Am J Physiol Regul Integr Comp Physiol 302: R929–R940, 2012.
First published February 8, 2012; doi:10.1152/ajpregu.00370.2011.

CALL FOR PAPERS | *Integrative and Translational Physiology: Integrative Aspects of Energy Homeostasis and Metabolic Diseases*

Hormonal responses to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups

Delphine Verrier,[1,2] Shannon Atkinson,[3] Christophe Guinet,[4] René Groscolas,[2] and John P. Y. Arnould[5]

[1]*Department of Zoology, University of Melbourne, Parkville, Victoria, Australia;* [2]*Institut Pluridisciplinaire Hubert Curien, Département Ecologie, Physiologie et Ethologie, UMR 7178 Centre National de la Recherche Scientifique (CNRS)-Université des Sciences, Strasbourg, France;* [3]*School of Fisheries and Ocean Sciences, Juneau Center, University of Alaska Fairbanks, Juneau, Alaska;* [4]*Centre d'Etudes Biologiques de Chizé, UPR 1934 CNRS, Villiers-en-Bois, France; and* [5]*School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, Australia*

Submitted 5 July 2011; accepted in final form 7 February 2012

Verrier D, Atkinson S, Guinet C, Groscolas R, Arnould JP. Hormonal responses to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups. *Am J Physiol Regul Integr Comp Physiol* 302: R929–R940, 2012. First published February 8, 2012; doi:10.1152/ajpregu.00370.2011.—Surviving prolonged fasting implies closely regulated alterations in fuel provisioning to meet metabolic requirements, while preserving homeostasis. Little is known, however, of the endocrine regulations governing such metabolic adaptations in naturally fasting free-ranging animals. The hormonal responses to natural prolonged fasting and how they correlate to the metabolic adaptations observed, were investigated in subantarctic fur seal (*Arctocephalus tropicalis*) pups, which, because of the intermittent pattern of maternal attendance, repeatedly endure exceptionally long fasting episodes throughout their development (1–3 mo). Phase I fasting was characterized by a dramatic decrease in plasma insulin, glucagon, leptin, and total L-thyroxine (T_4) associated with reductions in mass-specific resting metabolic rate (RMR), plasma triglycerides, glycerol, and urea-to-creatine ratio, while nonesterified fatty acids (NEFA) and β-OHB increased. In contrast, the metabolic steady-state of phase II fasting reached within 6 days was associated with minimal concentrations of insulin, glucagon, and leptin; unchanged cortisol and triiodothyronine (T_3); and moderately increased T_4. The early fall in insulin and leptin may mediate the shift to the strategy of energy conservation, protein sparing, and primary reliance on body lipids observed in response to the cessation of feeding. In contrast to the typical mammalian starvation response, nonelevated cortisol and minimal glucagon levels may contribute to body protein preservation and downregulation of catabolic pathways, in general. Furthermore, thyroid hormones may be involved in a process of energy conservation, independent of pups' nutritional state. These original hormonal settings might reflect an adaptation to the otariid repeated fasting pattern and emphasize the crucial importance of a tight physiological control over metabolism to survive extreme energetic constraints.

starvation; leptin; cortisol; thyroid hormones; insulin

TO SURVIVE PERIODS OF FOOD deprivation, animals make behavioral and metabolic adjustments leading to the adoption of a common energy-saving strategy and regulated changes in fuel selection (11, 13, 71). After a brief adaptive period (phase I) during which the diet-derived energy and limited carbohydrate stores are exhausted, fasting individuals enter phase II, a stage of economy characterized by *1*) a depressed metabolic rate reducing the amount of nutrients to be catabolized to meet maintenance costs; *2*) a high level of lipid utilization, providing the majority of energy expenditure; and *3*) a protein-sparing process protecting vital lean tissues from depletion (13, 71). Eventually, as fat reserves reach a critical threshold of depletion, energy metabolism may become primarily dependent on protein oxidation, marking the onset of phase III fasting, which may rapidly lead to compromised muscle function and irreversible starvation (13). Hence, an individual's ability to prolong the metabolic state of phase II determines its endurance to extended fasting (15).

The initiation of the metabolic adaptations of phase II fasting is thought to be associated with a typical cascade of endocrine changes among mammals. Insulin (e.g., the major antilipolytic hormone) falls, while counter-regulatory hormones (e.g., glucagon and epinephrine) rise, to trigger the switch to a fat-based metabolism (11, 36). Glucocorticoids also increase to promote energy supply through enhanced lipolysis, proteolysis, and gluconeogenesis (20), helping individuals cope with the stress of food deprivation (66). Concomitantly, thyroid hormones known to stimulate metabolism by increasing mitochondrial respiration and metabolic heat production (21) decrease dramatically as a possible means to promote energy conservation (26).

Over the past decades, evidence has grown that these hormonal changes might be orchestrated by the starvation-induced fall in circulating leptin (1, 2), a peripheral signal involved in the regulation of food intake, body adiposity, metabolic rate, and immune function in mammals, and thereby suspected to be the mediator of the adaptations to fasting (1). However, the current knowledge about the factors regulating those adaptations mostly originates from short-term starvation experiments carried out on laboratory animals that are not adapted to food deprivation. In fact, whereas long-term fasts are natural components of the life history of many vertebrates (13, 48, 71), few data exist on the hormonal changes associated with prolonged, active fasting in mammals in their natural habitat (40, 57, 58, 70). Understanding the links between nutritional state and free-ranging animal physiology is, however, crucial in understanding the mechanisms regulating energy balance and feeding behavior in free-ranging animals (18).

Address for reprint requests and other correspondence: D. Verrier, Centre de Primatologie, Centre International de Recherches Médicales de Franceville, B.P. 769, Franceville, Gabon (e-mail: ddlafouine@free.fr).

Pinnipeds (seals, sea lions, and walrus) naturally alternate periods of hyperphagia and fasting throughout their life cycle (10, 17). For instance, otariid (fur seal and sea lion) pups must undergo regular fasts during their development for their mothers alternate between short attendance periods ashore (1–4 days) and long at-sea foraging trips leaving their pup on land (typically, 2–6 days) throughout lactation (4 mo to 3 yr depending on species) (10, 17, 29). At Amsterdam Island (southern Indian Ocean), subantarctic fur seals (*Arctocephalus tropicalis*) represent the most extreme example of the otariid life history pattern. Lactating females undertake the longest foraging trips of any otariid seal due to the great distances they must travel to feed on myctophid fish in the subtropical front (up to 1,600 km away from breeding colony) (8, 31), forcing their pups to repeatedly endure exceptionally long fasting episodes throughout the 10-mo rearing period. At-sea periods for lactating females average 2 wk in summer (from birth to 3 mo post partum), when their pup's body mass is 5–12 kg, to 4 wk in winter (at 7–9 mo post partum) with regular records up to 2 mo, when their still-dependent pup has a body mass of 8–18 kg (68, 69). For pups to be able to cope with such energetic constraints, despite their small body size and the conflicting demands of growth and development, subantarctic fur seal pups have developed robust adaptations to food deprivation (68, 69) and, hence, provide an ideal model to investigate fasting energetics and their endocrine regulation in a species adapted to prolonged fasting.

It has been previously shown that, in response to the extreme fasts experienced, subantarctic fur seal pups adopt an extraordinarily efficient strategy of energy and lean body mass conservation, which is facilitated by their high body adiposity (an average of 48%) and the utilization of alternative fuels, such as β-hydroxybutyrate (β-OHB) and de novo synthesis of glucose from fat-released glycerol. This makes them one of the most advanced evolutionary adaptations of any mammal to conditions of no food and no water during development (69). In these animals, phase I fasting is of short duration (5–6 days), and phase II is characterized by markedly reduced mass-specific rates of body mass loss (>50% compared with phase I), metabolic rates (25%), and plasma levels of triglycerides (80%), glycerol (50%), and urea-to-creatinine ratio (U:C) (50%), accompanied by dramatic increases in the plasma concentrations of β-OHB and nonesterified fatty acids (NEFA) (69).

Furthermore, in marked contrast with the commonly observed pattern of growth in otariid seals, previous results have revealed the absence of any differences in body composition and fasting energetics between the sexes in subantarctic fur seal pups at Amsterdam Island (68, 69). Yet, in highly polygynous and sexually dimorphic species, such as otariid seals, there is strong selection for large body size in males to facilitate access to females and high reproductive success. Consequently, male otariid infants are expected to favor lean body mass protection and growth over fat mass storage, whereas female pups can afford to rely more on protein catabolism (4–6, 24, 53). While sex-based differences in plasma metabolite concentrations during phase I fasting indicate that subantarctic fur seal pups could follow the "typical" otariid pattern of divergent fuel utilization between the sexes in the "normal" postabsorptive state, both sexes shifted to a common metabolic strategy (e.g., based on primary body fat reliance and protein sparing, regardless of sex and body composition) as they entered phase II and prepared to face prolonged fasting (69). These observations support the theory of a convergent metabolic strategy that could have evolved to promote offspring survival in response to the evolutionary pressures exerted by repeated prolonged fasting (68, 69).

The trade-off between the need to conserve energy to survive repeated prolonged fasting and to allocate resources to growth and development infers a tight control over energy balance and the pattern of body reserve utilization. However, little is known of the endocrine regulations governing these extreme adaptations to recurrent food deprivation. The aim of the present study, therefore, was to investigate the hormonal responses to extreme fasting in subantarctic fur seal pups. To do so, we determined *1*) the changes in cortisol, thyroid hormones, insulin, glucagon, and leptin occurring throughout natural episodes of prolonged fasting in winter when periods of maternal absence are the longest; and *2*) how they correlate with the adaptive metabolic and biochemical changes previously described (69), as to infer on the potential role of these hormonal factors in mediating the adaptations to fasting. The influence of sex on these parameters was also investigated to determine to what extent sex differences in hormone concentrations could lead to the convergent metabolic strategy reported.

METHODS

Study site and animals. All procedures involved in the present study were approved by the Ethics Committee of the French Polar Institute and the Polar Environment Committee of Terres Australes et Antarctiques Françaises. They complied with the Agreed Measures for the Conservation of Antarctic and sub-Antarctic Fauna and current French laws.

The study was conducted at the breeding colony of subantarctic fur seals "La Mare aux Elephants" at Amsterdam Island (37°55'S, 77°30′E) during the austral winter of 2005. The present study was concurrent with investigations of fasting energetics in pups and involved the same animals (69). Animal handling and sampling thus followed the procedures previously described. Briefly, 20 known-age subantarctic fur seal pups (10 males, 10 females) were serially sampled throughout a single extended maternal absence. To detect fasting bouts in the study animals, individual maternal attendance patterns were monitored at least twice daily by visual inspection of the colony, following the standard protocol based on previous results on maternal care at the study site (8, 31, 69). In winter, because no food resource is available in the vicinity of the island and average foraging trip durations range from 15 to >50 days, the likelihood of mothers being away on a short foraging trip lasting <24 h that could not be detected with a minimum of two daily checks is very limited. Furthermore, at this period of the year, females need to recover from their long foraging trips and maximize the time spent nursing, as well as milk transfer to their offspring. Hence, they mostly remain on land in close contact with their pup throughout the winter visits and the likelihood of missing a reunion event is unlikely. In addition, since pups were weighed and bled regularly throughout the study, any missed nursing bout would have been detected from *1*) the inflection in the pup's body mass curve; *2*) the marked lipemic aspect of the plasma samples following suckling; and *3*) changes in the circulating concentrations of blood metabolites.

Study periods commenced at the end of a maternal attendance period ashore as the mother departed the colony on a foraging trip and continued until she returned to nurse again. At the time of first capture, pups were fully molted, aged 217 ± 2.6 days (range: 195–236 days), and weighed 16.1 ± 0.5 kg (range: 12.2–20.0 kg). Pups were captured on *days 0*, *1*, *2*, and *4* following maternal departure, and subsequently, every 4 days until the end of the natural fast. Pups were also opportunistically weighed and had blood samples collected in the

colony on *day 6*. Because the pups were left free ranging in the colony between sampling periods, not all animals could be located and captured on each sampling day. Furthermore, maternal absence durations varied between individuals, and the fasting periods covered were, therefore, of unequal durations between study animals.

Upon capture, animals were placed into a large Hessian bag to facilitate manual restraint, and a blood sample (5–10 ml, representing <1.5% of total blood volume) was collected by venipuncture of either an interdigital vein of the hind flipper or the brachial vein of the fore flipper within <10 min of capture. Blood was immediately transferred into lithium-heparin and EDTA-treated tubes and kept on ice for <3 h. Pups were then weighed in the bag using a spring scale (±0.05 kg) and transported to the field laboratory (300–600 m from pup location in the colony) to undergo resting metabolic rate (RMR) measurements using standard open-circuit respirometry, as previously described (69). Meanwhile, blood samples were centrifuged, and the plasma fractions were separated and stored at −20°C until analysis for metabolite and hormone concentrations (within 6 mo). Upon completion of measurements, pups were finally released in the colony at the site of capture.

Hormone measurements and metabolic data. In the laboratory, plasma hormone concentrations were measured in unextracted and undiluted subantarctic fur seal pup plasma collected in lithium-heparin tubes by direct assay using solid-phase radioimmunoassay (RIA). Commercially available RIA kits were used (Table 1), which had been validated previously in other pinnipeds, including northern elephant seals (*Mirounga angustirostris*) (54, 58), harbor seals (*Phoca vitulina*) (52), Steller sea lions (*Eumetopias jubatus*) (46, 49, 50), Australian fur seals (*Arctocephalus pusillus doriferus*) (7), and Antarctic fur seals (*Arctocephalus gazella*) (3). The assays for leptin, cortisol, insulin, glucagon, and free and total L-thyroxine (T_4), as well as free and total triiodothyronine (T_3) were specifically validated for use with subantarctic fur seals (see Table 1 for details). The immunoreactivity of subantarctic fur seal hormones in plasma was validated for each commercial kit by examining the degree of parallelism between a serially diluted pool of subantarctic fur seal pup plasma and the standard curves (Figs. 1 and 2). Slopes of the curves were compared statistically after linearization by log-transformation using analysis of covariance (ANCOVA). The model fitted related percent binding to group (standards vs. serially diluted subantarctic fur seal pup plasma pool), Ln(hormone mass), and the interaction between group and Ln(hormone mass), the latter testing the hypothesis of equal slopes. Accuracy was assessed by combining each standard with pooled subantarctic fur seal pup plasma (vol:vol) and testing the relationship between measured and added hormone concentrations. Results for parallelism, accuracy, and cold recovery for each hormone are presented in Table 1.

The RIAs were performed per manufacturer instructions with the exception that all volumes were halved (except plasma volume for free T_3) and an additional standard was added to the curve (i.e., one half of the lowest standard) to increase sensitivity. In addition, as previously found in Steller sea lions (46), the incubation period with primary antibody had to be doubled for the leptin RIA to obtain the necessary specificity and accuracy. Indeed, using the manufacturer's protocol, serial dilutions of subantarctic fur seal pup plasma pool [$y = -12.4 \ln(x) + 85.3$, $r^2 = 0.88$, $P = 0.017$] did not yield a displacement parallel to that of the leptin standard curve [$y = -21.1 \ln(x) + 91.1$, $r^2 = 0.97$, $P < 0.0001$; ANCOVA: $F_{1,11} = 6.56$, $P = 0.034$]. In addition, recovery of added leptin (0.5–25 ng/ml) was only 82.4% (SD = 34.2, CV = 41.6%; $y = 1.16x - 0.44$, $r^2 = 0.988$, $P < 0.0001$) vs. 97.8% (SD = 5.5, CV = 1.8%; $y = 0.99x - 0.08$, $r^2 = 0.9995$, $P < 0.0001$) after doubling the primary antibody incubation period. All samples were analyzed in duplicate and in batches to reduce interassay variation, which was measured from the high-, medium-, and low-quality controls supplied with the kits. Intra-assay variation was <8% for all assays. Leptin was expressed as human equivalent (HE).

Several hormones are known to respond to stress, including both handling and fasting stress. To reduce the potential restraint effect that could blunt the hormonal response to fasting (that of cortisol, in particular), all pups were handled in exactly the same manner, and blood samples were collected within <10 min of capture. In addition to the analytical validation described above, a physiological validation was also conducted for cortisol. For practical reasons, it was not possible to conduct an ACTH challenge to physiologically validate the cortisol response for subantarctic fur seals and establish whether the cortisol concentrations measured were indeed "basal" and not stressed values. However, ACTH challenges were previously conducted in captive juvenile Steller sea lions and showed that *1*) changes in serum cortisol in response to ACTH injection could be detected only after a minimum of 30 min; and *2*) peak values of circulating cortisol corresponding to a 2- to 3-fold increase compared with basal levels were obtained by 90 to 150 min poststimulus (46). Thus, in the present study, since blood samples were collected within <10 min of capture, we are confident that the potential deleterious effect of any stress caused by the collection procedure on the measured cortisol values was limited.

Furthermore, we were able to validate the cortisol assay biologically in two ways. First, we found that the cortisol concentrations measured in the blood samples that were excluded from analysis because they failed to be collected within 10 min of capture ($n = 21$ individual samples collected between 1–4 h of handling) were elevated (by 33–89%) compared with the values obtained at the same fasting day from other individuals, which could be efficiently bled within 10 min of capture. This suggests that pup HPA axis was, in fact, responsive. Second, we demonstrated that cortisol concentrations did increase significantly throughout fasting *1*) in a cohort of inexperienced, acutely fasted pups (2.6-fold increase in 1 wk-old pups experiencing their first fast following the perinatal attendance period: 111 ± 20 nmol/l vs. 292 ± 96 nmol/l at the beginning and end of the 5- to 6-day fast, respectively; paired *t*-test: $n = 5$, $t_4 = -3.967$, $P = 0.017$); and *2*) confirming previous results by Guinet et al. (33), in lactating females nursing their pups ashore (twofold increase: 419 ± 105 nmol/l vs. 824 ± 151 nmol/l at the beginning and end of the 4-day fast, respectively; paired *t*-test: $n = 5$, $t_4 = -3.494$, $P = 0.025$),

Table 1. *Characteristics of radioimmunoassay for subantarctic fur seal pup plasma*

		Parallelism			Accuracy						
Hormones	RIA Commercial Kits	*F*	*df*	*P*	Slope	r^2	*F*	*df*	*P*	% Recovery	Interassay CV, %
Cortisol, nmol/l	TKCO1 DPC, Los Angeles, CA	0.537	1,9	0.491	0.95	0.99	977	1,8	<0.001	94.0 (3.0)	5.4
Total T_4, nmol/l	TKT41 DPC, Los Angeles, CA	1.561	1,9	0.243	0.92	0.96	190	1,9	<0.001	100.9 (3.6)	12.1
Total T_3, nmol/l	TKT31 DPC, Los Angeles, CA	1.908	1,9	0.216	1.01	1.00	2836	1,9	<0.001	103.8 (3.8)	6.3
Free T_4, pmol/l	MP Biomedicals, Irvine, CA	0.510	1,10	0.498	1.00	1.00	7551	1,8	<0.001	100.1 (3.4)	5.3
Free T_3, pmol/l	TKF31 DPC, Los Angeles, CA	0.907	1,11	0.366	1.01	1.00	2455	1,11	<0.001	101.8 (1.9)	4.1
Insulin, pmol/l	SRI-13K Linco, St Charles, MO	0.162	1,9	0.701	0.93	0.99	1477	1,12	<0.001	99.0 (3.9)	11.7
Glucagon, pmol/l	GL-32K Linco, St Charles, MO	0.003	1,10	0.961	1.16	0.99	840	1,10	<0.001	98.9 (5.2)	11.0
Leptin, ng/ml HE	XL-85K Linco, St Charles, MO	0.037	1,11	0.852	0.97	0.99	1171	1,8	<0.001	97.8 (1.8)	10.4

Data for % Recovery are presented as means ± SE in parentheses. CV, coefficient of variation; HE, human equivalent.

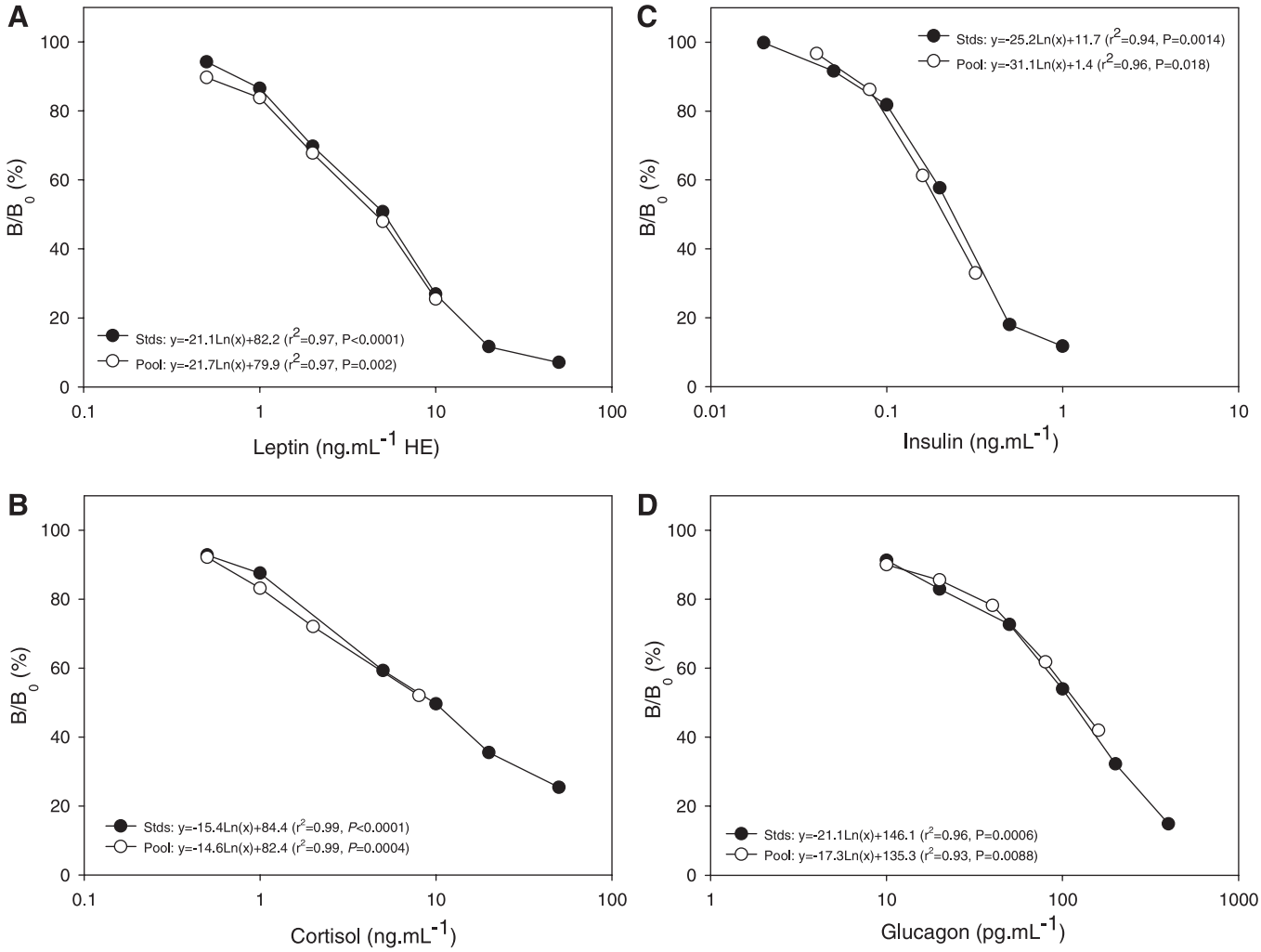

Fig. 1. Representative estimation of the percentage of cross-reactivity between subantarctic fur seal plasma and antibodies of the radioimmunoassays for leptin (*A*), cortisol (*B*), insulin (*C*), and glucagon (*D*). B/B_0, percent binding; Stds, standards; Pool, subantarctic fur seal pup plasma pool; HE, human equivalent.

which were handled identically to the pups subjected to the present study. These results, therefore, suggest that the cortisol values measured in the present study may reasonably be regarded as indicative of the physiological response to fasting in the study animals.

Pup RMR and circulating concentrations of blood metabolites were derived from previously published data on the same individuals (69). Changes in RMR were used to illustrate the changes in energy expenditure throughout fasting. Plasma concentrations of β-OHB were used as an indicator of lipid oxidation, and NEFA and glycerol were used as indicators of lipolysis. Plasma urea was used as an indicator of protein catabolism, whereas plasma creatinine was used as an indicator for skeletal muscle catabolism, and the plasma urea to creatinine ratio (U:C) were used as a corrected index of whole body protein catabolism (5, 51). U:C has also been shown to accurately predict glomerular filtration rate in fasting pinnipeds (19).

Data analyses. Statistical analyses were performed using SPSS (version 17.0 for Mac; SPSS, Chicago, IL). The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed, and an *F*-test was used to confirm homogeneity of variances. Data that failed the tests for normality or homogeneity of variances were log-transformed. Linear mixed models (LMM) were used to analyze data with a repeated-measure pattern (43). Individuals were used as random effect, and fasting days represented ranks for repeated measures. For each mixed model analysis, the covariance structure was examined, and the best fit was selected based on lowest Schwarz's Bayesian criterion. A R^2 statistic for the fixed effects in the LMM was calculated to assess the level of association between the independent and dependent variables of the models, as described elsewhere (27). Where means were compared by mixed analysis of variance (mixed ANOVA), Sidak adjustments were performed to allow for multiple pairwise comparisons. Sex- and fasting phase-related effects were systematically tested and removed from the model if they were not significant ($P > 0.05$) prior to rerunning the analysis. Values are reported as means ± SE, and results were considered significantly different at $P < 0.05$.

RESULTS

Changes in plasma hormone concentrations throughout fasting. Pups lost on average 21.5% of their initial body mass (BM) within an average fasting period of 33.4 ± 3.3 day (range: 15–73 days), with the majority of BM loss occurring during the first 6 days (phase I fasting). The metabolic changes previously reported throughout phase I (*days 0–6*) and phase II fasting (from *day 6* onward) in the study pups (69) were concomitant with marked changes in plasma hormone concentrations (Figs. 3 and 4 and Table 2).

In addition to the dramatic decreases in mass-specific rate of BM loss (>50%), metabolic rate (25%), and plasma levels of

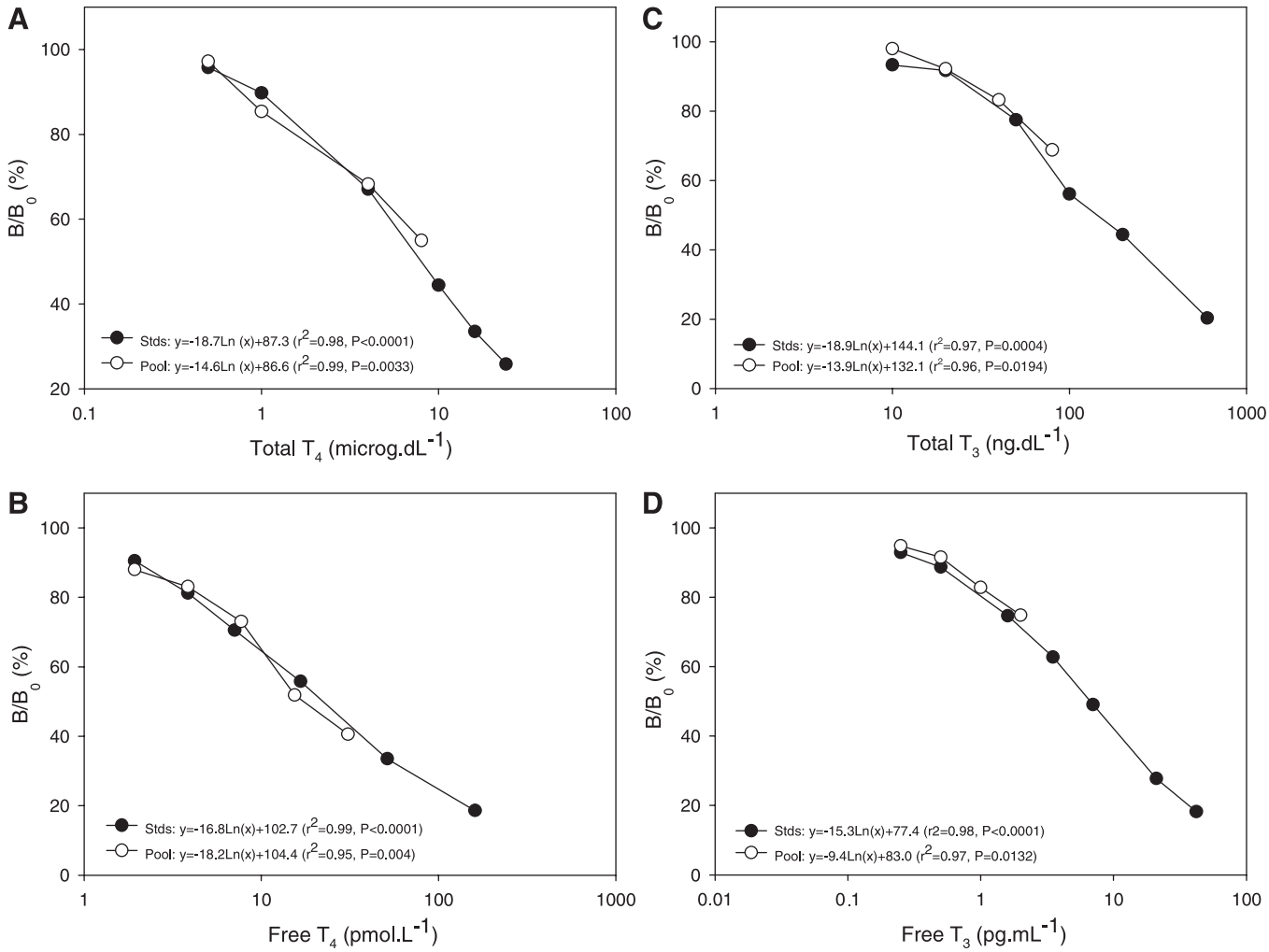

Fig. 2. Representative estimation of the percentage of cross-reactivity between subantarctic fur seal plasma and antibodies of the radioimmunoassays for total L-thyroxine (T_4) (*A*), free T_4 (*B*), total triiodothyronine (T_3) (*C*), and free T_3 (*D*).

triglycerides (80%), glycerol (50%), and U:C (50%), phase I of the fast was characterized by significant reductions in circulating insulin (67%), glucagon (60%), leptin (50%), and total T_4 (35%) (between *day 0* and *day 6*; $P < 0.05$ in all cases) (Figs. 3 and 4 and Table 2). In phase II, concomitantly with dramatic increases in the plasma concentrations of β-OHB and NEFA, plasma concentrations of insulin and glucagon stabilized at minimum levels (13.3 ± 0.7 and 12.5 ± 0.5 pmol/l, respectively) ($P > 0.05$ in both cases), while plasma leptin diminished further to reach an average of 3.5 ± 0.3 ng/ml HE at the end of the fasting periods ($P < 0.001$) (Fig. 3 and Table 2). Total T_4 concentrations rose back to initial level from *day 12* and free T_4 peaked at 60% above phase I level between *day 12* and *day 24* ($P < 0.05$) (Fig. 4 and Table 2).

In contrast, plasma cortisol (284 ± 20 nmol/l), total T_3 (0.59 ± 0.03 nmol/l), free T_3 (1.12 ± 0.11 pmol/l), and total T_3:total T_4 ratio (T_3:T_4, 1.12 ± 0.03%) were not significantly altered by fasting ($P > 0.05$ in all cases) (Fig. 3 and Table 2). Overall, the concentrations of insulin, free T_4, and total T_3 were consistently higher in female than male pups over the entire fasts ($P < 0.05$ in all cases) (Figs. 3 and 4 and Table 2).

Hormonal correlates of the physiological responses to fasting and hormone interrelations. The relationships between circulating hormone levels and fasting energetics were not influenced by sex ($P > 0.05$ in all cases) but differed between phase I and phase II fasting for some metabolic parameters ($P < 0.05$) (Table 3).

Changes in plasma insulin, leptin, and total T_4 concentrations significantly contributed to the decrease in mass-specific RMR observed in phase I ($P < 0.05$ in all cases) (Fig. 5 and Table 3). Decrease in leptin concentrations also partly explained the increase in plasma NEFA in phase I, as well as the increase in β-OHB concentrations and decrease in both plasma urea and U:C throughout the fast ($P < 0.05$ in all cases) (Fig. 5 and Table 3). The decrease in plasma glucagon significantly contributed to the increase in plasma β-OHB throughout phase I and the decrease in U:C throughout the fast ($P < 0.001$ in both cases) (Fig. 5 and Table 3). In contrast, while cortisol explained >25% of the changes in plasma β-OHB throughout phase I ($P < 0.001$) (Table 3), it did, however, influence neither plasma NEFA concentrations ($P > 0.5$ in both fasting phases) nor plasma U:C ($n = 171$, $F_{1,56} = 0.08$, $P = 0.777$)

Table 2. *Influence of prolonged fasting on circulating hormone concentrations in subantarctic fur seal pups at Amsterdam Island*

		Statistics				
Hormones	Factors	*n*	*F*	*df*	*P*	Results
Cortisol	Fasting day	183	1.51	16,102	0.110	n.s.
	Sex		0.02	1,27	0.877	n.s.
Leptin	Fasting day	144	22.67	15,117	**<0.001**	↓
	Sex		1.56	1,18	0.227	n.s.
Insulin	Fasting day	181	17.26	16,112	**<0.001**	↓
	Sex		7.71	1,17	**0.013**	**F>M**
Glucagon	Fasting day	177	16.37	16,116	**<0.001**	↓
	Sex		0.90	1,31	0.350	n.s.
Total T_4	Fasting day	184	2.19	16,91	**0.011**	**↓ then ↑**
	Sex		0.86	1,18	0.365	n.s.
Free T_4	Fasting day	184	1.84	16,84	**0.039**	**↑ then ↓**
	Sex		8.10	1,15	**0.012**	**F>M**
Total T_3	Fasting day	182	1.16	16,124	0.310	n.s.
	Sex		5.18	1,17	**0.028**	**F>M**
Free T_3	Fasting day	180	1.20	16,55	0.301	n.s.
	Sex		0.13	1,20	0.722	n.s.

Mixed ANOVA with individuals included as random effect and fasting days as ranks for repeated measures were used to account for the repeated-measure pattern of the data. The fixed effects tested were fasting day, sex, and fasting day×sex interaction. Results were considered statistically significant at $P < 0.05$ (indicated in bold). There was no significant interaction between sex and fasting day (not shown; $P > 0.117$ in all cases). n.s., nonsignificant ($P > 0.05$); ↓, decrease; ↑, increase; F, females; M, males.

throughout the fast. In addition, the decrease in glucagon, insulin, and leptin concentrations strongly contributed to the decrease in plasma glycerol throughout the fast ($P < 0.001$ in all cases), while changes in insulin and free T_3 ($P \leq 0.01$ in both cases), but not glucagon ($n = 93$, $F_{1,68} = 0.01$, $P = 0.919$), contributed to the slow linear decrease in glycemia observed throughout fasting in subantarctic fur seal pups (Table 3).

The molar insulin:glucagon ratio (I:G; 1.27 ± 0.06) remained steady with insulin and glucagon concentrations correlating positively throughout the fast ($n = 179$, $r^2 = 0.21$, $F_{1,80} = 90.91$, $P < 0.001$). Furthermore, plasma leptin was significantly positively related to insulin ($n = 147$, $r^2 = 0.73$, $F_{1,82} = 88.20$, $P < 0.001$), total T_3 ($n = 149$, $r^2 = 0.23$, $F_{1,139} = 12.99$, $P < 0.001$) and glucagon ($n = 141$, $r^2 = 0.21$, $F_{1,80} = 90.91$, $P < 0.001$) concentrations over the entire fast but not to total T_4 and cortisol levels ($F_{1,88} = 2.60$, $P = 0.111$ and $F_{1,49} = 0.00$, $P = 0.995$, respectively).

DISCUSSION

In mammals, nutritional deprivation generally leads to a cascade of hormonal responses to the crisis in metabolic fuel availability, which encompasses a decline in insulin, leptin, and thyroid hormones and a rise in glucocorticoids and glucagon. Despite similar metabolic and biochemical adjustments, results of the present study suggest that subantarctic fur seal pups adopt a fundamentally different pattern of hormonal responses during the extreme fasts they must endure throughout their postnatal development. The potential involvement of these endocrine responses in the regulation of pup metabolism and metabolic fuel use was investigated in view of their adaptation to extreme fasting.

Cortisol and nutritional stress. In mammals (20, 36), and more generally in vertebrates (37, 60, 63), food deprivation is typically associated with a significant elevation in glucocorticoids. This increase in glucocorticoids is thought to play a major role in helping individuals cope with the fuel supply crisis, in particular by enabling the maintenance of energy and biochemical homeostasis through increased lipolysis and gluconeogenesis (20). Although plasma cortisol has been shown to increase linearly in elephant seal pups during their postweaning fast (58) and exhibit a twofold rise within 3–4 days in fasting, lactating, subantarctic fur seals during maternal attendance periods ashore (33), no significant change was detected throughout prolonged fasting in subantarctic fur seal pups in the present study. The values obtained over the entire fasts were similar to serum cortisol baselines reported in fed captive juvenile Steller sea lions (140–330 nmol/l) (49), thus, indicating that, contrary to expectation, sustained food deprivation did not induce an acute stress response (i.e., stimulated cortisol secretion) in subantarctic fur seal pups. The cortisol values obtained in the present study are also consistent with values measured in free-ranging Steller sea lion pups (300–470 nmol/l) (49) and Australian fur seal pups (300 ± 30 nmol/l) (7).

By contrast with other mammals in which plasma glucocorticoid and leptin levels are generally inversely related (1), there was also no relation between cortisol and leptin during fasting, and the fasting-induced fall in leptin did not appear to trigger the expected response of the hypothalamic-pituitary-adrenal (HPA) axis (66). As the glucocorticoid stress response generally depends upon how harmful the stressor stimulus is interpreted (62) and subantarctic fur seals have to repeatedly endure sustained fasting periods from birth (68), by the time they reach 8–9 mo of age (present study), they may have acclimatized to the "routine" stress of both maternal absence and food deprivation, thus resulting in a blunted response of the HPA.

The hormonal correlates of metabolic economy. Plasma leptin is reduced in response to food restriction or deprivation in humans (41), rodents (2), ruminants (23), and bats (72), while refeeding increases it (41). In the present study, plasma leptin concentrations were also found to decrease markedly (five-fold) throughout prolonged fasting. This finding confirms the small but significant decreases previously noted in Antarc-

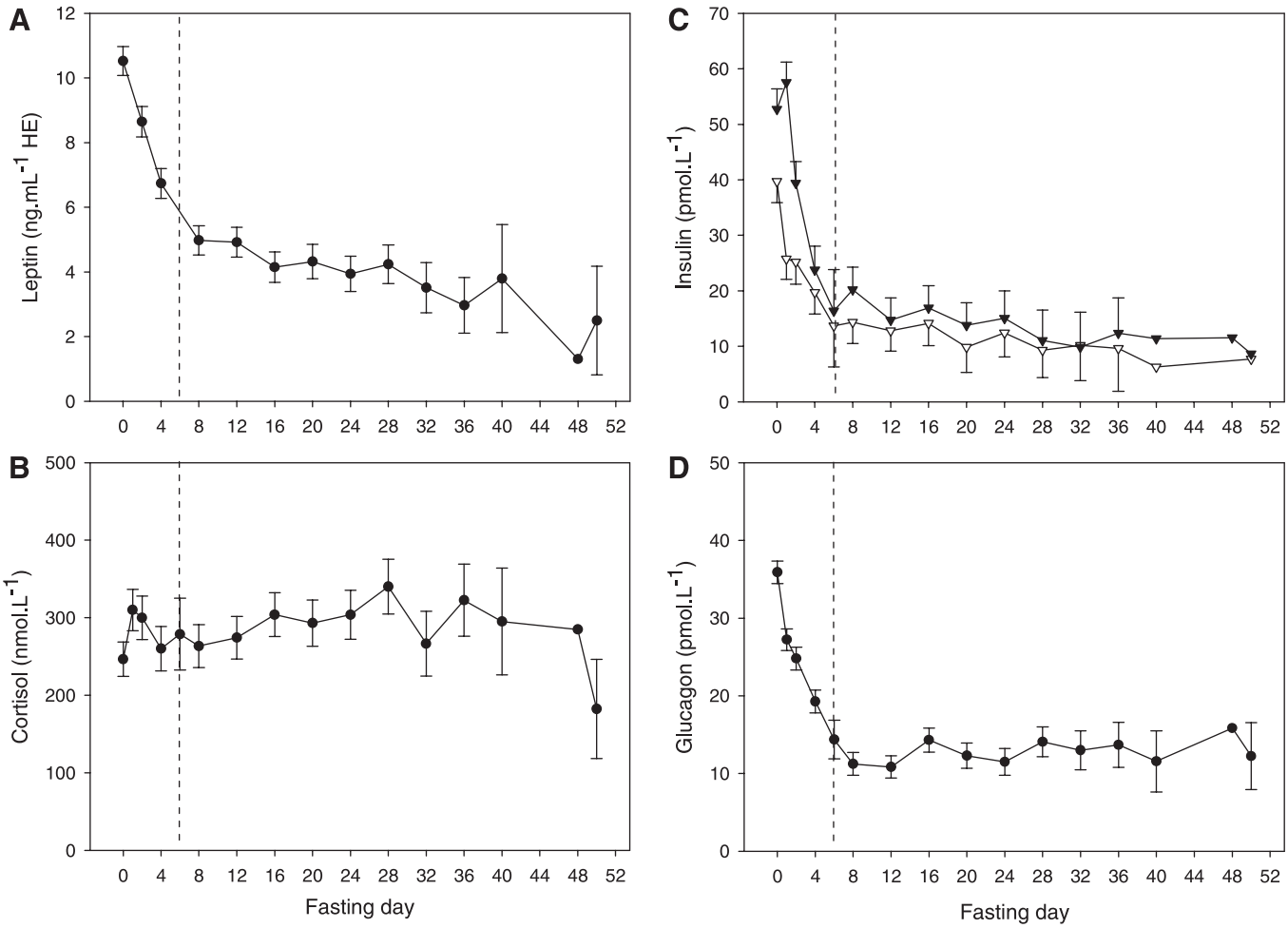

Fig. 3. Changes in plasma concentrations of leptin (*A*), cortisol (*B*), insulin (*C*), and glucagon (*D*) during prolonged fasting in subantarctic fur seal pups at Amsterdam Island. Data for male (open symbols) and female (closed symbols) pups are presented separately when significant sex-based differences between means were detected (mixed ANOVA: $P < 0.05$). In the absence of significant sex differences (mixed ANOVA: $P > 0.05$), data for pooled sexes are presented. Vertical dashed line indicates the transition between phase I and phase II fasting. Phase I (*days 0–6*) was characterized by a significant decrease in mass-specific rate of body mass (BM) loss (>50%) and mass-specific resting metabolic rate (RMR) (25%). In phase II (past *day 6*), mass-specific rate of daily BM loss was maintained at 7.3 ± 0.2 $g \cdot kg^{-1} \cdot day^{-1}$ and mass-specific RMR stabilized at 5.9 ± 0.1 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ (69).

tic fur seal pups over shorter fasting durations (3), but it is in marked contrast to previous reports in fasting adapted sea lions and phocids, in which no evident effect of fasting on circulating leptin could be found (28, 56, 58, 61). It was suggested that either an inappropriate detection method may be involved in the latter species (34) or a fundamental difference in the role of leptin in energy balance between terrestrial and marine mammals (56). We further hypothesize that fundamental differences in the evolution of body fat regulation and the role of leptin in such a regulatory system might exist between marine mammals also relying on fat for thermoregulation (cetaceans, sea lions, and phocids) and those relying primarily on their dense fur (fur seals, as in the present study).

As previously described in humans and rodents (2, 9), the rapid decrease of plasma leptin occurring in phase I fasting in subantarctic fur seal pups may act as a signal mediating the responses to fasting at that stage (2). Supporting this hypothesis, plasma leptin was found to contribute to the reduction in mass-specific RMR in phase I. Because leptin increases thermogenesis and energy expenditure when administrated to rodents (1), its dramatic reduction early in the fast could, indeed, participate to the reduction in RMR occurring in response to food deprivation (69). Plasma leptin is also known to depress immunity which, in turn, lowers energy expenditure and protein needs for the maintenance of immune function (44). Furthermore, this fasting-induced decrease in plasma leptin was concurrent with a rapid reduction in circulating insulin, which may also contribute to the response to fasting by enabling a thermogenic depression (65). Correspondingly, the dramatic decrease in insulinemia explained 24% of the reduction in mass-specific RMR in early fasting in the present study.

In mammals, starvation also generally causes a decline in plasma T_4 (the amplitude of which varies between species) and a marked decrease in plasma T_3 (21, 26). In the present study, however, all forms of thyroid hormones were either maintained or increased over the entire fasts. Only total T_4 concentrations displayed a moderate and transitory reduction that was restricted to phase I fasting and might partly contribute to the decrease in mass-specific RMR. This suggested that T_4 was the main thyroid hormone involved in the adaptive metabolic depression in response to fasting in subantarctic fur seal pups, as observed in king penguins (16). However, contrasting with

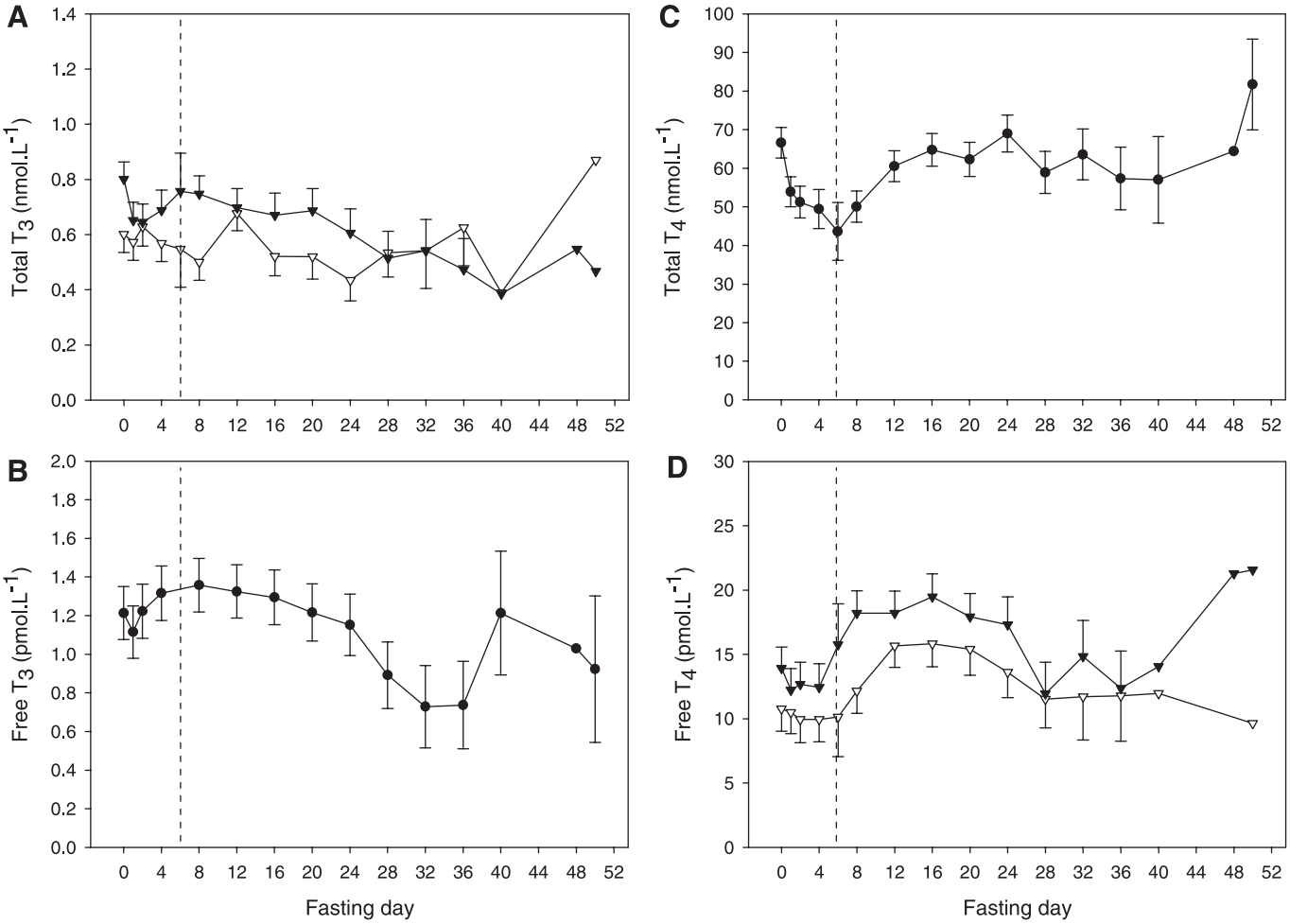

Fig. 4. Changes in plasma concentrations of total T_3 (*A*), free T_3 (*B*), total T_4 (*C*), and free T_4 (*D*) during prolonged fasting in subantarctic fur seal pups at Amsterdam Island. Data for male (open symbols) and female (closed symbols) pups are presented separately when significant sex-based differences between means were detected (mixed ANOVA: $P < 0.05$). In the absence of significant sex differences (mixed ANOVA: $P > 0.05$), data for pooled sexes are presented. Vertical dashed line indicates the transition between phase I and phase II fasting. Other indications are as in the legend of Fig. 3.

the general mammalian model and the situation of hypothalamic hypothyroidism in humans with anorexia nervosa (25, 26), whereby all forms of thyroid hormones decrease in response to fasting, the observations of the present study agree with previous reports in food-deprived manatees and elephant seal pups (55, 58), and deep-hibernating terrestrial mammals (45). In such situations, the lack of thyroid hormone reduction may not reflect increased synthesis and release of thyroid hormones but rather significant alterations in their clearance from circulation (26, 45).

In addition, the decrease in T_4 deiodination generally observed in fasting mammals (26, 55, 58) is viewed as a mechanism to protect target tissues from the oxygen-demanding processes induced by the cellular actions of T_3 (21). In the present study, although the total T_3:total T_4 ratio showed no consistent decrease in response to fasting, it was maintained consistently low, thus, possibly contributing to the low rates of energy expenditure displayed by subantarctic fur seal pups in winter, independently of their nutritional state (69). For instance, with metabolic rates two- to four-fold lower than pups of any other otariid species (68, 69), subantarctic fur seal pups exhibited total T_3:total T_4 ratios three-fold lower than those of free-ranging Steller sea lion pups (50).

The hormonal correlates of preferential lipid utilization and protein sparing. The shift to a lipid-based metabolism in response to food deprivation typically occurs in conjunction with specific changes in pancreatic hormone levels in mammals (11, 12). Plasma insulin levels decrease rapidly in response to food deprivation (12, 32, 36), which promotes lipolysis in adipose tissue and the use of fat as metabolic fuel during fasting (11). In the present study, the positive relationship between plasma concentrations of insulin and leptin concomitant with the negative relationship between NEFA and leptin in phase I fasting supports the hypothesis that the early fall in insulin and its permissive effects on lipolysis may mediate the starvation-induced decline in leptin levels (9) through the metabolic stress inflicted to adipocytes (35). In addition to promoting lipolysis, low insulin levels can lift inhibitory effects on gluconeogenesis and ketogenesis (38). This was verified in the present study with subantarctic fur seal pups exhibiting *1*) a dramatic decrease in insulinemia in proportion to the reduction in plasma glycerol [likely shuttled to hepatic recycling and gluconeogenesis (11)] in phase I fasting and *2*) minimal insulin levels throughout phase II, which explained 43%, 20%, and 10% of the observed variations in glycerol, glucose, and creatinine concentrations, respectively.

Table 3. *Influence of hormones on the metabolic responses to fasting in subantarctic fur seal pups at Amsterdam Island*

Fasting Responses	Hormones	Statistics							Phase
		n	*F*	*df*	*P*	r^2	Effect ± SE		
↓ msRMR	Insulin	53	15.17	1,48	<0.001	0.240	0.042	± 0.011	1
	Glucagon	53	10.28	1,44	0.002	0.188	0.054	± 0.017	1
	Leptin	53	6.54	1,46	0.014	0.125	0.160	± 0.062	1
	Total T_4	53	4.51	1,49	0.039	0.084	0.023	± 0.011	1
↓ urea	Leptin	142	37.01	1,84	<0.001	0.306	0.665	± 0.109	1 + 2
	Glucagon	175	41.48	1,140	<0.001	0.228	0.175	± 0.027	1 + 2
↑ creatinine	Total T_4	98	5.60	1,81	0.020	0.064	0.110	± 0.046	2
	Insulin	97	9.78	1,87	0.002	0.101	−0.415	± 0.133	2
↓ U:C	Leptin	144	72.94	1,79	<0.001	0.481	14.079	± 1.648	1 + 2
	Glucagon	171	57.92	1,169	<0.001	0.255	3.236	± 0.425	1 + 2
↓ glucose	Total T_3	85	6.65	1,77	0.012	0.079	−0.945	± 0.367	1
	Free T_4	179	10.70	1,177	0.001	0.057	0.034	± 0.010	1 + 2
	Insulin	97	14.10	1,58	<0.001	0.196	0.045	± 0.012	2
	Free T_3	95	7.13	1,55	0.010	0.114	0.376	± 0.141	2
↓ glycerol	Glucagon	151	80.99	1,83	<0.001	0.495	10.554	± 1.173	1 + 2
	Insulin	152	59.17	1,77	<0.001	0.433	6.816	± 0.886	1 + 2
	Leptin	141	44.90	1,80	<0.001	0.358	31.207	± 4.657	1 + 2
	Total T_4	97	15.04	1,39	<0.001	0.225	6.100	± 1.571	1
	Free T_4	97	6.02	1,52	0.018	0.103	0.042	± 0.017	1
↑ NEFA	Leptin	54	10.91	1,34	0.002	0.244	−0.028	± 0.008	1
	Free T_3	54	4.18	1,78	0.044	0.051	−0.122	± 0.060	2
↑ β-OHB	Cortisol	85	22.45	1,65	<0.001	0.255	0.285	± 0.060	1
	Glucagon	84	18.44	1,30	<0.001	0.378	−0.012	± 0.003	1
	Leptin	144	23.59	1,123	<0.001	0.161	−0.160	± 0.033	1 + 2
	Total T_3	97	11.32	1,62	0.001	0.153	−0.162	± 0.048	2

Linear mixed models with individuals included as random effect and fasting days as ranks for repeated measures were used to account for the repeated-measures pattern of the data. Results were considered statistically significant at $P < 0.05$.

In contrast to insulin, glucagon stimulates gluconeogenesis from lactate, amino acids, and lipolysis-released glycerol in mammals and also indirectly influences protein metabolism by stimulating the hepatic uptake of released amino acids (42). Glucagon response to fasting is, however, less consistent than that of insulin. Whereas glucagon does not change over the fast in rats (32) and dogs (22), it rises early in the fast in humans (36) and denning bears (59) and increases significantly across prolonged fasts in weaned elephant seal pups (14, 57) and long-term fasting penguins (16). In the present study, however, plasma glucagon concentrations fell dramatically throughout phase I and stabilized at minimal levels for the remainder of the fasting period. Initially elevated concentrations of glucagon may result from the stimulation of both glucagon and insulin release following the important intake of carbohydrate-free, protein-rich milk (30, 31) during the previous suckling bout (42). The following decrease may partly be attributed to the inhibitory effects of ketones on glucagon release (47) and, thus, on gluconeogenesis to protect protein stores, as suggested by the negative relationship between glucagon and β-OHB at this stage of the fast. Interactions with insulin could also intervene.

As a result, plasma I:G molar ratio remained unaltered and ≥1. This contrasts with the significant decrease in I:G ratio (<1) generally reported in response to prolonged fasting [e.g., in elephant seals (14), penguins (16), and humans (67)], which indicates an upregulation of catabolic processes and high rates of hepatic gluconeogenesis to maintain homeostasis (14, 42). In the present study, although glycemia remained above 6.0 mmol/l throughout the fast, indicating that mobilization of body stores provided sufficient gluconeogenic precursors and glucose substitutes, the lack of an I:G ratio decrease suggests a downregulation of gluconeogenesis and catabolic processes in general. This is consistent with the adoption of a finely regulated energetic strategy aiming to match pup metabolic needs, while delaying the critical depletion of their body reserves. Being 10 times smaller than elephant seal pups, which appear to overproduce glucose at the expense of their extensive body fat stores during their postweaning fast (14), subantarctic fur seal pups might, indeed, require a higher level of metabolic efficiency to be able to maintain homeostasis for similar extended fasting durations with finite and comparatively limited body reserves.

Furthermore, although glucocorticoids promote lipolysis in mammals (20) and fat mobilization in elephant seals during their postweaning fast (57), there was no such evidence in the present study. Cortisol is also known to increase proteolysis in vertebrates (20) and, while subantarctic fur seal pups have large body fat reserves, they have limited protein stores, which they need to preserve for their lean body mass development (69). Hence, the absence of stress caused by the routine fasts and the resulting maintenance of nonelevated cortisol levels would allow minimal body protein utilization throughout fasting. Because leptin stimulates hepatic gluconeogenesis, in particular, from amino acid precursors (64), decreased leptin levels could also contribute to depressed protein catabolism, as suggested by the positive relationship with plasma U:C levels and, thus, lead to the adoption of an efficient strategy of lean body mass preservation, allowing growth to occur in fasting pups. Correspondingly, the reverse relationships between plasma concentrations of leptin and lipid-derived metabolites (e.g., NEFA and β-OHB) suggested that leptin reduction was associated with an activation of lipid catabolism in response to starvation, as previously observed in fasting humans (41). Such effects are likely to be mediated through interactions between

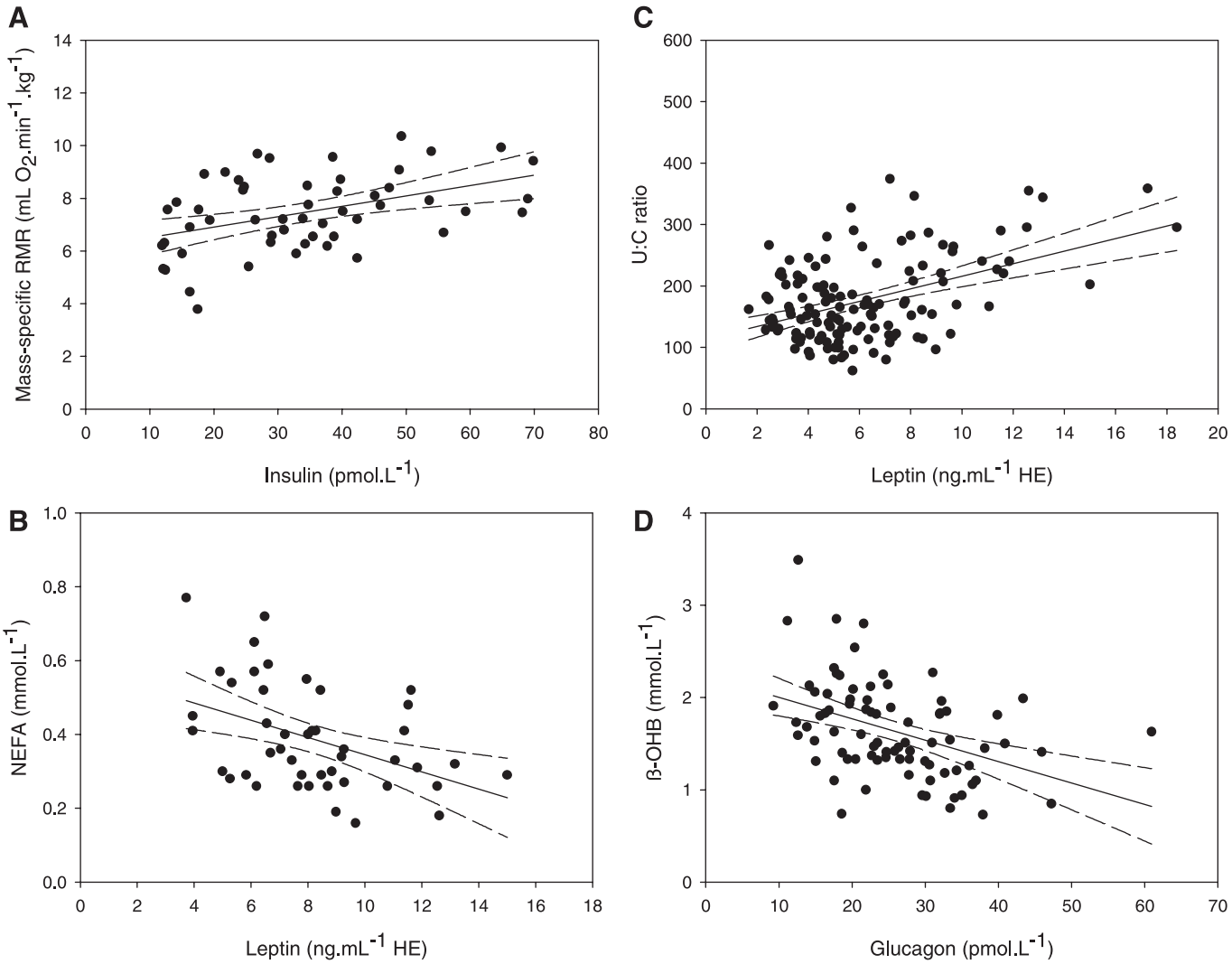

Fig. 5. Relationships between the metabolic responses to fasting and circulating concentrations of hormones throughout fasting in subantarctic fur seal pups at Amsterdam Island. *A*: relationship between mass-specific RMR and plasma insulin in phase I fasting. *B*: relationship between nonesterified fatty acids (NEFA) and plasma leptin in phase I fasting. *C*: relationship between plasma urea-to-creatinine ratio (U:C) and plasma leptin throughout the entire fasting period. *D*: relationship between β-hydroxybutyrate (β-OHB) and plasma glucagon in phase I fasting. Statistical results are presented in Table 3. Solid lines (trends) and dashed lines (95% confidence interval) are indicative and displayed for illustrative purposes only.

leptin and other hormonal systems (1, 2), in particular, through the interactions with total T_3, glucagon, and insulin found in the present study.

Overall, these findings greatly contrast with previous reports in elephant seals, in which no correlation was found between leptin and the pancreatic and thyroid hormones and the metabolic responses to fasting, while augmented cortisol was found to act as the major factor for body lipid mobilization (56–58). In addition, suckling elephant seal pups are in a state of marked hypoinsulinemia (39) and show no evidence of metabolic transition through phase I after the cessation of feeding (54). Such fundamental differences in the endocrine systems regulating fuel homeostasis between fasting phocids and fur seals could have evolved from the contrasting selective pressures exerted by their respective life history strategies, such as capital vs. income breeding and sustained vs. intermittent lactation.

Sex differences in hormone levels. Pinnipeds are highly sexually dimorphic animals, and numerous studies have documented sex-based differences in growth strategy in otariids, with male pups being typically heavier and leaner, and growing faster than females. Previous studies have shown this to be due to differences in energy expenditure and/or metabolic fuel use and, thus, growth efficiency between the sexes (4–6, 24, 53). In subantarctic fur seal pups, however, no sex differences in body composition and fasting energetics were found, suggesting the existence of a convergent metabolic strategy between the sexes (e.g., based on primary body fat reliance and protein sparing, regardless of sex and body composition) to face prolonged fasting (69).

In the present study, sex differences in hormone levels were found, with concentrations of insulin, total T_3, and free T_4 being significantly higher in female pups. Such sex differences could potentially lead to the convergent strategy reported. Higher insulin levels have been previously reported in fasting female elephant seal pups (57), but this differs from observations in humans, in which men generally exhibit higher glucose and glucagon concentrations than women with similar insulin

levels in response to fasting (36). Furthermore, whereas female mammals generally have higher leptin levels than males when matched by age, weight, or body fat, no difference in leptin levels was detected between the sexes in the present study. Since sexual dimorphism in leptin levels partly results from interactions with reproductive hormones (1), the lack of sex differences in subantarctic fur seal pups could reflect the immaturity of their reproductive system (3).

Perspectives and Significance

The results of the present study suggest that the hormonal changes associated with the striking adaptations to extreme fasting developed by subantarctic fur seal pups differ from the typical mammalian model, thus probably reflecting the unusual life history traits of this species. Overall, the hormonal settings reported in the present study appear like the keystone of an energetically efficient system promoting resistance to long-term fasting in a relatively small-sized developing animal and providing a physiological buffer of high survival value against infrequent and unpredictable maternal provisioning. Therein, subantarctic fur seal pups represent a fascinating model to investigate the relationship between behavior, fuel metabolism, and environmental factors, and further decipher the mechanisms controlling nutritional transitions and adipose tissue development in mammals. In particular, the importance of leptin in the regulation of energetic homeostasis in response to changes in nutritional state suggested by the results of the present study deserves further investigation. Whether leptin does play a major role as mediator of the response to fasting and sensor of energy balance, as well as a role in the regulation of the energetic trade-off between maintenance and immune function during prolonged fasting, in the study species should be further examined.

ACKNOWLEDGMENTS

We would like to thank Alexandre Allag, Bruno Dauteloup, Matthieu Authier, and Hervé Conseil for their help in the field, and Kendall Mashburn and Lisa Petrauskas for their help in the School of Fisheries and Ocean Sciences Endocrine Laboratory. We are also grateful to Lloyd Edwards for statistical advice and three anonymous reviewers whose helpful comments greatly improved the present manuscript.

GRANTS

The present work was carried out at the Martin du Viviès research station and was financially and logistically supported by the French Polar Institute through Programs 109 and 119, the Terres Australes et Antarctiques Françaises and Sea World Research and Rescue Foundation. D. Verrier was the recipient of an International Postgraduate Research Scholarship and Melbourne International Research Scholarship awarded by the University of Melbourne, Australia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. **Ahima RS, Flier JS.** Leptin. *Ann Rev Physiol* 62: 413–437, 2000.
2. **Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS.** Role of leptin in the neuroendocrine response to fasting. *Nature* 382: 250–252, 1996.
3. **Arnould JP, Morris MJ, Rawlins DR, Boyd IL.** Variation in plasma leptin levels in response to fasting in Antarctic fur seals (*Arctocephalus gazella*). *J Comp Physiol B* 172: 27–34, 2002.
4. **Arnould JPY, Boyd IL, Socha DG.** Milk consumption and growth efficiency in Antarctic fur seal (*Arctocephalus gazella*) pups. *Can J Zool* 74: 254–266, 1996.
5. **Arnould JPY, Green JA, Rawlins DR.** Fasting metabolism in Antarctic fur seal (*Arctocephalus gazella*) pups. *Comp Biochem Physiol A* 129: 829–841, 2001.
6. **Arnould JPY, Hindell MA.** Milk consumption, body composition and pre-weaning growth rates of Australian fur seal (*Arctocephalus pusillus doriferus*) pups. *J Zool* 256: 351–359, 2002.
7. **Atkinson S, Arnould JPY, Mashburn KL.** Plasma cortisol and thyroid hormone concentrations in pre-weaning Australian fur seal pups. *Gen Comp Endocrinol* 172: 277–281, 2011.
8. **Beauplet G, Dubroca L, Guinet C, Cherel Y, Dabin W, Gagne C, Hindell M.** Foraging ecology of subantarctic fur seals *Arctocephalus tropicalis* breeding on Amsterdam Island: seasonal changes in relation to maternal characteristics and pup growth. *Mar Ecol Prog Ser* 273: 211–225, 2004.
9. **Boden G, Chen X, Mozzoli M, Ryan I.** Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 81: 3419–3423, 1996.
10. **Bonner WN.** Lactation strategies in pinnipeds: problems for a marine mammalian group. *Symp Zool Soc London* 51: 253–272, 1984.
11. **Cahill GFJ.** Starvation in man. *Clin Endocrinol Metab* 5: 397–415, 1976.
12. **Cahill GFJ, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA, Kipnis DM.** Hormone-fuel interrelationships during fasting. *J Clin Invest* 45: 1751–1769, 1966.
13. **Castellini MA, Rea LD.** The biochemistry of natural fasting at its limits. *Experientia* 48: 575–582, 1992.
14. **Champagne CD, Houser DS, Crocker DE.** Glucose production and substrate cycle activity in a fasting adapted animal, the northern elephant seal. *J Exp Biol* 208: 859–868, 2005.
15. **Cherel Y, Groscolas R.** Relationships between nutrient storage and nutrient utilisation in long-term fasting birds and mammals. In: *Proceedings of the 22nd International Ornithological Congress, Durban*, edited by Adams NJ, and Slotow RH. Johannesburg, South Africa: Birdlife, 1999, p. 17–34.
16. **Cherel Y, Robin JP, Walch O, Karmann H, Netchitailo P, Le Maho Y.** Fasting in King penguin 1 Hormonal and metabolic changes during breeding. *Am J Physiol Regul Integr Comp Physiol* 254: R170–R177, 1988.
17. **Costa DP.** Reproduction and foraging energetics of pinnipeds: implications for life history patterns. In: *The Behavior of Pinnipeds*, edited by Renouf D. London: Chapman and Hall, 1991, p. 301–344.
18. **Costa DP, Sinervo B.** Field physiology: physiological insights from animals in nature. *Ann Rev Physiol* 66: 209–238, 2004.
19. **Crocker DE, Webb PM, Costa DP, Le Boeuf BJ.** Protein catabolism and renal function in lactating northern elephant seals. *Physiol Zool* 71: 485–491, 1998.
20. **Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M.** Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front Neuroendocrinol* 14: 303–347, 1993.
21. **Davis TA, Nguyen HV, Costa DP, Reeds PJ.** Amino-acid-composition of pinniped milk. *Comp Biochem Physiol B* 110: 633–639, 1995.
22. **de Bruijne JJ, Altszuler N, Hampshire J, Visser TJ, Hackeng WHL.** Fat mobilization and plasma hormone levels in fasted dogs. *Metabolism* 30: 190–194, 1981.
23. **Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A, Kann G.** Plasma leptin determination in ruminants: effects of nutritional status, and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *J Endocrinol* 166: 519–528, 2000.
24. **Donohue MJ, Costa DP, Goebel E, Antonelis GA, Baker JD.** Milk intake and energy expenditure of free-ranging northern fur seal, *Callorhinus ursinus*, pups. *Physiol Biochem Zool* 75: 3–18, 2002.
25. **Douyon L, Schteingart DE.** Effect of obesity and starvation on thyroid hormone, growth hormone and cortisol secretion. *Endocrinol Metab Clin North America* 31: 173–189, 2002.
26. **Eales JG.** The influence of nutritional state on thyroid function in various vertebrates. *Am Zool* 28: 351–362, 1988.
27. **Edwards LJ, Muller KE, Wolfinger RD, Qaqish BJ, Schabenberger O.** An R2 statistic for fixed effects in the linear mixed model. *Stat Med* 27: 6137–6157, 2008.
28. **Eisert R, Breier BH, Barrell GK.** Plasma IGF-1, but not leptin, concentrations fall during the lactation period in Weddell seals. In: *Proceedings*

of the Annual Scientific Meeting of the New Zealand Society of Endocrinology. Queenstown, NZ: New Zealand Society of Endocrinology, 2000.
29. **Gentry RL, Kooyman GL.** *Fur Seals: Maternal Strategies on Land and at Sea*. Guildford, NJ: Princeton University Press, 1986.
30. **Georges JY, Groscolas R, Guinet C, Robin JP.** Milking strategy in subantarctic fur seals *Arctocephalus tropicalis* breeding on Amsterdam island: evidence from changes in milk composition. *Physiol Biochem Zool* 74: 548–559, 2001.
31. **Georges JY, Guinet C.** Maternal care in the subantarctic fur seals on Amsterdam Island. *Ecology* 81: 295–308, 2000.
32. **Goodman MN, Larsen RP, Kaplan MM, Aoki TT, Young VR, NBR.** Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *Am J Physiol Endocrinol Metab* 239: E277–E286, 1980.
33. **Guinet C, Servera N, Mangin S, Georges JY, Lacroix A.** Change in plasma cortisol and metabolites during the attendance period ashore in fasting lactating subantarctic fur seals. *Comp Biochem Physiol A* 137: 523–531, 2004.
34. **Hammond JA, Bennett KA, Walton MJ, Hall AJ.** Molecular cloning and expression of leptin in gray and harbor seal blubber, bone marrow, and lung and its potential role in marine mammal respiratory physiology. *Am J Physiol Regul Integr Comp Physiol* 289: R545–R553, 2005.
35. **Harris RBS.** Leptin—much more than a satiety signal. *Annu Rev Nutr* 20: 45–75, 2000.
36. **Hojlund K, Wildner-Christensen M, Eshoj O, Skjaerbaek C, Holst JJ, Koldkjaer O, Jensen DM, Beck-Nielsen H.** Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. *Am J Physiol Endocrinol Metab* 280: E50–E58, 2001.
37. **Jeffrey DA, Peakall DB, Miller DS, Herzberg GR.** Blood chemistry changes in food-deprived herring gulls. *Comp Biochem Physiol A* 81: 911–913, 1985.
38. **Kaloyianni M, Freedland RA.** Effect of diabetes and time after in vivo insulin administration on ketogenesis and gluconeogenesis in isolated rat hepatocytes. *Int J Biochem* 22: 159–164, 1990.
39. **Kirby VL, Ortiz CL.** Hormones and fuel regulation in fasting elephant seals. In: *Elephant Seals: Population Ecology, Behavior and Physiology*, edited by Le Boeuf BJ, and Laws RM. Los Angeles, CA: University of California Press, 1994, p. 374–386.
40. **Kitao N, Fukui D, Shibata H, Saito M, Osborne PG, Hashimoto M.** Seasonality and fasting effect in raccoon dog *Nyctereutes procyonoides* serum leptin levels determined by canine leptin-specific enzyme-linked immunosorbent assay. *J Exp Zool A Ecol Genet Physiol* 315A: 84–89, 2011.
41. **Kolaczynski JW, Cosidine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF.** Responses of leptin to short-term fasting and refeeding in humans. A link with ketogenesis but not ketones themselves. *Diabetes* 45: 1511–1515, 1996.
42. **Kraus-Friedmann N.** Hormonal regulation of hepatic gluconeogenesis. *Physiol Rev* 64: 170–220, 1984.
43. **Littell RC, Henry PR, Ammerman CB.** Statistical analysis of repeated measures data using SAS procedures. *J Anim Sci* 76: 1216–1231, 1998.
44. **Lochmiller RL, Deerenberg C.** Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88: 87–98, 2000.
45. **Magnus TH, Henderson NE.** Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. I. Increased binding of triiodo-L-thyronine and L-thyroxine by serum proteins. *Gen Comp Endocrinol* 69: 352–360, 1988.
46. **Mashburn KL, Atkinson S.** Variability in leptin and adrenal response in juvenile Steller sea lions (*Eumetopias jubatus*) to adrenocorticotropic hormone (ACTH) in different seasons. *Gen Comp Endocrinol* 155: 352–358, 2008.
47. **Morio B, Wolfe RR.** Ketone bodies. In: *Encyclopedia of Life Sciences*. Chichester, UK: John Wiley & Sons, http://www.els.net.ezproxy.lib.unimelb.edu.au/ [doi:10.1038/npg.els.0003819], 2005.
48. **Mrosovsky N, Sherry DF.** Animal anorexias. *Science* 207: 837–842, 1980.
49. **Myers MJ, Litz B, Atkinson S.** The effects of age, sex, season and geographic region on circulating serum cortisol concentrations in threatened and endangered Steller sea lions (*Eumetopias jubatus*). *Gen Comp Endocrinol* 165: 72–77, 2010.
50. **Myers MJ, Rea LD, Atkinson S.** The effects of age, season and geographic region on thyroid hormones in Steller sea lions (*Eumetopias jubatus*). *Comp Biochem Physiol A* 145: 90–98, 2006.
51. **Nelson RA, Steiger DL, Beck TDT.** Neuroendocrine and metabolic interactions in hibernating black bear. *Acta Zoologica Fennica* 174: 137–141, 1983.
52. **Oki C, Atkinson S.** Diurnal patterns of cortisol and thyroid hormones in the Harbor seal (*Phoca vitulina*) during summer and winter seasons. *Gen Comp Endocrinol* 136: 289–297, 2004.
53. **Ono KA, Boness DJ.** Sexual dimorphism in sea lion pups: differential maternal investment, or sex-specific differences in energy allocation? *Behav Ecol Sociobiol* 38: 31–41, 1996.
54. **Ortiz RM, Houser DS, Wade CE, Ortiz CL.** Hormonal changes associated with the transition between nursing and natural fasting in northern elephant seals (*Mirounga angustirostris*). *Gen Comp Endocrinol* 130: 78–83, 2003.
55. **Ortiz RM, Mackenzie DS, Worthy GAJ.** Thyroid hormone concentrations in captive and free-ranging West Indian manatees (*Trichechus manatus*). *J Exp Biol* 203: 3631–3637, 2000.
56. **Ortiz RM, Noren DP, Litz B, Ortiz CL.** A new perspective on adiposity in a naturally obese mammal. *Am J Physiol Endocrinol Metab* 281: E1347–E1351, 2001.
57. **Ortiz RM, Noren DP, Ortiz CL, Talamantes F.** GH and ghrelin increase with fasting in a naturally adapted species, the northern elephant seal (*Mirounga angustirostris*). *J Endocrinol* 178: 533–539, 2003.
58. **Ortiz RM, Wade CE, Ortiz CL.** Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. *Am J Physiol Regul Integr Comp Physiol* 280: R790–R795, 2001.
59. **Palumbo PJ, Wellick DL, Bagley NA, Nelson RA.** Insulin and glucagon responses in the hibernating black bear. *Intl Conf Bear Res Manag* 5: 291–296, 1983.
60. **Peterson BC, Small BC.** Effects of fasting on circulating IGF-binding proteins, glucose and cortisol in channel catfish (*Ictalurus punctatus*). *Domest Anim Endocrinol* 26: 231–240, 2004.
61. **Rea LD, Nagy TR.** Changes in serum leptin levels during fasting and food limitation in Steller sea lions (*Eumetopias jubatus*). In: *Proceedings of the 3rd Biennial Symposium of the Comparative Nutrition Society*. Monterey, CA: Comparative Nutrition Society, 2000, p. 171–175.
62. **Romero LM.** Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol* 19: 249–255, 2004.
63. **Romero LM, Wikelski M.** Corticosterone levels predict survival probabilities of Galápagos marine iguanas during El Niño events. *Proc Natl Acad Sci USA* 98: 7366–7370, 2001.
64. **Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J, Wang J.** Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *J Biol Chem* 272: 27758–27763, 1997.
65. **Rothwell NJ, Saville ME, Stock MJ.** Role of insulin in thermogenic responses to refeeding in 3-day-fasted rats. *Am J Physiol Endocrinol Metab* 245: E160–E165, 1983.
66. **Sapolsky RM, Romero LM, Munck AU.** How do glucocorticosteroids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Rev* 21: 55–89, 2000.
67. **Streja DA, Steiner G, Marliss EB, Vranic M.** Turnover and recycling of glucose in man during prolonged fasting. *Metabolism* 26: 1089–1098, 1977.
68. **Verrier D, Groscolas R, Guinet C, Arnould JPY.** Development of fasting abilities in subantarctic fur seal (*Arctocephalus tropicalis*) pups: balancing the demands of growth under extreme nutritional restrictions. *Funct Ecol* 25: 704–717, 2011.
69. **Verrier D, Groscolas R, Guinet C, Arnould JPY.** Physiological response to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups: metabolic rates, energy reserve utilization and water fluxes. *Am J Physiol Regul Integr Comp Physiol* 297: R1582–R1592, 2009.
70. **Viscarra JA, Vazquez-Medina JP, Crocker DE, Ortiz RM.** Glut4 is upregulated despite decreased insulin signaling during prolonged fasting in northern elephant seal pups. *Am J Physiol Regul Integr Comp Physiol* 300: R150–R154, 2011.
71. **Wang T, Hung CCY, Randall DJ.** The comparative physiology of food deprivation: from feast to famine. *Ann Rev Physiol* 68: 223–251, 2006.
72. **Widmaier EP, Long J, Cadigan B, Gurgel S, Kunz TH.** Leptin, corticotropin-releasing hormone (CRH), and neuropeptide Y (NPY) in free-ranging pregnant bats. *Endocrine* 7: 145–150, 1997.