Lipid stores not only serve to provide energy via fatty acid oxidation but also contribute to meeting the energetic costs of glucose-dependent tissues.

Northern elephant seals (Mirounga angustirostris) undertake prolonged fasts of 1–3 mo. Adult females undertake two fasts each year, one while lactating and another while molting. Both lactation and molting incur energetic costs that place additional constraints on the utilization of body stores to meet the costs of maintenance metabolism. Maintenance metabolism of lactating and molting adult female elephant seals is primarily met through fat catabolism (17, 18). Protein is effectively spared until the end of lactation, when the strain on nutrient reserves requires additional input from protein stores (17). Shortly after the contribution of protein catabolism to the metabolic rate increases, the female abruptly weans her offspring and departs to sea. The relative contribution of fat and protein to maintenance metabolism in adult females undergoing molting has yet to be determined.

Protein catabolism in fasting adult female elephant seals is assumed to support the demands of glucose-dependent tissues by supplying amino acids to gluconeogenic pathways. The use of glycerol in hepatic gluconeogenesis has been proposed as a means to meet central nervous system requirements for glucose and obviate the need for ketoacid accumulation (14). By extension, substantive glycerol gluconeogenesis should contribute to minimizing the need for other gluconeogenic substrates (e.g., protein) to meet the substrate requirements of all glucose-dependent tissues (e.g., red blood cells). Circulating glucose levels in lactating and recently molted female elephant seals are high, ranging from ~6.7 to 7.8 mmol/l, and levels of endogenous glucose production (EGP) are similar to or higher than those observed in non-fasting-adapted species enduring fasts of similar duration (15). The contribution of glycerol to EGP has not been quantified in any fasting seal, and the effect that glycerol gluconeogenesis may have on protein sparing during simultaneous fasting and lactation/molting is unknown. Given the high rates of lipolysis that occur in fasting adult female elephant seals, and the effect of lipolytic rates on the conversion of glycerol to glucose (7), it seems feasible that glycerol is the primary gluconeogenic substrate in the fasting elephant seal.

This study attempted to quantify glycerol flux in fasting adult female elephant seals during the lactation and molting periods. Fluctuation was determined through the administration of radiolabeled glycerol, and the contribution of glycerol to glucose production was determined via the incorporation of radiolabel into glucose. By extension, rates of lipolysis were calculated and compared with the contribution of glycerol to glucose production.
gluconeogenesis. The goal of the study was to determine whether glycerol is a predominant substrate for gluconeogenesis in naturally fasting elephant seals and whether the proposed relationship between lipolysis and glycerol conversion to glucose is observed in this species.

MATERIALS AND METHODS

Subjects

Procedures were conducted at the Año Nuevo State Reserve, San Mateo County, California and were conducted during the winter breeding (January-March) and spring molting (May–June) periods. All procedures were approved by the Institutional Animal Care and Utilization Committee of Sonoma State University. During the winter breeding season, adult female elephant seals were marked with dye (Lady Clairol, Clairol, Stamford, CT) soon after arrival on shore to facilitate daily identification and observation. Females were observed for several days after parturition to determine which animals demonstrated strong mother-pup bonding. From these adult females, a group of 10 seals was selected for participation in the glycerol infusion study. Females from this group were studied twice during the lactation period at 5 and 21–22 days postpartum (referred to hereafter as early and late lactation, respectively). A second group of 10 adult females was selected during the spring molt. Females were selected based on apparent good health (e.g., adequate mass, no external wounds). The glycerol infusion study was conducted in each of these females within several days of completion of the molt.

Body Composition

Measurements of blubber thickness were determined ultrasonically (Ithaca Scanprobe, Ithaca, NY) at six sequential points along the dorsal, ventral, and lateral lengths of the seals. Standard morphometries (e.g., standard length, axillary girth) were taken at each corresponding point. Body mass was measured by weighing females from a tripod with a 1,000 kg suspension scale (MSI tension dynamometer, Seattle, WA) and levered hand winch. Body composition was determined for each subject by using the morphometric data to model the seal as a series of truncated cones (20). This method of determining body composition has been validated extensively in elephant seals through $^{3}$H.O dilution techniques and yields a mean absolute error in lipid content of adult females of $\approx$3% (40).

Glycerol Infusion

Adult female elephant seals were immobilized with intramuscular injections of tileamine/zolazepam (Telazol) at a dose of 1 mg/kg. After induction, vascular access to the extradural vein was obtained via an 18-gauge spinal needle set within the intravertebral space. Blood collection and administration of chemical immobilizing agents were made via the spinal needle. Chemical immobilization was maintained through 100-mg bolus injections of ketamine HCl. Intravenous injections of diazepam (5 mg; all drugs from Fort Dodge Laboratories, Ft. Dodge, IA) were administered as needed to control the occasional occurrence of tremors. During the infusion study, which typically lasted 3–4 h, the nose and face of the animal were manipulated to keep the animal awake and eupnic. The purpose of this process was to prevent compartmentalization of the vascular pool, as occurs during apnea, and thus minimize vascular dynamics that might impact on mixing of administered radiolabel into the vascular pool.

Baseline blood samples were collected into chilled serum and Na-heparin tubes and were subsequently placed on ice until further processing. A 14-gauge catheter was inserted into the extradural vein ~30 cm anterior of the insertion of the spinal needle and threaded forward of the animal. The catheter was checked for patency, back-filled with saline, and connected to a Pegasus VARIO micropipiston infusion pump (Instech Solomon, Plymouth Meeting, PA). Immediately after connection to the infusion pump a primed constant infusion of [2-14C]glycerol (250-μCi priming dose, 1.1–1.5 μCi/min) was started. Infusions were performed over a 3-h period. Blood samples were collected every 5 min for 30 min after the start of the infusion and every 15 min thereafter up to the 3-h period was completed. All blood samples were centrifuged for 20 min at 2,000 rpm and 4°C. Serum and plasma samples were withdrawn and stored at $\approx$80°C until they could be processed.

Sample Processing

Metabolite analysis. Plasma samples were analyzed for the hormones insulin and glucagon as well as the metabolites glucose, glycerol, lactate, β-hydroxybutyrate (β-HBA), and nonesterified fatty acids (NEFA). Insulin was assayed with a Sensitive Rat InsulinRIA kit (SRI-13K; Linco Research, St. Charles, MO), glucagon was assayed with a Glucagon RIA kit (GL-32K; Linco Research), and cortisol levels were measured with a Cortisol RIA kit (TKCO2, Diagnostic Products, Los Angeles, CA). All of these kits have been validated previously for this species (16, 33). The mean intra-assay coefficient of variation was 10.6%, 9.0%, and 6.0% for insulin, glucagon, and cortisol, respectively. Glycerol was analyzed in duplicate with either a Free Glycerol Determination Kit (FG0100; Sigma-Aldrich, St. Louis, MO) or a GM-7 Micro-Stat autoanalyzer (Analog Instruments, Lunenburg, MA), while β-HBA and lactate were assayed in duplicate with the GM-7 Micro-Stat autoanalyzer. Plasma glucose was measured in triplicate with a YSI 2300 glucose autoanalyzer (YSI, Yellow Springs, OH).

Determination of glycerol and glucose-specific activity. Plasma samples were thawed for deproteination and placed in borosilicate tubes in an ice-water bath. To each 1 ml of plasma to be deproteinized, 2 ml of chilled 0.3 N Ba(OH)$_2$ and 2 ml of chilled 0.3 N ZnSO$_4$ were added. Samples were vortexed, placed back into the ice bath for 20 min, and then centrifuged at 3,000 rpm for 20 min. After centrifugation, the supernatant was decanted and set aside for column chromatography.

Cation and anion exchange chromatography was performed on each deproteinated sample to remove charged particles and permit the passage of glucose and glycerol. Within a Pasteur pipette, a glass wool plug was placed and subsequently layered with 1 ml (~2 cm) of anion resin (Bio-Rad AG 1-X8, 200–400 mesh, formate form) and 1 ml of cation resin (Bio-Rad AG 50W-X8, 200–400 mesh, hydroxide form). Deproteinated plasma samples were added to the columns, and the eluate was collected. Columns were subsequently rinsed twice with several milliliters of double-distilled (dd)H$_2$O, and the eluate was again collected.

A second set of columns was established with Bio-Rad AG 1-X8 resin (100–200 mesh, hydroxide form) to separate the filtered plasma into glucose and glycerol fractions. Columns were created with 2 ml of resin over a glass wool plug and were then converted to a boronate form by running 2 bed volumes of 0.5 M boric acid through the columns. Columns were subsequently rinsed with ddH$_2$O until the pH of the eluate was ~5. Samples were then placed on the column, and the eluate was collected. The collected volume formed the glycerol fraction of the eluate. Each column was then rinsed with 2 bed volumes of ddH$_2$O, and the eluate was added to the glycerol fraction. The resin beds were then washed with 2 bed volumes of 1.0 M Tris·HCl buffer (pH 2.0), and the glucose eluate was collected. The columns were once again rinsed with 2 bed volumes of ddH$_2$O, and the eluate was added to the glucose fraction.

All samples were placed in reinforced glass vials, covered with Parafilm, and frozen at ~80°C. Samples were then lyophilized for 48 h. Both glucose and glycerol fractions were reconstituted in 1 ml of ddH$_2$O, and the glycerol concentrations of both the glucose and
glycerol fractions were determined either spectrophotometrically with a Free Glycerol Determination Kit or on a GM-7 Micro-Stat autoanalyzer. Glucose concentrations were determined for each of the fractions through the use of a YSI 2300 glucose autoanalyzer. A 200- to 400-μl aliquot of each of the fractions was then placed in a 20-ml scintillation vial with 10 ml of EcoLite scintillation cocktail (ICN, Irvine, CA). The radioactivity of each sample was determined via standard scintillation techniques, and the specific activity of the sample was determined from prior determinations of glycerol and glucose concentration. Glucose fractions were occasionally contaminated with labeled glycerol because of incomplete separation of the metabolites. Contaminated samples were not used in the kinetic analyses.

**Kinetic analysis.** Glycerol uptake from the blood pool is accomplished by the splanchic bed and kidneys (29); thus, since irreversible loss is restricted to the sampled compartment, analysis of glycerol kinetics was based on the single-pool model of substrate kinetics. Assuming that steady-state conditions existed across the interval over which kinetics were calculated, the rate of appearance (Ra) of glycerol was then determined as Ra/SAGly, where Ra is the rate of infusion of radiolabel in disintegrations per minute (dpm) per minute and SAGly is the plateau specific activity of glycerol in disintegrations per minute per millimole. The fraction of glucose derived from glycerol was then determined as SAGlu/2SAGly, where SAGly is the specific activity of glucose at isotopic equilibrium. The SA at equilibrium for [14C]glucose produced through lipolysis, three FFA molecules are presumed to be fatty acid (FFA) oxidation occurs (35, 37). Thus, for every glycerol-glycerophosphate, does not exist within the adipocytes where free glycerol kinase, the enzyme necessary for the formation of glycerol into a triglyceride backbone (i.e., α-glycerophosphate), does not exist within the adipocytes where free fatty acid (FFA) oxidation occurs (35, 37). Thus, for every glycerol produced through lipolysis, three FFA molecules are presumed to be released. Because of the potential contributions of mesenteric lipolysis to glycerol Ra, calculated rates of lipolysis are estimates and not absolute measurements.

![Fig. 1. Increase in the specific activity (SA) of glucose with the progression of a constant infusion of [2-14C]glycerol. The plateau SA was determined from the asymptotic value of a 1-phase exponential association model fitted to the growth in SA over time.](http://ajpregu.physiology.org/)

**Data analysis.** The determination of glycerol gluconeogenesis could not be determined for all of the test subjects in the study. This occurred because of infusion pump failure (n = 1), cross-contamination of labeled glycerol and glucose in processed samples (n = 5), or loss of catheter patency (n = 1) during constant infusions. Because the resulting data set was unbalanced, a linear mixed model with fasting state (postmolt, early lactation, and late lactation) as a fixed effect and a term included for individual seals was used to test for differences in kinetic values. Matched-sample t-tests were used for comparisons of metabolites between early- and late-lactation samples. Comparisons of mean values for early and late lactation and those obtained from postmortem animals were also made by using separate two-tailed t-tests for unequal sample sizes. Similar comparisons were made for mass by using one-tailed t-tests on the expectation of mass loss with time fasting and lactating. Within groups, regression analyses were used to investigate relationships between mass, body composition, metabolites, and kinetic analyses.

**RESULTS**

**Mass and Body Composition**

The average mass of females was 475 ± 51 kg early in lactation and 373 ± 61 kg during late lactation, representing a mean mass loss of 102 kg across ~16 days of fasting. The average mass of postmort females was 287 ± 67 kg. Variations in mass were reflected in changes in the proportion of mass due to lipid stores. Across lactation, females demonstrated a significant reduction in adipose tissue (paired t = 11.2, P < 0.001; as a percentage of the total mass the adipose compartment declined ~8.2%, from a mean of 38.0 ± 0.2% early in lactation to 29.8 ± 0.2% late in lactation. Mean adipose content of postmort seals was 32.6 ± 0.03% and was significantly less than that of females early in lactation (t = −4.97, P < 0.01) and significantly greater than that of females late in lactation (t = 2.44, P = 0.01).

**Metabolites and Hormones**

Plasma glucose levels were similar during early and late lactation (7.43 ± 0.54 and 7.23 ± 0.98 mmol/l, respectively; Table 1). Glucose levels during the postmolt period (6.18 ± 0.64 mmol/l) were significantly lower than early lactation (t = −4.74, P < 0.01) and late lactation (t = −2.85, P = 0.01) values. Lactate levels exhibited the opposite pattern: although levels were similar between the early and late lactation periods (2.47 ± 0.76 and 2.69 ± 0.68 mmol/l, respectively), levels from early and late lactation were significantly lower than the 3.65 ± 1.02 mmol/l observed after the molt (t = 2.93, P < 0.01 and t = 2.47, P = 0.02, respectively). Levels of β-HBA were similar across all fasting periods. Glycerol levels were lowest during early lactation (0.25 ± 0.06 mmol/l), more than double during late lactation (0.52 ± 0.22 mmol/l), and in between during the postmolt period (0.34 ± 0.08 mmol/l). Levels of NEFA followed a similar trend, and NEFA was significantly correlated with glycerol concentrations when each fasting period was considered independently (P < 0.01, r² = 0.69, early lactation; P < 0.01, r² = 0.60, late lactation; P < 0.01, r² = 0.63, postmolt).

Insulin concentrations were significantly higher during early lactation than in late lactation (Table 2; t = 2.46, P = 0.02), but neither of the lactation periods had levels that were significantly different than those of the postmolt period. In contrast, glucagon concentrations were similar among all three fasting
Table 1. Measured metabolite levels in each fasting state

<table>
<thead>
<tr>
<th>Fasting State</th>
<th>Glucose</th>
<th>Glycerol</th>
<th>Lactate</th>
<th>β-HBA</th>
<th>NEFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early lactation</td>
<td>7.43±0.54*</td>
<td>0.25±0.06†</td>
<td>2.47±0.76*</td>
<td>0.70±0.23</td>
<td>0.99±0.24‡</td>
</tr>
<tr>
<td>Late lactation</td>
<td>7.23±0.98†</td>
<td>0.52±0.22‡</td>
<td>2.69±0.68‡</td>
<td>0.76±0.21</td>
<td>3.25±0.82†</td>
</tr>
<tr>
<td>Postmolt</td>
<td>6.18±0.64*‡</td>
<td>0.34±0.08†‡</td>
<td>3.65±1.02†‡</td>
<td>0.63±0.08</td>
<td>1.37±0.34‡</td>
</tr>
</tbody>
</table>

Values (in mmol/l) are means ± SD. β-HBA, β-hydroxybutyrate; NEFA, nonesterified fatty acids. Significantly different values between fasting conditions are noted by similar superscripts. Values are significantly different at P < 0.05.

Table 2. Hormone levels measured in this study

| Fasting State | Insulin, pmol/l | Glucagon, pmol/l | Cortisol, nmol/l |
|---------------|----------------|----------------|----------------|---------|
| Early lactation | 16.9±3.5* | 16.4±5.3 | 144.2±68.4† |
| Late lactation | 13.2±3.1* | 15.6±3.8 | 246.8±83.2* |
| Postmolt | 14.8±2.7 | 13.4±2.3 | 295.8±184.6‡ |

Values are means ± SD. Significantly different values between fasting conditions are noted by similar superscripts. Values are significantly different at P < 0.05.

Table 3. Measured and calculated flux of metabolites

<table>
<thead>
<tr>
<th>Fasting State</th>
<th>Glycerol Ra, μmol·kg⁻¹·min⁻¹</th>
<th>Lipolysis, μmol·kg⁻¹·min⁻¹</th>
<th>Glucose Derived From Glycerol, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early lactation</td>
<td>8.0±3.1</td>
<td>24.1±9.2</td>
<td>2.8±2.2</td>
</tr>
<tr>
<td>Late lactation</td>
<td>7.4±6.0</td>
<td>22±17.9</td>
<td>2.4±1.5</td>
</tr>
<tr>
<td>Postmolt</td>
<td>2.9±1.1</td>
<td>8.8±3.2</td>
<td>0.8±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Ra, rate of appearance. *For the early lactation period, the percentage of glucose derived from glycerol has a different number of samples associated with the calculation of the mean than do glycerol Ra and rate of lipolysis. This occurred because of label contamination in the glucose extraction process. n = 10 for calculations of glycerol Ra and rate of lipolysis; n = 5 for percentage of glucose derived from glycerol.

**DISCUSSION**

The Ra of glycerol is high in simultaneously fasting and lactating adult female elephant seals. Values measured early and late in lactation (8.0 and 7.4 μmol·kg⁻¹·min⁻¹, respectively) are substantially higher than values observed in humans that are either postabsorptive (~3.1 μmol·kg⁻¹·min⁻¹) or fasted from 3–4 days to 3 wk (4.2–5.3 μmol·kg⁻¹·min⁻¹; Refs. 2, 7, 12). By contrast, although glycerol concentrations late in lactation are high (0.52 mmol/l), concentrations observed early in lactation are comparable to those observed in obese humans fasted for 3 wk (0.25 vs. 0.29 mmol/l; Ref. 7). Glycerol concentrations observed after the molt are intermediate between those observed early and late in lactation, but the mean glycerol Ra is nearly three times lower and similar to that observed in fasting humans, suggesting an increased need for NEFA availability during lactation. Milk delivery to offspring is consistent throughout lactation (up to 4 kg consumed per day) and milk energy content is high, with milk fat ranging from ~30% to 50% of the proximate composition (18, 25, 34). By contrast, after the molt, increased metabolic costs associated with pelage synthesis should be complete, and it is expected that this condition most closely reflects lipid dynamics without the additional constraint of lactogenesis (i.e., it is a better assessment of true maintenance metabolism while fasting).

Across all fasting periods, the average contribution of glycerol to glucose production was <3%. A fast of several days in humans raises the percentage of glucose due to glycerol from ~4.5% to ~10–22% (2, 7, 29). Similar results (i.e., 16% of glucose due to glycerol) have been observed in obese individuals placed on very low-calorie diets for several weeks (36). A strong relationship between the Ra of glycerol and the percentage of glucose that is derived from glycerol, and the NEFA concentration is observed in postabsorptive, short-term and long-term fasting humans (2, 7). This relationship has been offered as partial support for the idea that within the fasting state increases in lipolysis primarily serve to increase the availability of gluconeogenic substrate in the form of glycerol, not the
availability of NEFA for substrate oxidation (2, 41). A direct correlation between NEFA and glycerol concentrations existed during all fasting states of the adult female elephant seal, but no correlation between glycerol Ra and glycerol concentration was observed. A direct relationship between glycerol Ra and the percentage of glucose that is derived from glycerol was also observed in lactating elephant seals, but only late in lactation after the seal had been fasting for ~3 wk (Fig. 2). That this relationship is apparent only during late lactation may suggest a coupling of glycerol gluconeogenesis to diminishing nutrient reserves. It is during this time, when blubber stores are substantially depleted, that a negative correlation between glycerol Ra and mass is observed (Fig. 3). It is also during this time that protein catabolism increases, presumably to meet a greater demand for gluconeogenic substrates (17). Thus, as energy reserves near depletion, the metabolic regulation of glycerol gluconeogenesis may become more tightly coupled to remaining energy reserves and protein stores in particular species.

The magnitude of the relationship between glycerol Ra and the contribution of glycerol to glucose production is much reduced compared with that observed in previous human studies; the maximum glycerol Ra of 16.8 μmol·kg⁻¹·min⁻¹ observed late in lactation corresponded to a glycerol contribution of <5%, whereas the work of Baba et al. (2) on short-term fasted humans demonstrated a contribution >20% for a glycerol Ra < 7 μmol·kg⁻¹·min⁻¹. Relative to humans and terrestrial mammals, the low rate of glycerol gluconeogenesis in the fasting elephant seal is surprising given the duration of the fast. However, comparisons to non-fasting-adapted species are likely misleading, because phocid seals exhibit an elevated rate of glucose production during fasting relative to non-fasting-adapted mammals (15). Rates of EGP in fasting elephant seals, and other phocid seals, can be two to three times those observed in non-fasting-adapted mammals undergoing similar fasts (15, 16, 19, 31). Given the measured rate of EGP, the relative contribution of glycerol gluconeogenesis to EGP may suffice to meet the glucose oxidation demands of some glucose-dependent tissues. On the basis of previous studies of NEFA flux, it was estimated that fasting weanling elephant seals produce ~686 μmol glycerol/min and that conversion of 25% of this glycerol to glucose was sufficient to meet the estimated glucose requirements (~86 μmol/min) of the seal brain (14, 21). The estimated glycerol Ra for fasting pups is similar to the absolute glycerol Ra observed in postmolt females (759.5 μmol/min) but less than that observed in lactating females (3,817 μmol/min, early lactation; 2,774 μmol/min, late lactation). With previously determined rates of endogenous glucose production (3.9 and 3.3 mmol glucose/min, early and late lactation, respectively; Ref. 15) it is estimated that ~93–109 μmol of glucose is produced from glycerol each minute. Thus the contribution of glycerol to gluconeogenesis would be sufficient to meet the metabolic demands of the brain. By contrast, during the molt, glycerol gluconeogenesis would only account for ~31 μmol glucose/min, which would appear to be insufficient for meeting the glucose demands of the brain. Protein catabolism after the molt has not been quantified in adult female elephant seals, so it cannot be determined whether a shift in gluconeogenic substrate partitioning occurs once the constraints of lactation are removed, i.e., amino acid precursors could provide a greater relative contribution to gluconeogenesis during the molt.

Levels of NEFA were ~1.0–1.4 mmol/l during the early lactation and postmolt period, respectively, and >3.0 mmol/l during late lactation. Glucose levels remained >6.0 mmol/l across all periods. No relationship between metabolites and glucoregulatory hormones was observed. Past research on elephant seal weanlings fasted up to 8 wk demonstrated reduced sensitivity to insulin challenges and an impaired insulin response to glucose challenges (24). It is reasonable to suspect that similar impairments exist in adult females, and preliminary results of glucose tolerance tests and insulin challenges suggest that this is the case (Crocker DE and Houser DS, unpublished data). These characteristics are similar to the human diabetic condition, and it may be that the pathology of diabetes in humans is adaptive in a species that fasts for long durations and exists on a diet high in lipid and protein but devoid of carbohydrate. Short-term elevations of plasma NEFA have been shown to induce hepatic and peripheral insulin resistance and possibly increase EGP (6, 26, 27). Conversely, under constant circulating concentrations of insulin, glucagon, and cortisol, hyperglycemia (200% basal levels) has been demonstrated to reduce rates of lipolysis by up to 30% (13). Although glucose and NEFA levels are high in elephant seals relative to other fasting mammals, the relevance of short-term responses to hyperglycemia and hyperlipidemia to the fasting physiology of the northern elephant seal is questionable. Consuming a diet that is high in lipid and protein but negligible in carbohydrate content, the elephant seal metabolic profile is likely similar between fasting and feeding states, as has been proposed for suckling and fasting elephant seal pups (22, 24). Thus the regulation of glucose
metabolism and lipolysis likely does not correspond to the traditional insulin-glucagon model of substrate regulation.

Perspectives and Significance

An increasing amount of information on long-duration fasting in elephant seals demonstrates that these fasting-adapted animals do not conform to the traditional fasting model. Specifically, high fasting glucose levels, little reliance on glycerol for gluconeogenesis, and a high rate of EGP are counter to the expectation of glucose dynamics during long-duration fasts. Fasting elephant seals are also insensitive to the action of glucoregulatory hormones, suggesting that a diabetes-like condition is normal in these animals and that it serves an adaptive role in the management of nutrient reserves while fasting. Future efforts to understand the biochemistry of these fasting adaptations, as well as the mechanisms that prevent the pathologies observed in other animals with similar biochemical and hormonal states, should focus on additional studies of metabolite flux and the influence of additional hormones (e.g., cortisol, growth hormone) on metabolite dynamics.

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