of elevated carbon dioxide exposure to hindlimb suspended animals.

Growth: Both groups had steady growth rates over the thirty day period. Weight gain between the two groups remained identical throughout the experiment, averaging 3.5 g/day from day 1 to day 30 (Figure 1). Though food consumption was significantly lower in the experimental animals, fecal production remained similar between groups (Figure 1). Experimental animals also showed greater urine production over the control group, with no differences noted in water intake or urine osmolality (Figure 2). Thus, exposed animals revealed an increased net water flux. There were no significant differences in tissue weights between the exposed and control animals (Table 2).

Blood Samples: Exposed animals had significantly higher plasma carbon dioxide levels when compared to the control animals (Table 3). There were no significant differences in whole blood gases or chemistries between groups (Table 4).

Renal Function: Urinary pH values for both groups were slightly alkalotic throughout the study, averaging 7.7 ± 0.02 for the experimental group and 7.7 ± 0.04 for the control group, with no significant difference between groups (Table 5, Figure 3). Sodium, potassium, calcium, inorganic phosphorous and creatinine excretion remained

consistent, also showing no statistically significant differences between groups (Table 5). However, general increases in sodium, potassium and creatinine excretion from baseline values were observed in both groups over the course of the experiment.

Urine osmolality also increased throughout the study, with no significant differences noted between groups (Figure 2). In the control group, calcium excretion decreased significantly from baseline values by day 30, while the experimental group only reflected a decreasing trend, with no significant differences between groups (Table 5).

Though aldosterone and corticosterone excretion increased throughout the experiment, there were no significant differences between treatments (Table 5). When looking at the urine gases, exposed animals revealed a significantly elevated carbon dioxide and ammonia excretion over controls (Figure 3). Ammonia and carbon dioxide excretion for the experimental group increased significantly from baseline values, while control excretions did not indicate significant increases by the end of the experiment.

DISCUSSION

Expected Results from Hindlimb Suspension

The hindlimb suspension model serves as an effective ground based model to study the effects of microgravity on rodent physiology (21,33). It mimics spaceflight in various aspects, ranging from muscular-skeletal changes to fluid hemodynamics. Some of the most notable changes associated with this model are decreased body weight, decreased muscular and skeletal mass, and alterations in fluid balance (16,19,20,22,27,37,41). Similar changes were observed in both groups of suspended animals in our experiment relative to ambulatory controls (See Table 1). Tissue atrophy and a noticeable decrease in food consumption appears to be the most probable explanations for weight loss, since no significant differences were observed for rate of growth (See Table 1). It has been well documented that there is a dramatic decrease in bone and muscle weights in association with suspension due to atrophy from disuse (16,20,22,25,27,38,41). Also, it has been reported that suspension causes significant decreases in gross organ mass, e.g.; heart and testes (3,8,10). It is interesting to note, that when comparing normalized organ masses (per 100g body weight) in the present study, the heart is conversely heavier in the suspended animals. This trend showing an increased heart mass due to head-down tilt is supported in another paper examining arterial morphology in tail suspended rats (2). It is possible that the increased hydrostatic pressure resulting from the head-down tilt resulted in increased cardiac work and therefore cardiac hypertrophy.

Hindlimb unloading has also been associated with the deconditioning of bone, eventually leading to an increase in calcium excretion (20,25). Calcium balance studies have shown a reduction in net absorption of calcium across the intestine averaged over a 14 day suspension period, further supporting the expected increase in calcium excretion and deconditioning of bone (20,25). Renal function can also be affected, influencing filtration

rates and therefore, excretion rates (4,35,36,37). The cephalic fluid shift induced by the head-down tilt would result in an initial "sensed" hypervolemia followed by appropriate countermeasures, thus resulting in changes in filtration and excretion rates. Studies have also shown a decrease in blood P_{CO2} during the first weeks of suspension (34).

Expected Results from Elevated Carbon Dioxide Exposure

Elevated ambient carbon dioxide can significantly alter rodent physiology. Chronic high exposure results in a reduction in food consumption, possibly due to the lethargic effects of the increased carbon dioxide concentrations (13). Chronic high exposure may also result in respiratory acidosis and eventually compensatory metabolic alkalosis (18,28,31). Compensation includes the formation of excess ammonia and bicarbonate ion to serve as buffers and also release of calcium from bone stores into the blood and eventually into the urine (1,9,13,14,15,29). There is also a marked drop in urine pH followed by a gradual increase over the thirty day period indicative of compensatory mechanisms. Significant increases are also noted in potassium, creatinine and corticosterone excretion. Chronic high carbon dioxide exposure results in both elevated blood and urine carbon dioxide concentrations (13).

At a more moderate level of gas exposure (0.7%), previous studies have shown similar increases in urine production and a gradual rise in urine pH without the initial dip observed at higher exposure levels (13). Urinary carbon dioxide excretion is elevated without significant changes in other parameters, such as ammonia, calcium, sodium, potassium, creatinine, or corticosterone excretion. Rats exposed to the elevated ambient carbon dioxide showed greater urine production when compared to respective controls, yet there were no differences in water consumption. A possible explanation for this increased net water flux lies in the elevated humidity within the experimental chamber. Humidity was, on average, 20% higher in the environmental chamber than in the room where the

controls were housed (both the ambulatory controls and the hindlimb suspended controls). The chronic exposure to elevated ambient carbon dioxide resulted in an elevated blood concentration, and by the elevated urinary excretion. A moderate elevated exposure of carbon dioxide appears to stimulate metabolic compensatory mechanisms, as evidenced by the gradual rise in pH and the formation of ammonia, but does not appear to utilize the buffer supply, resulting in excess ammonia found in the urine. It is thought that at the more moderate level of (0.7%) exposure, rats are capable of compensation to the respiratory acidosis without decalcification of bone.

Expected Combined Effects (Elevated CO_2 and suspension relative to suspension controls)

In considering the spaceflight environment for long term animal housing, it is imperative to consider all elements involved. Microgravity and elevated ambient carbon dioxide concentrations are major factors that may compromise the integrity of science experiments conducted in space, and may even pose a threat to the health and safety of crew members (6,7,17,20,23,39,40). It appears that rodents exposed to moderate elevated levels of ambient carbon dioxide are capable of metabolic compensation, showing slight adjustments in renal function (9,13,14,15,18,31). Additionally, hindlimb suspension has been shown to alter renal function (4,35,36,37). Combining elevated ambient carbon dioxide and hindlimb suspension, we expected the animals' ability to compensate for the induced respiratory acidosis to be compromised, thus resulting in exaggerated effects similar to those observed following higher levels (2%) of carbon dioxide exposure alone. Relative to suspended controls, animals exposed to the moderate levels of gas (0.7%) should exhibit a relative increase in urine production. Carbon dioxide excretion should be elevated and urinary excretion of ammonia and calcium may increase due to the altered renal handling of electrolytes from suspension effects. Urine pH may exhibit a marked drop initially, as observed at the 2.0% level, indicating respiratory acidosis without the

immediate ability to compensate. Also, food consumption may be reduced due to the lower animal weights from suspension and the lethargic effects from exposure to elevated carbon dioxide.

Actual Results and Explanations

There appears to be no difference in growth or well-being due to elevated ambient carbon dioxide exposure to hindlimb suspended rats, as indicated by their stable growth rates (See Figure 1) . Experimental (HLS, 0.7% CO_2) animals exhibited the expected trend toward lowered food consumption, indicative of apathy due to elevated carbon dioxide concentrations. However, animal activity was not quantitated in this study. The reduced food consumption with no change in weight gain implies a more efficient metabolism. The similar normalized tissue weights between experimental and control groups support the idea of continued well-being of animals exposed to 0.7% carbon dioxide (See Table 2).

As observed in previous experiments at 0.7% CO_2, the exposed group exhibited an increased urine production. Assuming that the animals' respiration rates adapted to the increased carbon dioxide concentrations, then the presumed decrease in activity correlates to decreased respiration and consequently reduced water loss from respiration. The elevated humidity in the chamber may also play a role in limiting the amount of insensible water loss through evaporation, as previously mentioned. This retention of water may contribute to the increased urine volume without concomitant increased water intake. Unfortunately, as with activity, minute ventilation was not monitored.

Experimental (HLS, 0.7% CO_2) animals showed an expected increase in plasma and urine carbon dioxide concentrations from the 0.7% ambient exposure. Increased ammonia excretion from experimental animals correlated with compensatory metabolic alkalosis observed in previous elevated carbon dioxide studies, where the higher concentrations of carbon dioxide may stimulate ammonia production providing a supply of

buffer for the excess hydrogen ions from the respiratory acidosis (9,13). Since adequate compensation seemed to occur without the depletion of the buffer supply, residual ammonia appeared in the urine. The experimental group did not exhibit the expected increase in urine calcium excretion when compared to the suspended control group. It is possible that the majority of the calcium was excreted via the feces, however this was not measured.

With only minor changes produced by a 0.7% ambient carbon dioxide exposure alone, we expected the additional strain of hindlimb suspension on the renal function in the rats to induce results more similar to the previous positive control (2.0% exposure). An examination of the effects from elevated exposure of carbon dioxide at 2.0% and 0.7% on ambulatory animals from a previous study versus the 0.7% exposure to hindlimb suspended animals from this study revealed minor differences at the 0.7% exposure (Table 6). With the addition of hindlimb suspension, experimental animals exposed to a 0.7% carbon dioxide concentration were expected to show an amplified effect from the elevated gas exposure, focusing on indices such as urinary electrolyte excretions. Though hindlimb suspension had significant effects on animals, showing results consistent with data from past hindlimb suspension studies, the effects from the 0.7% carbon dioxide exposure were not exacerbated significantly, as initially expected.

This study indicates that the addition of hindlimb suspension does not appear to compromise the rats' ability to compensate for the induced respiratory acidosis produced by chronic exposure to ambient 0.7% carbon dioxide. The current proposed upper limit for ambient carbon dioxide (0.7%) in conjunction with the microgravity environment does not appear to adversely impact science experiments using rats, and should not jeopardize studies conducted aboard the International Space Station.

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APPENDIX

TABLE 1. Response to hindlimb suspension, a comparison of suspended animals (HLS, n=10) and ambulatory controls (AMB, n=10) over the thirty-seven suspended days. Values represent group means±SEM. Normalized values are represented as per 100 grams of body weight.
* Denotes a significant difference to AMB ($p \leq 0.05$).

	AMB	HLS
Body weight gain (g/day)	5.0 ± 0.1	3.8 ± 0.1 *
Food consumption (g/day)	18.6 ± 0.2	16.6 ± 0.2 *
(normalized value)	5.4 ± 0.03	5.4 ± 0.04
Fecal output (g/day)	6.5 ± 0.2	5.6 ± 0.1 *
(normalized value)	1.9 ± 0.06	1.8 ± 0.04
Water intake (ml/day)	35.6 ± 0.8	30.1 ± 1.0 *
(normalized value)	10.3 ± 0.24	9.9 ± 0.30
Urine output (ml/day)	12.9 ± 0.5	9.7 ± 0.6 *
(normalized value)	3.7 ± 0.15	3.2 ± 0.21 *
Right femur weight (g)	0.823 ± 0.011	0.661 ± 0.012 *
(normalized value)	0.256 ± 0.002	0.241 ± 0.003 *
Testes weight (g)	3.457 ± 0.088	1.039 ± 0.022 *
(normalized value)	1.078 ± 0.029	0.380 ± 0.006 *
Heart weight (g)	1.070 ± 0.010	0.957 ± 0.022 *
(normalized value)	0.334 ± 0.005	0.349 ± 0.004 *

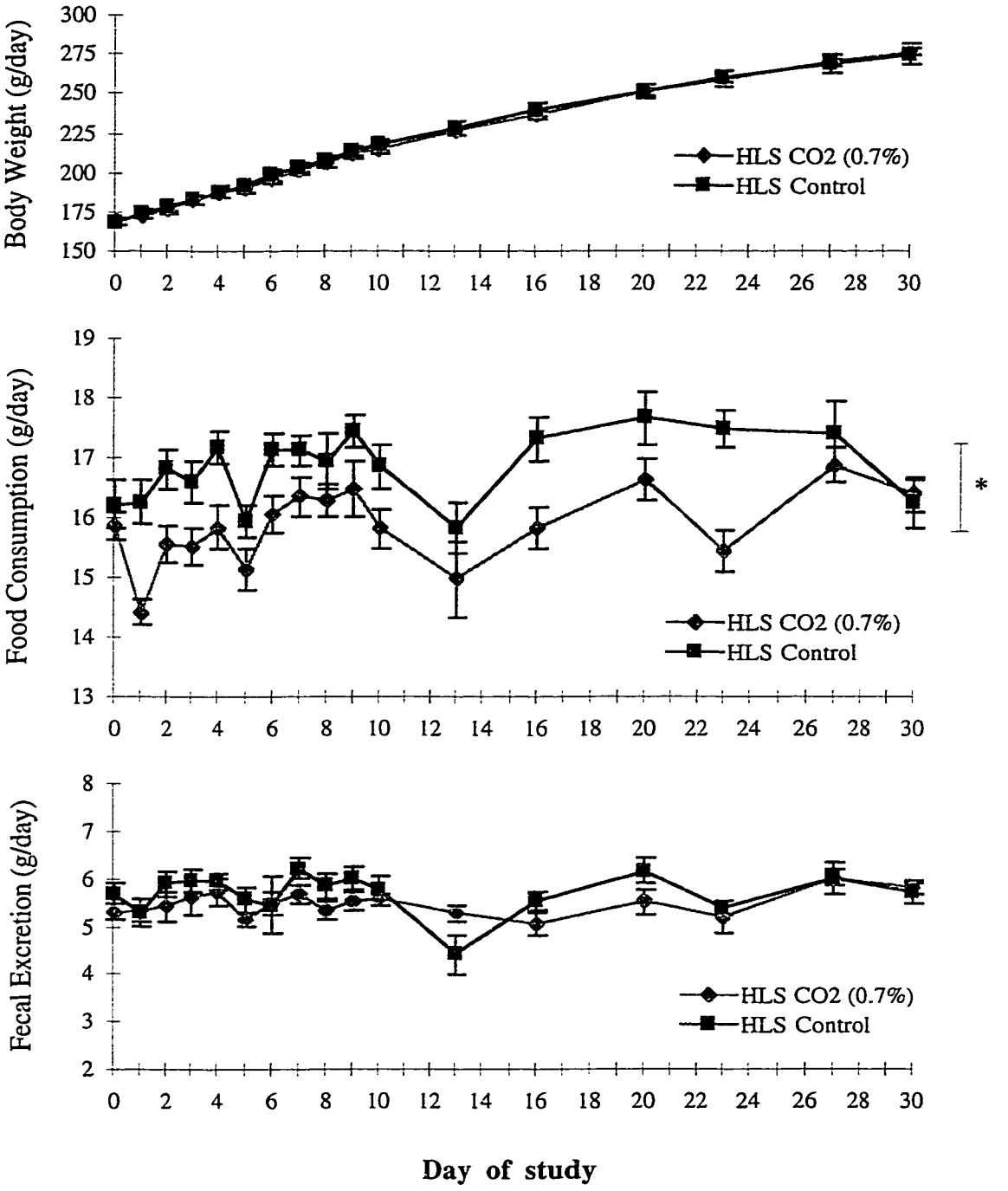


FIG. 1. Daily body weights, food consumption, and fecal excretion for experimental animals (HLS CO_2 0.7%) vs. control animals (HLS Control) over the experimental period. Values are group means±SEM.

* Significant group difference.

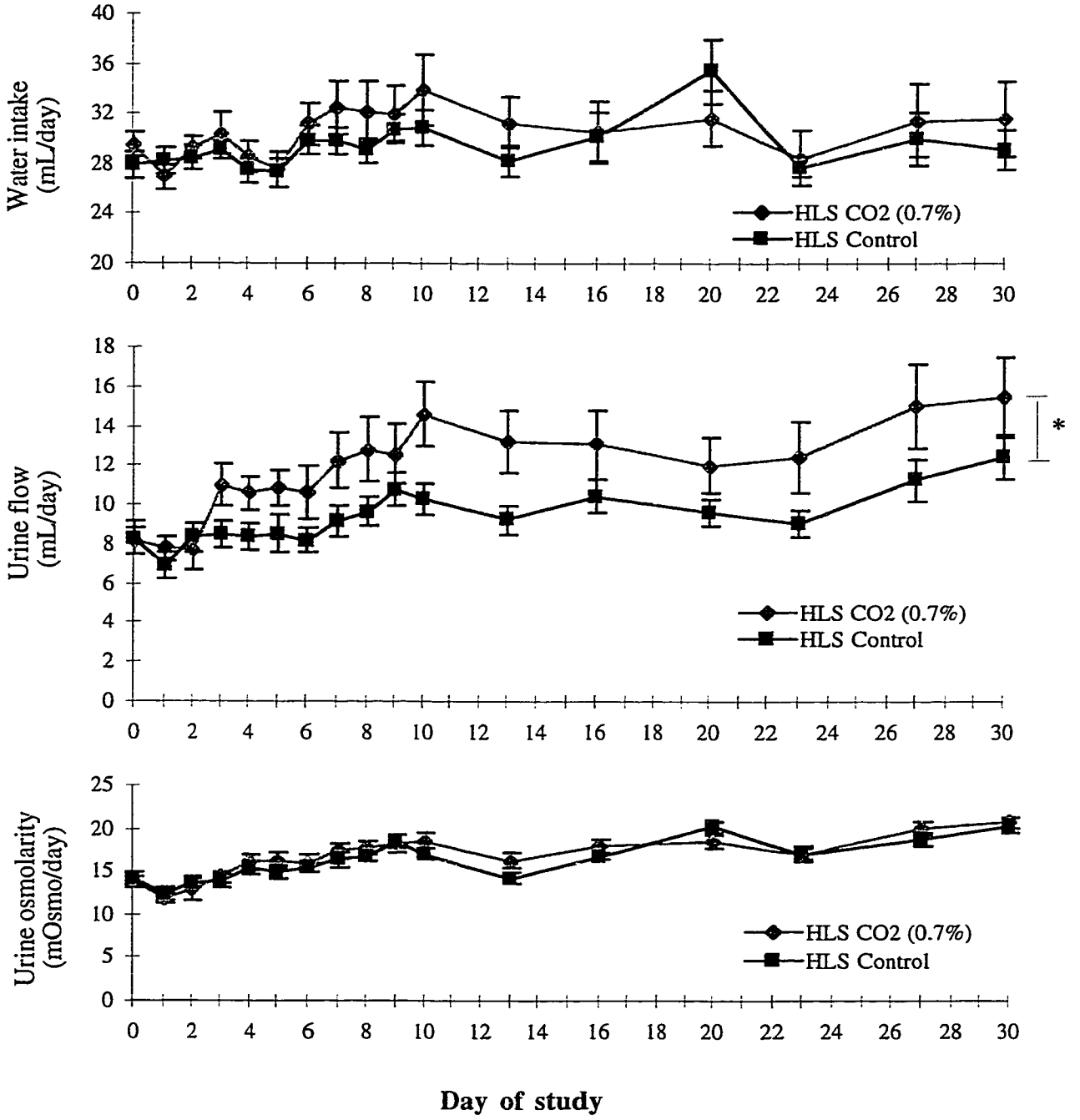


FIG. 2. Daily water intake, urine flow and urine osmolality for experimental animals (HLS CO_2 0.7%) vs. control animals (HLS Control) over the experimental period. Values are group means±SEM.

* Significant group difference.

TABLE 2. Comparison of absolute organ weights for experimental animals (HLS CO_2 0.7%) vs. Control animals (HLS Control) at the end of exposure, day 30 of the experimental period. Values are group means±SEM. Normalized values are not presented due to the lack of difference in body weights.

	HLS Control	**HLS CO_2 (0.7%)**
Body weight (g)	273.9 ± 5.82	275.6 ± 1.52
Heart (g)	0.957 ± 0.022	0.994 ± 0.017
Thymus (g)	0.532 ± 0.018	0.526 ± 0.020
Lung (g)	1.265 ± 0.032	1.236 ± 0.043
Kidneys, pair (g)	1.940 ± 0.049	1.953 ± 0.053
Spleen (g)	0.582 ± 0.021	0.610 ± 0.022
Liver (g)	9.153 ± 0.212	9.310 ± 0.148
Adrenals, pair (g)	0.041 ± 0.001	0.042 ± 0.002
E. fat pads, pair (g)	2.375 ± 0.071	2.501 ± 0.144
Testes, pair (g)	1.039 ± 0.022	1.051 ± 0.028
Femur, right (g)	0.661 ± 0.012	0.670 ± 0.013

TABLE 3. Comparison of arterial plasma chemistries for experimental (HLS CO_2 0.7%) vs. control animals (HLS Control) at the end of exposure, day 30 of the experimental period. Values are group means±SEM.

* Significant difference compared to control.

	HLS Control	HLS CO_2 (0.7%)
Sodium (mmol/l)	140.4 ± 0.8	141.7 ± 0.5
Potassium (mmol/l)	5.6 ± 0.1	5.3 ± 0.1
Calcium (mg/dl)	8.2 ± 0.2	8.4 ± 0.4
Phosphorous (mg/dl)	7.1 ± 0.2	6.8 ± 0.3
Creatinine (mg/dl)	0.38 ± 0.01	0.39 ± 0.02
Osmolarity (mOsmo/l)	294.3 ± 2.1	290.2 ± 0.9
Carbon Dioxide (mmol/l)	23.38 ± 0.61	25.41 ± 0.56 *
Ammonia (umol/l)	258.8 ± 93.2	226.6 ± 70.2
Corticosterone (ng/ml)	63.0 ± 13.5	63.5 ± 12.2
Aldosterone (pg/ml)	219.4 ± 51.3	307.3 ± 61.4

TABLE 4. Comparison of arterial whole blood gases and chemistries for experimental animals (HLS CO_2 0.7%) vs. control animals (HLS Control) at the end of exposure, day 30 of the experimental period. Values are group means±SEM.

	HLS Control	**HLS CO_2 (0.7%)**
pH	7.41 ± 0.02	7.40 ± 0.01
Base Excess (mmol/l)	-0.74 ± 0.29	-0.07 ± 0.62
HCO_3 (mmol/l)	23.0 ± 0.7	24.0 ± 1.0
pCO_2 (mmHg)	38.4 ± 2.4	40.4 ± 2.5
Total CO_2 (mmol/l)	24.1 ± 0.8	25.2 ± 1.0
pO_2 (mmHg)	100.9 ± 2.6	85.7 ± 7.6

TABLE 5. Comparison of urine parameters for experimental animals (HLS CO_2 0.7%) vs. control animals (HLS Control) at baseline^ and at the end of exposure, day 30 of the experimental period. Values are group means±SEM.

^ Baseline values are average of three days prior to exposure
† Significant difference compared to baseline.
* Significant difference compared to control.

	Time period	HLS Control	HLS CO_2 (0.7%)
pH	Baseline	7.7 ± 0.3	7.7 ± 0.3
	Day 30	8.1 ± 0.3	8.0 ± 0.3
Osmolarity (mOsmos/day)	Baseline	14.7 ± 0.7	14.3 ± 0.5
	Day 30	20.4 ± 0.9 †	20.9 ± 0.6 †
Sodium (mmol/day)	Baseline	1.3 ± 0.1	1.2 ± 0.0
	Day 30	1.5 ± 0.1 †	1.6 ± 0.0 †
Potassium (mmol/day)	Baseline	3.0 ± 0.1	2.9 ± 0.1
	Day 30	3.3 ± 0.1 †	3.5 ± 0.1 †
Calcium (mg/day)	Baseline	1.9 ± 0.2	1.6 ± 0.2
	Day 30	0.9 ± 0.1 †	0.9 ± 0.1 †
Phosphorous (mg/day)	Baseline	13.4 ± 1.3	12.8 ± 1.3
	Day 30	15.5 ± 1.3	14.5 ± 0.7
Creatinine (mg/day)	Baseline	6.2 ± 0.3	6.1 ± 0.2
	Day 30	10.6 ± 0.4 †	11.0 ± 0.3 †
Corticosterone (ng/day)	Baseline	132.7 ± 22.1	127.3 ± 25.0
	Day 30	287.4 ± 42.1 †	312.8 ± 36.3 †
Aldosterone (ng/day)	Baseline	9.9 ± 0.7	10.6 ± 1.5
	Day 30	14.8 ± 1.3 †	15.8 ± 2.1 †
Carbon Dioxide (umol/day)	Baseline	291.5 ± 100.5	285.2 ± 109.0
	Day 30	449.9 ± 88.6 †	604.3 ± 184.6 † *
Ammonia (umol/day)	Baseline	198.9 ± 14.8	193.6 ± 16.1
	Day 30	281.3 ± 33.0 †	343.6 ± 59.4 † *

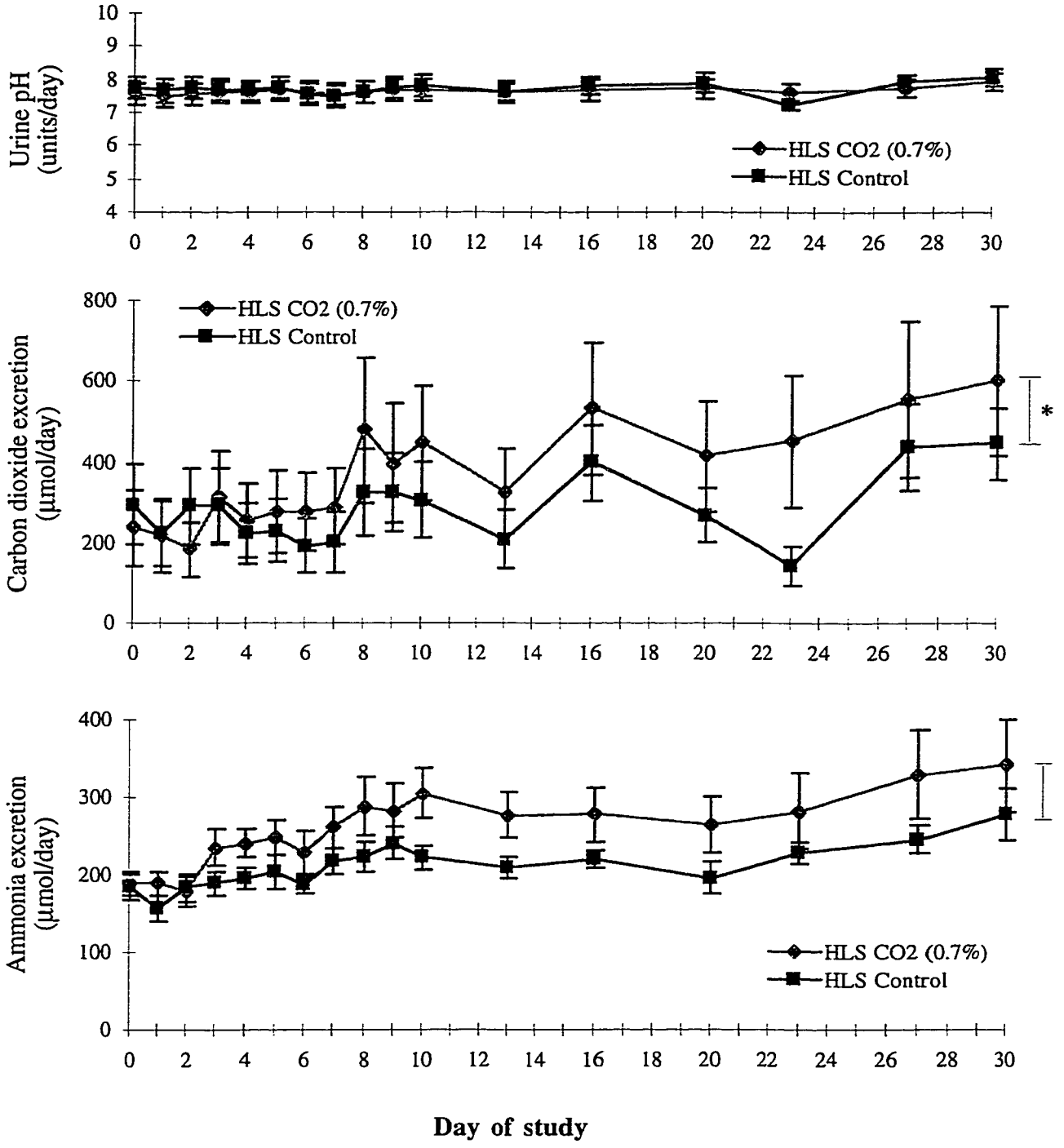


FIG. 3. Daily urine pH and carbon dioxide and ammonia excretion for experimental animals (HLS CO_2 0.7%) vs. control animals (HLS Control) over the experimental period. Values are group means±SEM.

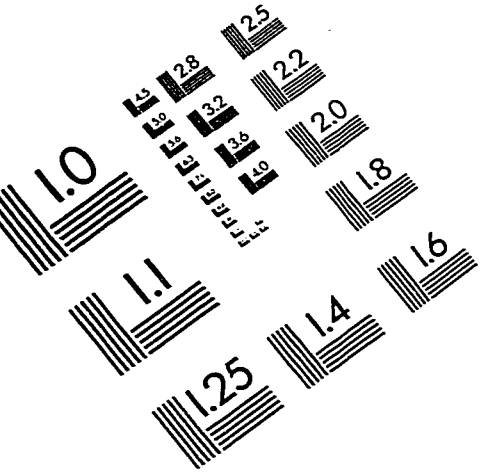
* Significant group difference.

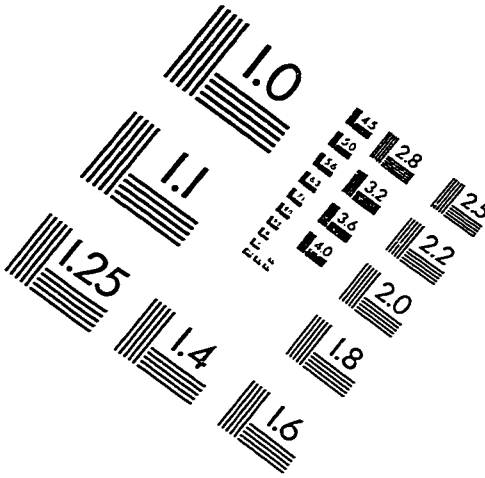
TABLE 6. Comparison of published studies, 2.0 % and 0.7% CO_2 vs. ambient controls (13) and this study, 0.7% CO_2 + HLS vs. HLS ambient controls, indicating significant differences when comparing the elevated carbon dioxide exposed group to their own control groups.

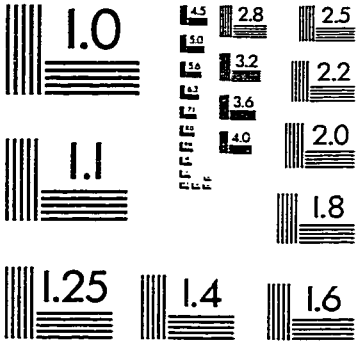
↓ significant decrease over the 30 day period compared to control
↑ significant increase over the 30 day period compared to control
No Δ no significant change over the 30 day period compared to control
↓ and ↑ initial significant decrease followed by a significant increase over the 30 day period compared to control

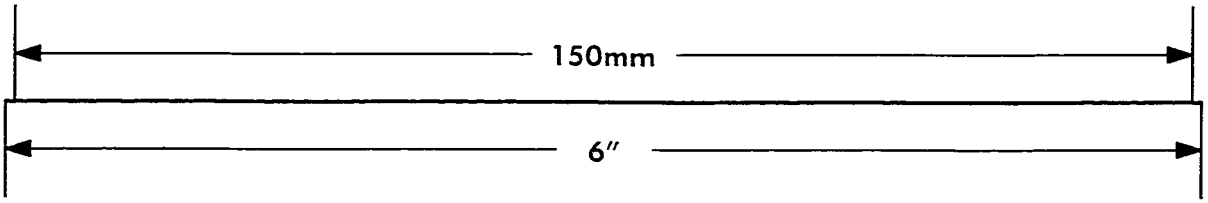
	2.0% CO_2	0.7% CO_2	0.7% CO_2 + HLS
Food Consumption (g/day)	↓	No Δ	↓
Urine Output (ml/day)	↑	↑	↑
Urine pH (units/day)	↓ and ↑	↑	No Δ
CO_2 excretion (mmol/day)	↑	↑	↑
Plasma CO_2 (mmol/L)	↑	No Δ	↑
NH_3 excretion (mmol/day)	↓ and ↑	No Δ	↑
Ca^{2+} excretion (mg/day)	↑	No Δ	No Δ
K^+ excretion (mmol/day)	↑	No Δ	No Δ
Creatinine excretion (mg/day)	↑	No Δ	No Δ

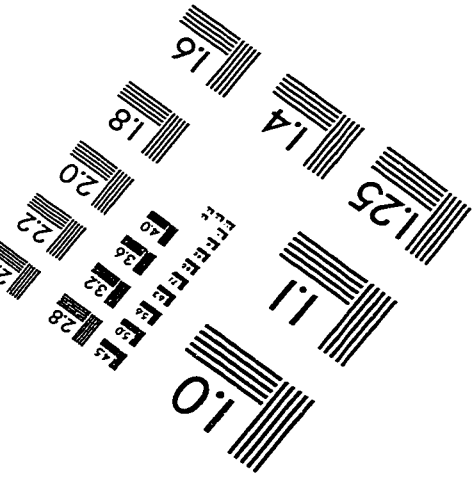
IMAGE EVALUATION
TEST TARGET (QA-3)

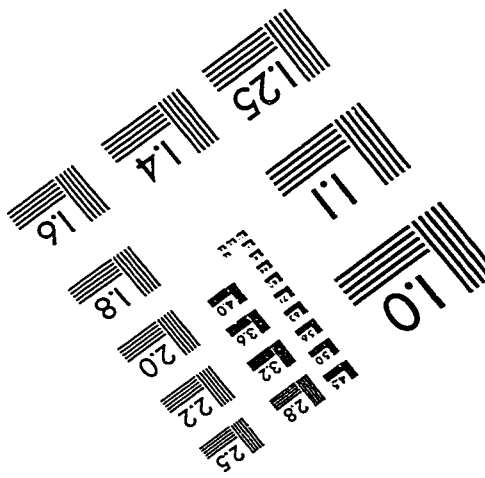












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