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## Physiological acclimation of a desert antelope, Arabian oryx (*Oryx leucoryx*), to long-term food and water restriction

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**Abstract** Desert mammals often experience scarcity of drinking water and food for prolonged periods. In this study, the first long-term acclimation experiment in a non-domesticated desert-adapted ungulate, we investigated the mechanisms used by the Arabian oryx *Oryx leucoryx*, to adjust its physiology to progressive food and water restriction over 5 months, an experimental regimen and time course chosen to mimic what it typically experiences between spring and late summer in the desert. At the end of the acclimation period, oryx consumed less than one and half of food and water of animals in the control group and lost  $8.2 \pm 2.6\%$  of their initial body mass. Experimental animals reduced their mass-specific resting metabolic rate (RMR) and total evaporative water loss (TEWL) by 16.2 and 25.7%, respectively, and maintained a digestive efficiency of about 70%. We found no support for the idea that reduced RMR in oryx correlated with a decreased thyroid

hormone concentration in plasma. At the end of the 5 months acclimation, oryx continued to mobilize fatty acids to fuel metabolism, and did not use protein breakdown as a major source of gluconeogenesis. Oryx in the experimental group reduced their water intake by 70% and maintained constant plasma osmolality. They adjusted their water budget by reducing mass-specific TEWL, increasing urine osmolality and reducing urine volume by 40%, and excreting feces with < 50% water content. Oryx have an unusually low TEWL compared with other arid-zone ungulates; both hydrated and water-deprived individuals have TEWL values, 51.7 and 39.3%, respectively, of allometric predictions for arid-zone ungulates.

**Keywords** Antelope · Arid environments · Phenotypic plasticity · Resting metabolic rate · Total evaporative water loss

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**Abbreviations** RMR: Resting metabolic rate · TEWL: Total evaporative water loss · WEI: Water economy index

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### Introduction

Formed at the end of the Oligocene, about 25 million years ago, the deserts of northern Africa and of the Arabian Peninsula are among the most austere of terrestrial environments (Gerson 1982). Classified as arid or hyperarid (Meigs 1953), these deserts have low rainfall, low humidity, high winds with blowing sand, and high ambient temperatures ( $T_a$ ), often in excess of 45°C during summer. One might regard these regions as unsuitable habitat for large ungulates (> 50 kg) because they cannot escape the extremes of daytime heat as do small mammals and because they require large quantities of vegetation to meet their daily energy and water requirements. Yet, as old world deserts developed in the Miocene, species of artiodactyls radiated to fill these niches. Currently two species of large wild ungulate can

be found in deserts of northern Africa, the addax (*Addax nasomaculatus*) and the scimitar-horned oryx (*Oryx dammah*), and one species in the Arabian Peninsula, the Arabian oryx (*Oryx leucoryx*). All three species are endangered and near extinction in the wild (Mallon and Kingswood 2001).

Early European explorers reported that the Arabian oryx ranged over most of the deserts of the Arabian Peninsula and Mesopotamia (Harrison and Bates 1991), but with the development of motorized vehicles, hunters eradicated the species from the wild in early 1970 s (Henderson 1974). From a genetically diverse collection of captive animals, the national wildlife research center (NWRC), Taif, Saudi Arabia reintroduced Arabian oryx into a 2,244 km<sup>2</sup> desert reserve called Mahazat as-Sayd in 1990. Now numbering nearly 700 animals, the population is the only self-sustaining herd of Arabian oryx in the World (Ostrowski et al. 1998; Gorman 1999). The free-living herd in Mahazat and the captive herd maintained by the NWRC offer a unique opportunity to study the ecological physiology of a large ungulate that has evolved the capacity to survive long periods in hot deserts without drinking (Williams et al. 2001; Ostrowski et al. 2002, 2003).

Oryx live in deserts that are characterized by long periods of drought, sometimes lasting 4–6 months, and high  $T_{as}$ , punctuated by brief periods of rain that can fall anytime in winter or spring (Fisher and Membery 1998). After rain, oryx have access to green vegetation, but throughout summer, intense heat steadily depletes the water and nutritional content of vegetation (Spalton 1999). Given this pattern of long periods of increasingly poorer quality food, one might predict that oryx have evolved the capability to adjust their physiology depending on resource abundance. However, whether they alter their physiology, and if they do, the magnitude of these changes, relative to variation in environment, is poorly known.

Free-living Arabian oryx can survive indefinitely without access to drinking water in the desert of Arabia (Williams et al. 2001; Ostrowski et al. 2002). Using doubly labeled water, Williams et al. (2001) reported that oryx decreased their field metabolic rate (FMR) from 22 mJ/day in spring to 11 mJ/day in summer, and their water influx rate (WIR) from 3.4 to 1.3 l/day; decline in FMR was among the largest reported for a eutherian mammal. Protein and water content of vegetation steadily declined throughout summer. We thought that oryx would increase their digestive efficiency in response to food shortage enabling them to obtain more energy from a given quantity of food as do Bedouin goats (*Capra hircus*; Brosh et al. 1986).

Because water is in short supply in deserts, one can envision selective pressures that enhance water conservation. Total evaporative water loss (TEWL), the sum of respiratory and cutaneous water loss, is the primary avenue of water loss in wild desert ungulates, exceeding losses in feces and urine combined (Wilson 1989). Adjustments in TEWL during periods of food and water

restriction may have a major effect on water balance of oryx and ultimately their survival.

Monitoring concentrations of organic molecules in the blood and urine can reveal homeostatic mechanisms used by animals to cope with food and water stress (Kaneko et al. 1997). White-tailed deer (*Odocoileus virginianus*) in North America can lose 30% of their mass during severe winters (DelGiudice et al. 1992). As forage quality and quantity progressively deteriorated during harsh winters, plasma protein and sometimes glucose concentration decreased, whereas plasma urea increased. Urinary urea/creatinine ratios increased indicating that deer were mobilizing tissue protein as an energy source (DelGiudice et al. 1987, 1992).

When in negative energy balance, ruminants shift to lipid catabolism to fuel oxidative phosphorylation, resulting in increased formation of ketone bodies such as  $\beta$ -OH butyrate (Chilliard et al. 1998). Triglycerides in adipose cells are broken down to non-esterified fatty acids (NEFA) that are released into the bloodstream and then used in other organs for production of acetyl-coenzyme A (Jungermann and Barth 1996). Changes in hormone concentration in plasma can signal initiation of homeostatic control such as increase in glucocorticoids to promote gluconeogenesis, a decrease in thyroid hormone production to reduce metabolic rate (Heimberg et al. 1985; DelGiudice et al. 1992; Chilliard et al. 1998), and a decrease in leptin production, a hormone of adipocytes that orchestrates a number of neural and hormonal responses to starvation (Ahima et al. 1996; Chilliard et al. 2001).

In this study, the first long-term acclimation experiment in a non-domesticated desert-adapted ungulate, we adopted an integrated approach to investigate the mechanisms used by Arabian oryx to adjust their physiology to progressive food and water restriction over 5 months, an experimental regimen and time course chosen to mimic what they typically experience between spring and late summer in Saudi Arabia. We hypothesized that oryx would decrease their resting metabolic rate (RMR), as governed by decrease in thyroid and leptin hormone concentrations in plasma, in response to restriction of food and water decreased by 15% every 3 weeks, and that digestive efficiency would increase. Further, we predicted that oryx would decrease their TEWL to promote conservation of water. After 5 months of progressive food restriction, we thought that oryx would have depleted their fat reserves forcing them to rely more on structural proteins as an energy source.

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## Materials and methods

### Animals and experimental design

We conducted this study at the NWRC, Taif, Saudi Arabia (21°17'N, 40°40'E) between April and August 2003. After selecting 14 adult non-pregnant Arabian

oryx females, we randomly assigned them either to a control group (CTRL;  $n=7$ ) or an experimental group (EXPT;  $n=7$ ). Oryx had similar body masses in both groups ( $93.6 \pm 7.2$  kg for CTRL,  $92.5 \pm 4.0$  kg for EXPT,  $t=0.35$ ,  $P=0.73$ ) and similar tarsus length ( $41.6 \pm 1.4$  and  $41.3 \pm 0.4$  cm for CTRL and EXPT, respectively,  $t=0.52$ ,  $P=0.61$ ). During the experiment, oryx were kept individually in  $40 \text{ m}^2$ -outdoor pens with shade available and weighed ( $\pm 0.2$  kg) every sixth day on a platform scale (Mod. 561 SG, GIM, Beauprout, France).

For 5 months, oryx in CTRL were provided with 2.0 kg/day of hay (Rhodes Grass, 17.2 MJ/kg dry matter and 9–10% crude protein) and 4.5 l/day of  $\text{H}_2\text{O}$ , quantities 10–15% above their average daily requirements (S. Ostrowski, unpublished). For the EXPT group, we gradually reduced their food and water by 15% every 3 weeks from CTRL levels down to 0.8 kg/day and 1.2 l/day, about a 60–70% reduction. The final ration of food provided the same metabolizable energy that we had calculated for the consumption of food by free-ranging oryx in summer, and the final allotment of water equaled that which free-living oryx obtained from their food in summer (Williams et al. 2001; Ostrowski et al. 2002). We waited 3.5 week after the final level of food and water was reached before taking measurements.

#### Metabolism and evaporative water loss

We measured minimum RMR and TEWL for oryx in both groups during the day, their resting phase, at the beginning and end of the acclimation period, using standard flow-through respirometry and hygrometry methods (Williams et al. 2001). Because of residual microbial activity in the rumen after 2 days of fasting, measurements of true basal metabolism may be difficult to achieve in ruminants (Blaxter 1989). Prior to measurements, we deprived oryx of food for 50 h, an appropriate fasting interval to achieve stable values of RMR (Williams et al. 2001).

Experimental apparatuses and equations for calculation of oxygen consumption and evaporative water loss are detailed elsewhere (Williams and Tieleman 2000; Williams et al. 2001). Briefly, we constructed a respirometry chamber ( $142 \times 180 \times 45$  cm) with sheets of galvanized steel welded to angle iron. In the chamber oryx stood on a steel-mesh floor, below which we positioned a tray containing a layer of mineral oil into which feces and urine fell, excluding both as a source of evaporative water. The chamber had a door fitted with a rubber gasket which, when bolted shut, rendered the system airtight. It was thermostatically controlled at  $26 \pm 1^\circ\text{C}$ , a temperature within the thermoneutral zone of many tropical ungulates (Parker and Robbins 1985);  $T_a$  within the chamber was monitored with a 28-gauge thermocouple and a data logger. During measurement of  $\text{O}_2$  consumption and TEWL, air under positive pressure

from a compressor coursed through two large ( $100 \times 21$  cm) drying columns containing Drierite (W. A. Hammond Drierite Company, Xenia, OH, USA), through a mass-flow controller set at 120 l/min (Model 2925 V, Tylan General Inc., San Diego, CA, USA, calibrated against a primary standard traceable to the NIST by Flow Dynamics Inc., Scottsdale, AZ, USA prior to measurements), then into the chamber. Exiting air was sampled by a pump, which routed air to a dew-point hygrometer (Model M4-DP, General Eastern, Wilmington, MA, USA; calibrated following Muñoz-Garcia and Williams 2005) and then to columns of silica gel, Ascarite, and silica gel (Thomas Scientific, Swedesboro, NJ, USA) before entering the  $\text{O}_2$ -analyzer (Model S3A-II, Applied Electrochemistry, Pittsburgh, PA, USA). Dry inlet air was assumed to be 20.95% oxygen. Outlet air had a relative humidity that was always below 25%. We allowed oryx to remain inside the chamber for 5–6 h before initiating our recording of fractional oxygen concentration and dew point at 1-min intervals onto a data logger (Model 21X, Campbell Scientific, Logan, UT, USA). When traces of  $\text{O}_2$  consumption were stable, we recorded data for at least 15 min and used them for calculations. Oxygen consumption was calculated using Eq. 4 of Hill (1972) and converted to heat production using 20.08 J/ml  $\text{O}_2$  (Schmidt-Nielsen 1990). The TEWL (g/day) was calculated from measurements of dew point of incoming and outgoing air using the equations of Williams and Tieleman (2000) and Williams et al. (2001) assuming a respiratory quotient of 0.71 (Robbins 1993). After respirometry measurements, we measured rectal temperature ( $T_b$ ) of oryx with a thermometer (Omega Engineering, Stamford, CT, USA) and a plastic coated 28-gauge thermocouple.

#### Parameters in blood and urine

At the beginning and end of the acclimation period, we collected blood from oryx between the hours of 6.00 and 6.30 A.M., prior to feeding them. Blood was drawn from the jugular vein, within 2 min after entering the oryx's pen, into glass tubes containing lithium-heparin and fluoride/oxalate (for glucose determination) (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Blood was centrifuged for 15 min at 2,500 rpm within 30 min of collection. Half of the plasma was frozen at  $-70^\circ\text{C}$  for determination of concentrations of hormones, of non-esterified fatty acids (NEFA) (Oliver et al. 1995) and of  $\beta$ -OH butyrate (McMurray et al. 1984). We made measurements of plasma concentration of total proteins, glucose, urea, and creatinine in duplicate within 2 h of collection (Vettest 8008, Idexx Laboratories Ltd., Chalfont St Peter, UK).

Leptin concentrations in plasma were determined with a competitive enzyme immunoassay previously validated in domestic herbivores (Sauerwein et al. 2004). Intra- and inter-assay variability was 6.3 and 13.9%, respectively (Sauerwein et al. 2004). Cortisol and corti-

costerone were extracted from plasma with diethylether and concentrations determined with an enzyme immunoassay validated for sheep and other herbivores (Palme and Möstl 1997; Dehnhard et al. 2001). Intra- and inter-assay variation was <13.5% for both hormones. Total thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) were determined using an automated immunoassay method (Immulate, Diagnostic Products Corporation, Los Angeles, CA, USA).

Following blood sampling, we collected urine from the bladder of each oryx by catheterization and analyzed it within 3 h. Osmotic pressures ( $\pm 1$  mOsm) of the plasma and urine were measured by a freezing-point depression osmometer (Type 13, Roebling, Berlin, Germany). We determined the concentration of urea in urine following Jung et al. (1975) and that of creatinine according to Jaffé (1886).

#### Construction of a water budget and calculation of food digestibility

At the end of the 5-mo acclimation period, we constructed a water budget for each oryx and measured their digestibility of hay. To quantify water/food intake and output, we housed oryx for three consecutive days in wood cages (155×153×51 cm) that had a wire mesh bottom with multiple layers and meshes permitting separation of urine and feces. Urine fell through the layers of wire onto an aluminum pan and was funneled into a glass vessel containing paraffin oil to prevent evaporation. Hay and water were provided in the same quantities that each group was receiving at the end of acclimation period and daily consumption was recorded. We measured total water intake (preformed water in the food + oxidation water + drinking water) and water loss in the feces and urine. We determined moisture content of hay by mass change after drying at 70°C to constant mass. The amount of water drunk was corrected for evaporation. We estimated metabolic water by assuming 0.028 ml of oxidative water produced  $\text{kJ}^{-1}$  of energy used (digested energy) (Schmidt-Nielsen 1990). We assumed that apparent digested energy approximated metabolized energy, but a sensitivity analysis showed that a 15% error in our estimate of metabolic water production would translate to an error of <2% in total water input. Urine and feces were collected daily, weighed to  $\pm 0.1$  g and dried at 70°C to constant mass. From these data, we calculated daily fecal water loss based on the total dry mass of feces produced and their water content. Here, we assumed water loss equaled water intake because body mass did not vary significantly between day 1 and 3 ( $F_{1,11} < 0.02$ ,  $P > 0.9$ ). We estimated TEWL by subtracting fecal and urinary water from total water intake.

To measure gross energy intake, neutral-detergent fiber, acid-detergent fiber, and crude cellulose in hay and feces, we dried both, and ground them in a Wiley Mill. Gross energy content was determined in an adiabatic

bomb calorimeter (Model C5000, IKA-Werk, Staufen, Germany), using benzoic acid as a standard. Total nitrogen was determined following Kjeldahl extraction procedure, and converted to protein content with a multiplier of 6.25 (Allen 1974). Neutral-detergent fiber was determined by the procedure of Goering and Van Soest (1970) after pre-treatment for 1 h with neutral dodecylsulfate and heat-stable  $\alpha$ -amylase (Ankom Technology, Macedon, NY, USA) to remove starch (Van Soest et al. 1991). Acid-detergent fiber was determined after pre-treatment for 1 h with cetyltrimethylammonium bromide in acid media (Goering and Van Soest 1970). Crude cellulose was determined according to Horwitz (1975). All analyses were run in duplicate and average values used in calculations. We estimated apparent digestion and digestibility of energy and nutrients of the food as: amount digested (g/day) = amount ingested (g/day) – amount defecated (g/day), apparent digestibility (%) =  $100 \times \text{amount digested} / \text{amount ingested}$  (Sibly 1981). The term “digestibility” is used for fiber because there is no endogenous source of fiber, but “apparent digestibility” is used for dry matter, crude protein, and energy to indicate that no correction was made for endogenous sources.

#### Statistical analysis

We verified the normality and homoscedasticity of variables with Kolmogorov–Smirnov goodness of fit and Levene’s tests, respectively (Zar 1996). Proportions were arcsine square root transformed before performing parametric statistics (Zar 1996). We used ANCOVA to test for difference in RMR and TEWL between groups. We ran post hoc Newman-Keuls multiple range tests to explore statistical differences between groups. To compare parameters between treatments and times we used either repeated measures ANOVAs or two-tailed  $t$ -tests after sequential Bonferroni correction in the level of significance (Sokal and Rohlf 1995). Significance was accepted at  $P = 0.05$ . Means are reported  $\pm 1$  SD. In addition, we consistently tested the interaction between covariates and fixed factors although we do not always report the results of insignificant interactions.

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## Results

### Body mass

After 5 months of progressive food and water restriction, oryx in CTRL weighed  $95 \pm 5.1$  kg, an insignificant change from the average mass at the beginning of the experiment ( $F_{1,6} = 2.42$ ,  $P = 0.17$ ). Those in EXPT weighed on average  $82.2 \pm 3.9$  kg, a loss of  $7.5 \pm 2.6$  kg or  $8.2 \pm 2.6\%$  of their initial body mass and were significantly lighter than oryx in CTRL ( $F_{1,12} = 19.6$ ,  $P = 0.008$ ).

## Minimum resting metabolic rate and body temperature

Before acclimation, RMR averaged  $8416 \pm 503$  kJ/day ( $n=6$ ) for animals in CTRL, and  $8317 \pm 355$  kJ/day ( $n=7$ ) in EXPT, values that did not differ significantly ( $F_{1,12}=0.01$ ,  $P>0.9$ ) (Fig. 1). After acclimation RMR for the control group averaged  $8787 \pm 646$  kJ/day ( $n=6$ ) and  $6506 \pm 601$  kJ/day ( $n=7$ ) for those in the experimental group. We could not measure RMR for one oryx of CTRL because of its restlessness in the metabolic chamber. An analysis of variance using body mass as covariate indicated that, after acclimation, RMR differed significantly between groups ( $F_{1,11}=12.2$ ,  $P=0.006$ ) (Fig. 1). Because some of the reduction in RMR for oryx in the EXPT group might be attributable to loss of body mass (Kleiber 1975), we calculated the difference between pre- and post-acclimation body mass as the independent variable and the difference in RMR as the dependent variable for each individual of both groups and tested for an interaction. Finding none ( $F_{1,9}=0.2$ ,  $P>0.6$ ), we reran the analysis with the interaction term removed and found no effect of the difference in body mass ( $F_{1,10}=0.002$ ,  $P=0.96$ ) but a significant effect of the treatment ( $F_{1,10}=11.5$ ,  $P<0.007$ ). Treatment alone explained 81.7% of the difference in RMR between groups ( $F_{1,11}=54.5$ ,  $P<0.001$ ). Corrected for body mass, RMR was 16.2% lower in food- and water-restricted oryx.

Pre-acclimation  $T_b$  averaged  $38.6 \pm 0.1$  and  $38.5 \pm 0.2^\circ\text{C}$  in CTRL and EXPT group, respectively, and did not differ between treatments ( $F_{1,12}=0.33$ ,  $P=0.57$ ). Post-acclimation  $T_b$  averaged  $38.6 \pm 0.2$  and  $37.5 \pm 0.4^\circ\text{C}$  in CTRL and EXPT group, respectively, and was  $1.0 \pm 0.51^\circ\text{C}$  lower in EXPT ( $F_{1,12}=36.6$ ,  $P<0.001$ ). The interaction between treatment and time

had a significant effect on  $T_b$  ( $F_{1,11}=17.9$ ,  $P<0.002$ ), and post hoc analysis showed that  $T_b$  of EXPT after acclimation was lower than that of any other group (Newman-Keuls,  $P<0.05$ ).

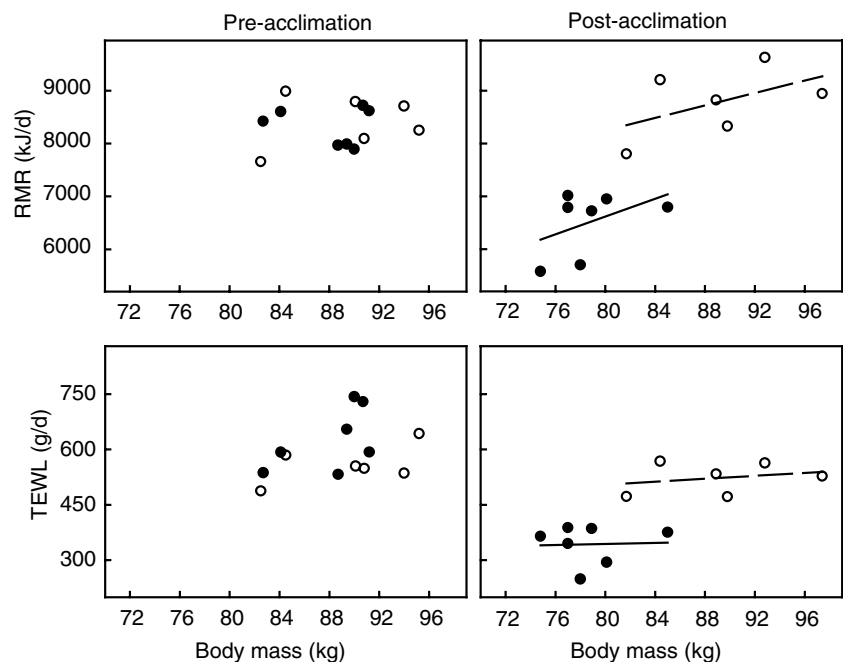
## Total evaporative water loss

Initial rates of TEWL at  $26^\circ\text{C}$  did not differ between groups ( $P>0.05$ ). After acclimation, TEWL of CTRL averaged  $522.8 \pm 42.3$  g  $\text{H}_2\text{O}/\text{day}$  ( $n=6$ ) whereas TEWL of EXPT was  $342.2 \pm 52.6$  g  $\text{H}_2\text{O}/\text{day}$  ( $n=7$ ). Analysis of covariance indicated that TEWL differed significantly between treatments ( $F_{1,11}=18.1$ ,  $P=0.002$ , Fig. 1). We also tested whether treatment alone explained the difference in TEWL. After finding no significant interaction term ( $F_{1,9}=0.9$ ,  $P=0.3$ ), we eliminated it and found no effect of the difference in body mass ( $F_{1,10}=0.10$ ,  $P=0.7$ ) but a significant effect of treatment ( $F_{1,10}=8.5$ ,  $P<0.02$ ) on difference of TEWL as we had done for RMR. Treatment alone explained 71.9% of the difference in TEWL between groups ( $F_{1,11}=31.7$ ,  $P<0.001$ ). The TEWL of oryx in EXPT after acclimation decreased by 25.7%.

## Blood and urine parameters

Plasma concentrations of total proteins, glucose, creatinine, urea and  $\beta$ -OH butyrate did not differ between treatment groups after acclimation ( $F_{1,12}<4.0$ ,  $P>0.07$ ). In addition, plasma osmolality remained unchanged. The interaction of treatment by time showed a significant effect on plasma concentrations of NEFA ( $F_{1,12}=10.7$ ,  $P=0.006$ ), with higher concentration in

**Fig. 1** Minimum resting metabolic rate (RMR) and total evaporative water loss (TEWL) as a function of body mass for Arabian oryx assigned to food and water restriction (solid symbols) or fed and watered ad libitum (open symbols), before (pre-acclimation) and after (post-acclimation) acclimation to the two different food and water regimens. Lines indicate regressions that were significantly different for groups acclimated to the different regimens



**Table 1** Mean  $\pm$  SD concentrations of plasma and urine biochemical variables, osmolality, and plasma hormones measured in Arabian oryx assigned to 5 months of progressive food and water restriction (experimental) or fed and watered ad libitum (control)

	Pre-acclimation		Post-acclimation		ANOVA $P^a$
	Experimental ( $n=7$ )	Control ( $n=7$ )	Experimental ( $n=7$ )	Control ( $n=7$ )	
<b>Blood parameters</b>					
Urea (mg/dl)	13.9 (1.2)	15.6 (4.9)	15.1 (2.5)	14.9 (3.6)	0.160
Creatinine (mg/dl)	1.2 (0.2)	1.2 (0.1)	1.3 (0.2)	1.2 (0.1)	0.618
Total protein (g/dl)	7.4 (0.3)	7.35 (0.4)	7.7 (0.4)	7.5 (0.2)	0.436
Glucose (mg/dl)	71.4 (3.1)	70.7 (7.9)	69.6 (8.3)	66.6 (5.3)	0.545
NEFA (mmol/l)	0.14 (0.05)	0.11 (0.06)	0.27 (0.11)	0.11 (0.02)	<b>0.006</b>
$\beta$ -OH butyrate (mg/dl)	2.1 (0.7)	2.1 (0.2)	2.3 (0.6)	1.9 (0.4)	0.158
<b>Urine parameters</b>					
Urea (mg/dl)	1,293.7 (110.0)	1,303.6 (231.7)	1,780.7 (217.4)	1,291.1 (230.5)	< <b>0.001</b>
Creatinine (mg/dl)	128.0 (16.6)	127.0 (22.5)	199.4 (19.5)	124.4 (31.8)	< <b>0.001</b>
Urea/creatinine	10.2 (1.2)	10.3 (1.7)	9.0 (1.4)	10.9 (3.2)	0.275
<b>Blood hormones</b>					
Total $T_4$ (nmol/l)	74.71 (6.98)	71.88 (10.88)	59.13 (22.29)	68.30 (14.58)	0.405
Total $T_3$ ( $\mu$ g/l)	0.88 (0.11)	0.87 (0.07)	0.75 (0.22)	0.83 (0.08)	0.135
Corticosterone (ng/ml)	0.80 (0.30)	0.78 (0.28)	0.67 (0.33)	0.61 (0.39)	0.859
Cortisol (ng/ml)	1.03 (0.29)	1.07 (0.23)	1.18 (0.48)	1.02 (0.50)	0.338
Leptin (ng/ml)	4.85 (1.10)	5.26 (1.10)	2.79 (0.95) <sup>a</sup>	5.21 (0.95)	<b>0.040</b>
<b>Osmolality</b>					
Plasma (mOsm)	306.7 (4.1)	306.1 (7.2)	316.1 (5.1)	310.8 (4.1)	0.139
Urine (mOsm)	1,449.3 (260.2)	1,436.3 (250.6)	2,362.7 (96.2) <sup>a</sup>	1,476.3 (173.6)	< <b>0.001</b>

$n$  number of Arabian oryx in the sample

<sup>a</sup>Statistical significance was determined by repeated-measures ANOVA after sequential Bonferroni correction (Sokal and Rohlf 1995). Significant effects are shown in boldface

EXPT group after acclimation (Newman-Keuls,  $P < 0.05$ ) (Table 1).

Thyroid and glucocorticoid hormone concentrations did not differ between groups for either pre- or post-acclimation. The interaction term for treatment and time was also insignificant ( $P > 0.05$  in all cases). Plasma concentrations of leptin did not differ between groups prior to acclimation ( $F_{1,12} = 0.48$ ,  $P = 0.5$ ), but the interaction of time by treatment had a significant effect on leptin concentration ( $F_{1,12} = 5.17$ ,  $P = 0.04$ ); the concentration of leptin in plasma was lower in the EXPT group after acclimation (Newman Keuls,  $P < 0.05$ ).

Urine concentrations of urea and creatinine, urea to creatinine ratio, and urine osmolality, did not differ between groups prior to acclimation ( $F_{1,13} < 0.02$ ,  $P > 0.9$ ), but the interaction of treatment by time had a significant effect on these parameters ( $F_{1,12} > 20.9$ ,  $P < 0.01$ ), except for urea to creatinine ratio. Post hoc analyses revealed that osmolality and concentrations of urea and creatinine were significantly higher in the urine of EXPT animals after acclimation (Newman Keuls,  $P < 0.05$ ).

#### Water budget and food digestibility

The total water income of oryx based on 3 day of balance measurements post-acclimation was  $3,872 \pm 453$  g/day in the CTRL group, partitioned as  $86.6 \pm 3.3\%$  drinking water,  $11.2 \pm 2.9\%$  metabolic water and

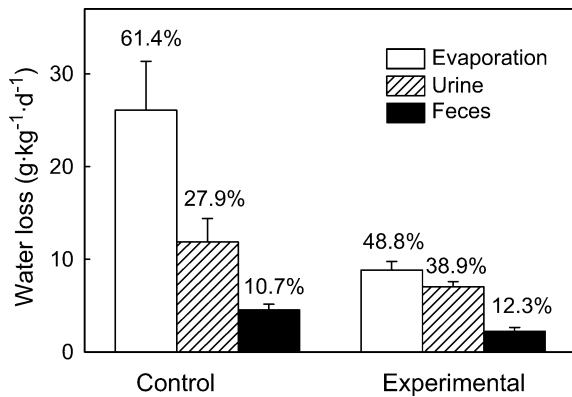
during the same period, before (pre-acclimation) and after (post-acclimation) acclimation to the two different food and water regimens

$2.2 \pm 0.4\%$  as pre-formed water. Total water influx in the EXPT group was  $1,478 \pm 15$  g/day,  $81.1 \pm 0.8\%$  as drinking water,  $15.7 \pm 0.9\%$  as metabolic water and as  $3.2 \pm 0.1\%$  pre-formed water. TEWL accounted for 61.0% of total water expenditure in the CTRL group, compared with only 48.7% in the EXPT group. Mass-specific TEWL averaged  $26.1 \pm 5.3$  g  $H_2O$   $kg^{-1}$   $day^{-1}$  in CTRL and  $8.8 \pm 0.9$  g  $H_2O$   $kg^{-1}$   $day^{-1}$  in EXPT, values that differed significantly ( $F_{1,12} = 97.7$ ,  $P < 0.001$ ).

Urine water volume averaged  $1,084 \pm 241$  g  $H_2O$ /day in CTRL group and  $577 \pm 48$  g  $H_2O$ /day in EXPT group ( $F_{1,12} = 30.2$ ,  $P < 0.001$ ). Mass-specific fecal water loss was significantly lower in EXPT animals ( $F_{1,12} = 24.7$ ,  $P < 0.001$ ). However, oryx in EXPT had a significantly higher proportion of mass-adjusted urinary water losses to their water output ( $F_{1,12} = 13.8$ ,  $P < 0.004$ ) accounting for  $38.9 \pm 3.2\%$  of total loss compared with  $27.9 \pm 6.0\%$  in CTRL (Fig. 2).

Although the percentage of total water loss represented by fecal water was similar between treatments, fecal water losses were higher in the CTRL group averaging  $412 \pm 47$  g  $H_2O$ /day compared with  $181 \pm 33$  g  $H_2O$ /day in EXPT group. Mass-specific fecal water losses varied significantly between treatments ( $F_{1,12} = 63.9$ ,  $P < 0.001$ ) (Fig. 2). Mean fecal water content was  $47.8 \pm 2.3\%$  in CTRL and  $42.6 \pm 1.7\%$  in EXPT, values that differed significantly ( $F_{1,12} = 20.14$ ,  $P < 0.001$ ).

Apparent digestibility of dry matter, crude energy, crude proteins and digestibility of crude cellulose, acid-



**Fig. 2** Relative contribution of mass-adjusted evaporative, urinary and fecal avenues of water expenditure in Arabian oryx fed and watered ad libitum or assigned to food and water restriction

detergent fiber and neutral-digested fiber ranged between 65 and 78% and did not differ between treatments ( $F_{1,12} < 1.7$ ,  $P > 0.2$ ) (Table 2).

## Discussion

Previous studies that investigated flexibility of physiological phenotype of wild ungulates to desert conditions were short-term experiments of several days to a few weeks, mostly involving acute water stress and high  $T_a$  (Wilson 1989). In these experiments arid-zone ungulates responded to water deprivation by reducing their evaporative water loss. Alterations in metabolic rate were not

studied in most cases, but studies on domestic arid-zone ungulates showed that organisms respond to short-term food restriction by lowering their metabolism (Brosh et al. 1986; Choshniak et al. 1995). The ability to extrapolate these findings to natural situations in deserts where both food and water supplies progressively deteriorate over the course of months remains uncertain. We provide the first data for physiological adjustments of a large non-domesticated desert ungulate, the Arabian oryx, to progressive food and water shortages over a long period.

A desert ungulate *par excellence*, Arabian oryx lost  $< 10\%$  of their body mass during long-term, progressive, food and water restriction that ended in them receiving less than one and half of what they would normally consume when fed ad libitum. To minimize mass loss, oryx reduced their RMR by 16.2% and maintained a digestive efficiency of about 70%, the latter a relatively high value for an ungulate feeding on medium-quality forage (Brosh et al. 1986). Oryx adjusted their RMR to compensate for dwindling resources thereby delaying reliance on catabolism of structural proteins for energy. Although the mechanisms that reduce RMR are unclear, two could be involved, either in tandem or separately. Visceral organs such as liver, heart and kidney might diminish in size, or tissue-specific oxygen consumption could be reduced (Krebs 1950; Canas et al. 1982). We found no support for the idea that reduced metabolism in oryx is mediated by a decrease in thyroid hormone production (Hulbert 2000), but this may not be surprising as these hormones are also involved in release of NEFA from adipocytes

**Table 2** Mean  $\pm$  SD digestibility of food constituents in Arabian oryx measured for three consecutive days at the end of 5 months of progressive restriction of food and water (Experimental), or without restriction (Control)

	Experimental (n = 7)	Control (n = 6)	ANOVA $P^a$
Dry matter			
Intake (g/d)	738.2 (7.6)	1,361.9 (159.7)	<b>&lt; 0.001</b>
Fecal dry matter output (g/d)	248.5 (38.3)	450.2 (50.1)	<b>&lt; 0.001</b>
Apparent digestibility (%)	66.4 (4.8)	66.6 (4.8)	0.321
Energy			
Intake (mJ/day)	12.7 (0.1)	23.4 (2.1)	<b>&lt; 0.001</b>
Fecal energy output (mJ/day)	4.4 (0.7)	7.8 (0.9)	<b>&lt; 0.001</b>
Apparent digestibility (%)	65.3 (5.0)	66.1 (5.2)	0.794
Crude protein			
Intake (g/day)	120.8 (1.3)	222.9 (26.1)	<b>&lt; 0.001</b>
Fecal protein output (g/day)	34.4 (8.4)	53.7 (5.9)	<b>&lt; 0.001</b>
Apparent digestibility (%)	71.6 (6.8)	75.6 (3.7)	0.210
Fibers			
Intake of cellulose (g/day)	235.0 (2.4)	433.5 (50.7)	<b>&lt; 0.001</b>
Fecal cellulose output (g/day)	54.0 (7.6)	93.0 (13.1)	<b>&lt; 0.001</b>
Digestibility of cellulose (%)	77.0 (3.1)	78.3 (3.8)	0.503
Intake of NDF (g/day)	503.7 (5.2)	929.5 (109.1)	<b>&lt; 0.001</b>
Fecal NDF output (g/day)	131.1 (18.8)	231.1 (28.7)	<b>&lt; 0.001</b>
Digestibility of NDF (%)	74.0 (3.5)	74.9 (3.7)	0.743
Intake of ADF (g/day)	258.9 (2.7)	477.8 (56.0)	<b>&lt; 0.001</b>
Fecal ADF output (g/day)	70.9 (9.4)	122.7 (12.7)	<b>&lt; 0.001</b>
Digestibility of ADF (%)	72.6 (3.4)	74.0 (3.9)	0.491

<sup>a</sup>Significant differences are shown in boldface  
NDF neutral-detergent fiber, ADF acid-detergent fiber

(Heimberg et al. 1985). Visceral organs of ruminants comprise 6–10% of body mass, but account for 40–50% of RMR (Webster 1981). When lambs were food restricted for 3 weeks, the masses of their liver and gut decreased by 10–33% (Burrin et al. 1990). In a study on the Arabian sand gazelle (*Gazella subguturosa marica*), a small (20 kg) ungulate that co-exists with oryx, we documented that, during a similar regimen of food restriction, gazelles reduced their mass-specific RMR compared with a control group by approximately 25%. Attendant to this decrease in RMR, we found a reduction in the dry lean mass of liver, heart, and muscle (S. Ostrowski, unpublished). These findings lead us to suggest the idea that the decrease in RMR of oryx can, at least partially, be attributed to shrinkage of visceral organs. Changes in RMR during food restriction could also result from differences in tissue-specific metabolism, but this hypothesis received no support when investigated in sheep (Burrin et al. 1990).

Because RMR make up only 25–45% of the daily energy expenditure of a mammal (Nagy 1987; Ricklefs et al. 1996), one might expect that oryx succeeded to survive a 50% decrease in energy expenditure by adjusting their behavior besides RMR reduction. In nature during summer, oryx forage only at night when ambient temperature values are moderate, and lie completely inactive beneath shade trees during daylight. Although we have not quantified activity budgets during the present study, it seems that food and water restricted oryx were usually inactive for most of daytime whereas those fed and watered ad libitum were only inactive during 3–4 h of maximal heat load.

The flexibility that we have found in RMR for oryx prompts caution when making broad-scale inter-specific comparisons among desert ungulates. The RMR measured under different planes of nutrition may differ not only because of genetic differences but also because of acclimation. An equation that relates RMR to body mass for 15 species of artiodactyls predicts a metabolic rate of 10,194 kJ/day for an oryx weighing 89.2 kg (Williams et al. 2001). Oryx in our control group had a RMR 13.8% less than the predicted value suggesting that they have only a slightly depressed metabolic rate. However, if we had measured this trait after a period of underfeeding we would have found that metabolic rate was 30% below allometric predictions for artiodactyls.

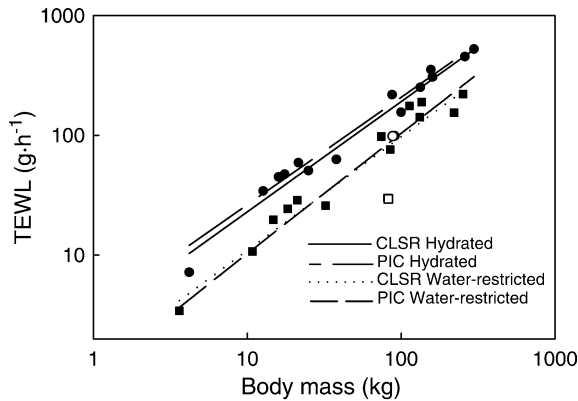
A decrease in RMR reduces TEWL because, with decreased oxygen requirements, oryx would breathe less frequently resulting in less water lost in respiration. Although the decrease in TEWL may be partly due to decreased ventilation rates, in food- and water-restricted oryx, the amount of evaporative water lost per unit of metabolism was  $0.053 \pm 0.008$  g H<sub>2</sub>O/kJ after acclimation, compared with  $0.063 \pm 0.025$  g H<sub>2</sub>O/kJ before acclimation. The post-acclimation ratio was significantly lower ( $t=3.70$ ,  $df=6$ ,  $P=0.005$ ), indicating that other mechanisms are possibly involved in the reduction of TEWL in oryx.

In mammals ranging in size from bats (15.8 g) to elephants (3,630 kg), measured at 18–29°C, Chew's (1965) equation predicts a TEWL of 2,526 g H<sub>2</sub>O/day for an 89.1 kg oryx, whereas our measurement for hydrated oryx was 522.8 g H<sub>2</sub>O/day, only 20.7% of the predicted value, suggesting that Arabian oryx have evolved a remarkably low TEWL.

To further test this idea and to compare TEWL of oryx with other water-deprived ungulates, we collated data for TEWL of 15 species (S1), and generated allometric equations of TEWL for hydrated and water-deprived arid-zone ungulates. We constructed a “best guess” phylogeny for arid-zone ungulates (S2 and S3) and explored the usage of statistical methods for historical bias correction. To test for the necessity of correction for relatedness of species, we used a test for serial independence (Abouheif 1999). We found that body mass and TEWL in both experimental sets were significantly correlated with phylogeny ( $P<0.01$ ). We generated independent contrasts and reran our test for serial independence. In all cases we found insignificant results ( $P>0.13$ ), indicating that this method adequately standardized traits (Abouheif 1999). The equations that resulted from these analyses, one using conventional least squares regression (CLSR) and the other using phylogenetic independent contrasts (PIC), for hydrated and water-deprived arid-zone ungulates, are presented in S4. Hydrated and water-restricted oryx had a TEWL equivalent to 53.9 and 34.6%, respectively, of predicted values based on equations using CLSR whereas they had a TEWL 51.7 and 39.3% of predicted values by PIC equations. These results confirmed that oryx have an unusually low TEWL (Fig. 3).

The adjustments made by oryx to maintain water balance during acclimation to food and water restriction should also reflect in the rate at which they process water. Expressed as the water flux relative to energy metabolism, the water economy index (WEI) could test this assumption (Nagy and Peterson 1988). The WEI of oryx fed and watered ad libitum was approximately 0.165 g H<sub>2</sub>O/kJ, within the theoretical expected value for a desert herbivore eating leaves of 62% water, and requiring no drinking water (Nagy and Peterson 1988). After acclimation, WEI decreased to 0.116 g H<sub>2</sub>O/kJ, close to the value measured in free-ranging oryx exposed to summer drought; 0.118 g H<sub>2</sub>O/kJ (Williams et al. 2001), suggesting that oryx employ, in their natural habitat, at least some of the physiological mechanisms for water conservation that we have described in the present study.

During water restriction, oryx increased urine osmotic concentration and reduced urine volume by about 40%. The increased concentrations of urea and creatinine in urine, but not in plasma, and the constant urea to creatinine ratios compared with hydrated oryx suggest that tubular reabsorption of water in the kidney was increased. However, under our experimental protocol, maximum urine osmolality rose to 2,504 mOsm, about 20% less than that reported for the camel (*Camelus*



**Fig. 3** Logarithmic plot of TEWL for hydrated (*round symbols*) and water-restricted (*square symbols*) arid-zone ungulates. *Unfilled symbols* represent Arabian oryx. Allometric equations obtained by the methods of conventional least squares regression (CLSR; *solid and dotted lines*) and phylogenetically independent contrasts (PIC; *long dash and short dash lines*) are detailed in S4

*dromedarius*) during complete water deprivation (3,100 mOsm; Maloiy 1973).

Arabian oryx in our control group excreted  $862.8 \pm 89.7$  g feces/day, containing  $47.8 \pm 2.3\%$  water, the lowest fecal moisture content reported for a hydrated ungulate species. Fecal water content for mammals generally exceeds 50%, only a few heteromyid rodents (Degen 1997) and the dik-dik antelope (*Rhynchotragus kirkii*) (Maloiy 1973) achieve a fecal water content of < 50%. When restricted in food and water, oryx excreted  $433.3 \pm 68.3$  g feces/day, and further decreased the moisture content of their feces by 5.2%, the latter a reduction that saved  $43.6 \pm 13.5$  g H<sub>2</sub>O/day per animal.

When their water intake was reduced by 70% over the course of 5 months, oryx maintained constant plasma osmolality and total proteins concentration. Decrease in leptin plasma concentration in food- and water-restricted oryx indicated, together with increased circulating NEFAs, that levels of body fat were reduced but not yet depleted at the end of the acclimation experiment. In camels, substantial changes in feeding level (17–134% of maintenance energy requirements) did not alter leptin concentrations (Delavaud et al. 2004). Because leptin levels increased in camel after 3 weeks of water deprivation (Chilliard et al. 2005), we attribute the alteration of leptin levels, we observed in oryx, to the mobilization of fat reserves rather than to water deprivation. The low level of plasma  $\beta$ -OH butyrate, a ketone body formed as a result of insufficient supply of oxaloacetate precursors to Krebs cycle, indicated that oryx could supply C4-carbon precursors in sufficient quantity for maintenance of the Krebs cycle. The absence of increased concentrations of urea and glucocorticoids in plasma, and the constant urea to creatinine ratio in urine suggest that oryx did not use protein breakdown as a major source of gluconeogenesis compared to lipid consumption (DelGiudice et al. 1992).

Understanding the extent and nature of physiological adjustments of large desert ungulates to environmental constraints is important to our comprehension of their ecology, a useful step towards their conservation (Treydte et al. 2001). Our data highlight the importance of physiological mechanisms in the ability to survive drought conditions, and therefore provides elements of prediction concerning the response of these endangered species to global processes such as climate change.

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