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COMMENTARY

Learning to starve: impacts of food limitation beyond the stress period

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ABSTRACT

Starvation is common among wild animal populations, and many individuals experience repeated bouts of starvation over the course of their lives. Although much information has been gained through laboratory studies of acute starvation, little is known about how starvation affects an animal once food is again available (i.e. during the refeeding and recovery phases). Many animals exhibit a curious phenomenon – some seem to 'get better' at starving following exposure to one or more starvation events – by this we mean that they exhibit potentially adaptive responses, including reduced rates of mass loss, reduced metabolic rates, and lower costs of digestion. During subsequent refeedings they may also exhibit improved digestive efficiency and more rapid mass gain. Importantly, these responses can last until the next starvation bout or even be inherited and expressed in the subsequent generation. Currently, however, little is known about the molecular regulation and physiological mechanisms underlying these changes. Here, we identify areas of research that can fill in the most pressing knowledge gaps. In particular, we highlight how recently refined techniques (e.g. stable isotope tracers, quantitative magnetic resonance and thermal measurement) as well as next-generation sequencing approaches (e.g. RNA-seq, proteomics and holobiome sequencing) can address specific starvation-focused questions. We also describe outstanding unknowns ripe for future research regarding the timing and severity of starvation, and concerning the persistence of these responses and their interactions with other ecological stressors.

KEY WORDS: Bioenergetics, Diet, Epigenetics, Fasting, Nutrition, Weight loss

Introduction: responses to starvation – the known knowns

Limitation in the availability of food is an omnipresent challenge faced by many animal populations. The durations of these events can be brief, and cause no apparent physiological impacts, or be moderate and cause measurable decrements in performance (e.g. reproduction, growth, immunity, etc.). In extreme (but not necessarily uncommon) cases, starvation bouts can be life threatening. A bout of food limitation in an adult animal involves the following three phases: starvation (complete or partial starvation; when body mass is lost owing to depletion of endogenous nutrient reserves), refeeding when food becomes available (during which body mass is regained), and finally recovery (the period after which body mass has stabilized and any starvation-induced damage is repaired) (Fig. 1A). Several reviews summarize the responses that humans and other animals have to fasting and/or starvation itself (Keys et al., 1950; Castellini and Rea, 1992; Wang et al., 2006; McCue, 2010, 2012; Secor and Carey, 2016), but we know much less about the responses and mechanisms involved during the refeeding and recovery phases.

We distinguish between the terms 'fasting' and 'starvation' in the sense that the former is caused by some intrinsic force (i.e. an animal forgoes eating when food is potentially available) and the latter is caused by some extrinsic force (*sensu* McCue, 2010). Animals may fast during hibernation (Mrosovsky and Sherry, 1980) or otherwise forgo feeding to protect territory or incubate offspring. Starvation occurs when no food can be found (perhaps because there is none to be found, or foraging attempts are unsuccessful) or when environmental conditions (e.g. acute cold exposure or drought) prevent the physiological processes responsible for ingestion or digestion. Most laboratory studies of starvation (including those done by the authors) lack an important component of biological realism by ignoring the fact that rather than seeing a single starvation event, wild animals often cycle through multiple periods of food limitation over the course of their lives (Carey et al., 2005; Ng'oma et al., 2017). These events may be predictable and regular (e.g. tidal, circadian, seasonal) or caused by unpredictable forces (e.g. caused by extreme weather events, fires, or intra- and interspecific competition).

Mounting evidence suggests that a single bout of starvation is sufficient to trigger a potentially adaptive suite of changes beginning in the refeeding phase, and in some cases extending through the recovery phase, that appear to somehow protect the individual from future bouts of food limitation. Usually this occurs by either retarding body mass loss during subsequent bouts of starvation or by increasing mass gain during subsequent refeeding periods (Fig. 1B). These potentially adaptive changes can persist into subsequent developmental/ontogenetic stages and even into subsequent generations (i.e. generating transgenerational effects). Specific examples of humans and other animals cycled through repeated bouts of fasting or food limitation suggest that these protective responses may be progressively enhanced with 'practice' (i.e. prior stress exposure). Notably, if this is the case, then it is possible that animals are capable of physiologically 'learning' to starve, or otherwise exhibiting some adaptive capacity to cope with future food limitation.

Evolutionary versus physiological adaptation to starvation

Given the ubiquity of food limitation among heterotrophs, both short-term responses through shifts in physiology and behavior and long-term evolutionary adaptations through natural selection are crucial to animal survival. Although our focus is short-term responses (e.g. within a lifespan or only a few generations), the impact of starvation through natural selection cannot be overlooked. It is clear that some species are 'well adapted' to fasting (*sensu* Castellini and Rea, 1992) and have evolved under conditions where

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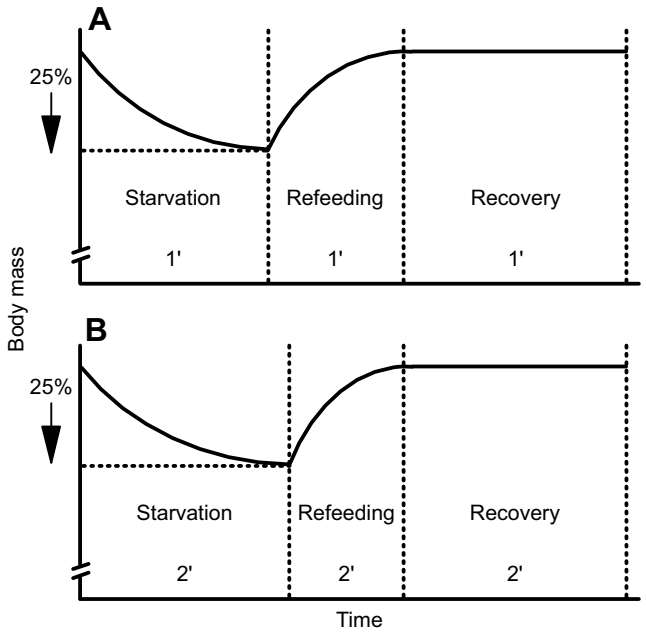

Fig. 1. Phases of food limitation in adult animals. (A) Phases of primary (1′) and (B) secondary (2′) exposures to food limitation in a hypothetical non-growing animal losing 25% of its initial body mass. Note that this animal shows reduced rates of mass loss and increased rates of mass gain during the secondary exposure.

fasting is part of the normal course of life; other species may never normally fast. While fasting-adapted species may also be quite tolerant of starvation, they are not necessarily adapted to starvation (*sensu stricto*). In contrast, species that have evolved amidst high probabilities of unexpected food limitation may be considered 'starvation adapted' even though fasting is not part of their natural history. This distinction between 'fasting adapted' and 'starvation adapted' is rarely considered in the literature, and warrants attention by evolutionary physiologists, yet is beyond the scope of this Commentary.

Specific mechanisms that increase starvation tolerance within broad taxonomic lineages are not uncommon, with the most striking example being the differences between endotherms and ectotherms; endotherms have metabolic rates that are an order of magnitude greater. The numerous examples of independent evolution of heterothermy from homeothermic endothermic ancestors is undoubtedly related to developing extended starvation tolerance (Munoz-Garcia et al., 2013; McAllen and Geiser, 2014; Nowack et al., 2017). Similarly, states of dormancy (e.g. hibernation, aestivation or diapause) or periods of quiescence (e.g. anhydrobiosis, torpor or other resting states) are additional examples of energy-saving mechanisms that can allow animals to survive long periods without food resources (Storey and Storey, 1990, 2004; Hahn and Denlinger, 2007). Moreover, in some species, the evolution of body size and baseline nutrient reserve levels might also be attributed to differences in starvation tolerance (Cherel et al., 1988; Arnett and Gotelli, 2003; Couvillon and Dornhaus, 2010; Champagne et al., 2012; Gergs and Jager, 2014; Knapp, 2016).

Several ectotherm lineages have evolved exceptional tolerance to starvation; examples include eels, lungfish, frogs and snakes (Larsson and Lewander, 1973; Delaney et al., 1974; Grably and Peiery, 1981; McCue, 2007) as well as several arthropods (Anderson, 1974; Benoit et al., 2005; DeVries et al., 2014) that can survive months to years without eating. Artificial selection in *Drosophila* indicates that starvation resistance can be extended greatly (Chippindale et al., 1996; Gibbs and Reynolds, 2012), but at the expense of other factors such as reproductive output or longevity. Thus, many animals might have evolved the effective mechanisms, or at least key elements thereof, that confer resistance to starvation, and short-term behavioral or physiological aspects can either enhance or blunt these long-term evolutionary responses.

Conserving mass and energy

The earliest scientific observations of animals subjected to repeated starvation events showed that they were consistently better able to defend against mass loss during subsequent bouts of starvation (Pashutin, 1902; Howe, 1910); but more recent studies show varied outcomes (Table 1). The most likely mechanisms that could account for reduced rates of mass loss include reduced resting metabolic rates (RMRs) and reduced activity levels. Although a discussion of behavioral adjustments to starvation are beyond the scope of this Commentary [e.g. starvation-induced hyperactivity is the one of the most common responses, where there has been significant focus on *Drosophila* (Yang et al., 2015)], several studies did not report differences in 'activity' in rats refeeding after starvation (Boyle et al., 1981; Björntorp and Yang, 1982; Archambault et al., 1989) – probably ruling out variation in locomotion-related energy expenditure as a significant mechanism for energy savings that spans across all animals.

Persistent reductions in RMR that are greater than expected from mass loss (absolute or fat-free) alone are documented in formerly obese humans (Rosenbaum et al., 2008; MacLean et al., 2011; Sumithran and Proietto, 2013). While obesity is perhaps not a common problem among wild animals (but see Schilder and Marden, 2006), it is instructive to use humans who routinely fast themselves (e.g. so-called 'yo-yo dieters'; *sensu* Prentice et al., 1992) as a starting point to generate hypotheses about which responses we might expect to see in other animals. Starvation-induced hypometabolism has been documented in a wide range of animal taxa (reviewed in McCue et al., 2012), but the

Table 1. Controlled studies reporting body masses in animals exposed to multiple bouts of starvation

Citation	Animal	Response
Reviewed by Pashutin, 1902	Dogs	Lost mass less rapidly during a second exposure
Reviewed by Pashutin, 1902	Rabbits	Lost mass less rapidly during a second exposure
Reviewed by Pashutin, 1902	Pigeons	Lost mass less rapidly during a second exposure
Howe, 1910	Dogs	Lost mass at the same rate after second exposure
McCue, 2017a	Rats	Lost mass at the same rate after second and third exposure
Wood and Bartness, 1996	Siberian hamsters	Lost mass less rapidly during a second exposure
Day et al., 1999	Siberian hamsters	Lost mass less rapidly during a second exposure
Brownell et al., 1986	Obese rats	Lost mass less rapidly during a second exposure
van Dale and Saris, 1989	Humans	Dieters with history of mass cycling lost mass at the same rate as controls
Archambault et al., 1989	Rats	Lost mass at the same rate after cycling periods of severe food limitation

Note the general lack of taxonomic diversity, or inclusion of species where starvation is a natural part of their life history.

persistence of these energy-saving mechanisms is rarely examined during the refeeding and recovery phases and even less frequently across generations.

Perhaps the most straightforward way to reduce RMR is to lower body temperature (T_b). Some ectotherms can do this through behavioral means by moving to a cooler environment (McCue, 2004; Bicego et al., 2007), but the greatest absolute energy savings during periods of food limitation could be realized by endotherms (Geiser, 1988). Rats previously exposed to multiple starvation cycles maintained lower T_b than naïve rats during starvation (McCue et al., 2017a). Japanese quail, known for their ability to progressively depress their night-time T_b with each additional day of starvation (Ben-Hamo et al., 2010; Hohtola, 2012), exhibited even deeper depressions in T_b after being exposed to multiple starvation cycles (Laurila et al., 2005). A study of formerly obese humans in the recovery phase showed reduced activities of enzymes involved in the glycerophosphate cycle, which is known to enhance heat production in the body (Bray, 1969). Even though T_b depression has been observed in multiple species that are not traditionally recognized to be heterothermic (*sensu* Ruf and Geiser, 2014), the specific mechanisms (e.g. neural or hormonal) responsible for shifts in T_b set-points remain unknown.

Regaining mass and energy

One of the most commonly documented responses during the refeeding phase is an increase in food conversion efficiency (FCE; this is similar to the concept of 'digestive efficiency' in some studies). FCE is expressed as 'gain in body mass per unit (e.g. mass or energy) of food ingested'. Several studies have reported a doubling in FCE during refeeding in previously starved rats (Reed et al., 1988; Archambault et al., 1989; Ilagan et al., 1993; Munch et al., 1993). It is possible that FCE progressively increases with repeated exposure to food limitation. For example, the FCE of obese rats was doubled during recovery from a first exposure to starvation, and then tripled during the recovery from a second exposure to starvation (Brownell et al., 1986).

One frequently proposed mechanism for increasing FCE is to reduce the energetic cost associated with processing a meal (or *specific dynamic action*, SDA) (McCue, 2006; Secor, 2009). Rats recovering from starvation exhibited reductions in SDA of 25% and 10% on the second and third day of refeeding, respectively (Boyle et al., 1981). A study of humans reports that the SDA response can even remain diminished for a year after mass loss (Rosenbaum et al., 2008). However, because SDA in endotherms usually accounts for <10% of ingested energy (Secor, 2009), it is unlikely to account completely for the enormous increases in FCE reported during the refeeding phase.

Body mass and stored energy are not equal

Comparisons of starvation-induced mass loss, refeeding-induced mass gain or changes in metabolic rates usually depend on the assumption that body composition remains constant. This is an unrealistic expectation for obese animals losing large amounts of body mass. It could also be problematic for interpreting physiological measurements on animals undergoing repeated mass cycling – but the current evidence is mixed. The relative muscle lipid content in pigs recovering from starvation decreased in some, but not all, major muscle groups (Gondret and Lebret, 2007). The whole bodies of rats that had recovered from starvation had a slightly higher relative protein content than that of control rats (Björntorp and Yang, 1982). Three bouts of starvation caused rats to increase the adipose mass and adipocyte cell counts in their retroperitoneal and parametrial depots (Reed et al., 1988); however, another study showed that rats consuming a high-fat diet and undergoing three cycles of starvation did not differ in relative lipid content (internal or subcutaneous), protein content or water content from age-matched controls raised on the same high-fat diet (Ilagan et al., 1993). These disparate outcomes underscore the complexity of the issue of body composition.

Most of the aforementioned examples of repeated exposure to food limitation and mass cycling employed humans or laboratory rats. Collectively, they could provide a basis for developing general hypotheses about how non-model organisms might respond to repeated exposure to food limitation – but there are no substitutes for direct observations. Specifically, animals adapted for prolonged starvation might exhibit distinct mechanisms to tolerate food limitation. Moreover, the conflicting experimental outcomes and the gaps in mechanistic understanding of many of the observed responses to food limitation highlight opportunities for rigorous hypothesis testing. Below, we provide examples of unexploited ways to combine modern analytical approaches with experimental designs that better reflect the biological reality of food limitation in animals.

Questions for future research

Here we begin by proposing what we consider to be promising research questions related to the potential mechanistic underpinnings of starvation-induced physiological responses, and continue by posing questions concerning the cues, timing and persistence of such responses. Each of these avenues is significant for understanding the adaptive role of starvation and nutrient deprivation on animal performance and fitness. It is increasingly clear that an understanding of organizational levels at which such responses occur, and how these are integrated to achieve organismal homeostasis, is central to advancing comparative and evolutionary physiology research (Mykles et al., 2010; Ng'oma et al., 2017), while information surrounding the cues (triggers), duration, speed of response and persistence of induced responses are significant for understanding phenotypic plasticity, systemic constraints or trade-offs, and the evolution thereof (Altimiras and Anderson, 2016; Sgro et al., 2016).

Mechanisms, responses and traits affected by repeated starvation

How is body composition altered by repeated starvation events? Changes in body mass are a coarse measure of how an animal responds to starvation or refeeding, and thus cannot inform us about subtle changes in body or organ composition or whether biochemical pathways of energy metabolism have been altered (e.g. between lipid and protein catabolism). Dual X-ray absorptiometry (DEXA) and more recently developed technologies such as quantitative magnetic resonance (QMR) allow researchers to measure lean (i.e. protein+carbohydrate) and lipid pools in the body with higher precision than ever (Taicher et al., 2003; Tinsley et al., 2004; McGuire and Guglielmo, 2010; Gerson and Guglielmo, 2011). Because these are non-destructive measures, they would be ideal for prospective, longitudinal studies of individual organs (Resnick et al., 2003; McWilliams and Whitman, 2013) during starvation as well as during the recovery phase and beyond for individual animals. If sacrificing a large number of animals, such as *Drosophila* or other invertebrates, is possible, exact measurements of lipids, proteins and other nutrient reserves can be made through destructive methods, such as spectrophotometric assays, at multiple points throughout the starvation, refeeding and recovery cycle

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(Djawdan et al., 1998; Schwasinger et al., 2009; Foray et al., 2012). The results of either destructive or non-destructive approaches can then be used to inform quantitative models about the timing and extent of macronutrient mobilization (*sensu* Caloin, 2004; Hall, 2012).

Do sources (fuel types) and sinks (tissues or activities) of energy resources vary as the number of starvation bouts increases? Changes in respiratory exchange ratios (RERs) have traditionally been used to document shifts among carbohydrate, lipid and protein catabolism, but the approach is increasingly recognized as unreliable for quantitative purposes when fuel shifts are subtle (McCue et al., 2015b; Treberg et al., 2016; Levin et al., 2017b). Recently developed techniques in ^{13}C-breath testing allow the isotopic labeling of selected nutrient pools (e.g. lipids or proteins) in the bodies of invertebrate (McCue et al., 2015c, 2016; Levin et al., 2017a) and vertebrate (McCue et al., 2013, 2017b; McCue and Pollock, 2013; Kirschman et al., 2017) animals, and can be used to reveal comparatively small changes in fuel ratios. In addition, next-generation techniques, such as RNA-seq, proteomics and metabolomics could be used in parallel to examine specific molecular and biochemical changes with progressive increases in the frequency or severity of food limitation. Changes could also be expressed through the animal diverting energy away from expensive tissues (e.g. gut–brain trade-offs) to limit perturbations in metabolic or growth rates (Tsuboi et al., 2015; Liao et al., 2016). Indeed, a recent study of honeybees showed that bees starved as larvae were able to shift more quickly to new fuels as adults when facing a subsequent bout of food limitation, thus stabilizing hemolymph sugar levels (Wang et al., 2016).

What physiological mechanisms underlie improved FCE? FCE is a gross measure that does not account for the possible lower energy requirements of the animal. Even moderate decreases (e.g. ∼10–20%) in metabolic expenditure could not explain the doubling and tripling of FCE reported in animals that have been previously exposed to starvation (see above). The ‘everted-sleeve’ technique can reveal differences in intestinal nutrient transport and paracellular uptake rates *in vitro* (Karasov and Diamond, 1988; Secor, 2005; Tracy et al., 2010). ^{13}C-Breath testing approaches using stable isotope tracers that are integrated into the diet of animals could also non-invasively track changes in nutrient routing among tissues/organs (McCue and Welch Jr, 2016; Welch Jr et al., 2016) or nutrient oxidation to fuel SDA *in vivo* (McCue et al., 2015a, 2016) during the refeeding or recovery periods.

One potential explanation for the improved FCE is that starvation-induced reductions in metabolic rate simply reflect reduced T_b set-points. Previous studies that document changes in metabolic rates during the refeeding or recovery phases did not monitor T_b. The metabolic rate of endotherms is tightly coupled with T_b, and even small adjustments in T_b of ±1°C, can have substantial consequences for the energy budget over extended periods. Implantable data loggers or temperature-sensitive radio frequency identification (RFID) tags are minimally invasive, and would allow researchers to determine if apparent reductions in metabolic rate are directly driven by changes in T_b or if they occur independently of one another (*sensu* Toien et al., 2011). Although these devices are sufficiently small to be used in mouse-sized animals, they would be suitable to complement non-invasive approaches such as infrared thermography (e.g. Tattersall, 2016) to permit more detailed investigations of regional T_b in rats or larger animals.

How does starvation adjust transcript/protein/metabolite levels, and do these changes underlie altered phenotypes during subsequent starvation bouts? Starvation results in substantial shifts in gene expression (Harbison et al., 2004, 2005; Moskalev et al., 2015), presumably to regulate the use of the limited nutrient stores and allow for shifts in metabolic investment in specific biological processes during starvation. The terminal stages of starvation when lipid and protein reserves are depleted show increased expression of genes associated with autophagy (Fujisaki, 2010), thereby tagging particular tissues for catabolism that are not required for immediate survival (e.g. digestive and reproductive tissues). Few studies outside of *Drosophila* have utilized next-generation methods to measure gene expression throughout starvation (but see Khudyakov et al., 2015a,b; Yang et al., 2017). In whole-body studies on *Drosophila*, ∼25% of the genome had coding sequences with differential transcription during starvation (Harbison et al., 2005), underscoring the significant biological shifts necessary under food limitation.

That there is a large shift in gene expression is supported by transgenic studies, where ∼40% of the examined lines have altered starvation tolerance (Harbison et al., 2004), indicating a deep and far-reaching genetic component related to starvation resistance. This genetic element is further supported by the ability to substantially increase starvation tolerance through prolonged selection with food limitation (reviewed by Gibbs and Reynolds, 2012). In general, there is increased gene expression associated with protein and organelle degradation and a suppression of transcripts associated with reproduction (Harbison et al., 2005; Faradian et al., 2012). Furthermore, genes related to immune function are downregulated (Faradian et al., 2012), supporting previous observations that immunity becomes compromised during starvation (Chandra, 1983; Lochmiller and Deerenberg, 2000; Valtonen et al., 2010). Comparisons among previous transcriptional-response studies (i.e. RNA-seq and microarray) related to starvation for two insects (*Drosophila melanogaster* and the soybean aphid *Aphis glycines*) revealed overlapping gene ontology (GO) categories associated with the large-scale shifts in metabolism likely to permit the catabolism and mobilization of energy reserves during starvation (Harbison et al., 2005; Enders et al., 2015).

Even though these studies have provided the groundwork on gene expression during starvation, extensive time-course analyses as starvation progresses or expression during the refeeding and recovery phases are lacking. Such studies will be crucial in linking gene expression with biochemical and physiological changes as starvation progresses followed by refeeding and recovery. For *Drosophila*, the number of genes with differential expression increased by >75% as the duration of starvation progressed from 24 to 48 h (Faradian et al., 2012). Of interest is that changes at 48 h of starvation are not solely an increase in the response at 24 h – rather, some of the same genes are upregulated 24 h into starvation and decreased at 48 h. In more detail, many of these genes showing differential expression are associated with the head, suggesting a major shift in neurological components as starvation progresses.

Recovery from starvation is not as simple as merely the reversal of the differential expression of transcripts according to other studies on stressed animals (Lopez-Martinez et al., 2009). Recovery probably represents a unique molecular response that has yet to be examined at the cellular level. These prolonged changes that persist in the recovery period could be key factors underlying the improved resistance of an animal to subsequent periods of starvation. The transcriptional differences following single or multiple bouts of starvation are unknown, but will probably be significant as single versus repeated bouts of stress or periods of acclimation (sub-lethal

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periods of stress) yield divergent transcriptional responses (Zhang et al., 2011; MacMillan et al., 2016). Of particular interest will be the utilization of combined next-generation approaches [e.g. transcriptomics (Khudyakov et al., 2015a,b) proteomics, phosphoproteomics and metabolomics (Olmstead et al., 2017); reviewed in Ng'oma et al., 2017] in future studies, as these will provide a clear picture of the molecular mechanisms underlying starvation responses. Combined next-generation studies have been used in many systems to more precisely establish critical biological responses through identification of enriched pathways overlapping between methods (e.g. MacMillan et al., 2016; Rosendale et al., 2016; Dong et al., 2017; Wu et al., 2017).

How does starvation alter responses to other stressors? Starvation might be the most effective way to exhaust nutrient reserves, but the presence of other stressors can increase the rates at which it occurs. Bouts of dehydration and cold exposure in ectotherms have been shown to alter rates of water loss and metabolism (Boardman et al., 2013) and reduce the reserves of lipid and protein (Benoit et al., 2010; Marshall and Sinclair, 2011; Teets and Denlinger, 2013; Rosendale et al., 2017). As the severity of stress exposure increases, decreases in nutrient reserves decline concomitantly, eventually leading to reduced reproductive output or other negative physiological aspects. In the American dog tick, the severity of dehydration stress can drastically increase reductions in lipid and protein reserves when compared with shorter, more manageable periods of dehydration stress (Rosendale et al., 2017). In addition, there appears to be overlap in gene expression between starvation and exposure to other stressors (Moskalev et al., 2015), suggesting that stressors other than starvation could act as a primer for increasing subsequent tolerance to food limitation (i.e. the possible presence of a general stress phenotype; Sørensen et al., 2017). These results indicate that periods of stress could either act to exacerbate starvation or serve in priming animals to tolerate starvation more effectively. Importantly, revealing the dynamics between stress exposure and food limitation is crucial to understanding the impact of single or multiple starvation bouts under field conditions as food limitation is often accompanied by other environmental stressors (Olson et al., 2017).

Improved starvation resistance following previous food limitation probably occurs at a cost to other physiological performance variables. Two of the most likely trade-offs are reduced reproductive output and increased susceptibility to other stressors (abiotic and biotic factors). In general, prolonged starvation is known to reduce the resistance of animals to thermal and water stress, but the extent to which these negative effects persist after refeeding is unknown. Similarly, an immune response is suppressed during starvation, and vice versa (Valtonen et al., 2012); however, similar to the case of abiotic stress, there has been little focus on this area. One recent study that examined this notion tested whether prior single bouts of either desiccation, starvation or the combination of both stressors altered the thermal tolerance of Mediterranean fruit flies in a predictable or additive way (Mitchell et al., 2017). Somewhat surprisingly, thermal limits appear relatively insensitive to these stressors, as has been shown in *Drosophila* (Overgaard et al., 2012), despite the fact that some theoretical expectations specifically argue for resource depletion mechanistically governing thermal stress resistance (Rezende et al., 2010). Carefully designed experiments will be necessary to elucidate how multiple bouts of starvation–refeeding–recovery impact organismal responses to other stressors. Moreover, consideration of multiple performance traits is required as it has implications for understanding niche evolution and fitness trade-offs, and consequently the evolution of starvation resistance in heterogeneous environments.

How do gut microbiota respond to repeated cycling of food availability? The role that the gut microbiome has on nutrition, immunity and even energy balance is being increasingly described (Turnbaugh et al., 2009; Nehra et al., 2016), and, given the recent improvements in sequencing technologies, additional crucial roles will soon be identified. Diet type as well as starvation have been shown to affect the microbiota community structure in several species (Carey et al., 2013; Kohl et al., 2014; Sonnenburg and Sonnenburg, 2014; Xia et al., 2014) – yet we have no idea how these communities respond to repeated starvation events, nor do we understand the physiological implications of such changes for the host animal. Recent studies that have replaced the gut microbiome of normal mice with communities from obese individuals caused mass gain, suggesting that shifts in the microbiome could allow increased retention of nutrient resources (Turnbaugh et al., 2008, 2009; Jayasinghe et al., 2016). Of interest is whether starvation bouts will alter components of the microbiome that protects against subsequent starvation by either slowing gut passage or maximizing digestion and nutrient uptake (i.e. promoting obese-like phenotypes).

Cues, timing and persistence of changes induced by repeated starvation

What are the persistent impacts of starvation during life? There is significant evidence that starvation (along with exposure to other stressors) is likely to have a prolonged effect that can extend to subsequent developmental stages (Heijmans et al., 2008; Zaldua and Naya, 2014; McCue et al., 2017a). These effects are likely under epigenetic controls through mechanisms such as DNA methylation, histone modification, and/or expression of small RNAs and dependent upon factors such as species and stress type (Ho and Burgrren, 2010; Rechavi et al., 2014). Exposure to

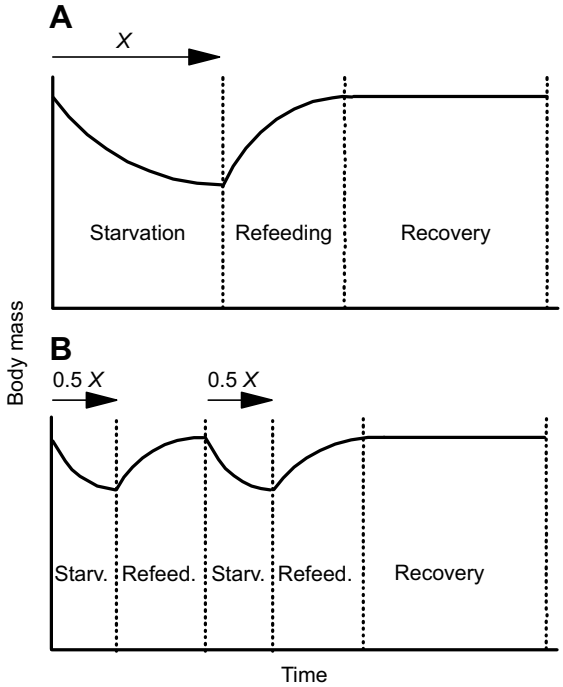

Fig. 2. Effects of starvation bouts. Contrasting the potential responses during and after (A) a single, severe bout of starvation of duration '*X*' and (B) two, less severe bouts of starvation each lasting half as long. Note that for simplicity, the rates of mass loss and mass gain do not differ with subsequent starvation events as in Fig. 1.

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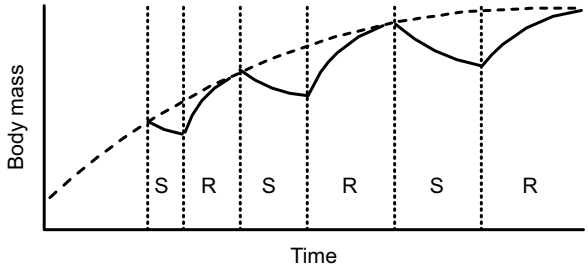

Fig. 3. Hypothetical cycling of starvation and refeeding bouts in a growing animal that exhibits complete compensatory growth.
S, starvation; R, refeeding. The dashed line represents the response of individuals that have not been exposed to food limitation. Note that at each starvation event the animal loses 20% of its initial body mass but the time required for this to occur takes longer each time. Moreover, the refeeding time required for compensatory growth also increases. This allometric framework could also be applied across species.

starvation early in life has been documented to have both positive and negative effects related to adult fitness, depending on the species and the environmental conditions (Chaby et al., 2015a,b; Jobson et al., 2015). An important outstanding issue is to determine how long the effect of a starvation bout persists and whether the responses are additive or non-linear (Fig. 2). For example, are two bouts of mild starvation equivalent in eliciting a similar response under subsequent starvation? From the rat studies discussed above, this seems not to be the case. One can think about this in terms of the 'dose' of starvation and estimate whether the impacts of two equivalent lethal doses (e.g. LD_{25}) of starvation add up (LD_{25}+LD_{25}=LD_{50}); so does 1+1=2 or is it more complicated than that?

What life stages are the most sensitive to starvation programming? Food limitation could occur at any point in life; most of our previous examples focused on non-growing (adult) organisms, but it is unlikely that all life stages are equally sensitive to starvation. The dynamics of starvation and refeeding probably change ontogenetically (Fig. 3), but we have very limited understanding of how starvation responses differ across life stages and developmental windows (Ziegler, 1991; Siegert et al., 1993). For some young animals (e.g. otariid pinnepeds; Champagne et al., 2012), periodic starvation lasting for days is common when the mother is on foraging trips; but this would not be tolerated by most other mammals, reinforcing the need to interpret observations within the context of a species' natural history. Carefully designed experiments aimed at determining the interactions (including the physiological and the genomic responses) between both the timing and severity of starvation will be required to fully appreciate persistent effects.

What are the impacts of starvation in subsequent generations? Documenting the responses to starvation across generations will require different experimental techniques from studies examining acute exposure, and may preclude, at least initially, species that are long-lived. Nevertheless, transgenerational impacts of starvation have been identified in all animal systems that have been studied (Heijmans et al., 2008; Carone et al., 2010; Ho and Burgrren, 2010; Veenendaal et al., 2013; English et al., 2014; Öst et al., 2014; Rechavi et al., 2014; Jobson et al., 2015). Both maternal and paternal transgenerational roles have been noted to have an impact on starvation resistance during the subsequent generation when varying nutrition levels are provided to juvenile or adults of the subsequent generation (Carone et al., 2010; Ho and Burgrren, 2010; Valtonen et al., 2012; Jobson et al., 2015). The longevity (i.e. number of generations) that these effects persist for has not been fully established, but other stress-induced changes have been noted to persist for over 12 generations (Klosin et al., 2017). The developmental stage most sensitive to epigenetic changes that yield this transgenerational priming is not yet known and will probably vary among species. Furthermore, will a single, more severe bout of starvation have a greater impact in relation to epigenetic changes than multiple, less severe bouts of starvation?

Concluding remarks

Starvation is a naturally occurring phenomenon with consequences that transcend several research areas, from molecular biology and physiology to ecology and evolution. We have still to answer several fundamental questions about how animals cope with starvation, yet it is clear that the way forward should include efforts to examine not only acute bouts of starvation, but also the longer-term post-starvation responses to food limitation. Our aim here has been to highlight areas that have been severely understudied in relation to starvation biology that could be fruitful for understanding the mechanisms utilized by animals to survive during times of food limitation. Importantly, response to food limitation must be examined from a perspective that considers both physiological and molecular changes across all phases (i.e. starvation, refeeding and recovery) in relation to the holobiont (host and symbionts) as well as previous and current exposure to eco-physiological stressors.

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Competing interests

The authors declare no competing or financial interests.

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