

COVER SHEET

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Subtitle: Physiological Resilience

Principal Investigator: Michael S. Davis

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CONTENTS

COVER SHEET.....	1
TITLE SHEET	2
ABSTRACT.....	3
CONTENTS.....	4
FIGURES	6
TABLES	9
SUMMARY	10
INTRODUCTION	11
September 2011 Deployment Simulation	11
Conditioning and Deconditioning	11
Metabolic Heat Management and Heat Acclimatization	11
Hydration and Water Requirements	12
June 2012 Deployment Simulation	12
METHODS AND PROCEDURES.....	13
September 2011 Deployment Simulation	13
Conditioning/Deconditioning Studies	14
Metabolic Heat Management and Heat Acclimatization	15
Hydration and Water Requirements	16
June 2012 Deployment Simulation	16
SITE DESCRIPTION	19
RESULTS AND DISCUSSION	20
September 2011 Deployment Simulation	20
Results.....	20
Discussion	24
Conditioning/Deconditioning Studies	27
Results.....	27
Discussion	29
Metabolic Heat Management and Heat Acclimatization	31
Results.....	31
Discussion	36
Hydration and Water Requirements	39
Results.....	39
Discussion	39

June 2012 Deployment Simulation	41
Results.....	41
Discussion.....	47
Conclusions.....	50
Recommendations.....	55
Conditioning.....	55
Medication.....	56
Nutrition	56
Hydration.....	56
Hyperthermia/Heat Management	56
Additional Research	57
References.....	58
Symbols, Abbreviations, and Acronyms	60
Glossary	61

FIGURES

Figure 1: VO ₂ determination in dogs	15
Figure 2: Effect of 5 day deployment simulation on muscle glycogen concentrations. N = 6. Pre-exercise muscle glycogen was measured in biopsies obtained 2 days prior to the start of the 5 day exercise; post-exercise muscle glycogen was measured in biopsies obtained within 4 hrs of completion of the 5 th day of exercise.	21
Figure 3: Serum creatine phosphokinase concentrations (mean ± SD) during September 2011 deployment simulation. N = 6 dogs except for Day 1 Post samples which had N = 5. “Pre” samples were obtained in the morning, at least 12 hr after any off-leash exercise. “Post” samples were obtained in the evening, approximately 2 hr after the off-leash activities of the day.	22
Figure 4: Serum aspartate transferase concentrations (mean ± SD) during September 2011 deployment simulation. N = 6 dogs. Samples were obtained in the morning, at least 12 hr after any off-leash exercise.	22
Figure 5: Serum protein concentrations (mean ± SD) during September 2011 deployment simulation. N = 6 dogs. Samples were obtained in the morning, at least 12 hr after any off-leash exercise.....	23
Figure 6: Serum cortisol concentrations during September 2011 deployment simulation. N = 5 dogs. Samples were obtained in the morning, at least 12 hr after any off-leash exercise.....	23
Figure 7: Example of gastric endoscopic severity score = 3, characteristic of post-deployment simulation gastric endoscopy.	24
Figure 8: Effect of conditioning program on post-exercise serum CPK concentrations. Serum samples were obtained within 2 hr of completing a single day deployment simulation (9 hr intermittent off-leash exercise).....	28
Figure 9: Effect of conditioning on post-exercise rectal temperature. Values are mean ± SD. All dogs (n=12) were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance capacity.	31
Figure 10: Effect of conditioning on static heat tolerance. All dogs (n=12) were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance capacity.	32
Figure 11: Effect of a cooling pad on body temperature during static heat stress. N = 6 dogs/group. Ambient conditions were 100°F, 60% RH. Cooling pad was perfused with 39°F water.	33
Figure 12: Effect of endurance conditioning on serum concentrations of HSP 27. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.....	34
Figure 13: Effect of endurance conditioning on serum concentrations of HSP 40. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.....	34

Figure 14: Effect of endurance conditioning on serum concentrations of HSP 60. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.....	35
Figure 15: Effect of endurance conditioning on serum concentrations of HSP 70. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.....	35
Figure 16: Effect of endurance conditioning on serum concentrations of HSP 90. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.....	36
Figure 17: Miles (mean $\pm$ SD) covered during each day of June 2012 deployment simulation by IDDs. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	41
Figure 18: Odor detection performance during June 2012 deployment simulation. Data (mean $\pm$ SD) are expressed as the percentage of the group means for a particular day. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	42
Figure 19: Pre-exercise body weight (mean $\pm$ SD) of IDDs during June 2012 Deployment Simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	43
Figure 20: Exercise-induced weight loss (mean $\pm$ SD) during June 2012 deployment simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	43
Figure 21: Daily voluntary water intake (mean $\pm$ SD) during June 2012 deployment simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	44
Figure 22: Effect of 5 day deployment simulation on serum CPK concentrations. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	45
Figure 23: Effect of 5 day deployment simulation on serum AST concentrations. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	45
Figure 24: Effect of 5 day deployment simulation on serum LDH concentrations. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	46
Figure 25: Post-exercise serum CPK concentrations (mean $\pm$ SD) in IDD during June 2012 deployment simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).	46

Figure 26: Effect of 5 day deployment simulation on muscle protein synthesis (mean $\pm$ SD).

Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 4);
IDD 2.0: Exercising IDD 2.0 dogs (n=4). 47

TABLES

Table 1: Conditioning programs used in IDD 2.0 experiments. Dogs performed standard IDD 1.0 conditioning tethered to an ATV. Dogs performed revised IDD 2.0 conditioning exercise as off-leash orbits and road clearing.	14
Table 2: Distance traveled (miles) of dogs during deployment simulation. Sandy did not participate in the first day of the simulation. Distance was recorded using a Garmin DC40 GPS tracking collar. A portion of the distance logged on 9/9/11 is estimated due to overwriting of the record on 9/10/11.....	20
Table 3: Pre- and Post-deployment simulation body weights of dogs in September 2011. Body weights were obtained in the morning, after consumption of the morning meal and before exercise.	20
Table 4: Effect of physical conditioning on serum chemistry and electrolyte values.	28
Table 5: Effect of Gatorade on hydration parameters and performance during deployment-style activity.....	39

SUMMARY

Improvised explosive device detection dogs (IDDs) are used by dismounted Marine Corps patrols to facilitate stand-off detection of explosive devices. Deployment experiences have suggested that these activities result in physiological stresses beyond that which the dogs have been conditioned to tolerate, and as a result the utility of the IDD has been adversely impacted. The purpose of the studies reported herein was to identify and quantify the physiological stresses of deployment on IDDs and develop recommendations for mitigation of these stresses. The studies quantified the range of metabolic demands associated with typical IDD activities, including caloric and water requirements, documented the improvement in endurance exercise capacity resulting from a revised physical conditioning program, and evaluated selected measures intended to improve voluntary water intake and hydration. The resulting revised management approach for the IDDs was tested against the existing management approach in order to confirm that the new management programs would result in improved performance of the dogs over the current management program. These studies found that the deployment activities resulted in substantially greater requirements with respect to daily exercise and energy expenditure than would be supported in a sustainable manner by the current IDD management program, resulting in a large daily net caloric deficit, fatigue, muscle damage, and gastric ulceration. Implementation of new conditioning and feeding practices largely alleviated these issues, although additional study is needed to identify more precisely the feeding strategy that will result in long-term sustainability of IDD activities. Studies on hydration found that adequate hydration could be accomplished through ad libitum oral water intake, and no support was found for the necessity of parenteral fluid administration to maintain hydration in normal, healthy, and properly conditioned dogs performing deployment-type activities. Finally, studies on heat acclimatization and thermal tolerance found clear evidence that endurance conditioning of the dogs results in both increased capacity to dissipate heat during exercise, as well as increased tolerance to high body temperatures at rest and exercise. These adaptations are expected to facilitate greater physiological resilience in the current operational areas of IDDs.

INTRODUCTION

September 2011 Deployment Simulation

The central charge for the physiological resilience portion of the IDD 2.0 program was to improve the conditioning of the IDD dogs to better prepare the dogs for deployment and to reduce fatigue and weight loss during deployment. The type of physical activity (duration, distance, speed, metabolic stress, thermal stress, and prevalence of exercise-related disease) for which the conditioning was to prepare the dogs was unclear at the onset of the IDD 2.0 program. Therefore, the initial study in September 2011 was constructed to be a descriptive 5 day deployment simulation intended to quantify the metabolic stresses of deployment as an IDD as well as the clinical consequences of those stresses.

Conditioning and Deconditioning

The results of the September 2011 deployment simulation indicated that the current conditioning programs stipulated for the IDD dogs did not result in sufficient fitness for the intended activities. Physical conditioning largely is activity-specific (i.e., conditioning activities only prepare subjects for activities similar to the conditioning activities), and the prevailing suspicion was that the conditioning program provided in the current IDD Program Manual provided for too little exercise relative to the expected deployment activities. Therefore, a study was conducted to test the hypothesis that a conditioning program specifically designed to produce fitness for long-duration multiday endurance activities would result in less perturbation of homeostasis during those activities than the current conditioning program.

The results of the conditioning experiments were very encouraging, with clear improvement in the dogs' fitness for endurance exercise. The immediate question then became how long that fitness would be preserved during a period of inactivity that is inevitable in the deployment preparation cycle. Therefore, following completion of the conditioning program, the scheduled activity of the dogs was sharply curtailed to determine the persistence of the conditioning-induced changes in physiology.

Metabolic Heat Management and Heat Acclimatization

An important limiting factor for canine exercise is the ability of the dog to shed metabolic heat while tolerating a degree of hyperthermia without adverse effects. Metabolic heat dissipation proceeds down an enthalpy gradient from the fully humidified air on the respiratory surfaces of the dog to the environment, where the enthalpy is the overall heat content dictated by the heat and water content (humidity) of the air. Early studies demonstrated that under relatively moderate enthalpy conditions, dogs performing typical deployment activities reached sustained rectal temperatures of 105°F or higher. Although there were no incidents of heat-related illness or injury, the dogs were judged to need extended rest in order to control metabolic heat accumulation. Additionally, the overall performance of the dogs was diminished coincident with the periods of hyperthermia. The tolerance of a dog to increased body temperature is not a fixed aspect of physiology, but can be changed through acclimatization. Anecdotal evidence indicates that heat acclimatization (the ability of the dog to tolerate high body temperatures) often develops in association with increased fitness, but there are no studies explicitly documenting a change in heat tolerance in dogs with increased fitness. Therefore, we conducted a series of studies to evaluate heat responses in dogs at different levels of fitness in order to test the hypothesis that increased fitness is associated with increased tolerance to hyperthermia.

Hydration and Water Requirements

Dogs primarily rely on evaporative cooling in order to dissipate metabolic heat. The need for evaporative cooling for thermoregulation places the dogs at risk for dehydration and subsequent performance failure. As a result, a great deal of attention is placed on maintaining hydration, even to the point of using pre-exercise parenteral fluids to hyperhydrate the dogs. There are no data describing the normal water requirements for IDD and whether parenteral fluid administration is necessary to maintain hydration. Therefore, a series of studies were conducted to assess the water requirements and whether they can be met using voluntary water intake.

June 2012 Deployment Simulation

The studies following the September 2011 deployment simulation resulted in revised conditioning and management plans intended to improve IDD performance during deployment. These revisions included a physical conditioning program that emphasized off-leash endurance exercise, increased mass of food divided into multiple meals, substitution of Gatorade sports drink for water during deployment activities to increase energy intake, and preventative administration of omeprazole to prevent exercise-induced gastritis and improve voluntary consumption of food and water. The purpose of the June 2012 deployment simulation was to compare the performance of dogs under the revised conditioning and management plans to dogs conditioned and managed under the current IDD guide.

METHODS AND PROCEDURES

September 2011 Deployment Simulation

Six dogs were selected from the pool of IDD candidate dogs available at K2 Solutions, Inc. These dogs had been purchased as candidates for deployment as IDDs, but had been rejected from the program after the initial conditioning and certification process due to either performance failures during certification or medical issues identified by DoD veterinarians. All dogs were clinically healthy at the time of the study, and were of comparable fitness to other IDDs.

The deployment simulation consisted of 5 days of combined road-clearing, orbit, and point-to-point activities lasting approximately 9 hrs/day. Actual distance covered by the dogs was recorded using individual GPS collars recording the location of the dogs every 3 seconds. Daily records were downloaded and filtered to exclude periods and distances during which the dogs were being moved using motor vehicles.

Total energy expenditure was measured using the doubly-labeled water technique. Precisely-measured doses of deuterium oxide (D_2O , 0.3 g/kg of 99% atomic excess) and ^{18}O -labeled water (2 g/kg of 10% atomic excess) were administered by gavage (during pre-exercise gastroscopy, see below) 48 hr prior to the start of the deployment simulation. Daily serum samples were obtained each morning for measurement of specific isotope concentrations in body water, and the difference in the decay rates between D_2O and ^{18}O -labeled water was used to calculate total energy expended during the examination period.

Total energy intake was measured by recording the mass of food provided to a “phantom” dog and the nutritional content of a sample of food obtained from the same bag of food used during the study. Any food refused by the dogs during the study was weighed and the estimated food consumption was adjusted to reflect these food refusals.

Muscle glycogen was measured in muscle biopsies obtained from the semimebranosus muscle 48 hr before the start of the deployment simulation and within 4 hr of completion of the last day of exercise.

Total body water was measured 48 hr prior to the start of the deployment simulation using a blood sample obtained 4 hr after administration of the D_2O described above for measurement of total energy expenditure. Water turnover was measured using the rate of elimination of the first dose of D_2O .

Body weight was recorded daily prior to the start of the simulated patrol activities.

Blood for clinical chemistry measurement and urine for measurement of electrolytes and fractional clearances of electrolytes was collected each morning prior to the start of the simulated patrol activities. Post-exercise creatine phosphokinase activity was measured in blood samples obtained between 2 and 4 hrs post-exercise each day.

Body temperature was measured by rectal thermometer intermittently throughout the 5 day exercise as feasible without interfering with the planned activities.

Gastroscopy was performed 48 hr before the start of the deployment simulation and within 4 hr of completion of the last day of exercise.

Conditioning/Deconditioning Studies

Twelve dogs were used to evaluate the redesigned conditioning program. Six of the dogs were dogs that completed the deployment simulation, and had been allowed to rest (no formal exercise) for 6 weeks. The additional six dogs were purchased through the same channels used to purchase candidate IDD for training and conditioning. Dogs were randomly assigned to either the standard conditioning program described in the Marine Corps IDD manual (IDD 1.0 Conditioning, condensed for the purposes of this study since no other training activities were necessary) or to the redesigned conditioning program (IDD 2.0 Conditioning). The specific activities of the conditioning programs are listed in Table 1.

Table 1: Conditioning programs used in IDD 2.0 experiments. Dogs performed standard IDD 1.0 conditioning tethered to an ATV. Dogs performed revised IDD 2.0 conditioning exercise as off-leash orbits and road clearing.

Week	IDD 1.0 Conditioning			IDD 2.0 Conditioning		
	Daily Duration	Speed/ Distance	Weekly Frequency	Daily Duration	Speed/ Distance	Weekly Frequency
1	15 min	6 mph/1.5 mi	3X	60 min	4.5 mph/4.5 mi	3X
2	25 min	6 mph/2.5 mi	3X	2 x 60	4.5 mph/9 mi	3X
3	35	6 mph/3.5 mi	3X	3 x 60	4.5 mph/13.5 mi	3X
4	40	7 mph/4.7 mi	3X	4 x 60	4.5 mph/18 mi	3X
5	40	8 mph/5.3 mi	3X	5 x 60	4.5 mph/22.5 mi	3X

Peak aerobic output (VO_{2peak}) was measured using an open-flow calorimetry system with the dog running on a highspeed treadmill. Dogs were fitted with a loose-fitting “helmet” that was connected to a precision flow generator that drew room air through the mask at 1200 l/min in order to capture all of the exhaled breath of the dogs (See Figure 1). The flow through the flow generator was subsampled to a set of precision gas analyzers for determination of oxygen, carbon dioxide, and water vapor content. Treadmill determination of VO_{2max} was determined using a graduated exercise test. The treadmill speed was set at the maximal speed at which the dog appeared comfortable, then the treadmill was incrementally inclined until the dog was unable to maintain position on the treadmill despite verbal encouragement. Valid examinations required progressive step-wise increments in work load and the decision by the investigators that the dog had fatigued physically, but did not require successive increments in work with no change in VO_2 .

Serum chemistry values and electrolyte concentrations were measured before and after the conditioning program. Blood samples were obtained in the morning, prior to any exercise scheduled that day and at least 36 hr following the most recent exercise activity.

Total body water was measured prior to and at the conclusion of the respective conditioning programs using deuterium oxide (0.3 g/kg 99% atomic excess), with serum samples obtained immediately prior to and 2 hrs following the intravenous injection of D_2O . Extracellular fluid volume was measured using sodium thiocyanate (22 mg/kg administered as a 20% w/v solution), with serum samples obtained immediately prior to and every 15 min following intravenous administration of the NaSCN and ECF volume calculated from the hypothetical Time 0 concentration determined through regression of the post-injection sample concentrations vs time. Plasma volume was measured using Evan’s Blue (3 mg/kg administered as a 2 mg/ml solution),

with serum samples obtained immediately prior to and every 15 min following intravenous administration of the Evan's Blue and plasma volume calculated from the hypothetical Time 0 concentration determined through regression of the post-injection sample concentrations vs time.

At the conclusion of the conditioning program, a single day of the previously-described deployment simulation (9 hrs of off-leash patrol activity) was performed, and post-exercise CPK was measured in a blood sample obtained within 2 hr after completion of the exercise to evaluate muscle fitness for the proscribed exercise.



Figure 1: VO₂ determination in dogs

Activity was reduced for 8 weeks after completion of the conditioning program. Activity was limited during the initial 4 weeks to approximately 50% of the activity proscribed during the last week of the redesigned conditioning program (2 episodes of 3-1 hr sessions each week). During the second 4 weeks, activity was limited to 25% of the activity proscribed during the last week of the redesigned conditioning program (1 episode of 3-1 hr sessions each week). Pre- and post-exercise CPK was measured every 2 weeks, and VO₂peak was measured every 4 weeks.

Metabolic Heat Management and Heat Acclimatization

Twelve dogs were used in the study which spanned the course of an endurance conditioning program (IDD 2.0 Conditioning, Table 1). Heat tolerance was assessed at the beginning of the conditioning program, midway through the program, and at the conclusion of the conditioning

program. Heat acclimatization was assessed using 3 methods: Static heat testing, in which the dogs' respiratory rate and rectal temperature was measured for 60 minutes while acutely exposed to high environmental heat (100°F); exercise heat testing, in which the dogs' rectal temperature was measured before and after moderate exercise (6 mph for 15 min) in moderate heat (85°F); and measurement of serum heat shock proteins using commercially-available ELISA assays and samples obtained from the previous conditioning experiments.

Hydration and Water Requirements

An initial study was conducted to determine the magnitude of voluntary water intake during a single day of off-leash deployment-style activity. Total body water was measured prior to and at the conclusion of the single day of exercise using deuterium oxide (0.3 g/kg 99% atomic excess), with serum samples obtained immediately prior to and 2 hrs following the intravenous injection of D₂O. During the exercise, dogs were prevented from accessing sources of water in the patrol area but were allowed to drink *ad libitum* during their rest periods (approximately every hour for about 30 min). Individual intake of water was recorded for each dog.

Three interventions (Gatorade vs plain water, omeprazole vs no medication, and Canine Forte vs plain water) hypothesized to improve water intake were tested using a crossover design with 1 week washout between experiments. For all studies, dogs were weighed prior to and at the conclusion of the daily exercise, and voluntary fluid intake was recorded throughout the exercise. For the studies evaluating the effect of Gatorade on voluntary water intake, Gatorade was mixed according to the manufacturer's directions (80 calories/ounce). In addition, total body water was measured before and after the conclusion of the single day of exercise using deuterium oxide (0.3 g/kg 99% atomic excess), with serum samples obtained immediately prior to and 2 hrs following the intravenous injection of D₂O. For the studies evaluating the effect of omeprazole, the drug was administered 20 mg once daily *per os* for 3 days before and the morning of the exercise activity. For the studies evaluating the effect of Canine Forte, the product was prepared using the lowest concentration recommended by the manufacturer.

June 2012 Deployment Simulation

Twelve IDD 2.0 dogs and 12 IDD 1.0 dogs were used for this study. The IDD 2.0 dogs were dogs that had been used as experimental dogs for the preceding 6-12 months, and the 12 IDD 1.0 dogs were dogs that had recently returned from overseas deployment as IDs assigned to patrols. Eight IDD 2.0 dogs and 4 IDD 1.0 dogs were assigned to an experimental protocol designed to evaluate energy balance, and 4 IDD 2.0 and 8 IDD 1.0 were assigned to an experimental protocol designed to evaluate protein synthesis. Differences between IDD 2.0 and IDD 1.0 dogs were as follows:

- IDD 2.0 dogs completed an 8-week conditioning program consisting of progressive increases in off-leash exercise duration 3 times weekly; IDD 1.0 dogs were maintained using roading and sprints as outlined in the IDD Marine Corps Manual.
- IDD 2.0 dogs received omeprazole (20 mg per os daily) to prevent exercise-induced gastric irritation; IDD 1.0 dogs did not receive any preventative medication.
- IDD 2.0 dogs were provided standard kibble at a rate of approximately 312 kcal/kg^{0.75}/day (the rate of energy expenditure in the September 2011 deployment simulation); IDD 1.0 dogs were provided standard kibble at a rate of approximately 218 kcal/kg^{0.75}/day (the amount fed during the September 2011 deployment simulation as

instructed by the current IDD Marine Corps Manual. In order to accommodate the larger feeding volume, the IDD 2.0 dogs received the same amount of food as the IDD 1.0 dogs during the morning and evening feedings, and received the additional volume of food as 2 “snacks” provided during patrol rests throughout the day.

- IDD 2.0 dogs received Gatorade (mixed according to the manufacturer’s instructions) in place of regular water during the patrol activities; IDD 1.0 dogs were offered regular water.

The deployment simulation consisted of 5 days of combined road-clearing, orbit, and point-to-point activities lasting approximately 9 hrs/day. Body weight was recorded daily prior to the start of the simulated patrol activities and at the end of the daily patrol activities.

Total energy intake was measured by recording the mass of food provided to each dog, as well as the mass of any feed refusals. The nutritional content of a sample of food obtained from the same bag of food used during the study was measured by a commercial laboratory. IDD 2.0 dogs received Gatorade in place of plain water during the patrol activities, and the calories contributed by the Gatorade were included in the total caloric balance. Muscle glycogen was measured in muscle biopsies obtained from the semimebranosus muscle 48 hr before the start of the deployment simulation and within 4 hr of completion of the last day of exercise. Blood for clinical chemistry measurement was collected each morning prior to the start of the simulated patrol activities. Post-exercise creatine phosphokinase activity was measured in blood samples obtained between 2 and 4 hrs post-exercise each day. Body temperature was measured by rectal thermometer intermittently throughout the 5 day exercise as feasible without interfering with the planned activities. At the conclusion of each day, an odor lane was created and the time from the start of the search to covering on the concealed odor source (4 oz ammonium nitrate) was recorded for all dogs. The scope of the study necessitated two different patrol locations and a new odor lane each day. In order to normalize for these different odor lanes, the elapsed time for an individual dog was expressed as a percentage of the mean for all dogs on a given odor lane on a given day.

Dogs participating in the energy balance portion of the experiment were evaluated for distance travelled, total energy expenditure, and amount of water consumed. Actual distance covered by the dogs assigned to the energy balance protocol was recorded using individual GPS collars recording the location of the dogs every 3 seconds. Daily records were downloaded and filtered to exclude periods and distances during which the dogs were being moved using motor vehicles. Total energy expenditure was measured in the energy balance protocol using the doubly-labeled water technique. Precisely-measured doses of deuterium oxide (D_2O) and ^{18}O -labeled water were administered intravenously 48 hr prior to the start of the deployment simulation. Daily serum samples were obtained each morning for measurement of specific isotope concentrations in body water, and the difference in the decay rates between D_2O and ^{18}O -labeled water was used to calculate total energy expended during the examination period. Total body water was measured 48 hr prior to the start of the deployment simulation using a blood sample obtained 4 hr after administration of the D_2O described above for measurement of total energy expenditure. Water turnover was measured using the rate of elimination of the first dose of D_2O .

Dogs participating in the protein turnover portion of the study received drinking water that contained 8% enrichment with deuterated water to maintain a constant level of enrichment in the body water pool. In doing so, small amounts of the deuterium will be progressively incorporated

into amino acids and those amino acids into muscle proteins. The difference in amino acid (specifically alanine) deuterium enrichment in skeletal muscle proteins between pre- and post-exercise muscle biopsies will provide an estimate of muscle protein synthesis.

SITE DESCRIPTION

All studies were conducted at K2 Solutions Inc Canine Training Facility, located outside Jackson Springs, North Carolina. Dogs were housed in sheltered outdoor runs measuring 3' x 12'. Routine husbandry of the dogs included periods of free exercise in a 24' x 40' outdoor exercise yard twice daily. Dogs were fed twice daily to maintain normal body condition and were provided with ad libitum water.

The environmental exercise chamber used for many of the studies is a 20' x 24' room constructed to achieve temperature extremes as needed for studies. Included in the room is a 3' x 12' highspeed treadmill specifically designed for conducting exercise studies under varied environmental conditions.

The grounds used for the deployment simulations and off-leash conditioning studies are semi-wooded fields and agricultural properties in the Jackson Springs/Pinehurst area of North Carolina.

Sample analysis was conducted at Oklahoma State University or at commercial scientific laboratories as appropriate.

RESULTS AND DISCUSSION

September 2011 Deployment Simulation

Results

The deployment simulation was conducted in Pinehurst, North Carolina from Sept 6-10, 2011. Midafternoon environment conditions averaged 83°F and 50% RH. One dog was removed from the study during the morning of the first day of exercise due to a torn cruciate ligament, and was replaced with another dog for the remaining 4 days of exercise. Activities on the 3rd day of exercise were terminated after 4 hr of exercise due to profound fatigue and the overall poor condition of the dogs. With the additional rest provided on the 3rd day, the dogs were able to complete the planned activities on Days 4 and 5. Dogs traveled an average of 20.6, 18.3, 12.5, 24.8, and 20.4 miles on each day of the deployment simulation (Table 2). The decrease in distance traveled on the 3rd day of the study was a function of the abbreviated activities on that day due to the excessive fatigue observed in the dogs.

Table 2: Distance traveled (miles) of dogs during deployment simulation. Sandy did not participate in the first day of the simulation. Distance was recorded using a Garmin DC40 GPS tracking collar. A portion of the distance logged on 9/9/11 is estimated due to overwriting of the record on 9/10/11.

Dog/Date	9/6/11	9/7/11	9/8/11	9/9/11	9/10/11	Total
Green	20.7	18.3	11.7	27.0	21.2	98.9
Moss	21.5	18.7	13.4	25.6	20.3	99.5
Sandy		16.5	10.7	25.3	19.9	72.4
Audi	24.5	18.8	12.5	24.2	21.6	101.6
Freda	17.9	19.7	15.1	26.2	21.0	99.9
Jimmy	18.2	17.9	11.6	20.6	18.5	86.8
Average	20.6	18.3	12.5	24.8	20.4	96.6

Total energy expenditure data was unavailable for 2 dogs due to errors in the initial administration of the labeled water. The remaining 4 dogs had a daily energy expenditure of 3246 ± 401 kcal/day. Total energy intake was estimated at 2114 ± 403 kcal/day ($165 \text{ kcal/kg}^{0.75}/\text{day}$) for 5 of the dogs. One dog refused to consume all of the food offered to him on Days 2-4 of the deployment simulation, and after correction for the mass of unconsumed food, this dog's total energy intake was estimated at 1686 ± 418 kcal/day ($136 \text{ kcal/kg}^{0.75}/\text{day}$).

In general, dogs maintained their body weight, losing on average only 0.9 ± 2.1 lb over the duration of the study (Table 3).

Table 3: Pre- and Post-deployment simulation body weights of dogs in September 2011. Body weights were obtained in the morning, after consumption of the morning meal and before exercise.

Dog	Pre-simulation weight (lb)	Post-simulation weight (lb)	Difference (lb)
Green	63.1	64.2	+1.1
Moss	56.4	54.2	-2.2
Sandy	79.0	75.5	-4.5
Audi	73.5	73.7	+0.2
Freda	72.3	71.4	-0.9
Jimmy	53.2	53.9	+0.7

The 5 days of exercise was associated with a 43% decrease in muscle glycogen content (231 ± 38 vs 132 ± 23 mmol glucose units/kg dry muscle) which was statistically-significant ($p=0.0001$, Figure 2).

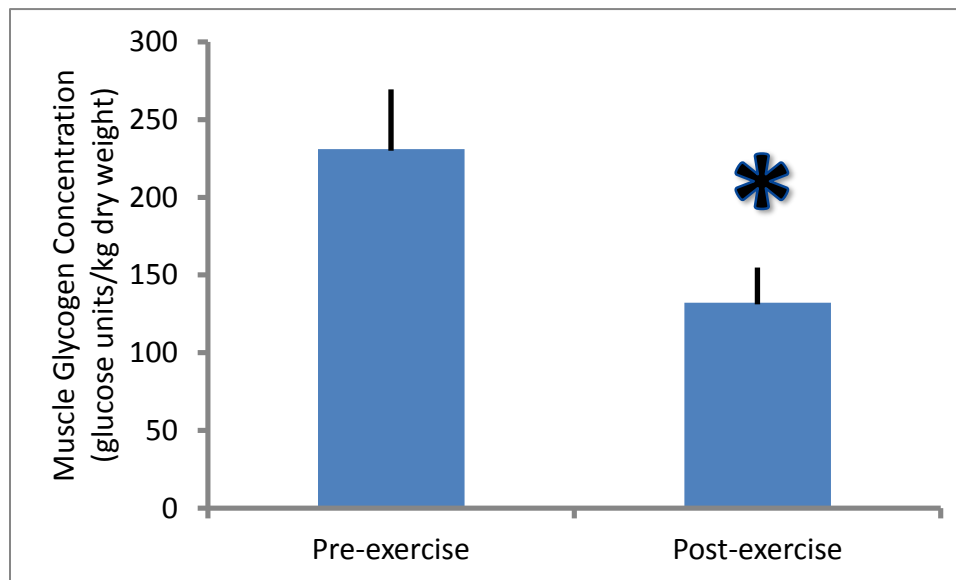


Figure 2: Effect of 5 day deployment simulation on muscle glycogen concentrations. N = 6. Pre-exercise muscle glycogen was measured in biopsies obtained 2 days prior to the start of the 5 day exercise; post-exercise muscle glycogen was measured in biopsies obtained within 4 hrs of completion of the 5th day of exercise.

Total body water was 18 ± 2 L (61% of body mass), and daily water turnover was 4.6 ± 0.4 L (25% of the total body water).

All dogs had normal clinical chemistry and electrolyte values at the beginning of the study, and the only analytes that did not remain within published normal ranges were CPK and AST, both of which increased (Figure 3, Figure 4). Post-exercise CPK was also higher than published normal ranges for resting dogs. The magnitude of deviation from normal peaked after the second day of exercise. Serum total protein decreased throughout the study (Figure 5), and serum potassium concentrations increased, but all of these remained within normal ranges. Serum cortisol was unaffected by the exercise activity, with 4 of the 5 dogs maintaining serum cortisol concentrations of approximately 12 ng/ml (Figure 6). However, one dog consistently had serum cortisol concentrations below 0.5 ng/ml, which were also not affected by exercise.

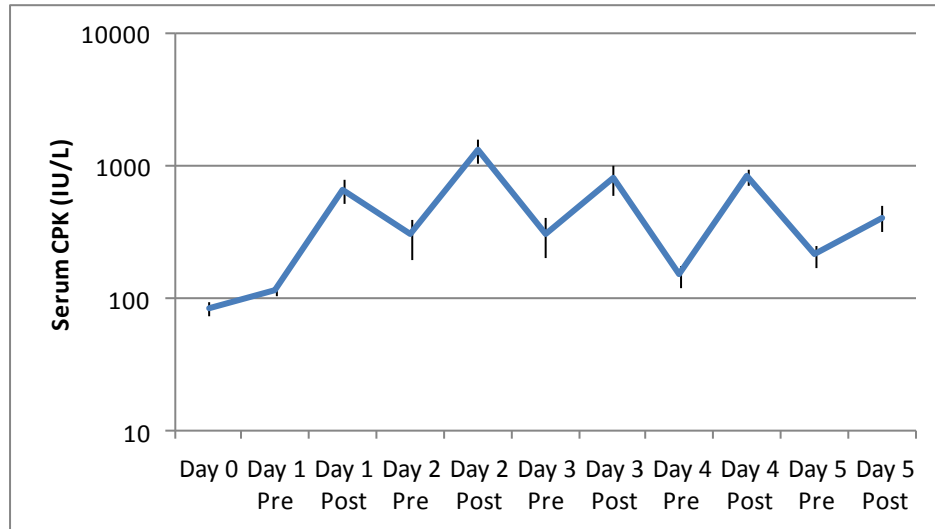


Figure 3: Serum creatine phosphokinase concentrations (mean $\pm$ SD) during September 2011 deployment simulation. N = 6 dogs except for Day 1 Post samples which had N = 5. “Pre” samples were obtained in the morning, at least 12 hr after any off-leash exercise. “Post” samples were obtained in the evening, approximately 2 hr after the off-leash activities of the day.

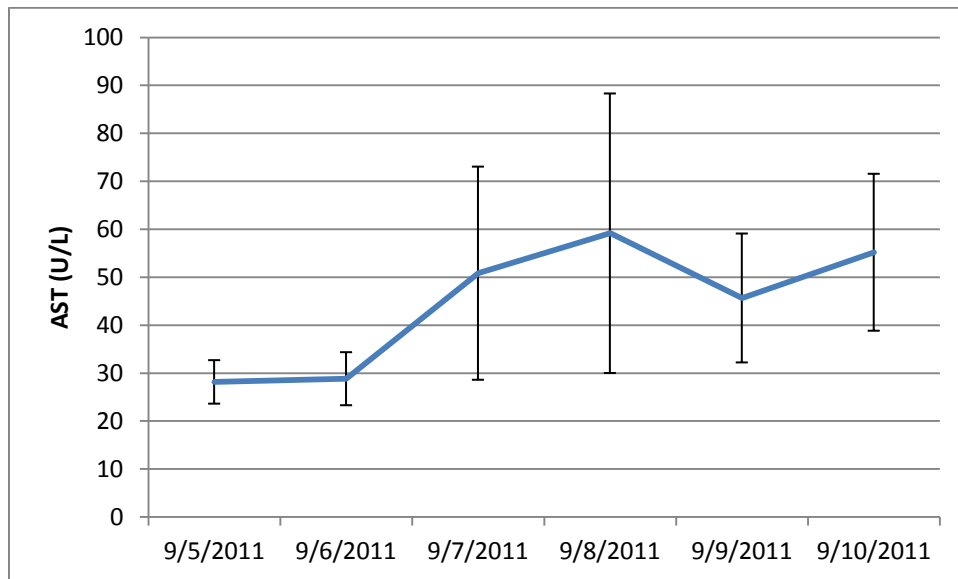


Figure 4: Serum aspartate transferase concentrations (mean $\pm$ SD) during September 2011 deployment simulation. N = 6 dogs. Samples were obtained in the morning, at least 12 hr after any off-leash exercise.

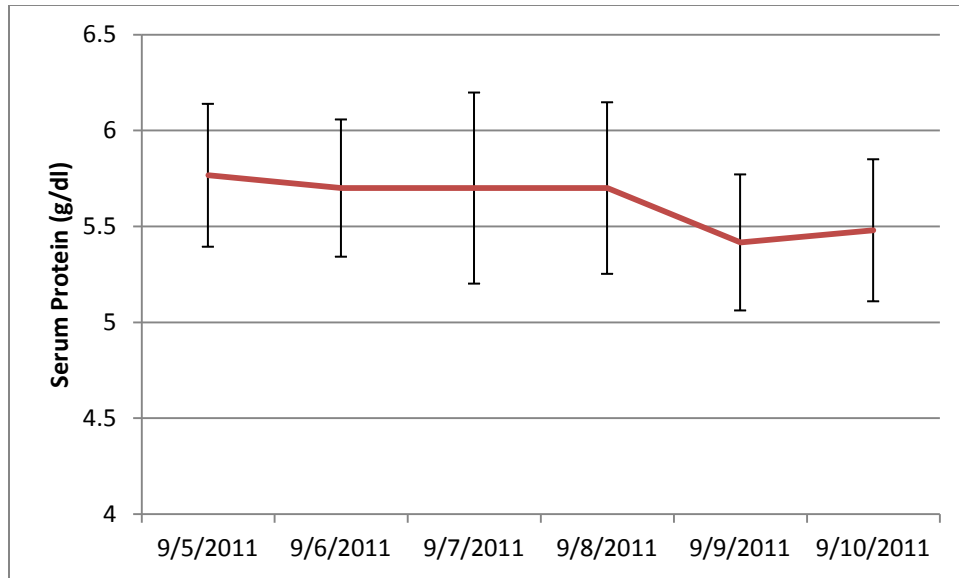


Figure 5: Serum protein concentrations (mean $\pm$ SD) during September 2011 deployment simulation. N = 6 dogs. Samples were obtained in the morning, at least 12 hr after any off-leash exercise.

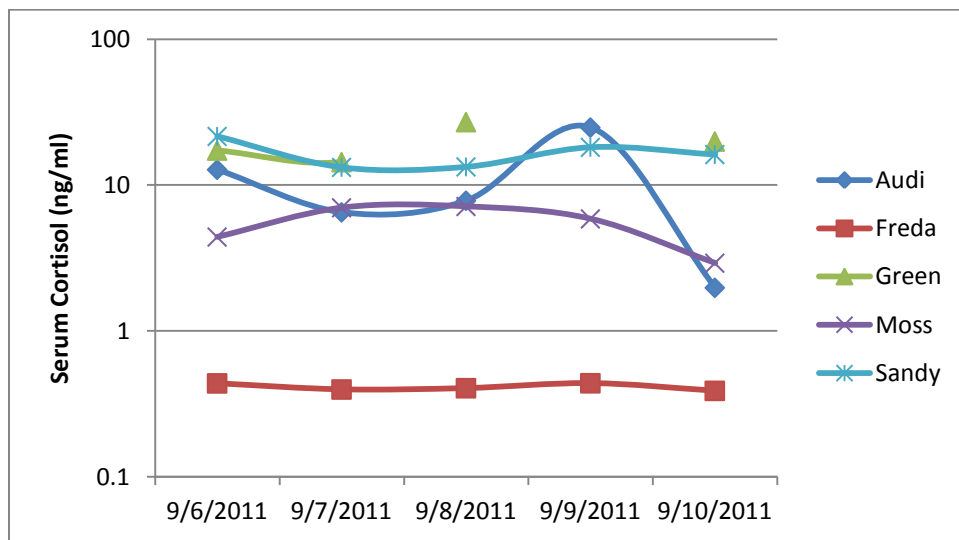


Figure 6: Serum cortisol concentrations during September 2011 deployment simulation. N = 5 dogs. Samples were obtained in the morning, at least 12 hr after any off-leash exercise.

Urine specific gravity was 1.030 ± 0.020 and did not vary in a systematic manner throughout the study, although 2 of the dogs (Audi and Sandy) consistently had low urine specific gravity values. Water turnover values were not available for Sandy, and Audi had a water turnover value during the study that was in the middle of the recorded values. Average fractional excretion of sodium was $0.20 \pm 0.32\%$, and fractional excretion of potassium was $\pm 0.02\%$; similar to urine specific gravity neither fractional excretion values varied systematically during the study, but the dogs with the unusually low urine specific gravity also tended to have higher fractional excretion values.

Pre-exercise gastroscopy was generally unremarkable, with one dog receiving a score of 0, one dog receiving a score of 2, and the rest receiving scores of 1. Post-exercise endoscopy was striking in the severity and prevalence of lesions. Five of the six dogs received scores of 3 (Figure 7), with a single dog unchanged from her pre-exercise gastroscopy (Severity Score = 1: 1-2 mild erosions, not clinically significant). Interestingly, the dog without post-exercise gastric disease was also the dog that maintained serum cortisol concentrations below 0.5 ng/ml.



Figure 7: Example of gastric endoscopic severity score = 3, characteristic of post-deployment simulation gastric endoscopy.

Discussion

The results of the September 2011 deployment simulation demonstrate that the maintenance program currently required for Marine Corps IDD is insufficient for the expected physical demands of deployment. For exercise to be sustainable, there should be no consumption of reserve resources (or at the very least, complete replenishment of those resources during rest), no tissue damage, and no loss of capacity or performance with repeated exercise. In short, there should be no difference in the dog between the first and any subsequent day of exercise. The current maintenance program fails in all of these areas: muscle glycogen (the primary intramuscular energy reserve was depleted during the simulation, there was substantial evidence of muscle damage, the dogs lost weight during the simulation (although the weight loss was not statistically significant, the profound gap between caloric intake and caloric expenditure strongly suggests that weight loss would eventually be manifested), and the dogs were unable to maintain their level of performance throughout the 5 day study.

The deficiencies of the current management program for the Marine Corps IDD's can be distilled down to 3 key areas: Conditioning, nutrition, and preventative medicine. The failure of the conditioning program is the result of violation of a basic principle of exercise physiology: conditioning is activity-specific. In other words, the physiological adaptations that result from conditioning only are those needed to perform the conditioning activities. An athlete (dog or human) will not develop the physiological capacity to run 25 miles/day by running 8 miles/day. Rather, they will develop the conditioning necessary to run 8 miles/day and likely will not be fully capable of performing 2.5-3X that exercise. In this study, the dogs did complete the first and second days of exercise, but developed evidence of muscle damage (increased serum CPK and AST, Figures Figure 3 & Figure 4, respectively). It is important to note that at a physiological level, the athlete's body does not distinguish whether an activity is part of conditioning or part of the actual "event", and as a result, the actual event may result in conditioning activity if the athlete has not already developed the necessary capacity for that activity. A suggestion of that effect can be seen in some of the deployment simulation data, particularly the serum CPK data. It is possible that, despite the severity of the mismatch between conditioning and required activity, the dogs did not develop outright muscle disease and were able to start adapting to the new physiological requirement during the course of the deployment simulation.

The conditioning program currently recommended for IDD's was developed based on the best available knowledge and with consideration to the overall logistical demands of maintaining this element of a combat unit, and this criticism should not be interpreted as an indictment of previous efforts. However, the information produced in this study relative to expected physiological stresses and relative preparedness now represents the best available information and should be used to re-evaluate and redesign the conditioning and maintenance programs that apply to Marine Corps IDD's.

The nutritional deficiencies measured in this study, though unanticipated in their magnitude, are not surprising given the concurrent information on the magnitude of exercise being performed by the dogs. Work with other canine athletes provides the crude estimates (for a 25kg dog) of approximately 1500 kcal/day basal requirement and approximately 70 kcal/mile when that exercise is performed in a cold environment¹⁻³. With an average of about 20 miles/day, this calculates favorably with the actual measured energy expenditure (20 miles x 70 kcal/mile = 1400 kcal + 1500 kcal/day basal requirement = 2900 kcal/day calculated vs. 3246 kcal/day measured); the 300-400 kcal/day difference likely is attributable to the increased energetic cost of thermoregulation through panting in this study. With a mean difference between energy intake and energy expenditure of over 1000 kcal/day, it is not surprising that muscle glycogen was depleted (a 25 kg dog having only about 1500 kcal total of stored glycogen between muscle and liver). Fat is the other major stored energy source in mammals, having approximately 7000 kcal/kg. Thus, if we assume the caloric deficit was met by increased fat catabolism, the dogs would have lost less than 1 kg over the 5 day deployment – a magnitude of body weight change that would be difficult to detect statistically in the small number of dogs used in this study, but if this rate of energy reserve catabolism were to continue, obvious weight loss would eventually be manifested. However, this magnitude of caloric deficit would be expected to result in a hormonal cascade intended to reduce the overall energy demands of the body, resulting in reduced tissue maintenance, reduction in metabolic rate, and reduced performance. A suggestion of the beginning of this process can be seen in the slight decrease in plasma protein concentration

(plasma protein being the most labile of protein reserves and the first to be catabolized in the face of caloric deficits and demand for gluconeogenesis). It is noteworthy that these changes are consistent with reports from deployed units describing the physiological resiliency of their dogs, making relative malnutrition in the face of a conditioning/activity mismatch the likely cause for poor field performance in these dogs.

The issue of preventative medicine is centered on the propensity for athletes to develop gastrointestinal disease as a result of strenuous exercise. Five of six dogs (83%) had clinically significant gastric lesions after completion of the 5-day exercise. Gastritis and gastric ulcers are a common finding in human, canine, and equine athletes⁴⁻¹⁰. The most common symptoms in humans is abdominal discomfort and inappetance, with more severe signs like vomiting/regurgitation, melena, or severe blood loss relatively rare. Nevertheless, overt clinical signs WERE present in at least one dog that displayed inappetance, and the failure of this dog to consume the food offered resulted in more severe caloric deficit than was already present during this study. It is likely that the reports of periodic inappetance, weight loss, and overall loss of condition in deployed IDD's are due, at least in part, to the development of exercise-induced gastric disease.

The inability of animals to articulate symptoms results in a condition in which overt clinical signs of disease represent the "tip of the iceberg" relative to the overall prevalence of the problem. As a result, veterinarians are likely to underestimate the actual prevalence of disease unless other, more sensitive examinations such as gastroscopy are conducted. Numerous studies of racing sled dogs have demonstrated that in the absence of effective prevention of gastritis, 50-60% of clinically normal dogs will have moderate to severe gastric disease with as little as a single day of exercise^{5;9;11-13}. Similar prevalence values also have been reported in racehorses^{7;8;10;14-16}. In all cases, the prevalence of exercise-induced gastric disease has been substantially higher when gastroscopy is used to diagnose the condition than when clinical signs are the diagnostic criteria. Thus, it is not surprising that this condition had not been heretofore recognized in field-deployed IDD's. Despite the lack of overt clinical signs in the majority of affected athletes, occult exercise-induced gastric disease does appear to have a deleterious impact on performance, as illustrated by studies in racehorses in which racing performance improved with the implementation of effective prevention, even in the absence of clinical signs of disease¹⁶. These data provide ample justification for implementation of a targeted program for prevention of exercise-induced gastric disease in deployed IDD's. Fortunately, this condition can be easily and effectively prevented with over-the-counter medications^{12;13;17}, and with the implementation of this prevention program the importance of this condition can be minimized.

Conditioning/Deconditioning Studies

Results

GPS collars were used to record the miles traveled by the dogs participating in the IDD 2.0 conditioning program. The average speed of the dogs while exercising off-leash was remarkably stable at approximately 4 miles/hr. Thus, distance traveled tended to be a function of time, with distances covered during weeks 1-5 of 5.6 ± 0.6 , 12.8 ± 1.2 , 15.3 ± 1.6 , 21.2 ± 1.8 , and 27.7 ± 2.9 miles/day. Overall coefficient of variation among dogs was approximately 10%, illustrating the high uniformity of the dogs when exercised as a group.

Determination of VO_2max was problematic, with many dogs unwilling to perform to their maximum capability while instrumented (demonstrably more exercise tolerant without calorimetry equipment). In addition, the calorimetry equipment was non-functional at the onset of the study, thus precluding the establishment of pre-conditioning baselines. In many cases, the absolute criterion for designating the oxygen consumption measured as VO_2max (increases in treadmill work without an increase in oxygen consumption) was not achieved, and therefore the data are more correctly designated as VO_2peak (i.e., the maximal amount of oxygen consumption measured during the examination). The limited numbers of valid data sets suggest that the dogs are capable of developing considerable aerobic fitness (VO_2 peak: 133.5 ± 23.7 ml/kg/min IDD 1.0; 140.0 ± 29.7 ml/kg/min IDD 2.0), but there was no significant difference between the different conditioning programs in aerobic capacity ($p = 0.35$).

Conditioning the dogs resulted in moderate weight loss, with the mean bodyweight decreasing from 28.56 ± 3.82 kg to 27.36 ± 3.35 kg ($p < 0.001$). This was largely attributable to dogs participating in the IDD 2.0 conditioning program, having lost significantly more weight than those participating in the IDD 1.0 conditioning program (1.94 ± 0.74 vs 0.45 ± 0.51 kg, $p = 0.001$).

Plasma volume at the beginning of the study was 1.68 ± 0.30 L or 5.92 ± 0.96 % of bodyweight. Conditioning did not result in a change in plasma volume as either an absolute number (1.60 ± 0.23 L, $p = 0.14$) or as a percentage of bodyweight ($5.87 \pm 0.76\%$, $p = 0.42$). There was no difference in the change in plasma volume as a function of the different conditioning programs (IDD 1.0: -0.03 ± 0.17 L; IDD 2.0: -0.15 ± 0.36 L, $p = 0.23$), (IDD 1.0: $0.03 \pm 0.69\%$ BW; IDD 2.0: $-0.15 \pm 1.23\%$ BW, $p = 0.38$). ECF volume at the beginning of the study was 13.58 ± 2.20 L or 47.47 ± 3.58 % of bodyweight. Conditioning did not result in a change in ECF volume as either an absolute number (13.83 ± 3.00 L, $p = 0.36$) or as a percentage of bodyweight ($50.62 \pm 8.81\%$, $p = 0.12$). There was no difference in the change in ECF volume as a function of the different conditioning programs (IDD 1.0: 0.99 ± 1.18 L; IDD 2.0: 0.79 ± 0.93 L, $p = 0.38$), (IDD 1.0: $4.51 \pm 4.67\%$ BW; IDD 2.0: $6.31 \pm 3.16\%$ BW, $p = 0.23$).

All dogs had serum chemistry and electrolyte values within normal ranges at the beginning of the conditioning study. Conditioning of the dogs resulted in a significant increase in serum glucose concentration, and significant decreases in serum inorganic phosphorus and sodium (Table 4: Effect of physical conditioning on serum chemistry and electrolyte values. Table 4). These changes were not judged to be biologically significant. There was no effect of the conditioning programs on serum chemistry values or electrolyte concentrations. When dogs performed a single day deployment simulation at the end of the conditioning period, the IDD 2.0 conditioning

program resulted in significantly lower post-exercise CPK values compared to the IDD 1.0 conditioning program ($p = 0.02$, Figure 8).

Table 4: Effect of physical conditioning on serum chemistry and electrolyte values.

	Pre-Conditioning	Post-Conditioning		Effect of Conditioning	Effect of Conditioning Program
Parameter		Group A	Group B		
LDH (U/L)	72±44	50±0	54±11	$p=0.08$	$p=0.2$
AST (U/L)	32±6	37±14	33±5	$p=0.2$	$p=0.3$
BUN (mg/dl)	25.8±4.6	24.1±5.0	25.4±1.8	$p=0.3$	$p=0.3$
tCa (mg/dl)	10.3±0.4	10.2±0.2	10.1±0.4	$p=0.2$	$p=0.4$
CPK (U/L)	114±59	92±20	111±31	$p=0.3$	$p=0.1$
Creat (mg/dl)	0.9±0.1	0.8±0.3	0.8±0.1	$p=0.1$	$p=0.3$
Alb (g/dl)	3.5±0.2	3.5±0.2	3.5±0.2	$p=0.4$	$p=0.4$
Tbili (mg/dl)	0.3±0.4	0.3±0.1	0.3±0.1	$p=0.4$	$p=0.1$
Tprot (g/dl)	5.7±0.3	5.5±0.3	5.6±0.3	$p=0.1$	$p=0.2$
Glu (mg/dl)	85±12	103±11	102±9	$p<0.01$	$p=0.4$
GGT (U/L)	15±6	11±1	10±4	$p=0.01$	$p=0.4$
iPhos (mg/dl)	4.8±0.6	3.3±0.3	3.4±0.3	$p<0.01$	$p=0.3$
Na (meq/l)	149±1	146±2	147±1	$p<0.01$	$p=0.5$
K (meq/l)	4.3±0.2	4.0±0.3	4.3±0.4	$p=0.1$	$p=0.1$
Cl (meq/l)	130±4	128±6	129±3	$p=0.2$	$p=0.4$

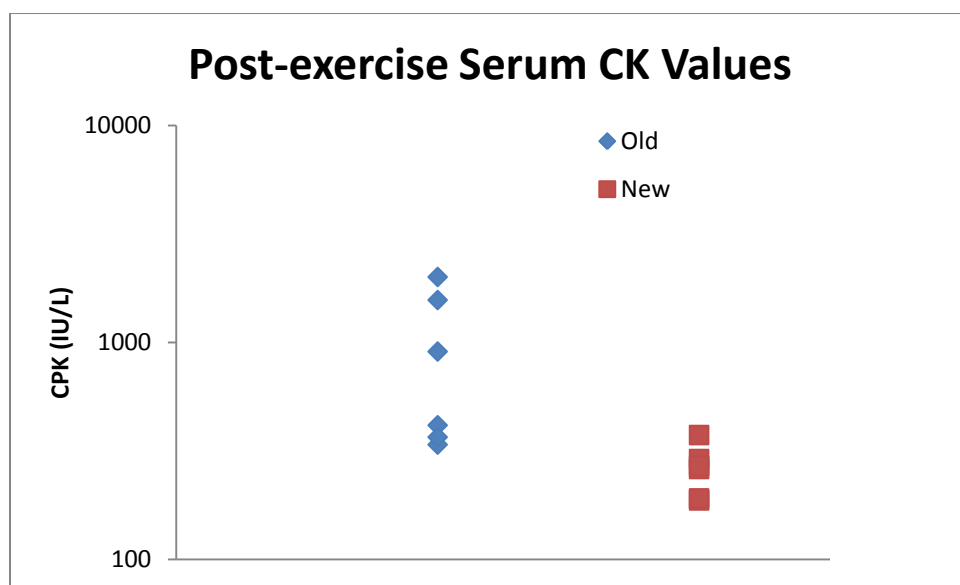


Figure 8: Effect of conditioning program on post-exercise serum CPK concentrations. Serum samples were obtained within 2 hr of completing a single day deployment simulation (9 hr intermittent off-leash exercise).

Loss of aerobic capacity was rapid upon reduction of activity. After 8 weeks of reduced activity, VO_2max was 84% of peak value measured during the conditioning program (VO_2 peak: 137.1 ± 26.0 ml/kg/min at the start of deconditioning; 115.4 ± 31.6 ml/kg/min after 8 weeks of deconditioning, $p = 0.003$). Deconditioning also resulted in a reduction of voluntary exercise performance. After 8 weeks of deconditioning, dogs performing a single day deployment simulation covered only 19.5 ± 3.7 miles (compared to 27.7 ± 2.9 miles at the end of the conditioning period, $p = 0.0001$). Though there was a trend towards increased serum CPK as a result of the single-day deployment simulation, post-exercise serum CPK concentrations were within normal limits (Pre-exercise 121 ± 45 ; Post-exercise 145 ± 54 IU/ml, $p = 0.07$). This result must be interpreted with caution in light of the reduced exercise performance of the dogs.

Discussion

The results of the conditioning studies were somewhat surprising, in that many of the expected cardiovascular adaptations to exercise (increased plasma volume, increased extracellular fluid volume^{18;19}) did not occur. It is possible that the relatively high variability in these measurements (coefficient of variation of 15-20%) precluded the detection of a conditioning effect. However, the expected change (20-25% of basal value) is large and even with the inherent variability of the measurements, the study was sufficiently-powered to detect changes of this magnitude with a high likelihood. These data suggest that the dogs used in this study (and perhaps athletic dogs in general) possessed sufficient basal plasma and extracellular fluid volume to support the exercise in either conditioning program. Cardiovascular fitness is most closely tied to aerobic exercise capacity (moderate duration, high intensity work) because the ability of the athlete's cardiovascular system to supply oxygen to the working muscle is the primary limiting factor. However, as described in the VO_2max examinations, the deployment simulation GPS records, and the respective conditioning programs, the dogs are conditioning and performing at speeds considerably slower than that approximating VO_2max , and therefore their ability to perform that type of exercise is not being limited by those features of their exercise physiology that define maximal aerobic capacity. As a result, it is logical that the conditioning programs used in this study did not result in increased cardiovascular capacity because no improvement in cardiovascular capacity was needed.

Endurance exercise lasting for hours to days, such as that expected of IDD during deployment, is limited primarily by their ability to transport combustible fuels (fats and carbohydrates) into the muscle, thus sparing the limited energy reserves stored in the muscle. Muscle cells maintain a basal level of fuel transport using fat and carbohydrate-specific transporters that is sufficient to sustain the cell at rest. A large number of additional transporters, representing additional transport capacity, are stored inside the cell and can be mobilized to an active state in response to contraction. Contraction-induced increases in fuel transport are limited by the overall abundance of transporters, and exercise intensities that exceed the overall capacity for fuel transport result in progressive depletion of the limited amounts of fuel (primarily glycogen) stored in the muscle. Given the finite amount of fuel stored inside muscle, exercise intensities that require utilization of this fuel are, by definition, unsustainable. As the stored intramuscular fuel is depleted, the muscle becomes energy-depleted, and fatigue and muscle damage occurs. Conditioning can increase the capacity for transport of fuel into the muscle through increased abundance of transporters and possibly increased efficiency of the contraction-mediated transport mechanism, and as a result, increases the intensity of exercise that can be sustained without the utilization of intramuscular reserves.

The ability of the muscle to transport sufficient fuel to sustain a given level of exercise is difficult to measure directly, and as a result surrogate markers are used instead. In the September 2011 deployment simulation, we relied on measurement of muscle glycogen before and after the 5 day exercise to provide indirect evidence of a failure of the working muscle to take up adequate amounts of combustible fuel, and also considered the release of muscle enzymes (CPK, AST) into the blood as evidence of muscle damage secondary to a relative energy deficit. In this study, we elected to rely entirely on serum CPK to determine whether a dog's muscle was able to maintain homeostasis in the face of the exercise demand due to the excellent association between the changes in serum CPK concentrations and the depletion of muscle glycogen, as well as the desire to avoid the risks of general anesthesia in order to obtain the muscle biopsies. Based on the post-exercise CPK values, a conditioning program that mimics the actual expected exercise activity is superior for preparing the dogs for that activity, as would be expected by the principle of activity-specific conditioning. Furthermore, the near-complete lack of overlap between the post-exercise values of the dogs performing the new conditioning program compared to the old conditioning program highlights the strong discrimination power of this diagnostic tool, and makes it a leading contender for evaluating the successful conditioning of dogs in the operational IDD program.

Metabolic Heat Management and Heat Acclimatization

Results

The tolerance of the dogs to exercise-induced hyperthermia and the capacity for regulating exercise-induced heat accumulation showed a clear improvement in response to conditioning. During the initial exercise-induced heat stress tests, conducted before the onset of conditioning, the dogs struggled to complete the 15 min treadmill exercise, with 3 dogs unable to complete the full test. All 3 of these dogs had rectal temperatures below the average of the entire group at the conclusion of their abbreviated exercise. All dogs were able to complete the exercise tests on the subsequent examination dates, and post-exercise rectal temperatures progressively decreased as conditioning progressed ($p = 0.01$, Figure 9).

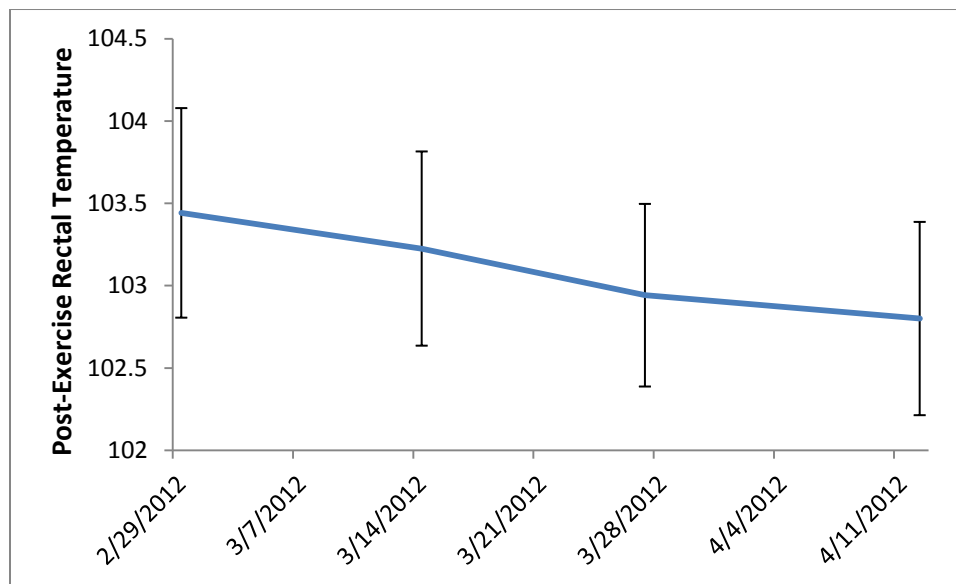


Figure 9: Effect of conditioning on post-exercise rectal temperature. Values are mean $\pm$ SD. All dogs (n=12) were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance capacity.

Static heat tolerance testing revealed a statistically significant increase in the rectal temperature of the dog when exposed to high ambient temperatures. During the initial testing, dogs were highly intolerant of the procedure and became acutely distressed, necessitating removal from the environmental chamber. Only 3 of dogs were able to remain in the environmental chamber for the entire 60 min of the exposure for the initial examination after 2 weeks of conditioning. As a result, the analyzed data from this examination is the peak rectal temperature measured prior to removing the dogs from the chamber. By the 4th week of conditioning, all dogs were able to tolerate the full 60 min exposure. Peak tolerated rectal temperature significantly increased with conditioning at each examination, with an overall gain of 1°F over the conditioning period ($p = 0.001$, Figure 10).

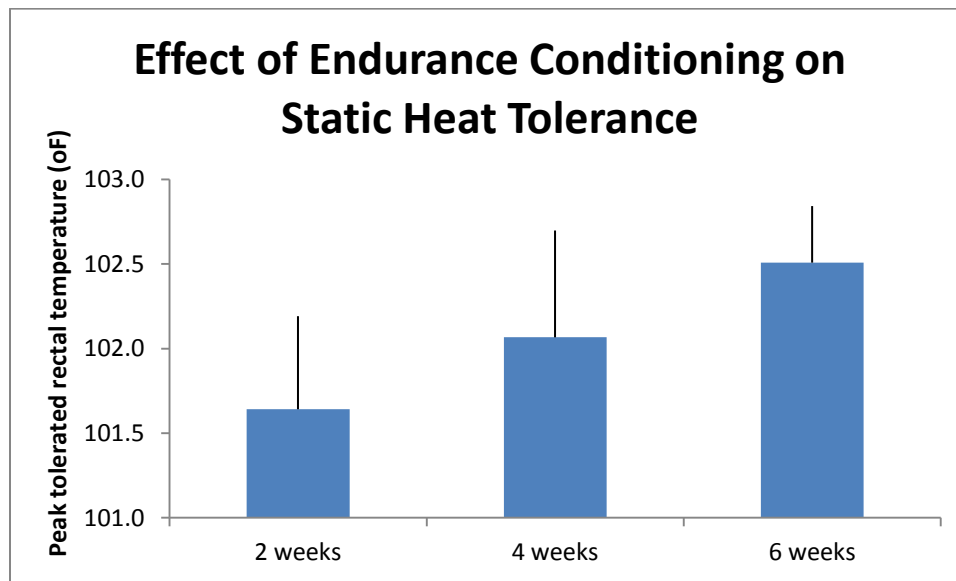


Figure 10: Effect of conditioning on static heat tolerance. All dogs (n=12) were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance capacity.

Allowing the dogs to rest on a cooling pad perfused with water at 39°F resulted in a slight benefit to the dogs (Figure 11), with a mean temperature (over the course of the 60 min heat stress exposure) of 101.6 vs 101.9°F without the cooling pad ($p = 0.03$). However, there was no difference in the peak temperature of the dogs during the 60 min static heat stress test (101.9 vs 102.1°F, $p = 0.23$).

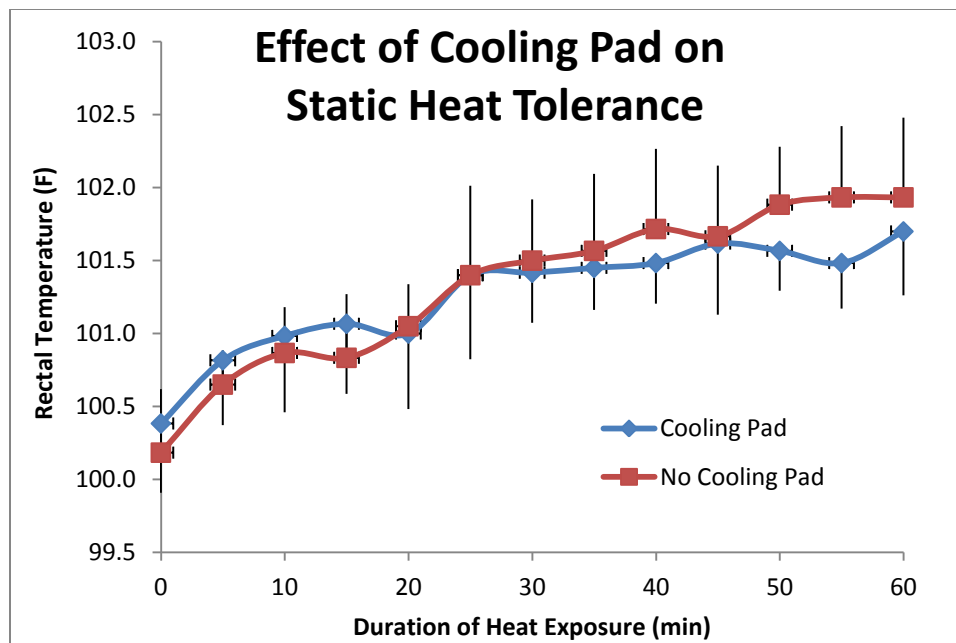


Figure 11: Effect of a cooling pad on body temperature during static heat stress. N = 6 dogs/group. Ambient conditions were 100°F, 60% RH. Cooling pad was perfused with 39°F water.

Heat shock proteins were measured in samples collected during the Conditioning/Deconditioning study to determine whether these blood-borne compounds could be used as markers of heat acclimatization. HSP 60 (Figure 14) and HSP 70 (Figure 15) showed no consistent pattern, either as a function of conditioning ($p = 0.24$, $p = 0.17$, respectively) or as a function of conditioning program ($p = 0.29$, $p = 0.70$, respectively). HSP 27 (Figure 12) and HSP 40 (Figure 13) did not vary based on specific conditioning program ($p = 0.46$, $p = 0.43$, respectively), but did vary between the beginning and end of the overall conditioning period ($p = 0.03$, $p = 0.008$, respectively). Whether this difference was due to conditioning or another temporal event (mean ambient temperature, for example) could not be determined in this study, but the overall variability of these markers likely preclude their use to determine relative heat acclimatization on an individual dog basis. HSP 90 (Figure 16) did not vary between conditioning program ($p = 0.34$), but decreased in a highly significant manner ($p < 0.001$) during the conditioning period. The relatively low magnitude of population variability provides encouragement for further investigation of this marker as a sensitive and specific diagnostic tool for quantifying heat acclimatization in dogs.

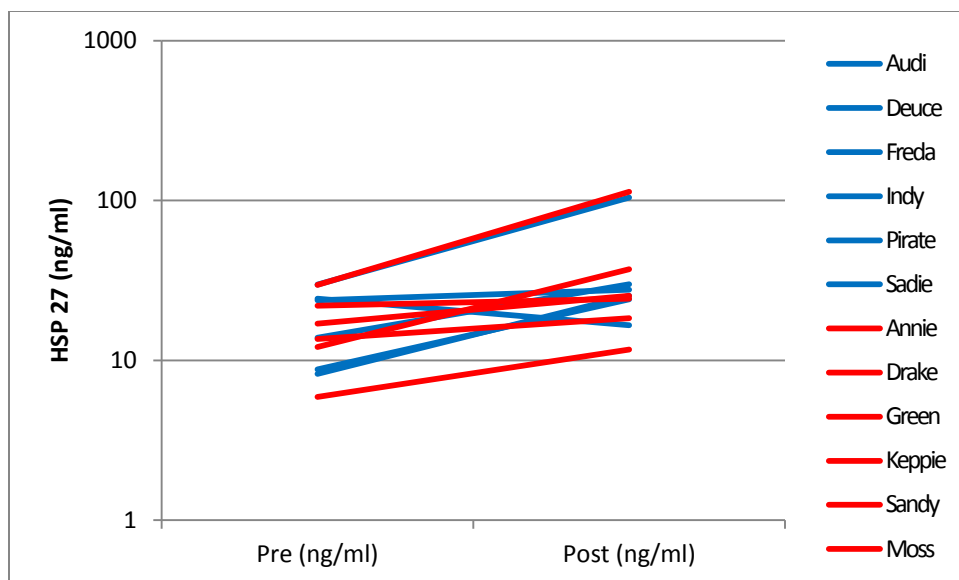


Figure 12: Effect of endurance conditioning on serum concentrations of HSP 27. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.

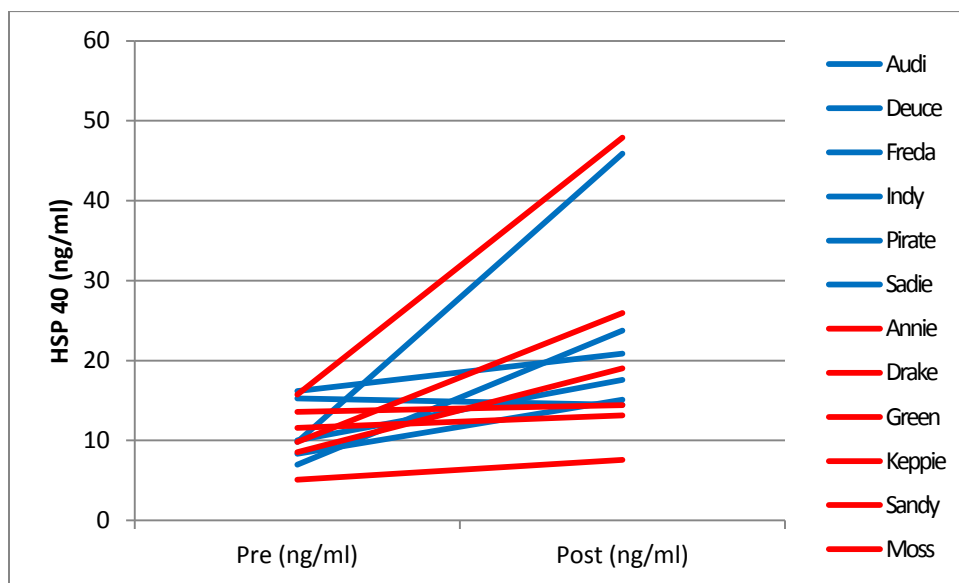


Figure 13: Effect of endurance conditioning on serum concentrations of HSP 40. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.

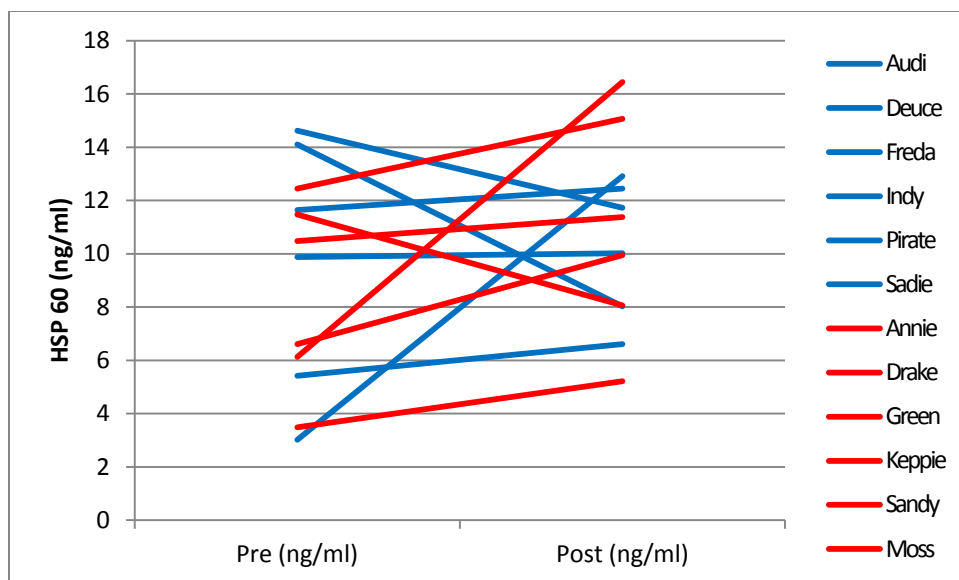


Figure 14: Effect of endurance conditioning on serum concentrations of HSP 60. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.

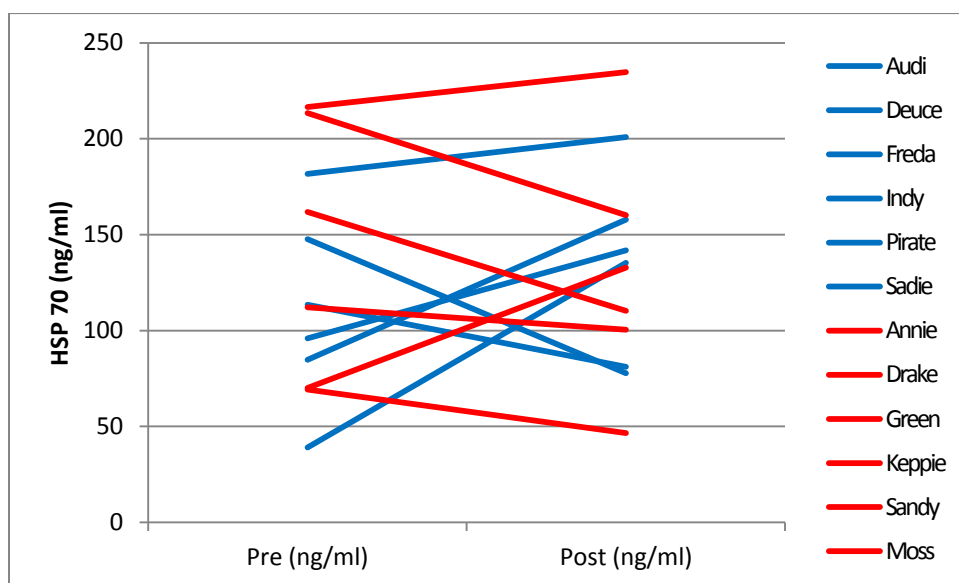


Figure 15: Effect of endurance conditioning on serum concentrations of HSP 70. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.

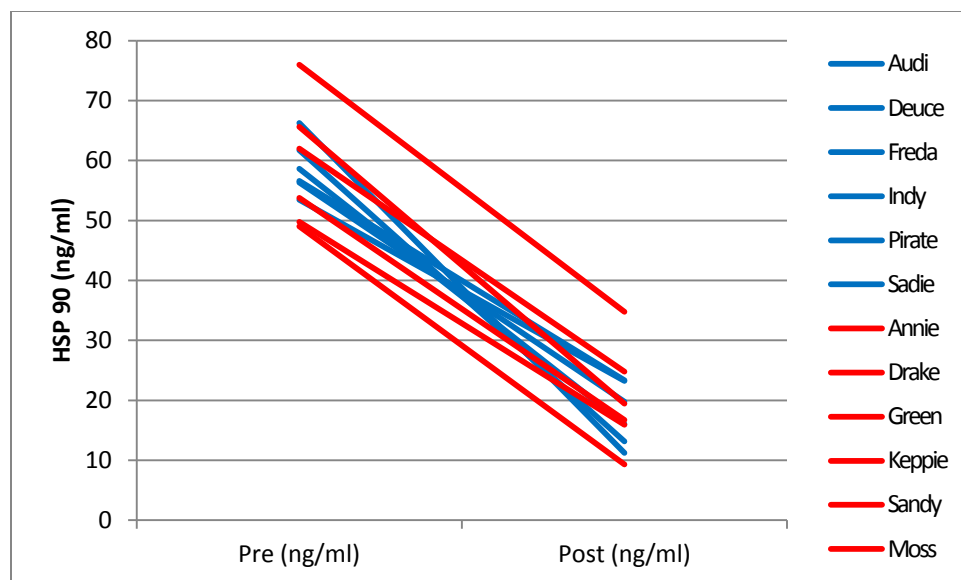


Figure 16: Effect of endurance conditioning on serum concentrations of HSP 90. “Pre” samples were obtained at the beginning of the conditioning program, and “Post” samples were obtained at the conclusion of the conditioning program. Dogs displayed in blue were conditioned using the standard IDD 1.0 conditioning program, and dogs displayed in red were conditioned using the modified IDD 2.0 conditioning program developed to improve endurance performance.

Discussion

The process of heat acclimatization falls into two broad conceptual processes: increased ability to shed heat (heat dissipation), and tolerance of hyperthermia. Heat dissipation has many elements, including the rate at which heat is moved to the dissipation surfaces, the rate of air movement across the dissipation surfaces, and the enthalpy gradient between the dissipation surfaces and the ambient air. The rate at which heat is moved to the dissipation surfaces is a function of cardiac output and the ability to simultaneously perfuse both the sites at which the heat is being produced AND the sites of dissipation. During exercise, when regional demand for cardiac output is high, the competition for the limited amount of blood flow can result in reduced perfusion of dissipation surfaces and impaired movement of heat from the site of generation (working muscle) to the oral mucosa. As a result, hyperthermia develops. However, physical conditioning can be expected to improve cardiac output capacity, so that at equivalent exercise intensities, there is less competition for blood flow. This permits adequate perfusion of oral mucosa and more effective shedding of heat. This is the likely explanation for the reduction of end-exercise temperature in the exercise-induced hyperthermia studies (Figure 9) – the rate of heat production was held constant by the specific treadmill workout, and gradient down which the heat was dissipated from the dog was held constant by the conditions in the environmental chamber. Thus, the only variable that was allowed to change and could account for the difference in the dogs’ temperature was the rate of heat transfer from the site of generation to the dissipation surfaces. As the dogs’ fitness increased, so did their ability to transport heat through their bodies and as a result, less hyperthermia developed. This simple experiment serves to underscore the important role of conditioning on heat tolerance during exercise, particularly in environments with high thermal stresses.

Passive tolerance to hyperthermia is an important adaptive process that is closely linked to the more active processes of thermoregulation. It is important to consider that, regardless of the relative capacities; the efforts of the dog to move heat to the oral surfaces for dissipation and to increase the airflow across those surfaces are energy intensive. Thus, the intensity with which these processes are activated is related to the PERCEIVED need to increase the rate of heat dissipation. For example, a dog may have a normal resting body temperature of 100°F, and that temperature represents the balance between heat production and heat dissipation. If that dog is placed in a situation in which heat dissipation is impaired (a hot, humid environment), then body temperature will rise. However, the dog will not immediately increase efforts to dissipate heat until its core temperature has increased sufficiently that there is a homeostatic need to prevent further increases. Furthermore, efforts to increase heat dissipation will be recruited incrementally as body temperature rises, and a new core temperature will stabilize, representing the balance between the degree of hyperthermia and the energetic costs of further increasing heat dissipation. This process is simplified conceptually as a series of thermoregulatory “set-points” – body temperatures that result in recruitment of specific, quantifiable processes related to heat dissipation (for example, the core temperature that causes a dog to start panting) or conversely, the steady-state temperature of a dog under a set of specific conditions. The process of tolerance to hyperthermia is quantified by the determination of these set-points, and represents a physiological phenomenon of reduced requirement to maintain a normal body temperature when doing so would require expenditure of energy or the use of other limited resources.

An increase in enthalpy gradient (the difference between the heat content of fully-humidified air at body temperature and the actual temperature and humidity of the ambient air) will increase the rate of heat dissipation without the expenditure of additional energy (and the creation of additional metabolic heat that needs to be dissipated). This aspect of thermoregulation forms the basis for heat acclimatization through tolerance to hyperthermia: if the subject can tolerate higher body temperature, then the enthalpy gradient down which metabolic heat is dissipated is increased, and a greater rate of heat production can be sustained without further increases in body temperature. Furthermore, to the extent that tolerance to hyperthermia permits an increase in the core body temperature, it delays the implementation of the more energy-intensive aspects of heat dissipation (distribution of cardiac output to oral mucosal vascular beds and panting), thus reducing the portion of overall metabolic heat production due to efforts to dissipate heat. As a result, proportionally greater amount of the overall tolerable rate of heat production is due to exercise work.

The effect of conditioning on tolerance to hyperthermia is illustrated by the static heat stress testing (Figure 10). In this test, the increase in ambient temperature reduced the enthalpy gradient down which the heat dissipates from the dog to the environment. As a result, heat begins to accumulate in the dog, serving to increase body temperature and partially restore the enthalpy gradient. In addition, the dog begins panting to restore the normal rate of heat dissipation to match the rate of basal metabolic heat production. The inability of the dogs to completely restore the normal rate of heat dissipation during the initial testing early in the conditioning process resulted in clinical distress in the majority of the dogs due to their body temperature exceeding a tolerable temperature. At that point, all processes available to maximize heat dissipation had been recruited – the dogs were panting heavily and their oral mucosal surfaces were fully exposed (mouths wide open, cheeks retracted) at a body temperature of approximately 101.7°F. In other words, the dogs perceived a body temperature of 101.7°F to

be sufficiently dangerous that ALL efforts to increase heat dissipation were needed. This is in contrast to the end of the conditioning period. Under the same experimental conditions, the same tolerated a body temperature of 102.6°F, and this higher temperature did not merit maximal recruitment of heat dissipation mechanisms. In other words, physical conditioning increased their thermoregulatory set-point, allowing a greater degree of hyperthermia before recruitment of active cooling processes is required.

The importance of tolerance to hyperthermia is not just in the delay in recruitment of energy-intensive processes for heat dissipation. Hyperthermia also helps maintain the enthalpy gradient down which metabolic heat must dissipate – a feature that is highlighted by the small enthalpy gradients encountered in the current operational theater. For example, assuming an ambient temperature of 120°F and 30% relative humidity (106 kJ), a dog with a normal body temperature (100°F) has an enthalpy gradient relative to the ambient air of approximately 42 kJ. The ability of the dog to tolerate a body temperature of 102°F during exercise results in a 16% increase in enthalpy gradient (50 kJ). However, an ability to tolerate a body temperature of 106°F during exercise, as has been observed in well-conditioned dogs in this program as well as other canine sporting events, results in an enthalpy gradient increase to 69 kJ (64% increase), thus dramatically increasing the permissible rate of metabolic heat production while still maintaining a stable body temperature.

Hydration and Water Requirements

Results

The determination of daily water requirements was performed under temperate conditions (Dec 16, 2011 in Pinehurst, NC). Initial Total Body Water was $68.9 \pm 3.7\%$ of bodyweight. At the conclusion of the single day of exercise, dogs had lost 1.2 ± 1.0 kg bodyweight ($p < 0.001$), but water loss was not significant (0.3 ± 0.6 L, $p = 0.08$) and there was no change in total body water as a % of bodyweight ($69.3 \pm 3.4\%$, $p = 0.30$). Daily water requirement, calculated as the sum of water intake and change in total body water, was 1.9 ± 0.7 L, of which 1.6 ± 0.5 L was met using voluntary water intake. The remaining 300 ml deficit represented less than 2% of total body water and did not result in clinical signs of dehydration. These results suggest that in well-conditioned dogs performing sustained aerobic exercise under temperate conditions, voluntary water intake is sufficient for meeting the dogs' hydration requirements. However, these results should be extended with caution (or not at all) to situations involving less adequate conditioning or greater heat stress, both of which may increase the water requirements of the dog and also decrease the willingness for voluntary water intake.

Gatorade as a water-baiting method had no effect on water consumption or hydration status. Dogs provided Gatorade instead of plain water had no difference in distance covered, water intake, body weight, and total body water as both absolute values and as a function of bodyweight. Overall weight loss during the single day of exercise was minimal (0.4 ± 1.2 kg), and overall change in total body water was 0.0 ± 1.9 L, indicating that similar to the previous single day study, voluntary water intake is sufficient to meet the daily water requirements of well-conditioned dogs performing exercise in moderate heat (Table 5). The sole benefit identified in substituting Gatorade for plain water during daily exercise is the intake of an additional 357 ± 197 kcal. Given the previously-demonstrated caloric deficit experienced by the dogs during deployment-type activities, the use of Gatorade in the drinking water should be considered as part of the overall metabolic and nutritional management of the dogs.

Table 5: Effect of Gatorade on hydration parameters and performance during deployment-style activity.

Parameter	Plain Water	Gatorade	p-value
Body weight	27.5 ± 3.3	27.3 ± 3.2	$p = 0.46$
Total body water (L)	19.3 ± 2.7	19.3 ± 2.7	$p = 0.49$
Total body water (%)	70.2 ± 3.1	70.5 ± 3.6	$p = 0.40$
Water intake	1.8 ± 0.8	1.6 ± 0.9	$p = 0.33$
Distance covered	17.6 ± 2.8	17.5 ± 2.7	$p = 0.25$

Premedication with omeprazole at a dose of 20 mg orally once daily per dog did not result in a difference in voluntary water consumption (omeprazole 1.371 ± 619 , no treatment 1.371 ± 733 ml/day, $p = 0.5$) during the single day deployment activity.

Discussion

A stated concern from the dog handlers in the deployment areas is the adequate maintenance of hydration in the IDD. Reports include insufficient water intake and the need for parental fluid administration in order to maintain hydration. However, we did not encounter any problems with serious dehydration in any studies conducted as part of the IDD 2.0 development process.

When water was offered during rest periods, dogs drank sufficient amounts to largely maintain hydration, and any reduction in total body water measured in these studies was not considered clinically significant.

There are numerous potential reasons for the discrepancy between the reports from the field and our observations. First, the ambient temperatures of our studies were lower (in some cases, substantially lower) than the worst-case conditions found in the deployment theater. This aspect not only increases the reliance of the dog on evaporation to cool, but also increases the temperature of the water being offered for voluntary intake, potentially making it less palatable. Second, with few exceptions, all of our water consumption studies were conducted using well-conditioned dogs, and it is possible that severe fatigue will decrease voluntary water intake, all other things being equal. However, in the two studies in which unconditioned dogs were used (the first and second deployment simulations), there was no difference noted in the water intake of well-conditioned and poorly-conditioned dogs. Third, the behavior being attributed to dehydration in theater is being misinterpreted, instead being the result of fatigue and/or caloric deficiencies. We do not have the data necessary to clearly refute or confirm any of these possibilities.

The primary value of these series of studies is the quantification of the water intake required for the average IDD during deployment. Combining all studies, the data suggest that the dogs have a daily requirement of approximately 4.5 liters/day, with approximately 40% of that (1.8 liters) being required to maintain hydration during a standard 9-hr patrol. Our data suggest that a well-conditioned IDD with an appropriate level of nutrition will voluntarily drink that volume over the course of a 9 hr patrol, thus maintaining normal hydration.

June 2012 Deployment Simulation

Results

The deployment simulation was conducted in Pinehurst, North Carolina from June 8-14, 2012. One IDD 2.0 dog in the energy expenditure study was unable to begin the study due to an injury obtained during the week prior to the study. Two IDD 1.0 dogs in the energy expenditure study were unable to continue with exercise activities after the first day due to severe fatigue, anorexia, and muscle soreness.

IDD 2.0 dogs averaged 20.9 miles/day, whereas the two remaining IDD 1.0 dogs averaged 18.7 miles/day ($p = 0.056$). IDD 2.0 dogs covered significantly more miles than IDD 1.0 dogs on the first 3 days of the simulation, but not on the last 2 days (Figure 17). Figure 17: Miles (mean $\pm$ SD) covered during each day of June 2012 deployment simulation by IDs.

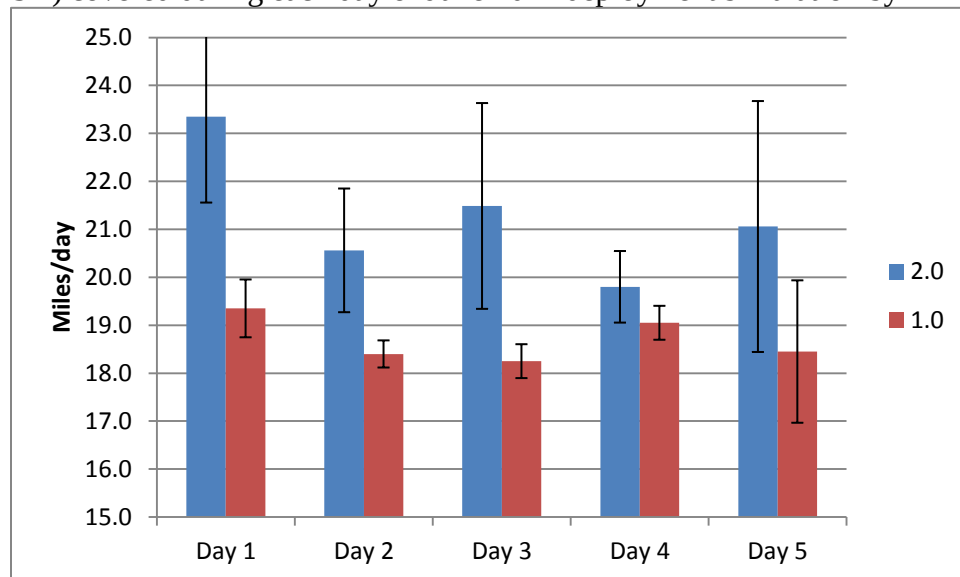


Figure 17: Miles (mean $\pm$ SD) covered during each day of June 2012 deployment simulation by IDs. Resting: Sedentary IDD 1.0 dogs ($n = 4$); IDD 1.0: Exercising IDD 1.0 dogs ($n = 8$); IDD 2.0: Exercising IDD 2.0 dogs ($n=11$).

Odor times were highly variable despite the methodology used to minimize this variability. There was a significant difference in odor times between IDD 1.0 and IDD 2.0 prior to the start of the 5 day exercise, and a strong trend towards continued difference at the end of the first day. However, although as a group IDD 2.0 dogs had consistently superior performance compared to IDD 1.0 dogs, odor times on Days 2-5 were not significantly different between groups, in part due to the magnitude of variability within the groups, as well as progressive reduction in the difference between the two groups (Figure 18).

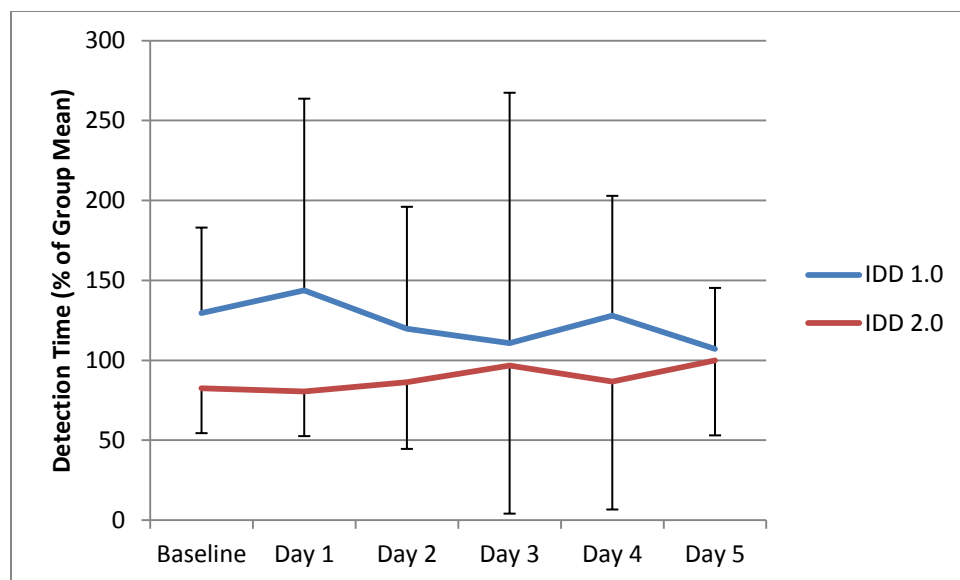


Figure 18: Odor detection performance during June 2012 deployment simulation. Data (mean $\pm$ SD) are expressed as the percentage of the group means for a particular day. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

IDD 2.0 dogs weighed significantly less than IDD 1.0 dogs (28.6 ± 3.9 vs 33.6 ± 3.9 kg), which were subjectively overweight at the beginning of the study (Figure 19). Total body water was 19.48 ± 2.41 L ($68 \pm 4\%$ of body mass) for IDD 2.0 and 20.10 ± 3.50 L ($58 \pm 5\%$ of body mass) for IDD 1.0, the difference in the relative amount of body water reflecting the difference in body condition (higher fat mass in IDD 1.0). There was no significant difference in total body water in absolute terms ($p = 0.39$), indicating that the two groups of dogs did not differ in overall lean body mass and that the significant difference in body weight was attributable to fat (obesity). IDD 2.0 dogs remained significantly lighter than IDD 1.0 dogs until the 5th day of the deployment simulation, at which point the average exercising IDD 1.0 had lost enough weight to approach the bodyweight of the IDD 2.0 dogs. IDD 2.0 dogs maintained (and even slightly increased) their bodyweight, whereas IDD 1.0 dogs lost an average 1.7 kg over the duration of the deployment simulation. IDD 2.0 dogs lost less weight during each of the daily patrols than IDD 1.0 dogs (2.3 vs 3.1 kg) – this was statistically significant on the first two days of the simulation but not on subsequent days (Figure 20).

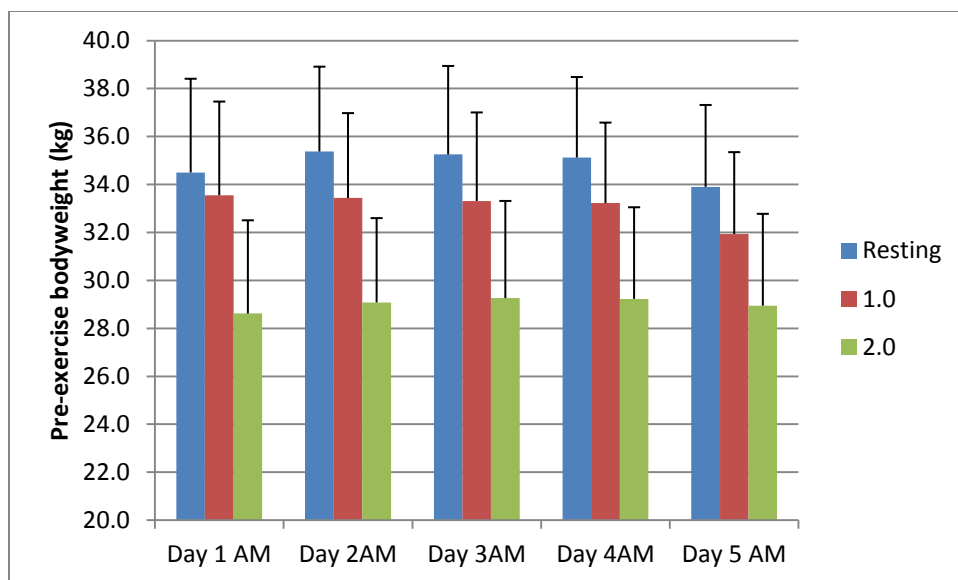


Figure 19: Pre-exercise body weight (mean $\pm$ SD) of IDD dogs during June 2012 Deployment Simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

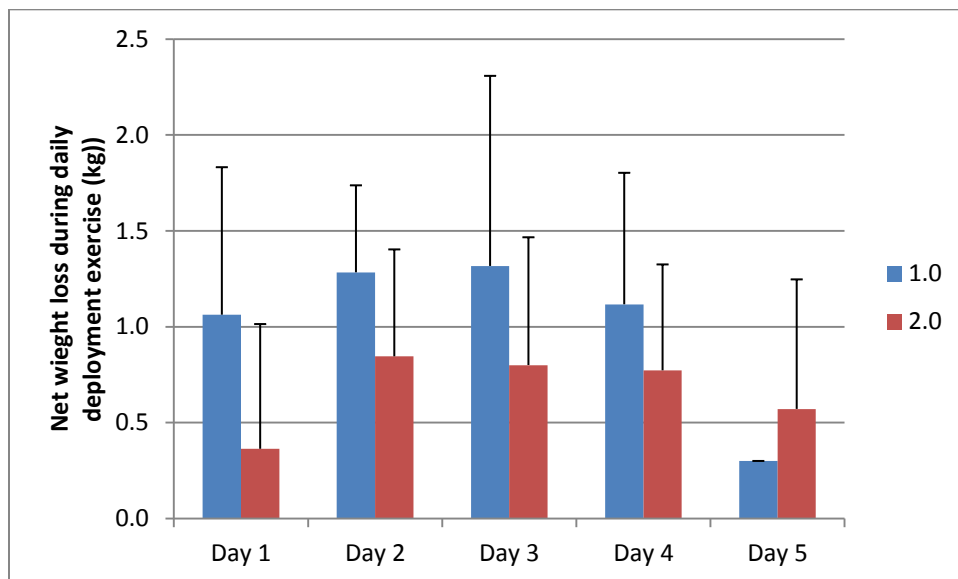


Figure 20: Exercise-induced weight loss (mean $\pm$ SD) during June 2012 deployment simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

Average voluntary water intake over the entire exercise was not different between IDD 1.0 and IDD 2.0 dogs (2310 ± 127 vs 2112 ± 875 ml/day). Daily water intake was significantly different between IDD 1.0 and IDD 2.0 on the first day of exercise, with the difference largely the result of a single IDD 1.0 that drank over 5 L of water on that day. This dog was also one that was unable to continue with the deployment simulation on subsequent days. Daily water turnover rates were identical between the two groups (IDD 2.0: 4.9 ± 0.9 vs IDD 1.0: 4.9 ± 0.3 L/day),

indicating that the dogs took in just under half of their daily requirement during the 9 hr measurement period.

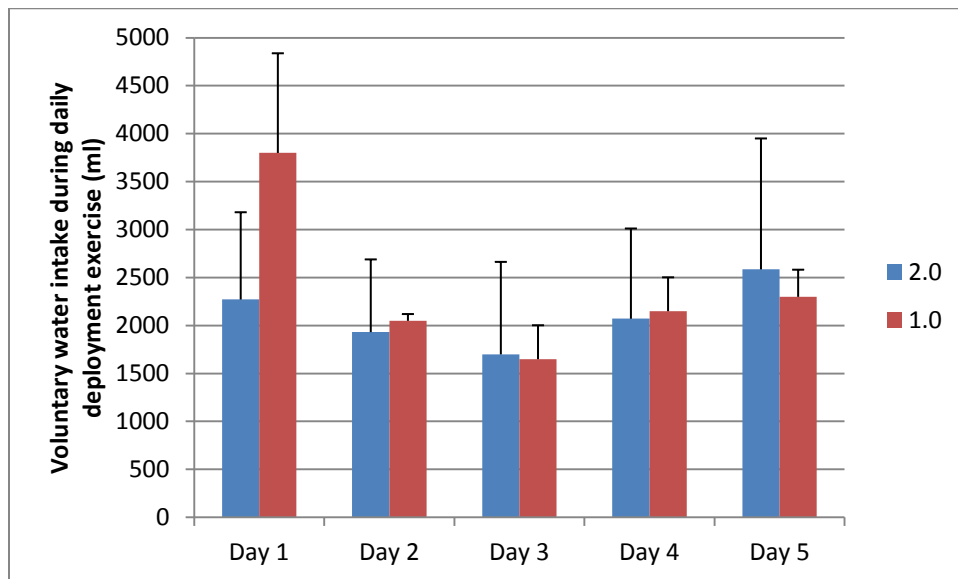


Figure 21: Daily voluntary water intake (mean $\pm$ SD) during June 2012 deployment simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

Total caloric intake of IDD 2.0 dogs was 310 ± 17 kcal/kg^{0.75}/day, of which 257 ± 22 kcal/kg^{0.75}/day were from kibble and the remainder from the Gatorade provided during the daily exercise period, or just slightly less than double the intakes recorded during the September 2011 deployment simulation. Gatorade provided approximately 17% of the total energy consumed by IDD 2.0 dogs during the study. IDD 1.0 dogs 209 ± 12 kcal/kg^{0.75}/day entirely from kibble. Total energy expenditure of the IDD 2.0 dogs was 500 ± 160 kcal/kg^{0.75}/day, compared to 364 ± 58 kcal/kg^{0.75}/day ($p = 0.15$). Though this comparison was not statistically significant, it is important to note that due to premature failures by the IDD 1.0 dogs, TEE was calculated from only 2 dogs for this group. As a result, this comparison is substantially underpowered. Overall, energy balance for these dogs, calculated as the proportion of caloric demands that were met by caloric intake, was 62% for IDD 2.0 and 57% for IDD 1.0.

The 5 days of exercise was associated with a 30% decrease in muscle glycogen content (171 ± 15 vs 120 ± 39 mmol glucosy units/kg dry muscle) in IDD 1.0 dogs, compared to a 10% decrease in muscle glycogen content (211 ± 71 vs 192 ± 40 mmol glucosy units/kg dry muscle). IDD 2.0 had significantly more muscle glycogen at the end of the 5 day deployment exercise compared to IDD 1.0 ($p = 0.03$).

All dogs had normal clinical chemistry and electrolyte values at the beginning of the study. The only analytes that differed between groups in a systematic manner were CPK, AST, and LDH (Figure 22, Figure 23, Figure 24). For all three analytes, the exercising IDD 1.0 dogs had significantly higher values than either the resting IDD 1.0 dogs or the IDD 2.0 dogs. These increases were at a maximum on the morning after the first exercise day and the second exercise day, but returned to baseline values for the remaining days of the study. Post-exercise CPK was also higher than baseline in both exercising groups on all exercise days, but the magnitude of

increase was significantly greater in the IDD 1.0 dogs compared to the IDD 2.0 dogs on all 5 days (Figure 25). The magnitude of increase in serum CPK post-exercise decreased with successive days of work in both groups, with the IDD 2.0 dogs approaching baseline values (i.e., minimal exercise-induced increase) by the 5th day of exercise.

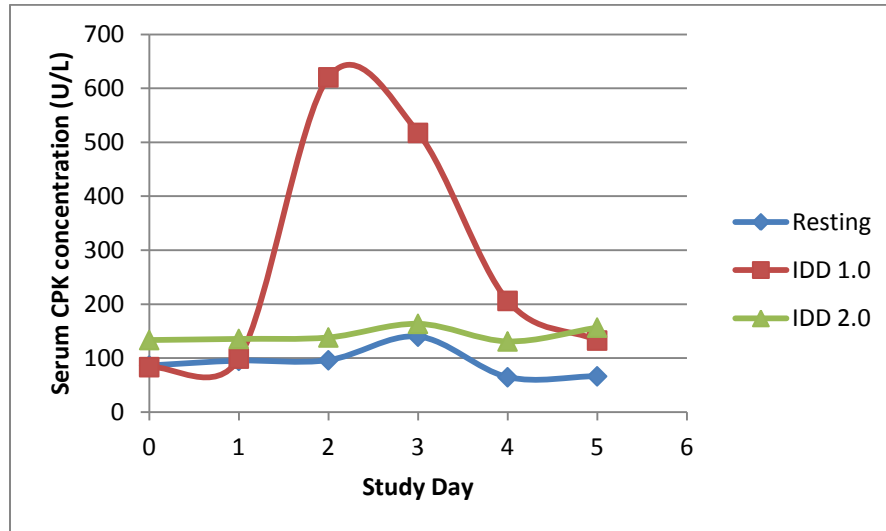


Figure 22: Effect of 5 day deployment simulation on serum CPK concentrations. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

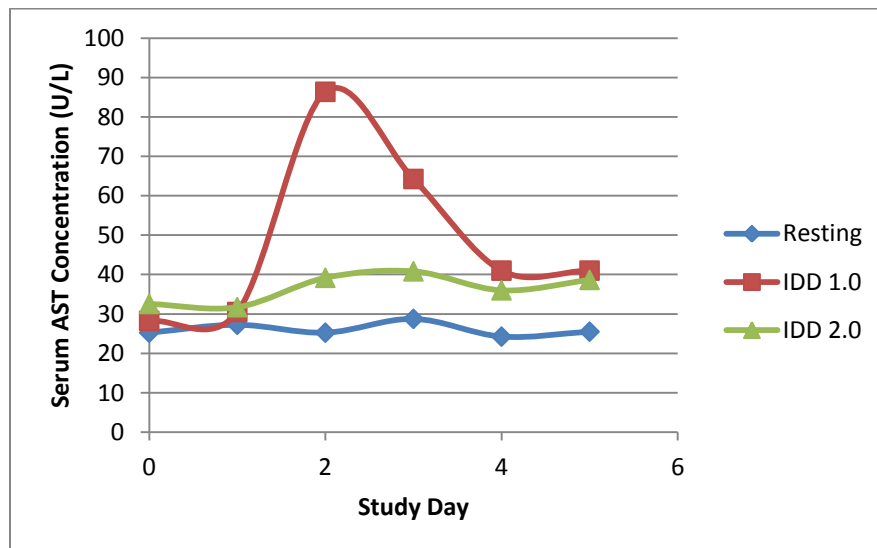


Figure 23: Effect of 5 day deployment simulation on serum AST concentrations. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

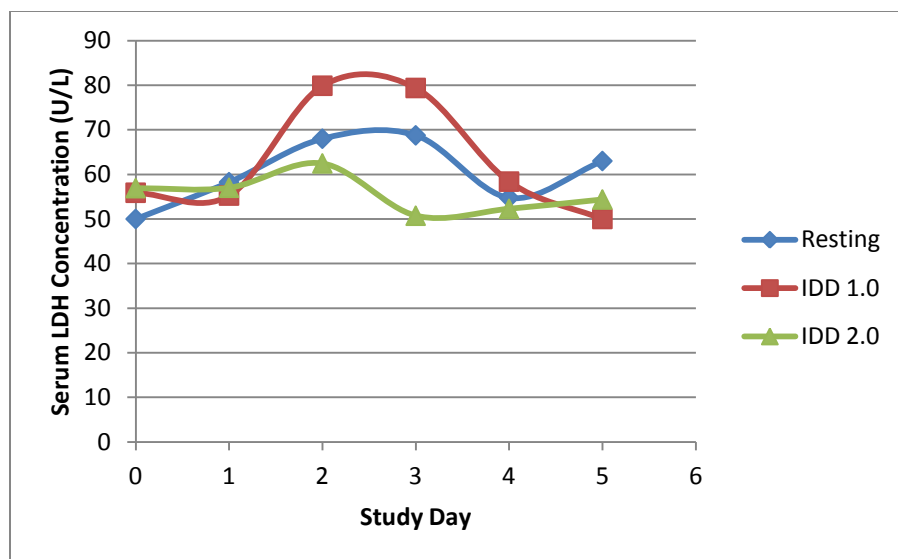


Figure 24: Effect of 5 day deployment simulation on serum LDH concentrations. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

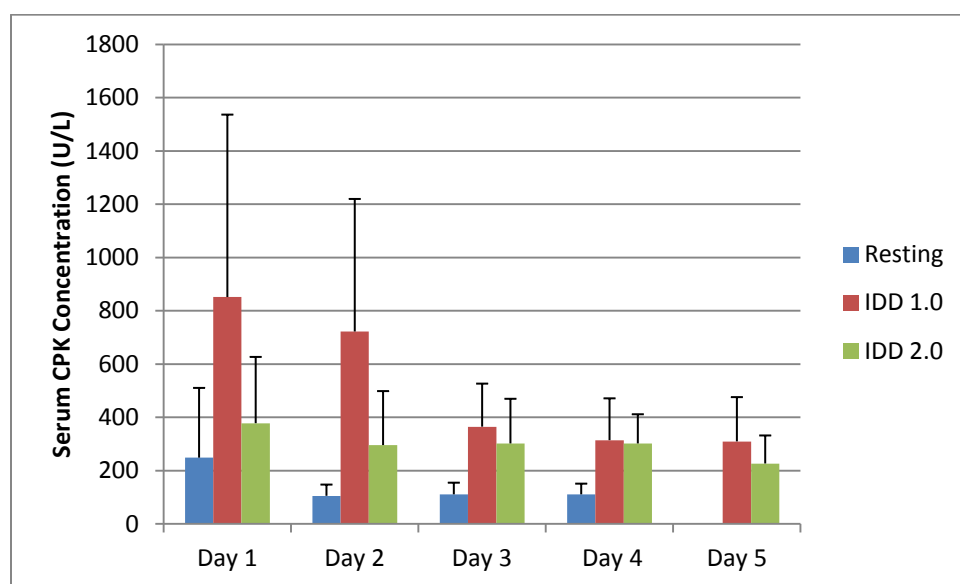


Figure 25: Post-exercise serum CPK concentrations (mean $\pm$ SD) in IDD during June 2012 deployment simulation. Resting: Sedentary IDD 1.0 dogs (n = 4); IDD 1.0: Exercising IDD 1.0 dogs (n = 8); IDD 2.0: Exercising IDD 2.0 dogs (n=11).

Electrolytes and acid-base parameters fluctuated on a daily basis across all three treatment groups, suggesting a strong influence of testing conditions. However, none of the measured parameters demonstrated clear between-treatment differences. The source of the systematic day-to-day variability is unknown, but may have served to obscure biologically-relevant changes in electrolytes or acid-base status in the dogs.

Muscle protein synthesis was successfully measured in 4 IDD 2.0, 4 exercising IDD 1.0 and 4 sedentary IDD 1.0 dogs. Synthesis of cytosolic proteins was relatively uniform across all 3 groups of dogs at 8-9% ($p = 0.70$). There were significant differences between groups with

respect to synthesis of mitochondrial proteins, with mitochondrial protein synthesis in the IDD 2.0 dogs ($12.170 \pm 1.830\%$) being significantly lower than in exercising ($15.929 \pm 1.625\%$, $p = 0.01$) or sedentary ($17.836 \pm 2.199\%$, $p = 0.004$) IDD 1.0 dogs (Figure 26). The lower protein synthesis in the IDD 2.0 dogs is likely a result of lower metabolic stress on these dogs during the deployment activities, and thus less demand for *de novo* adaptation, than in the IDD 1.0 dogs. However, it is unclear why the sedentary IDD 1.0 dogs had significantly higher rates of protein synthesis, and the contribution of the overall energy and nutrient demands of the exercise in reducing the rate of protein synthesis in both exercising groups cannot be excluded. Because of this aspect of the data, additional research will be required to determine whether protein intake requirements can be modified in conditioned dogs that are no longer actively adapting to exercise stresses.

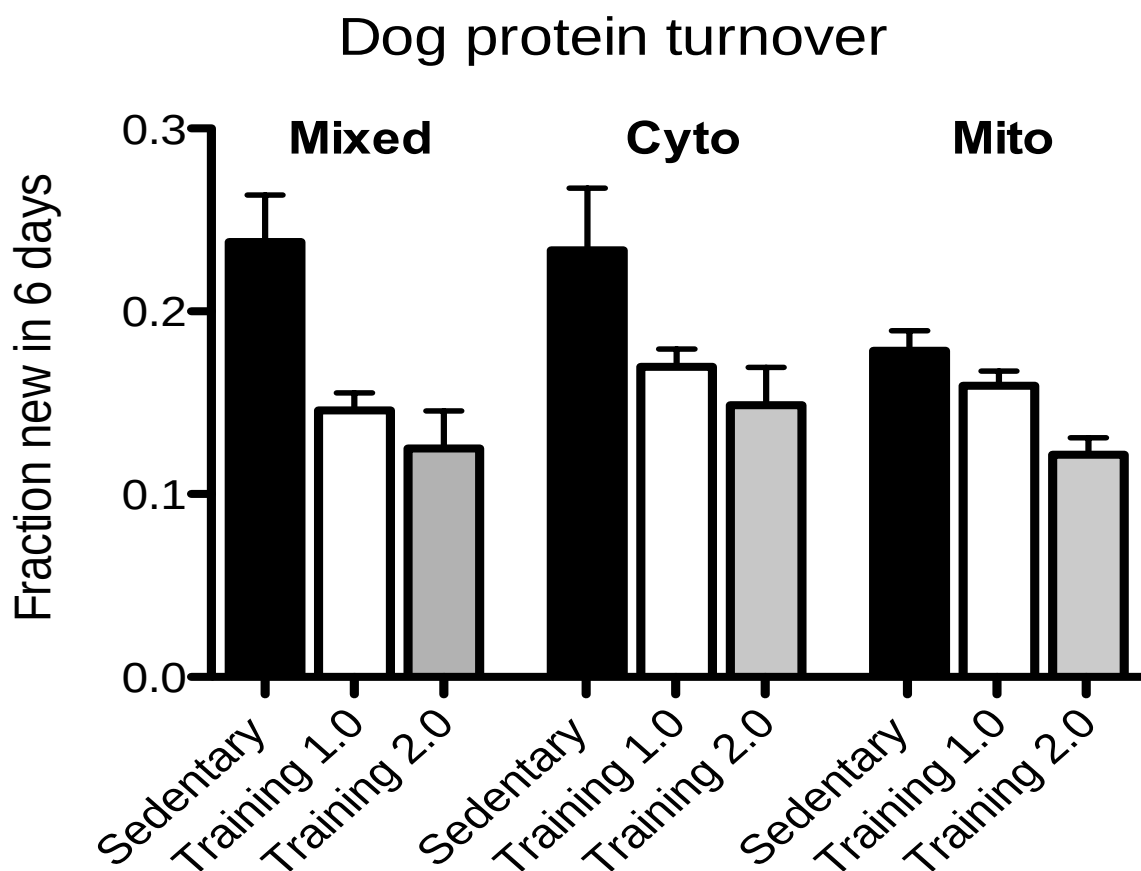


Figure 26: Effect of 5 day deployment simulation on muscle protein synthesis (mean $\pm$ SD). Resting: Sedentary IDD 1.0 dogs ($n = 4$); IDD 1.0: Exercising IDD 1.0 dogs ($n = 4$); IDD 2.0: Exercising IDD 2.0 dogs ($n=4$).

Discussion

The June 2012 deployment simulation defined a stark contrast between IDD 1.0 dogs and the proposed IDD 2.0 dogs. IDD 2.0 dogs outperformed IDD 1.0 dogs in nearly every performance and health metric, starting with the simplest and most important metric: survival. Eight IDD 1.0

dogs started the exercise portion of the study, but 2 of them (25%) were physically unable to continue beyond the first day. These dogs were monitored throughout the remainder of the study but were not allowed to exercise, and it was not until the end of the study (4 days post-exercise) that they were clinically normal. Even with the removal of these two dogs from the statistical comparisons of performance, IDD 2.0 dogs logged more miles and faster odor detection times than did IDD 1.0 dogs, at least during the initial stages of the exercise. The fact that the IDD 1.0 dogs “closed the gap” during the later days of the study likely is a testament to the adaptability of dogs to a novel physiological stressor, similar to what was observed in the first deployment simulation. These observations provide even more striking evidence for the conclusion drawn from the first deployment simulation in September, 2011 that the current program for conditioning and maintaining IDD dogs is not sufficient to ensure immediate readiness for the physical stresses of deployment.

The results of the caloric balance portion of this study were in some instances unexpected. The feeding rate of the IDD 1.0 dogs was expected to be insufficient to meet the energy requirements of the exercising dogs, based on the results of the September 2011 deployment simulation. However, the daily difference of over 2000 kcal was substantially larger than expected, and was the result of higher than expected values for total energy expenditure. This difference was also present in the IDD 2.0 dogs which were being fed at a rate intended to match their energy expenditure (based on the data from the Sept 2011 deployment simulation), but recorded substantially higher values for total energy expenditure than the 1.0 dogs in either deployment simulation. There are a number of factors that need to be considered when interpreting these data. First, the environmental conditions of the June 2012 study, compared to the September 2011 study, were hotter and more humid. As a result, the dogs could be expected to pant harder in order to eliminate the same amount of metabolic heat. This could account for at least some of the additional energy expenditure in both groups. Second, it is possible that measured energy expenditure in the IDD 1.0 dogs represents the energy required for exercise, partially offset by downregulation of basal metabolism as a result of the negative caloric balance, and that the true energy cost of full basal metabolism plus exercise is at least that measured in the IDD 2.0 dogs (or even higher if this hypothesized downregulation is still present to some degree in the IDD 2.0 dogs – a possibility that is raised by the protein balance studies discussed later). Like the September 2011 deployment simulation, the June 2012 deployment simulation was too brief to reliably detect weight loss as a result of insufficient caloric intake, although it is noteworthy that statistically-significant weight loss was detected in the IDD 1.0 dogs. While it is clear that the more aggressive feeding strategy of the IDD 2.0 dogs was an improvement over the IDD 1.0 feeding program, we cannot conclude that the former is sufficient for long-term maintenance of health in the face of sustained daily exercise.

The results of this study illustrate that the conditioning program recommended for IDD 2.0 prepares the dogs to perform the anticipated deployment activities in a sustainable fashion; that is, while maintaining tissue health and without excessive consumption of reserves. Similar to the results of the September 2011 deployment simulation, IDD 1.0 dogs were not capable of maintaining the supply of combustible fuel to the working muscle, resulting in consumption and depletion of intramuscular glycogen. In contrast, IDD 2.0 dogs largely maintained their pre-exercise concentration of muscle glycogen, with the 10% difference between pre-exercise and post-exercise value well within the inherent variability of this analytical technique. As a result of the preservation of muscle energy, IDD 2.0 dogs did not have the serum biochemical evidence of

muscle damage that was consistently observed in IDD 1.0 dogs, both in the September 2011 deployment simulation as well as the June 2012 deployment simulation. These observations reinforce the sustainability of this type of exercise in dogs receiving IDD 2.0-style conditioning.

The protein synthesis portion of the study provides an interesting glimpse at what level of support may be needed for true sustainability. Our hypothesis was that exercising IDD 1.0 dogs, being unconditioned relative to the requirements of the exercise, would have a higher rate of protein synthesis than the exercising IDD 2.0 dogs, with the latter having a rate of protein synthesis that was close to baseline (i.e., simply maintaining the metabolic machinery that was already in place to support the exercise demands). However, BOTH groups of exercising dogs had LOWER protein synthesis. The single most likely cause for protein synthesis being lower than the basal resting rate is if the subject is receiving insufficient nutrition and as a result, has adopted a conservation mode of metabolism that reduces or eliminates processes that are not absolutely and immediately necessary. Given the apparent caloric deficit present in both groups of exercising dogs, it is likely that this mode of metabolism was adopted by these dogs, and as a result, protein metabolism was reduced. However, it is also apparent that it was reduced to a greater extent in the IDD 2.0 dogs compared to the IDD 1.0 dogs, despite having comparable relative levels of nutrition. Our interpretation of this difference is that, consistent with our hypothesis, the properly-conditioned IDD 2.0 dogs had less of an immediate requirement for protein synthesis due to the fact that they had less of stimulus to actively adapt to a novel physiological stressor. These interpretations require a considerable amount of conjecture, and a repeat of this (or similarly-designed) study with more intensive caloric support is indicated to provide clarity and more reliable, direct data.

Conclusions

The IDD program was conceived and created as a highly-novel approach to the detection of concealed explosives, and thus by definition the developers of this program lacked even basic knowledge regarding exactly what sort of physiological demands would be encountered by the dogs during deployment. As a result, it is not surprising that the program was not ideal, and it certainly should not be considered a strike against the developers that various elements of the program needed adjustment. Indeed, even after several years of deployment, the exact demands placed on the dogs were not sufficiently known to permit the appropriate modification of the program. The design and execution of the initial deployment simulation provided the required information on the physiological stressors faced by deployed IDD dogs, while simultaneously highlighting the specific areas of conditioning, nutrition, hydration, and metabolic heat management that currently are the most important physiological limitations to IDD performance.

It is dogmatic in the field of exercise physiology that conditioning is activity-specific. Conditioning is the process of reprioritizing limited body resources to improve exercise performance. The fact that these resources are limited means that reassignment of these resources only takes place when clearly necessary, and only to the extent that it is necessary to facilitate the specific conditioning requirements. If a certain activity can be performed successfully and without deviation from an acceptable homeostatic range, then no reallocation of resources is necessary and no conditioning will occur. Similarly, if the activity is never performed unsuccessfully, then no requirement for reallocation has been demonstrated and no conditioning to that activity will occur. These principles form the basis of the failure of the current IDD 1.0 conditioning program to prepare dogs for deployment activities, as well as the basis for the successful development of a revised conditioning program (IDD 2.0).

The simplest and most reliable means of conditioning for a specific activity and determining whether sufficient fitness exists for a specific activity is to perform that activity. Depending upon the intensity of the physiological stress relative to basal resilience, it may even be possible to go straight from basal fitness to the desired activity. This is essentially what was done in conducting the first deployment simulation and in the case of the IDD 1.0 dogs in the second deployment simulation – rather than building incrementally from their existing level of fitness to a level of fitness that would support the desired activity, the dogs were asked to perform exercise for which they were not prepared. Two outcomes were possible, and both were observed. If the difference between the existing level of fitness and the required level of fitness was excessive, then the dogs failed, as was seen in 2 of the 8 IDD 1.0 dogs in the June 2012 deployment simulation. If the difference between the existing level of fitness and the required level of fitness was not excessive, then the dogs would manage to complete the required task, but stray outside sustainable homeostasis. As long as they didn't fail, then their efforts constituted a conditioning stimulus and they began to adapt to the exercise demands. Dogs are remarkable in the speed at which they can adapt to exercise challenges, at least in comparison to humans. Evidence of their adaptation is clearly found towards the end of both deployment simulations in the dogs that were able to continue to exercise in the progressive decrease in markers of muscle cell damage (serum CPK). These observations have guided the development of the revised conditioning program, which has been constructed to take advantage of the dogs' ability to condition rapidly, but also provide slow enough progression to avoid injuring dogs by excessive conditioning challenges.

The specific areas or systems that must be altered by conditioning can be identified by considering the specific type of exercise being performed by the dogs. Sprint-type activity, defined as activity that lasts seconds to minutes, is limited by the number of contractile units that can be activated (i.e., functional muscle mass). Aerobic activity, defined as activity that lasts minutes to hours, is limited by the ability to perfuse the working muscle, supplying oxygen and removing carbon dioxide and heat. Endurance activity, defined as activity that lasts hours to days, is limited by the ability to move combustible nutrients into the working muscle. Based on reports from deployed and post-deployed IDD handlers, the majority of the work being done by the dogs is endurance in nature – patrols lasting hours and being done on consecutive days. As a result, the key element of conditioning is the development of a robust capacity for muscle uptake of carbohydrate and fat, thereby eliminating the need to utilize finite intramuscular stores of these same compounds. We know from studies of other athletic dogs that consumption of intramuscular energy stores and muscle cell damage are closely and inversely related^{2;3;20}. In racing sled dogs, the first few days of high mileage racing results in depletion of muscle glycogen and a concurrent increase in serum CPK. However, as the dogs adapt to this new level of exertion, the daily use of muscle glycogen decreased, with a concurrent reduction in serum CPK levels. Furthermore, studies have demonstrated that serum CPK in actively-training sled dogs can predict the suitability of individual dogs for multiday endurance racing, presumably by identifying those dogs for which training is no longer resulting in depletion of muscle reserves and subsequent muscle damage²¹. This same relationship was found in these studies and provides an easy and readily-available means of indirectly assessing fitness for endurance-type exercise through the post-exercise measurement of a blood-borne marker of muscle damage (CPK). In the absence of a post-exercise rise in CPK, it is reasonably certain that consumption of intramuscular energy reserves largely was spared by the transport of extramuscular energy substrates at a rate that met the overall energy requirements of the exercise, and the subject being assessed possesses the key conditioning features to support that level of endurance exercise. We also know from studies of other athletic dogs that this specific adaptation is both slower to develop and slower to recede upon removal of the conditioning stimulus compared to other conditioning adaptations such as increased capacity for cardiac output. The slowness to develop increased capacity for transport of energy into the muscle mandates a somewhat prolonged conditioning program (something that is unlikely to be problematic given the prolonged imprinting program already required), whereas the tendency for this adaptation to linger after when activity is reduced is fortuitous given the likelihood that conditioned dogs will experience periods of inactivity resulting from overall unit logistical demands.

Assessment of conditioning requires not only a means of quantifying the response to the exercise challenge, but also a means of quantifying or controlling the challenge itself. Current conditioning programs use tethered exercise (either dogs harnessed to an all-terrain vehicle or to a rotary hot-walker), and thus the speed, distance, and duration are precisely controlled and uniform. Unfortunately, this method of conditioning fails to reproduce the intended activity adequately, and does not result in the development of the appropriate level and type of fitness. Extending the duration of tethered conditioning may not be practical due to the possibility of dogs becoming bored with long hours of tethered exercise. Furthermore, the consistency of the tethered exercise is different, perhaps importantly, from the stop-and-start nature of the off-leash exercise. Thus, the revised conditioning program uses off-leash exercise, with duration as the sole means of controlling the relative intensity of a given workout. While exercising as a group,

the dogs were surprisingly uniform in the overall distance covered – typically a group of dogs exercising together had a coefficient of variation of approximately 10%. However, this level of variability is too large to be able to record the distance of a representative dog in an exercising group and extrapolate to the group at large. For example, during a standard all-day deployment simulation, during which the dogs are “patrolling” for 9 hrs (5 hrs of active movement and 4 hrs of rest/cool down/hydration/snacking), the mean distance travelled will be approximately 24 miles/day (depending on ambient conditions). The 95% confidence interval is 19 – 29 miles, meaning it is reasonably likely that within that group, a dog may only travel 19 miles (a 20% decrease compared to the mean). This highlights the need to record the mileage on each individual dog during an exercise session that is being quantified for the sake of fitness assessment, rather than assuming that all dogs are performing similar exercise. Furthermore, the confidence interval of mileage covered by conditioned dogs has a substantial overlap with the reduced mileage performed by partially deconditioned dogs. Therefore, if a dog with low mileage relative to the target has a normal value for post-exercise CPK, the possibility of the low mileage being due to insufficient conditioning cannot be excluded. For this reason, a strict minimum distance for any assessment must be established, and the exercise performance of each individual dog must be measured in order to assess the relative fitness of the dog.

Nutrition of working dogs primarily is directed at providing sufficient calories to match the number of calories expended during exercise, and secondarily providing sufficient non-energy nutrients (protein, macro- and microminerals) to replace those lost. Unfortunately, the goals are simpler in concept than they are in practice, in part due to the difficulty in assessing nutritional balance of an individual dog over the short-term. Bodyweight is an exceedingly crude tool to measure dietary sufficiency in the short term, and subjective body condition score is even more imprecise. A pound of mammalian fat contains approximately 3500 kcal; hence a dog’s intake/expenditure balance can fall short by that amount (nearly double the daily basal requirement) and the dog will only lose a single pound of bodyweight. By the time the average dog has lost sufficient weight to be obvious by appearance, the caloric deficiency has been substantial and long-term. This method of assessing dietary sufficiency should be a last-resort, and feeding recommendations need to be based on more sensitive methods.

Current feeding guidelines fall dramatically short of anticipating the magnitude of caloric requirements of the dogs (2000 kcal/day fed vs 3000 kcal/day or more expended), perhaps primarily due to the lack of prior knowledge as to the amount of exercise actually being performed by the dogs. As a more informed approximation, the caloric requirements established in the first deployment simulation seemed in line with estimates that would have been provided had the exercise quantity been more accurately predicted. However, the results of the second deployment simulation raise the question as to whether the new feeding guidelines are sufficient, in that even after having dramatically increased the rate of caloric intake, there was still a substantial difference between intake and expenditure. These data suggest that, in the face of an untenable deficit between caloric expenditure and intake, the dogs adopted a quasi-starvation metabolic strategy and reduced or eliminated metabolic processes that were not absolutely required in the short-term. The efforts in the second deployment simulation to reduce this deficit by increased caloric intake resulted in the reduction of the shift to starvation conservation, in turn resulting in greater energy expenditure for the equivalent amount of work. Evidence in the form of reduced protein synthesis in both exercising groups of the second deployment simulation

suggests that the new caloric imbalance was still sufficient to trigger a reduction in long-term homeostasis, and raises the possibility that, despite the overall improvement observed with increase nutritional intake, the amount of intake relative to the amount of work is still not sufficiently matched to be considered indefinitely sustainable. Further work is required to determine the levels of nutrition necessary to permit off-leash patrol exercise in an indefinitely-sustainable fashion (i.e., without any interruption in basal metabolic activities).

There is near-uniform appreciation among the users of military working dogs, including IDD, of the importance of maintaining hydration and avoiding dehydration in order to maintain the health and performance of the dogs. However, the tactics for maintaining hydration are variable, and there is little verifiable evidence of success, failure, or relative efficacy of the different approaches. The studies in this report add considerable data to this area of interest, including data on water requirements, voluntary intake, and the relative benefits of different forms of water baiting to improve intake. Multiple studies demonstrated that the daily requirements for water in the average working IDD (approximately 25-28 kg bodyweight) is approximately 4.5 liters of water, and approximately 40% of that requirement (1.8 – 2 liters) is needed to maintain hydration during a typical 9 hr patrol. There was surprisingly minimal variation in those estimates based on relative fitness, and only a small amount of variation based on the ambient conditions in which the exercise was being performed. Therefore, these estimates should be considered representative of field/deployment requirements for water intake, and to the extent that water intake can be quantified, should be valuable to help guide hydration assessments of the IDDs during deployment.

Reports from deployed units have cited the need to provide subcutaneous fluids immediately prior to a daily patrol in order to maintain hydration due to lack of voluntary water intake. Details regarding this lack of intake, such as how frequently are dogs being allowed to drink, what volume is being offered, and what is the evidence that they are becoming dehydrated during the patrol) are not available to this investigator, so identification of a specific deficiency is impossible. However, it is important to note that in ALL instances in which water balance and hydration specifically were measured as an element of the experimental design, there were no instances in which dogs failed to maintain adequate hydration through voluntary water intake, with or without baiting. Reconciling these observations with the reports from deployed units will require further data collection from the dog handlers on the details of water balance management in the field.

Metabolic heat management is a concern for any athlete, but more so for dogs since they have less capacity for shedding metabolic heat than other animal athletes since they have minimal capacity to sweat. Instead, dogs shed metabolic heat primarily through evaporation of water from the oral cavity and respiratory tract, and increase their rate of heat dissipation by increasing the movement of air over their oral and respiratory mucosa (panting) and by increasing the exposed surface area of the oral cavity (swollen tongue, retracted cheeks). The greater the exposure of the oral mucous membranes, through the retraction of the cheeks and lips, the greater the perceived heat stress and efforts to improve heat dissipation. In this regard, exposure of the 4th lower premolar and 1st lower molar (when viewed in profile) is common in dogs that are hyperthermic, but not dangerously so. Exposure of the 2nd lower premolar indicates substantial heat stress and the need to temper the rate at which heat is being produced and/or

provide additional cooling. Exposure of any of the upper arcade is rare, but usually indicates an immediate danger for serious illness or death resulting from overheating.

The experiments in this project illustrate both the importance of improving the capacity of the dogs to dissipate heat, as well as the need for acquired tolerance to hyperthermia. Both adaptations are a product of a proper conditioning program, and the principles of exercise conditioning apply equally as well to thermal conditioning. There is no reason for a subject to become acclimatized to a body temperature of 102.5°F if they have not experienced that stressor previously. A dog will only develop the ability to tolerate higher body temperatures if they are exposed to those temperatures. Conditioning improves tolerance to hyperthermia because it causes controlled hyperthermia, but the dog must be carefully observed to ensure that the hyperthermia is only incrementally greater than that to which the dog is acclimatized. It is extremely important to note that these exposures must be carefully administered and increased gradually due to the narrow margin of error between stimulating an adaptive response and causing life-threatening heat illness.

Monitoring the dog's response to exercise and hyperthermia is critical, but the phenomenon of tolerance to hyperthermia limits the usefulness of the simple determination of body temperature. Depending on the degree of tolerance present in any given dog, a body temperature of 103.5°F may either represent dangerous hyperthermia or a normal working temperature. Instead, dog handlers must become skilled at interpreting the dog's behavior in order to assess whether a particular body temperature is dangerously elevated in THAT dog at THAT time. The key points of behavior interpretation are related to the dog's own efforts to increase the rate of heat dissipation – how fast is the dog panting, how swollen and distended is the dog's tongue, and how exposed are the dog's oral mucous membranes. Of these, the exposure of the dog's mucous membranes (as described in previous paragraphs) has the potential for the greatest sensitivity and specificity, as this feature is unlikely to be affected by other elements of the dog's physiology such as fatigue or hydration.

Recommendations

Conditioning

The following conditioning program is recommended:

	IDD 2.0 Conditioning		
Week	Daily Duration	Speed/Distance	Weekly Frequency
1	60 min	4.5 mph/4.5 mi	3X
2	2 x 60	4.5 mph/9 mi	3X
3	3 x 60	4.5 mph/13.5 mi	3X
4	3 x 60	4.5 mph/13.5 mi	3X
5	4 x 60	4.5 mph/18 mi	3X
6	4 x 60	4.5 mph/18 mi	3X
7	5 x 60	4.5 mph/22.5 mi	3X
8	5 x 60	4.5 mph/22.5 mi	3X

Individual dogs can enter the conditioning program at whatever level matches their current physical training routine. For example, current IDD 1.0 dogs have a maintenance physical conditioning prescription that is the same approximate intensity as Week 1 of the new conditioning program, and thus could start with Week 2.

The specific goal of each program step is a function of distance/time. Healthy off-leash dogs that are not extremely fatigued or hyperthermic will elect to move at approximately 4.5 miles/hr. Thus, if the target distance is 18 miles in a single day, the dogs will require a minimum of 4 hrs of off-leash activity. Depending on the environmental conditions, this duration of activity may be completed in two 2-hour sessions (in cool conditions where hyperthermia is less of a concern) or as many as five or six 40 min sessions (in hot, humid conditions that require frequent breaks to allow the dogs to cool down). Breaks should only be long enough to allow the dogs to cool back to a safe body temperature in order to preserve the metabolic stress on the muscle that is necessary to stimulate adaption and to preserve elevated body temperature necessary to stimulate tolerance to hyperthermia.

Dogs should receive their appropriate conditioning prescription with no more than 2 consecutive days of work and no more than 2 consecutive days of rest.

Dogs should be assessed continuously during the conditioning sessions by the handler for signs of muscle soreness, overheating, or reluctance to move. If these signs develop, exercise should be stopped and the dog examined by a veterinarian. Dogs should be examined by a veterinarian experienced in canine sports medicine following the second or third conditioning session in weeks 4, and 6 to determine whether the dogs are sufficiently conditioned to move to the next conditioning level (Intermediate certification of fitness) and week 8 (Final certification of fitness). These examinations should include measurement of the individual dog mileage for that day (using a GPS collar) and post-exercise CPK. GPS collar mileage should be within 10% of the target mileage and post-exercise serum CPK is <400 IU/L for the dogs to continue in the conditioning program. There are circumstances unrelated to a dog's fitness that can cause the dog to either fail to cover the required mileage or have a serum CPK value above the mandated threshold. Therefore, fitness certifications can be repeated twice at any level in order to avoid failing a dog that has the reasonable potential to meet the fitness certification requirements. If a

dog fails to meet the certification requirements at any level 3 times, then it is unlikely to be successful in this program.

Medication

Dogs performing patrol-type exercise are at high risk for exercise-induced gastric disease. Although this disease does not result in overt clinical signs in the majority of affected subjects, it has the potential to produce subtle and vague signs such as ill-thrift, inappetance, anemia, and lack of stamina. Research in other athletic subjects (humans, dogs, and horses) has demonstrated clearly that blockade of gastric acid secretion prevents the development of exercise-induced gastric disease. Therefore, administration of omeprazole at a dose of 20 mg orally once daily is recommended for dogs performing activities that have been proven to place them at risk for exercise-induced gastric disease. It is possible that long-term administration of acid suppression therapy may help to preserve the willingness of the dog to eat and drink, thereby reducing the logistical challenges of enhanced nutrient and water intake that is required by the patrol activities.

Nutrition

The increased caloric requirements of patrol activities must be recognized proactively, rather than reacting to a loss of body weight or condition. A 9-hour patrol increases the caloric requirements 2-3 fold over baseline, and failing to meet this requirement will result in a hormonal cascade that results in consumption of body reserves, including the breakdown of muscle protein in order to support energy requirements. Consumption of this magnitude of food will require multiple meals and intermittent snacking during the exercise periods. Additional calories can be provided in the water consumed during the patrol through the use of sports drinks (depending on palatability and the dog's individual preferences).

Hydration

Dogs should be provided with the opportunity to consume at least 2 liters of water during a full-day patrol. Water consumption patterns observed during these studies suggest that the majority of the water will be consumed early in the patrol, which should reduce the impact of the additional weight on the patrol logistics. If appropriate for the individual dog, caloric supplementation in the form of sports drink mix can be added to the water to help maintain caloric balance.

Hyperthermia/Heat Management

Proper conditioning results in both the ability to more effectively transport heat in the body for dissipation, as well as relative tolerance to hyperthermia. Therefore, conditioning must be developed and maintained. During deployment, there is no physiological difference between exercise purely for the sake of conditioning and the same duration and intensity of exercise conducted as part of a patrol. Therefore, to the extent that the dogs successfully develop the appropriate conditioning for patrol activities, regular patrols (combined with adequate nutrition, hydration, and medication) will serve to reinforce and maintain the needed conditioning, including the aspects of conditioning that support metabolic heat management.

It is vital to note that the phenomenon of tolerance to hyperthermia will result in dogs with body temperatures that would be life-threatening to unconditioned dogs that have not developed

tolerance to hyperthermia. Therefore, the same standards and protocols relative to body temperature of unconditioned dogs are not appropriate for highly-conditioned dogs that have developed a tolerance to high body temperatures. The ability of a dog to tolerate hyperthermia is mandatory in the current operational environment in order to have a large enough enthalpy gradient between the dog and the environment to support a high rate of heat dissipation (and by extension a high rate of heat generation through exercise). However, this requirement places a premium on the ability of the handler to examine the dog in a subjective manner to determine whether the dog has maximized its ability to dissipate heat and needs to reduce the rate of heat production in order to maintain an acceptable margin of error between a safe and unsafe body temperature. Specific handler lessons are required in this area during training and retraining to ensure the safety of the dogs.

Additional Research

Certain areas of this project were inconclusive or incomplete due to constraints of time, resources, or the emergence of unexpected findings. These areas warrant additional investigation in order to maximize the physiological resilience of IDD's:

Nutrition: Based on the repeated deployment simulations, it is not clear that the current nutritional recommendations, although an improvement over past recommendations, are ideal. Specific projects are indicated to determine what level of caloric and/or protein intake is necessary to permit consecutive days of patrol activity while maintaining normal rates of protein synthesis/turnover. The goal is to identify the nutritional intake that permits this type of activity in a truly sustainable manner – without any change in body composition or reprioritization of body resources.

Hydration: The perceived requirement for parenteral fluid administration in order to maintain hydration remains in conflict with the data produced in this program that demonstrated the ability of the dogs to maintain hydration through oral intake. This discrepancy may be a result of differences in conditioning, hydration management, or perception of dehydration. A more thorough review of deployment practices and observations is warranted and, if necessary, additional studies to confirm (or refute) the need for parenteral fluid administration as a routine hydration practice.

Olfactory performance: Although outside the scope of this specific segment of the IDD 2.0 program, it is strongly recommended that the mandated changes in the management of IDD's be assessed with respect to their olfactory performance. This aspect of the program was addressed briefly in the second deployment simulation, but more rigorous testing is needed.

Objective markers of fatigue and tolerance to hyperthermia: These are areas that are vitally important for the safety of the dogs, but are currently only assessed in a subjective manner. This manner of assessment provides the opportunity for bias and variability among the assessors (in the field, the handlers). Improved assessment techniques, to include objective markers, would reduce variability and the opportunity for critical error.

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Symbols, Abbreviations, and Acronyms

°F: Degrees Fahrenheit
AST: Aspartate amino-transferase
ATV: All-terrain vehicle
CPK: Creatine phosphokinase
D₂O: Deuterium oxide
dl: deciliter
ECF: Extracellular fluid
g: gram
GPS: Global positioning satellite
hr: hour
HSP: Heat shock protein
IDD: IED detection dog
IDD 1.0: IDD produced under current NEDP guidelines (circa 2011)
IDD 2.0: IDD produced under guidelines revised using data produced in 2011-2012 (unapproved as of the time of this report).
IED: Improvised explosive device
IU/L: International units per liter
Kcal: kilocalorie
kg: kilogram
kJ: kilojoule
L: Liter
LDH: Lactate dehydrogenase
meq: milliequivalent
mg: milligram
min: minute
ml: milliliter
mmol: millimoles
NaSCN: Sodium thiocyanate
ng/ml: nanogram per milliliter
RH: Relative humidity
SD: Standard deviation
VO₂: Rate of oxygen consumption
VO₂max: Maximum capacity (rate) of oxygen consumption
VO₂peak: Maximum rate of measured oxygen consumption

Glossary

1. Improvised explosive device: a device fabricated or placed in an improvised manner, incorporating lethal, noxious, pyrotechnic, or incendiary materials designed to destroy, incapacitate, harass, or distract. It may incorporate military parts, but is normally constructed from nonmilitary components.
2. Metabolic stress: A condition in which the physiological demands placed upon a cell or organism cannot be met with the current cellular or organism capabilities, resulting in disruption in homeostasis and a requirement for altered capabilities in order to survive.
3. Homeostasis: a state of equilibrium or a tendency to reach equilibrium, either metabolically within a cell or organism or socially and psychologically within an individual or group.
4. Hyperthermia: elevated temperature of the body.
5. Enthalpy: a thermodynamic function of a system, equivalent to the sum of the internal energy of the system plus the product of its volume multiplied by the pressure exerted on it by its surroundings. In this document, is considered synonymous with total heat content of the system.
6. Heat acclimatization: process by which the body becomes physiologically more tolerant to high environmental temperatures.
7. Thermoregulation: the mechanisms and control systems used by the body to balance thermal inputs and thermal losses so as to maintain its core temperature nearly constant.
8. Parenteral fluid: Fluids provided to an organism through a route other than the gastrointestinal tract.
9. Omeprazole: medication used to treat symptoms of gastroesophageal reflux disease (GERD) and other conditions caused by excess stomach acid. It is also used to promote healing of erosive esophagitis (damage to your esophagus caused by stomach acid).
10. Gastritis: an inflammation of the lining of the stomach.
11. Deuterium oxide: an isotopic form of water with composition D₂O.
12. Gavage: forced feeding, especially through a tube passed into the stomach.
13. Creatine phosphokinase: an enzyme found mainly in the heart, brain, and skeletal muscle. The enzyme is released into the circulation when these cells are damaged, and is thus frequently used as a diagnostic marker for cell damage.
14. Gastroscopy: the visual inspection of the interior of the stomach by means of a gastro scope inserted through the esophagus.

15. Sodium thiocyanate: the chemical compound with the formula NaSCN. This colorless deliquescent salt is one of the main sources of the thiocyanate anion. As such, it is used as a precursor for the synthesis of pharmaceuticals and other specialty chemicals.
16. Evans's Blue: an azo dye which has a very high affinity for serum albumin. Because of this, it can be useful in physiology in estimating the proportion of body water contained in blood plasma.
17. ad libitum: At the discretion of the performer. Used chiefly as a direction giving license to alter or omit a part.
18. Basal: pertaining to or situated near a base; in physiology, pertaining to the lowest possible level.
19. Cortisol: known more formally as hydrocortisone, is a steroid hormone, more specifically a glucocorticoid, produced by the zona fasciculata of the adrenal gland. It is released in response to stress and a low level of blood glucocorticoids. Its primary functions are to increase blood sugar through gluconeogenesis; suppress the immune system; and aid in fat, protein and carbohydrate metabolism.
20. Aspartate transferase: a salt of aspartic acid, or aspartic acid in dissociated form that catalyzes the transfer of a functional group (e.g., a methyl or phosphate group) from one molecule (called the donor) to another (called the acceptor). In mammalian systems, it is an enzyme commonly found in the liver and is released into the circulation in instances of liver damage.
21. Melena: the passage of dark stools stained with altered blood.
22. Inappetance: lack of appetite or desire.
23. Heat shock proteins: a class of functionally related proteins involved in the folding and unfolding of other proteins. Their expression is increased when cells are exposed to elevated temperatures or other stress. This increase in expression is transcriptionally regulated. The dramatic upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (HSF).
24. de novo: anew; afresh; beginning again; from the start.
25. Subcutaneous: beneath the skin.