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# Environmental risk factors in puppies and kittens for developing chronic disorders in adulthood: A call for research on developmental programming

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Many dogs and cats are affected by chronic diseases that significantly impact their health and welfare and relationships with humans. Some of these diseases can be challenging to treat, and a better understanding of early-life risk factors for diseases occurring in adulthood is key to improving preventive veterinary care and husbandry practices. This article reviews early-life risk factors for obesity and chronic enteropathy, and for chronic behavioral problems, which can also be intractable with life-changing consequences. Aspects of early life in puppies and kittens that can impact the risk of adult disorders include maternal nutrition, establishment of the gut microbiome, maternal behavior, weaning, nutrition during growth, growth rate, socialization with conspecifics and humans, rehoming and neutering. Despite evidence in some species that the disorders reviewed here reflect the developmental origins of health and disease (DOHaD), developmental programming has rarely been studied in dogs and cats. Priorities and strategies to increase knowledge of early-life risk factors and DOHaD in dogs and cats are discussed. Critical windows of development are proposed: preconception, gestation, the suckling period, early growth pre-neutering or pre-puberty, and growth post-neutering or post-puberty to adult size, the durations of which depend upon species and breed. Challenges to DOHaD research in these species include a large number of breeds with wide genetic and phenotypic variability, and the existence of many mixed-breed individuals. Moreover, difficulties in conducting prospective lifelong cohort studies are exacerbated by discontinuity in pet husbandry between breeders and subsequent owners, and by the dispersed nature of pet ownership.

## KEYWORDS

behavior, epigenetics, microbiota, nutrition, obesity, chronic enteropathy, developmental programming

## Introduction

There is increasing awareness that aspects of early life in puppies and kittens, especially nutrition during gestation and early growth, impact the risk of neonatal mortality (1–3) and the development of chronic diseases in adulthood (4, 5). In many mammalian species, early-life and parental experiences have been investigated as potential contributors to the developmental origins of health and disease (DOHaD). The concept of DOHaD encompasses the observations that environmental exposures during development can drive epigenetic changes that modify, or “program” the expression of genes, affecting structural and functional development, with rapid or delayed risks to health. In humans, the first 1,000 days of life, which approximates to gestation plus 2 postnatal years, have been identified as a critical period when developmental programming sets the foundations for optimal neurodevelopment, growth and health (6). Much of what is known about DOHaD and the epigenome is derived from laboratory animal models (7), but experimental knowledge has also accrued for ruminants, pigs and even horses (7–9).

In dogs and cats, most research into the etiology of chronic adulthood conditions has focused on adult environmental predictors and risk factors, without investigating whether these have developmental origins. Literature searches in PubMed<sup>®</sup> (31 July, 2022) with the broad search string (epigenetics OR “developmental programming” OR DOHaD OR “developmental origins of health and disease”), combined with “Dogs” or “Cats” as Medical Subject Headings, retrieved 218 articles. After screening titles and abstracts for relevance, 51 articles relating to dogs and 6 relating to cats remained; the most apparent topics of interest were epigenetic modifications in cancer cells [32 articles (56%)], and epigenetic aspects of breed phenotype. Overall, there has been inadequate consideration in dogs and cats of the extent to which the environment, during different stages of growth and maturation, can influence the subsequent occurrence of adult conditions and behavioral traits, even if these environmental factors are chronologically distant. Research in domestic carnivores has been led mainly by experts in specialized fields of veterinary medicine, including nutrition, reproduction, gastrointestinal microbiology, and behavior, with a paucity of expertise in DOHaD that crosses the relevant disciplines.

This review provides an overview of the main environmental risk factors in puppies and kittens that can affect the occurrence of obesity, chronic enteropathy (CE) and behavioral problems in adulthood. These chronic disorders are common in domestic carnivores, challenging to treat, and have major deleterious effects on health, quality of life and potentially longevity (10–12). Difficult behavior can lead to a break-down in the human–animal bond, and may result in abuse, relinquishment or euthanasia of pets (13–15). It is possible that some of the modifiable variables explored may represent ongoing risks that commence or become apparent in early life, and some may be

manifestations of DOHaD, with changes in the epigenome at periods of developmental plasticity. There is also the potential for exposures to unmask the effects of DOHaD. Suggested research priorities are discussed for each condition, based on existing research in puppies and kittens, factors in adult dogs and cats known to be associated with the condition and hypothesized to become established during early life, and on knowledge of developmental programming in other species. Research strategies are proposed to increase our understanding of the long-term impact of early environment and life events for dogs and cats. Such strategies must include studies to determine the role of DOHaD as has been done for other species. These studies might ultimately allow the generation of guidelines to inform disease prevention from as early as preconception. This is not only important for animal welfare, but should be considered in the broader economic and societal context of dog and cat ownership.

Building upon evidence in dogs and cats, humans and laboratory animals, we propose a timeline of key exposures and developmental milestones in puppies and kittens that shape and define “early life.” Early life in this review is not intended to relate to a fixed chronologic age or necessarily to the same period of development classically considered in DOHaD studies in other species. It is used to describe the periods preceding adulthood in which the physiological and psychological maturation of puppies and kittens can be affected for good or bad, or modifiable risk factors for later chronic disease emerge. This is intended to help frame future research and to encourage breeders, owners and veterinarians to take a holistic, integrated and proactive approach to promoting the long-term health of pets (10–12).

## The context for research on early-life development of dogs and cats

### Societal

Dogs and cats are cherished as family members in many households, making their long-term health a high priority for owners. Societal benefits of dog and cat ownership include the promotion of human health and wellbeing (16, 17); dogs also work in a wide variety of service roles. While these factors, combined with a general concern for animal welfare, provide a rationale for advancing our understanding of early-life risk factors for chronic diseases, they also mean that acceptance of invasive research in these species is limited; this is likely to be one reason for the relatively slow advancement of DOHaD knowledge in dogs and cats.

With respect to large-scale observational studies, the dispersion of the pet population makes studying connections between early and late exposures and events particularly

challenging. There is no coherent network of the relevant parties throughout a pet's life. For example, each individual dog or cat may have a different breeder who is responsible for its prenatal and first 2–3 postnatal months of life, and each pet may subsequently be homed with a different owner, who in turn may have a different veterinarian.

## Economic

The size of the pet population and the direct economic significance of pets are tangible measures that help to contextualize the importance of pursuing avenues for preventive medicine. There are an estimated 92.9 million dogs in Europe (25% of households; 2021 data) (18), and 83.7–88.9 million in the USA (45% of households; 2020 data) (19). The total population of cats is estimated to be more than 113.6 million in Europe (26% of households; 2021 data) (18) and 60.2–61.9 million in the USA (26% of households; 2020 data) (19). Sales of pet food products were €27.2 billion in Europe in 2021 (18). In the USA in 2021, market sales were \$50.0 billion for pet foods and treats, \$34.3 billion for veterinary care and product sales, and \$9.5 billion for other services outside of veterinary care, such as boarding, grooming and insurance (20).

## Biological

### Breed

Large phenotypic variability within the canine species, and to a lesser extent the feline species, contributes to the complexity of research in companion animals. The canine species exhibits the widest morphological and weight differences between breeds of all terrestrial mammalian species. More than 350 breeds of dogs are recognized by the International Cynological Federation (21). Adult weights range from 1 kg, for a Chihuahua, to more than 100 kg, for an English Mastiff. Moreover, many pet dogs (up to 40% in the UK) are a mix of breeds (22). Age at which adult body weight is attained correlates with dog breed size, ranging from ~9 to 10 months for toy, small and medium-sized breeds, to 11–15 months for large and giant breeds (23). Size diversity is less pronounced in the cat population, in which 45 breeds are recognized (24) and only 5–15% of cats are pedigreed (25). Adult cat weights range from ~2 kg for a Munchkin to 10 kg for a Maine Coon (25).

### Reproductive biology

Understanding early-life risk factors for adult diseases and the potential for developmental programming requires a knowledge of species-specific biology of conception and fetal and neonatal development (summarized for dogs and cats in [Supplementary Figure 1](#), [Supplementary Tables 1, 2](#)). This

allows the timing of environmental exposures to be related to the differentiation of cell types and the development of specific tissues and organs. Overall embryonic and fetal development is similar between dogs and cats (26) with the exception of oocyte maturation and ovulation. Ovulation in cats is typically induced by coitus (26), although spontaneous ovulation seems to be more than anecdotal (27). Oocytes are released in metaphase II, so fertilization can occur as soon as they reach the oviduct (28). In dogs, there is spontaneous ovulation of immature oocytes at prophase I. Oocyte meiosis resumes after ~ 48 h in the oviduct, and fertilization occurs from 90 h after ovulation (28, 29). Another difference in dogs is that follicles undergo preovulatory luteinization, so serum progesterone concentrations are already high at ovulation (28, 29).

### Milestones of early life

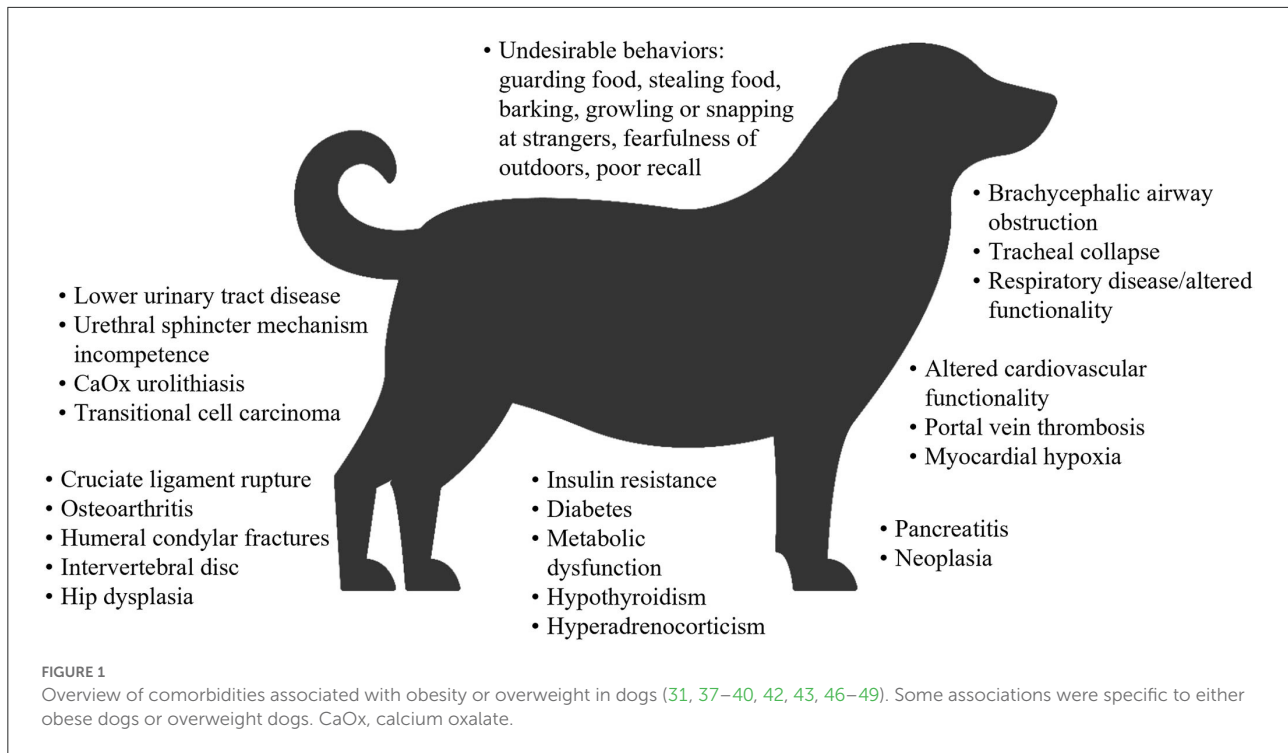
Dogs and cats share many major biological milestones with other species, but the timing and biological details differ. These milestones include embryonic and fetal events ([Supplementary Figure 1](#); [Supplementary Tables 1, 2](#)), neonatal survival, transition to solid foods and neutering. Periods of organ and organ/system development and maturation in which external factors can modify its developmental trajectory are numerous. These critical periods represent different windows of opportunity to promote development beneficial to long-term health.

## Early-life environmental exposures and events as risk factors for selected disorders in adult dogs and cats

### Obesity in dogs and cats

As stated by Kopelman (2000), “obesity can be defined as a disease in which excess body fat has accumulated such that health may be adversely affected” (30). Obesity is defined as a chronic relapsing disease, which itself can predispose to other non-communicable diseases, such as diabetes mellitus, cardiovascular diseases and cancer in dogs and cats (31). In the field of veterinary medicine, over 20 national and international veterinary and associated organizations support the classification of obesity as a disease (32), which is regarded as the number one health problem in companion animals (33).

Overweight and obesity in both dogs and cats is generally measured by determining the body condition score (BCS), which correlates well with adipose tissue mass (32–35). On this basis, a study of dogs at family pet shows in the UK reported that 65% of adult dogs were overweight or had obesity, and 9% had obesity (10). In the 2018 obesity prevalence survey in the USA conducted by the Association for Pet Obesity Prevention



(APOP), veterinarians assessed 36.9% of dogs as overweight, and 18.9% as having obesity (36). Obesity and/or overweight in dogs are associated with many comorbidities, functional impairments (37–43), a shorter lifespan (44), and a poorer quality of life (45) (Figure 1). In the APOP 2018 survey for cats, the prevalence of overweight and obesity were 26 and 34%, respectively. The prevalence of overweight or obesity in adult cats at vaccination visits in New Zealand was 22 and 3%, respectively (50). As for dogs, overweight and/or obesity in cats is associated with an increased risk of a wide range of co-morbidities (46, 51, 52), a reduced lifespan and a higher risk of death (severe obesity only) (53, 54), and some, but not all data, suggest a reduced quality of life (55) (Figure 2).

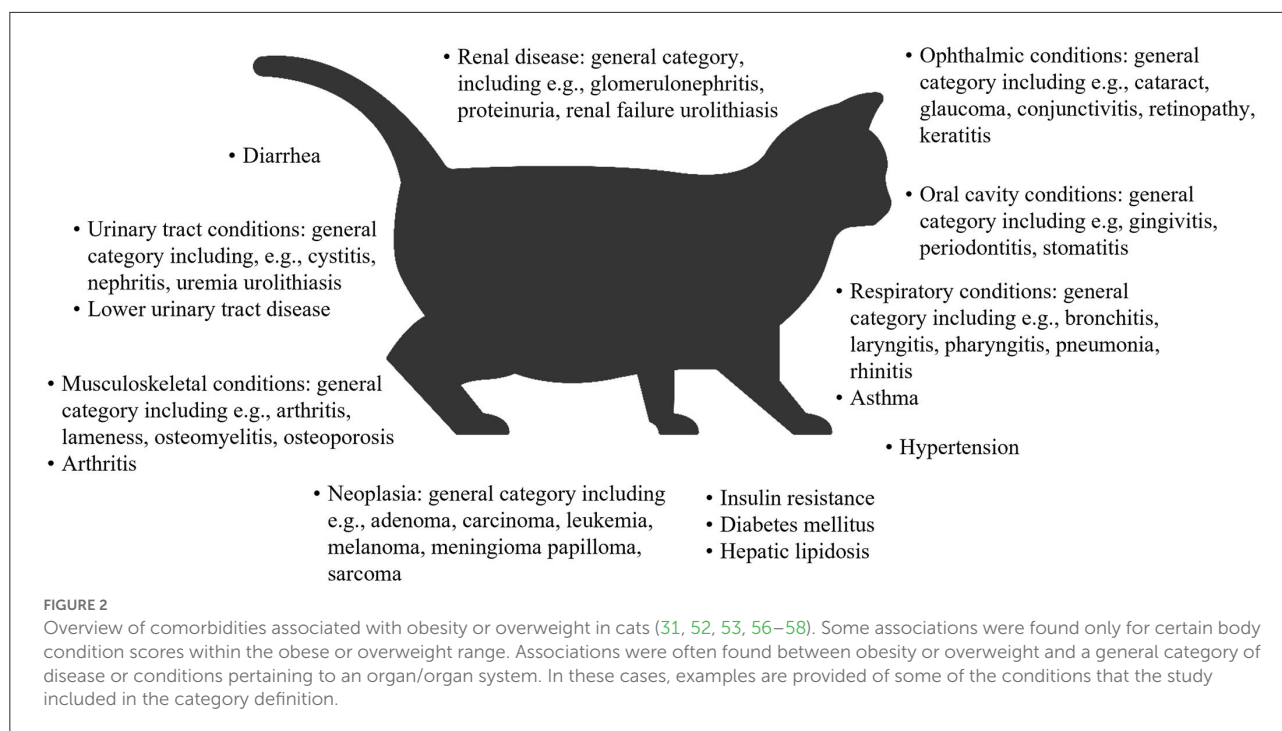
### Early-life risk factors for obesity in adult dogs

Risk factors for obesity can be identified in early life, as early as the fetal period. For example, low birth weight in Labrador Retrievers has been associated with overweight in adulthood even after adjusting for age and neuter status: 70% of dogs with birth weights below the median were overweight as adults, compared with 47% of dogs with birth weights above the median (5). No association was found between adult obesity and growth rate between birth and Day 2 or between Day 2 and Day 15. In contrast, in a study of female Beagle colony dogs raised in controlled environmental conditions, birth weight did not correlate with adult overweight status, but fast growth rate from birth to 2 weeks was a predictor for adult overweight at 2 years

of age (4). By the age of 7 months, BCS discriminated between dogs that would be overweight as adults and those that would be slightly overweight or ideal weight (4). No significant difference was found between adult weight groups in their energy intake or resting energy expenditure corrected for metabolic bodyweight at the age of 4 months. Resting energy balance between the age of 7 and 10 months was significantly higher in puppies who were overweight compared with ideal weight in adulthood. During this study, dogs were fed *ad libitum* (time-restricted after weaning) with a diet formulated for growth, or for neutered adults, as appropriate. In a different study, a retrospective analysis of veterinary practice records found that dogs that were obese by 3 years of age (127 breeds, 93% neutered) had faster growth in body weight between 12 and 60 weeks of age than that modeled in healthy dogs in ideal body condition (59).

Neutering is common for dogs and cats and is often performed before puberty while they are still developing. The exact timing varies between countries, species and breeds, and is still a controversial issue. Neutering is well established as one of the most significant risk factors for obesity in adults (46, 60–65). Prepuberty or peripuberty neutering in the context of this review is considered an early-life environmental exposure of relevance to the risk of obesity, albeit during the later stages of development. The potential impact of sex (60, 66, 67) and age of neutering on the effects of neutering on adult obesity are unclear because findings differ by study. A prospective cohort study of Golden Retrievers identified an ~42% greater risk of obesity in dogs neutered between 6 and 12 months





of age compared with those neutered at >1 year of age, but no difference in risk between neutering at <6 months compared with 6–12 months and >1 year (66). Conversely, in a retrospective study of veterinary records, age at neutering (ages ≤6 months, >6 months to ≤1 year and >1 to ≤5 years of age) was not associated with the risk of obesity (67). When growth patterns of pet dogs from the same proprietary data source were examined, neutering before and after 37 weeks was associated with slight upward or downward shifts in growth trajectory, respectively; however, these shifts were small, suggesting limited overall impact on weight gain and, therefore, future obesity (68). Differences in study design and dog breeds might explain apparent inconsistencies between these studies. Indeed, interactions between breed size, age at neutering and number of veterinary visits per year were reported to affect the risk of overweight (67).

Hormonal changes resulting from neutering could have a direct effect on the risk of obesity. Neutered dogs have lower metabolizable energy requirements than sexually intact dogs (69, 70), and neutering can increase indiscriminate appetite (71). Evidence suggests that neutering could also unmask or augment the effects of environmental exposures in the younger animal. Increases in the bodyweight of female Beagle dogs after neutering between the ages of 7 and 10 months were higher in dogs retrospectively identified to be at risk of adult obesity by having a higher neonatal growth rate than their contemporaries of an ideal adult bodyweight (4). The growth of Labrador Retrievers between 2 and 21 days of life was associated with risk of obesity at adulthood, but only in neutered dogs (72).

### Early-life risk factors for obesity in adult cats

In humans, breast feeding has a protective effect against childhood obesity compared with feeding formula milk (73), which might in part be associated with the presence of leptin in breast milk. Leptin is a hormone produced by adipose tissue that inhibits food intake and modulates glucose metabolism; the main source for neonates may be maternal milk (74). In a small study in cats, the odds for overweight in adulthood were 3 times less in kittens suckled for >6 weeks compared with <6 weeks, and there was a predisposition for overweight with suckling duration of 11 weeks or less (75). The investigators hypothesized that a short suckling period might lead to perturbations in the development of control mechanisms for fat accumulation and body composition through curtailment of leptin intake. A fast growth rate in cats is a key risk factor for obesity. A comparison of *ad libitum*-fed colony cats that were overweight with those of ideal weight at a median of 8.5 years of age, showed a significant association between growth rate between 3 and 12 months and later overweight status (76). In a different study that modeled the growth of colony cats fed *ad libitum* from weaning, early growth rate indicated by weight at 15 weeks of age was a significant predictor of being overweight at 9 years (77). Hypotheses to explain these associations include genetic, epigenetic and *in utero* factors, in addition to physical activity, food quality, feeding behavior and the gut microbiome (76).

Faster growth rate, smaller litter size, lower birthweight, and maternal overweight before pregnancy were associated with a predisposition of kittens to be overweight at 8 months of age in a study focused on genetic factors, and designed to reduce the

potential for non-genetic confounders and epigenetic differences (78). Despite the study design, some of the findings suggested that developmental programming might have played a role. The authors speculated that epigenetics might underlie the weak but significant negative correlation of litter size with overweight at 8 months. Also, epigenetic differences might have contributed to the observation that, although both overweight mothers vs. lean and variable-weight mothers, and male vs. female sex were associated with faster weight gain of kittens, this relationship became statistically significant for the maternal phenotype later than the sex difference.

As in dogs, neutering of male and female kittens is a risk factor for adult obesity (65, 79, 80). Whilst neutering is associated with increased appetite and food intake (81–83), it is also associated with reduced maintenance energy requirements (70, 81, 84). There is insufficient evidence in cats to know whether the age at neutering is associated with risk of obesity. However, differential changes in appetite have been associated with age of neutering; acute hyperphagia was observed in female cats neutered at 31 weeks of age but not in those neutered at 19 weeks of age (85). These behavioral changes may be associated with the effects of neutering on appetite-related hormones such as ghrelin, leptin, adiponectin and glucagon-like peptide-1 (86). For example, in a study of adult male cats, serum concentrations of adiponectin rapidly decreased after neutering, and within 7 days, there was a significant increase in serum concentration of ghrelin (83).

## Potential research priorities

### Nutrition

The role of early-life nutrition in the development of adult obesity demands more extensive and diverse research. In puppies and kittens and their parents, nutrition is relatively easy to modify in both research and “real-world” settings, and is likely to have multiple impacts on factors associated with obesity (87–91). At its simplest, chronic excessive calorie intake that starts at a young age results in progressive accumulation of body fat that ultimately manifests as adult obesity. However, a wealth of evidence in other species, including humans, shows that nutritional insults both *in utero* and postnatally can program later obesity and other metabolic disorders (92, 93). Models of obesity in polytocous species demonstrate that poor maternal nutrition (quantitative and/or qualitative) can modulate aspects of fat deposition and energy homeostasis in offspring through epigenetic mechanisms (89, 94, 95). Alterations in the development of the offspring’s hypothalamus-adipose tissue axis are believed to be particularly important for obesogenic traits, manifested as structural changes, mal-programming of appetite regulation favoring orexigenic pathways, central leptin and insulin resistance, and alterations in noradrenergic innervation of adipose tissue (96, 97). Research is needed to determine if low birth weight in puppies, as a risk factor for adult obesity, is an

example of fetal programming of a “thrifty” phenotype, whereby a metabolic profile set to cope with inadequate nutrition during pregnancy, later becomes a risk factor for obesity in the context of abundant postnatal nutrition. Paternal nutrition in laboratory animal models can also program obesogenic traits in the offspring (98), but this does not appear to have been researched yet in companion animals.

Obesogenic traits can also be sensitive to postnatal nutritional environment as development of organs and hormonal pathways continues after birth in mammals (87). The literature on postnatal maturation of domestic carnivores is limited, and as in other species, the timing depends on the organs involved (Supplementary Tables 1, 2). For example, changes in the morphology of organs such as the adrenal gland can occur during the first year (99), functional maturation of digestive processes may not occur until 3 months (100) and the immune system may not attain all adult characteristics until 12 months (101). Myelination of the neocortex continues to increase until ~9 months after birth (102). Nevertheless, it is reasonable to hypothesize that developmental plasticity is concentrated in the suckling period. Research is needed in dogs and cats to determine the effects of diet in the pregnant and lactating dam on the quantity and quality of colostrum and milk, and whether these effects have consequences for the offspring’s adult body composition and metabolism. The evaluation of the impact of food intake and nutritional interventions of the first days of life is particularly relevant for low birth weight puppies and kittens when considering nutritional interventions; rapid catch-up growth is associated with an increased risk for adult obesity in other species (97).

### Growth

In both puppies and kittens, higher growth rates have been associated with adult obesity (59, 77). It is unclear if and how aspects of energy balance regulation during growth predispose adults to be obese or of ideal weight. Postprandial decreases in acylated ghrelin, an orexigenic gut hormone, are delayed in 7-month old female Beagles already identified as being on a trajectory to adult overweight, and this may promote excess food intake (4). The basal plasma concentration of leptin is positively associated with adiposity but does not appear to be an early predictor of weight gain. In humans; evidence suggests that leptin’s main role is to signal low body fat stores in situations of negative energy balance (4, 103). Research is needed in larger study populations with different breeds and sexes to characterize further the dynamics of energy balance during growth associated with adult obesity, and to evaluate any role of developmental programming and the environmental triggers. Ideally, studies of growth and obesity should evaluate body composition. However, whilst for practical reasons BCS is most commonly used to evaluate adiposity, the scoring scales have only been properly validated in adult dogs and cats. Puppies and kittens have different body composition profiles

and morphologies compared with adult dogs and cats (104–106), which makes diagnosing overweight status with BCS scales designed for adults unsatisfactory. Greater objectivity and more uniformity between studies might easily be achieved by evaluating growth against growth rate standards now available for a comprehensive range of different breed sizes from 12 weeks of age (68, 107). These standards will be valuable in facilitating DOHaD research in obesity by identifying rapid or slow growth at an early age, and for case ascertainment in body composition and metabolic studies.

### Gut microbiota

Differences in the gut microbiota and/or microbiome of obese vs. lean adult dogs and cats have been observed (108–112) and changes characterized in obese dogs and cats during diet-driven weight-loss studies (108, 113, 114). However, associations between diet, gut microbiota, enteroendocrine hormones and metabolic disturbances are complex, with studies reporting contrary findings (113, 115, 116). When the effects of macronutrient ratios in diets fed to both dams and their kittens were evaluated, composition of the pre-weaning diet did not affect the profile of bacterial populations in kitten feces at 8 weeks, but did modulate expression levels of genes in the glucose and metabolic pathways in blood samples taken at 18 weeks (117). The findings were reversed for a comparison between two post-weaning diets. What has not been investigated directly is any association between gut microbiota as it is developing in the puppy and kitten and adult obesity, and the potential for early nutrition to influence this. Research in other species on developmental programming suggests that could be a fruitful avenue of research (118, 119).

In mice, gut microbiota mediate changes in global histone acetylation and methylation of DNA both locally in cells of the colon and distally in tissues such as liver and white adipose tissue (120). These microbe-mediated changes have been demonstrated in species other than dogs and cats during early life at a time when the gut microbiota is developing (121). Microbial metabolites have a direct role in epigenetic modifications, and the composition of the gut microbiota is relevant because the profile of metabolic byproducts of dietary constituents such as short-chain fatty acids (SCFAs) may differ between bacterial species (120, 122). Factors such as suckling vs. bottle feeding, lifestyle, environment and exposure to antimicrobials may also impact obesogenic traits through their effects on the emergent microbiota (123, 124).

### Neutering

The strength of neutering as a modifiable risk factor for obesity in both dogs and cats demands a greater understanding of the interactions between sex hormones and diet on appetite-related hormones and blood metabolites (86). The impact of neutering at different stages of development (early vs. late) needs to be dissected to resolve differences between studies and explore

sex, species and breed differences. The impact of environmental exposures such as nutrition and growth rate during the first days/weeks/months on the effects of subsequent neutering is under-researched, but existing data warrant further longitudinal prospective studies (4). One question to be addressed is whether neutering unmasks or potentiates the effects of developmental programming puppies or kittens.

### Interaction of environmental exposures with genetic susceptibilities to overweight and obesity

The interaction of genetic risk factors with modifiable variables in development can increase or decrease the likelihood of particular phenotypes. In humans, genes enriched or only expressed within the central nervous system have a central role in the biology of obesity (125). Knowledge of genetic susceptibilities can help researchers design studies on developmental programming and interpret their results.

Dog breeds including Pug, Beagle, Golden Retriever, English Springer Spaniel, Border Terrier, Labrador Retriever, and Cavalier King Charles Spaniel are at a higher risk for overweight than crossbred dogs (126, 127), whilst domestic short-hair cats have an increased risk of obesity (127). Candidate genes for genetic variants suspected to increase the risk of obesity in dogs include *POMC*, *FTO*, *PPARG*, *MC4R*, and *MC3R*, *INSIG2*, *GPR120* (127). Genetic variants may be restricted to a small number of breeds, e.g., a 14 base-pair deletion in *POMC* associated with obesity and food motivation found in Labradors and Flat-coated Retrievers (128, 129). Genetic risk factors need to be a consideration in studies investigating the impact of early-life environment on obesity. Genome-wide association studies could help elucidate the genetic background of obesity in companion animals and there is potential value in both within breed and large-scale across-breed approaches.

## Chronic enteropathy in dogs and cats

Chronic enteropathy is an overarching term that encompasses subgroups of chronic intestinal disorders based on treatment response: immunosuppressant-responsive enteropathy [IRE, previously known as idiopathic inflammatory bowel disease (IBD)], food-responsive enteropathy, and antibiotic-responsive enteropathy (12). The prevalence of CE reported in different studies ranges from 1 to 18% (12). In cats, IRE frequently coexists with small cell lymphoma (130), which is considered to fall under the umbrella of CE in this species (131). Although the underlying etiology of each subtype of CE is unclear, and may not be the same, they are chronic inflammatory conditions, and the pathogenesis reflects interactions between the gut microbiota and gut immune systems in the context of environmental factors such as diet, and genetic susceptibilities in some breeds.



## Early-life risk factors for chronic enteropathy in adult dogs

There are very few data on early-life risk factors for CE in dogs; however, these limited data implicate a diverse range of variables that warrant full investigation. Puppies that had historically presented in the acute stages of canine parvovirus infection at a median of 12 weeks of age, had a greater risk of owner-reported chronic gastrointestinal signs in later life than control dogs that presented at the veterinary clinic either for a routine check or for signs not associated with parvovirus [odds ratio 5.33 (95% CI: 2.12–14.87)] (132). A similar study also found that previous parvovirus enteritis was a risk factor for persistent gastrointestinal signs, and among dogs that had recovered from parvovirus infection, markers of disease severity were associated with that risk (133). In another study, early modifiable risk factors for CE in adulthood included vaccination of the dam during pregnancy, type of solid food fed to puppies during the first 6 months, and the puppy's body condition ("slim" rather than "normal weight") (134). These results should be interpreted with caution because of methodological limitations such as retrospective owner questionnaires, participant bias and broad diet types that were not nutritionally controlled. In a retrospective review of veterinary records from a medical teaching hospital in the USA, neutering was associated with an increased odds of IBD in males and especially female dogs (odds ratios for neutered vs. sexually intact 1.43 and 2.0, respectively,  $p < 0.05$  for both) (135). The authors hypothesized that the same anti-inflammatory and antioxidant effects of estradiol demonstrated in murine models could be protective against IBD in dogs.

## Early-life risk factors for chronic enteropathy in adult cats

Although chronic enteropathy in cats is well described in the literature (131, 136), no studies were found that have investigated modifiable risk factors in kittens.

## Potential research priorities

Disruption to the maturing gut microbiota, which might be due to diet or antimicrobials, is associated with increased risk of later IBD in humans or experimental colitis in animal models (137). Research suggests that epigenetic modifications underlie interactions between diet, the immune system and the microbiota in the development of chronic diseases including IBD (121, 138).

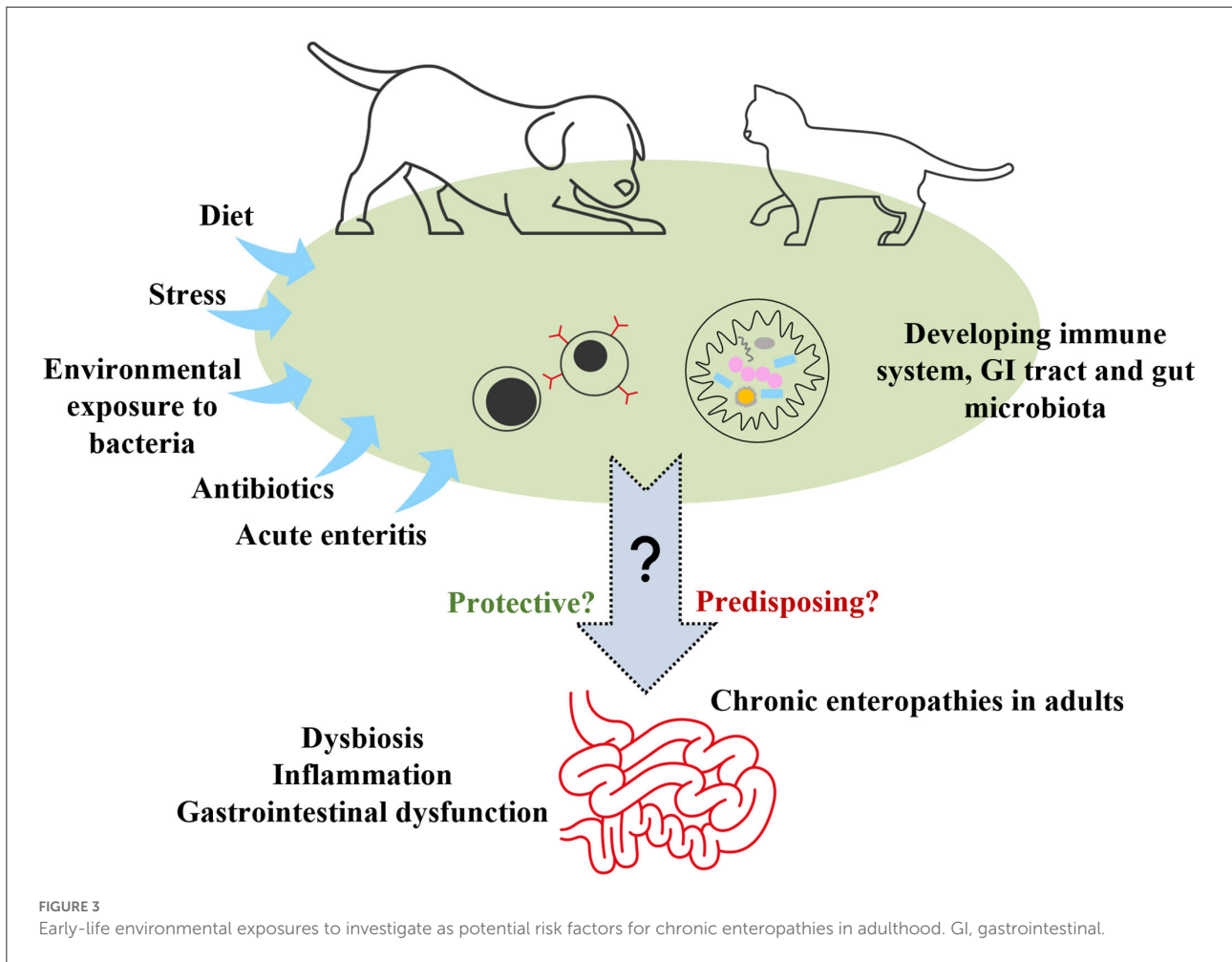
We suggest that investigation of any association between gut microbiota in puppies and kittens and development of CE in adulthood should be a research priority (Figure 3). There are complex interrelationships between gut microbiota, host metabolism, the immune system, intestinal inflammation and gastrointestinal health or dysfunction (139). Is there a

relationship between the gut microbiota that develops in puppies and kittens and that found in adult dogs and cats with CE, which differs from that in healthy adults? Do perturbations in the developing gut microbiota affect the maturation of the immune system and acquisition of tolerance in ways that predispose puppies and kittens to later CE? Do the effects of microbiota dysbiosis on the gut metabolome in these pets epigenetically program susceptibility to future CE and/or dysbiosis? Pieces of the puzzle have been characterized in puppies and kittens (140, 141), and separately in adults with CE (139, 142, 143), but the existence of a link between these has not yet been established.

Bacterial dysbiosis is defined as alterations in the composition of the bacterial gut microbiota leading to functional changes in the microbial transcriptome, proteome or metabolome, and/or decreased bacterial diversity (139, 144, 145). It is reported that 72%–79% of dogs and 76% of cats with CE have dysbiosis as evaluated by dysbiosis indices (142, 146, 147). Research in humans and animal models suggest that the role of dysbiosis in the pathogenesis of IBD could be causative (148, 149). This makes the development of the gut microbiota in puppies and kittens, and perturbations of this, of particular interest as a potential risk factor for CE.

The possibility in dogs of intra-uterine bacterial transfer from dam to fetus is controversial, but after birth, data suggest that the dam seeds the initial bacteria and her individual microbial profile plays a fundamental role in shaping the gut microbiota of her litter (150). The richness of bacterial species in the neonatal gut increases from day 2 after birth, and the gut microbiota changes significantly with age during the suckling and weaning period (151). The greatest changes to the microbiota of healthy puppies had occurred by 5–6 weeks of age in one investigation (151), although differences between the microbiota of offspring and dam were still apparent at 8 weeks in another study (152), and small changes might feasibly occur until 1 year of age (153). As with puppies, the gut microbiota of healthy kittens develops substantially during suckling and weaning, although the adult profile might not be fully achieved in those periods (154, 155). In a study in kittens, changes in the microbiome were still evident at 18–30 weeks of age, but had stabilized by 30–42 weeks (156). In another study, the microbiome was relatively stable in kittens aged 8–16 weeks (141, 157). Further longitudinal investigation is clearly needed. It is contended that the microbiota established in puppies and kittens is likely to be generally stable during healthy adult life as for humans, but this needs to be verified (139, 151, 152).

The relevance of dietary influences on gut microbiota and the gut microbiome in puppies and kittens to later gastrointestinal health status is likely to be multifactorial. Existing research needs to be extended to investigate early diet as a potential risk or protective factor for CE. For example, pre- and probiotic supplementation of Great Danes in the last week or last 4 weeks of pregnancy reduces the risk of neonatal gastroenteritis in their offspring (158). It is hypothesized that this protective



effect is conveyed *via* the entero-mammary link, given that in other studies feeding dams with pro and/or prebiotics improved the immune properties of their colostrum (159, 160). Another hypothesis (not mutually exclusive) is that the effect is mediated by the selection of health-promoting bacteria in the dam that then colonize the neonates.

The most profound disturbances to gut microbiota are those caused by antibiotic use. It is hypothesized that antibiotic use could be a major priming factor in puppies for later CE. In humans, antibiotic treatment in the first postnatal year is associated with an increased risk of later development of IBD (161–163). Acute diarrhea is common in puppies, and it is often treated with antibiotics. The fecal microbiota changes in dogs with acute diarrhea and the bacterial groups involved are not consistently reported to be the same as in chronic diarrhea, although reduction in fecal concentrations of SCFAs is a shared finding (139, 145, 164). In a prospective controlled study, metronidazole (a common antibiotic treatment for acute diarrhea) significantly altered the fecal microbiome and metabolome of healthy dogs, including

a decrease in the abundance of Fusobacteria, which are key SCFA-producing bacteria, and the main bile acid converting bacterium *Clostridium hiranonis* that was associated with a reduction in secondary bile acids (165). Changes persisted in nearly half of the dogs for at least 4 weeks. The long-term effects of such treatment in puppies still establishing a normal gut microbiota needs to be explored in studies on developmental programming. A course of antibiotic treatment (20 or 28 days) in 2-month old cats with upper respiratory tract disease was shown to delay the maturation of their gut microbiota compared to healthy untreated cats (166). The duration of effects differed between antibiotics; the impact of amoxicillin-clavulanate on the microbiome occurred mainly during treatment, whereas the impact of doxycycline was observed from 1 to 3 months after antibiotic withdrawal (166). Research should extend to the use of antibiotics in pregnant dogs; data in humans and mice suggest that this is a risk factor for gastrointestinal disease in the offspring (167, 168).

It is not known whether the gut microbiota influences early development of the gastrointestinal tract and susceptibility to

chronic disease through epigenetic modifications in puppies and kittens. Data from mouse studies however, point to the importance of gut microbiota in modulating post-natal development of the gut through DNA methylation of genes in intestinal epithelial cells associated with immunity, metabolism, and vascular regulation (122, 162). Changes in bacterial metabolites associated with CE in dogs are known in other species to influence epigenetic modifications affecting immune and inflammatory pathways. For example, decreased fecal abundance of *Fusobacterium* and *Faecalibacterium* in dogs with CE is associated with reduced fecal concentrations of the SCFA propionate (147, 169). Short-chain fatty acids can regulate epigenetic modifications by inhibiting histone deacetylases (HDACs) and contributing acetyl donors for DNA or histone modifications.

Abnormalities or deficiencies in immune responses to environmental antigens, together with genetic susceptibilities, appear to play central roles in the development of CE in dogs (170). The possibility that some of the immunopathogenesis in dogs with CE has origins in epigenetic changes was raised by an investigation of the reduced intestinal expression of mucosal IgA found in these dogs (171). Hypermethylation of the gene for TACI was negatively associated with expression of mucosal IgA; the authors hypothesized that such changes in methylation status might have been induced by inflammatory mediators and exposure of the gut to an altered intestinal microflora (171). Such mechanisms might therefore be a link between environmental exposures during development and risk of later CE.

Across all the avenues of research suggested, developmental periods of particular interest in puppies and kittens include initial colonization of the neonatal gut, weaning, and the transition from breeding facilities to new owners, when diarrhea is common, coinciding with changes in diet, stress and exposure to different microbial environments. Large populations need to be studied to understand interindividual variations in microbiota—there may not be a single “normal,” “healthy” microbiota. Robust studies are needed that use nutritionally specific diets and record only veterinarian-diagnosed CE. Those conducting research in developmental programming must of course consider breed susceptibilities and breed-independent genetic associations with disease. Dog breeds susceptible to CE include Weimaraner, Rottweiler, German Shepherd, Border Collie and Boxer (12, 172).

## Behavioral problems in dogs and cats

Behavioral problems in dogs and cats are common and can affect their welfare and quality of life (173), their relationship with humans, and their suitability for assistance work (174). Difficult behavior is frequently cited by owners as being at least one of the reasons for them relinquishing their pets to animal rescue centers, being the primary reason for 10% of dogs in

a recent Canadian study (13), and the sole reason for 27% of dogs and 19% of cats in a US study (14). They can also drive some owners to seek elective euthanasia for their pets (15). Although the nature of behavioral problems is wide ranging, such as aggression toward humans and other animals, separation anxiety, and soiling in the house, at least some adverse behavior traits detrimental to the long-term future of dogs and cats can be attributed to their early-life environment.

Most neurological development occurs during fetal life; it continues rapidly in the neonate, but myelin formation and maturation continues until at least 36 weeks of age in dogs (175). Regions of the brain develop at different rates throughout early life, potentially therefore remaining susceptible to environmental exposures (175–177). The development of behavioral and cognitive traits can be considered in different phases: gestation, the neonatal period including feeding, neurological stimulation and mothering in the first 3 weeks, early socialization from ~3 to 12 weeks of age, late socialization from 12 weeks up to 6 months, and the enrichment period, which may extend to 1 year of age (177, 178). It is believed that experiences during each period have cumulative effects on trainability, health and performance (177, 178).

## Early-life risk factors for behavioral problems in adult dogs

No research in dogs investigating the effects of maternal stress or diet during pregnancy on the behavior of offspring was identified, except a mention that puppies of malnourished dams were extremely nervous in addition to displaying physical abnormalities (179).

Poor maternal care and socialization before 3 months of age have been associated with fearfulness in dogs, and poor maternal care alone was also associated with a combination of fearfulness, noise sensitivity and separation anxiety (180). These data were derived from a survey of owners, but other studies with more objective measures show that the level of mothering can affect the performance of dogs in cognition tests, stress responses, and temperament in later life. However, some research findings appear to be contradictory as to whether an environmental exposure has a positive or negative effect. For example, in one prospective study, guide dogs that had experienced more intense mothering had poorer problem-solving abilities and showed higher levels of anxiety at 14–17 months of age, both of which were associated with a significantly greater risk of failing the guide dog training program (174). In contrast, a benefit of greater maternal care was demonstrated in male and female Beagle puppies; the mean duration of daily maternal care in their first 3 weeks was positively correlated with exploration and latency of the first yelp, and negatively correlated with stress in isolation tests at 8 weeks of age (181). A study with long-term follow up found that a higher level of maternal care of male and female German Shepherd dogs was associated

with greater physical and social engagement (e.g., ball retrieval, positive acceptance of handling) as well as aggression in young adults at 18 months of age (182). In summary, stimuli in the suckling period appear to effect some behaviors of adult dogs, but the direction of reported associations is not always intuitive or consistent, perhaps reflecting the complexity of the biology as well as interstudy differences in behavior tests, ages, and breeds (181).

In a review of seven observational studies on dogs originating from high-volume commercial breeding establishments and sold either online or through pet shops, risk factors were highlighted for later behavioral and psychological problems (183). In the largest of these seven studies, UK dogs acquired from sources such as pet stores and the internet were 1.8 times more likely to show aggression toward humans than dogs acquired directly from breeders (183, 184). Across studies, aggression was the most common problem behavior associated with commercial breeding establishments or puppy farms and pet stores. Although causative factors were not investigated, potential causes discussed included stress in the dam, insufficient or excessive neonatal stimulation, early weaning and maternal separation, and social isolation between the age of 3 and 12 weeks.

By the early socialization period, the central nervous system has developed to a stage that allows conditioning and associated learning (177). Socialization of puppies with familiar conspecifics is important for the development of communication competency, and early interactions with non-familiar conspecifics may influence the risk of aggressiveness in adult life (177, 185). For example, restriction of a puppy's contact with conspecifics in the 8 weeks after their first exposure to other dogs in a public setting was found to be associated with aggression toward unfamiliar dogs when they were 1–3 years old (185). Early socialization with humans is important for later responses to handling, leash training and stress tests (186).

Behavioral traits and non-social cognitive abilities continue to develop in puppies during the late socialization and enrichment periods (187–189). In young candidate working dogs, measures of inhibitory control, attention and spatial cognition improved between 3 and 12 months of age (187). In a second longitudinal study, performance of cognitive tasks improved between the age of ~ 9 weeks and 21 months, and the adult phenotype for some traits could be predicted from test results in puppyhood (188). However, little is known about specific exposures in these periods that might influence the course of brain development, and the general environmental context, breed and sex are also likely to play a role (189). Questionnaires completed by foster carers of puppies from ~ 2 months of age until the initiation of formal guide-dog training, showed a positive behavioral effect of growing up in a household with another dog and with more experienced puppy raisers (189). Puppies that had been attacked or threatened by an unfamiliar dog showed significantly higher “dog-directed fear”

and “stranger-directed aggression” at the age of 12 months old compared with puppies that had not experienced that trauma and had worse training outcomes (189). However the age at which the trauma had occurred was not specified.

A link between epigenetic changes and human-directed social behavior in dogs was found in one study (190). The DNA methylation of the promoter region of the oxytocin receptor gene (*OXTR*) was measured by bisulfite pyrosequencing followed by methylation-specific PCR in mouth epithelial cells obtained from various Canidae. Four differentially methylated 5'-cytosine-phosphate-guanine\_3' (CpG) sites were identified. They were subsequently studied in a large population ( $n = 217$ ) of Border Collies. Not only did DNA methylation status differ between females and males, it was also associated with their response in a “threatening approach” test in a sex-dependent manner. For example, more methylation at a specific CpG site in female dogs tended to correspond with a greater likelihood of appeasing behavior in the test, whereas the opposite relationship was found in male dogs. In addition, CpG sites differed in whether promoter methylation increases or decreased *OXTR* expression levels (190), both neuter status itself and the interaction of sex with neuter status did not predict methylation levels at the three CpG sites investigated. This study highlights the complexity of relationships between epigenetic modifications and behavior in dogs, and the need for research on environmental factors that influence the epigenetics of the *OXTR* gene.

## Early-life risk factors for behavioral problems in adult cats

There is a dearth of knowledge on the effect of the maternal environment during pregnancy and subsequent behavioral problems in adult feline offspring. However, one study showed that when the kittens of dams that had been malnourished throughout pregnancy were fostered onto non-food deprived cats, both physical and behavioral development were delayed (179, 191). The behaviors affected included time spent playing and the use of a litter tray. Moreover, in adulthood the cats displayed marked antisocial behavior and alternation between dominant and submissive behaviors. These observations could potentially represent classic DOHaD in cats. In other research, protein restriction of cats during late gestation and lactation adversely affected the attachment processes in both dams and kittens in the first 12 days after birth (192).

There is ongoing neurological development of kittens during the first 3 postnatal months (193). This is observed, for example, in increasingly sophisticated ability to respond to sound, development of visual placing and binocular coordination, and gross behaviors during interactions with siblings (193). Early life experiences of cats affect behavior in adulthood (194). For example, human handling of kittens during the sensitive period for socialization has been associated with more friendly behavior

toward humans at the age of 1 year (195, 196). The extent to which epigenetic modulation and/or genetic differences contribute to these observations is unknown.

### Potential research priorities

We suggest that research into the risk factors for behavior problems in dogs and cats needs to diversify to include assessments *in utero* and more studies of environmental influences in animals aged 6–12 months. This should not be at the expense of further work in neonates when brain development is particularly plastic. Neonatal studies are facilitated by the relative ease with which the environment can be controlled for related individuals in the same litter, although this does not allow for the distinction between genetic and environmental effects. Apparently contradictory results for the effect of maternal care on stress responses need to be explored, perhaps by comparing different stress challenges and intensities at various ages in animals kept in standardized environments. More long-term studies are required to determine the durability and reversibility of the effects of early-life environment on adult behavior.

The extent to which epigenetic modulation drives risk factors for behavioral disorders in dogs and cats is not known. The few existing data linking epigenetic changes to dog behavior (190) highlight the complexity of the relationships involved. Environmental factors influencing known epigenetic variation of *OXTR* are especially important to explore. Polymorphisms in the gene for the dopamine receptor 2 (*DRD2*) are associated with fearful behavior in some breeds of dog (197), and a variant haplotype in this gene is associated with anxiety separation in Golden Retrievers (198). *DRD2* could therefore be another gene of interest to study for epigenetic changes affecting behavior.

Studies in other species provide a rich source of developmental behavioral data and hence hypotheses for dogs and cats. Various cognitive, behavioral and emotional disturbances in children have been associated with stress during development (199, 200). Prenatal stress can result in structural and functional changes in multiple regions of the developing fetal brain, including the hypothalamus-pituitary axis (199, 201). DNA methylation of the glucocorticoid receptor gene and *OXTR* are examples of mechanisms believed to link childhood experiences with psychiatric disorders and temperament, respectively (202, 203). Preconception experiences of parents may also be relevant and can affect anxiogenic responses of their offspring and subsequent generations of descendants (204, 205). Rodent studies highlight the importance of the exact timing of environmental exposures and sexual dimorphism in the developmental sequelae (206). Stresses that may affect the behavior of offspring include, for example, preconception psychological trauma of either parent, maternal diet during pregnancy, early separation of offspring from their dam, and mothering behaviors (201, 205, 207, 208). Evidence from

various species also links the composition of the gut microbiota with neurocognitive and behavioral development, building the concept of a microbiome-gut-brain axis (138, 209, 210).

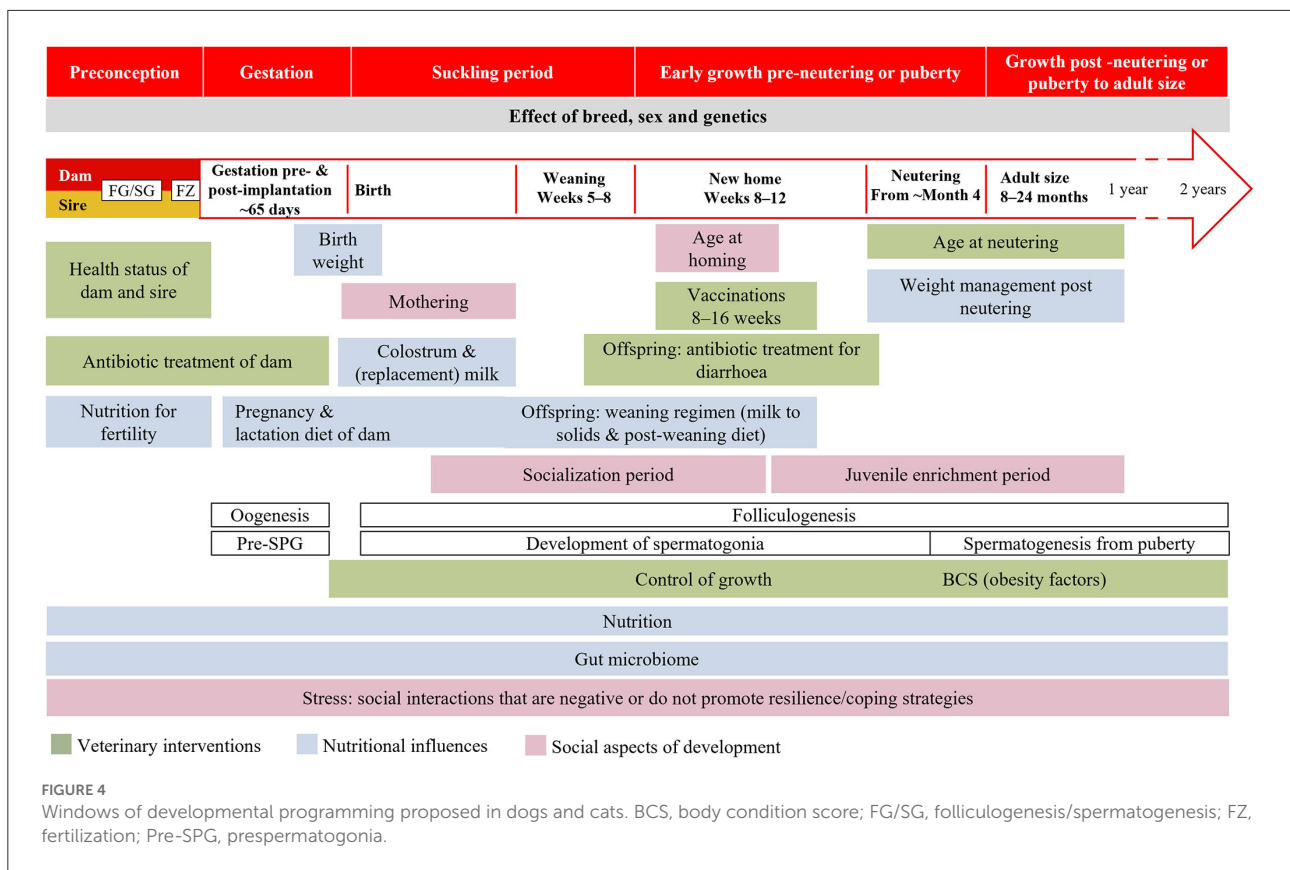
### Strategies for research to understand early-life risk factors for chronic diseases and behavioral problems in dogs and cats, and the potential role of developmental programming

The developmental origins of health and disease have not yet been confirmed in pets, but the examples discussed suggest that developmental programming is likely to be as important as in other species. We recommend a concerted multidisciplinary approach to explore developmental programming in dogs and cats and to close the large knowledge gaps compared with other species.

Human research and information campaigns on DOHaD are conducted in the context of a species-specific critical window of development. We believe that the overall critical window of time implicated in the development of dogs and cats extends from preconception to the end of growth, comprising five periods: preconception, gestation, the suckling period, early growth pre-neutering or pre-puberty, growth post neutering or post puberty to adult size (Figure 4). Within this window, there will be different and sometimes overlapping critical periods for different aspects of health and disease according to the developmental plasticity of the relevant tissues. The upper age limit of this “window” will depend upon species and breed. Variables of particular interest for DOHaD research in dogs and cats, both individually and in combination, include the maternal and neonatal environment, nutrition and associated weight gain and/or growth rate, the gut microbiota, weaning stress, and neutering (Figure 4). Work in other species highlights the need for studies to consider the periconception period (211), the exact timing of environmental exposure, differences in programming between the sexes (212–214), the role of the placenta (215), paternal influences on the offspring’s epigenome, breed and genetic variation (216–218).

It is critical for the whole scientific research community to be able to access large datasets encompassing high quality “whole of life” data and biobanks of tissue samples in order to explore the long-term impact of exposures in early life. Research colonies of cats and dogs with internal breeding programs are useful to address the effect of individual interventions, but they are uncommon and do not reflect the situation of household pets, which are exposed to many non-controlled, interacting environmental factors. Analysis of large prospective observational cohorts of privately owned animals, perhaps spanning up to 15 years to encompass whole lifespan, may identify effects of programming that are small in the individual





and very variable between individuals. A particular challenge to be addressed is obtaining longitudinal data for a dog or cat that follows both parents in the preconception period and extends through gestation and neonatal life to adulthood and end of life. This might need data from two breeders (one for the sire and one for the dam), at least one owner, and probably at least three veterinary surgeons (one each for the sire, dam and puppy or kitten). These stakeholders need to be engaged with the potential wide-ranging and long-term implications of DOHaD and the many possible opportunities for intervention. Institutions that breed and train dogs for service roles, and subsequently monitor their progress, e.g., guide dogs, can provide collaborative opportunities for researchers.

Although there are ongoing large, prospective and observational longitudinal studies, these primarily target dogs after they have left the breeder, and so will lack data from the first 2 months after birth. For example, the Generation Pup project operated by the Dogs Trust in the UK was initiated in 2016 to use owner and veterinary data from up to 10,000 dogs to identify modifiable risk factors during development that impact adult health and welfare (219). The research will investigate relationships between genotype, environment, and health and behavior outcomes at different life stages. There are also breed-specific longitudinal cohort studies such as the

Golden Retriever Lifetime Study run by the Morris Animal Foundation in the USA, which is collecting data on the lifestyle, environment, behavior and health of 3,000 dogs recruited between 2012 and 2015, including annual biological samples (66, 220, 221). The ongoing Dogslife epidemiological project (University of Edinburgh, University of Manchester, University of Liverpool and the Kennel Club) recruits UK pedigree Labrador Retrievers born after January 2010 ( $n = 6,084$  dogs by December 2015) (222, 223). Owners complete questionnaires each month for the first year of their dog's life and every 3 months thereafter (222, 223). In the USA, the ongoing Dog Aging Project (University of Washington and Texas A&M University) has recruited tens of thousands of companion dogs to explore aspects of "health-span" i.e., the period of life spent free from disease (224). The Norwegian School of Veterinary Science cohort established to investigate skeletal disease in four large dog breeds ( $n = 700$  puppies recruited 1998–2001), is notable as an example of a longitudinal cohort in which dog litters were recruited from the time of the dam's mating, and data were obtained from breeders, owners and veterinarians (225, 226).

Longitudinal cat registries and cohort studies appear to be scarce. The landmark Bristol Cats Study led by the University of Bristol is the first reported birth cohort study of kittens

(227). Cats were registered between the ages of 8 and 16 weeks, and owners complete questionnaires at set intervals. Analyses reported to date include the prevalence of and risk factors for obesity, and owner-reported lower urinary tract signs (228–230). The Cat Phenotype and Health Information Registry in the USA (UC Davis Veterinary Medicine) collects DNA samples from healthy and diseased cats with long-term follow-up where possible (231).

Data on epigenetic mechanisms linking early-life experiences to adult disorders in dogs and cats are scant; they are needed to help confirm and understand developmental programming, and unravel the effects of genetic background. Methods for profiling genome-wide DNA methylation are well established, and much can be achieved before attempting to identify the specific genes responsible for an epigenetically determined phenotype (232, 233). Epigenetic mechanisms other than DNA methylation should also be studied, such as histone modifications, including but not limited to methylation and acetylation, and non-coding RNA that can regulate gene expression during cell differentiation and development (234). A publicly available repository of canine epigenomic data (BarkBase) has recently been established, comprising the results of RNA sequencing and assays determining chromatin accessibility across the genome (235). The database includes 27 different adult tissues and five fetal tissue types at four embryonic timepoints. The Royal Veterinary College has instituted the Companion Animal Brain Bank—a standardized collection of brain tissue and other biological samples from dogs and cats euthanized with neurological conditions, together with appropriate controls. Although such tissue banks could be used to investigate changes in the epigenome, including those associated with disease, without corresponding data on environmental aspects of pregnancy and early life, they will not provide evidence of developmental programming.

The effect of early-life experiences and developmental programming on at least some physiological characteristics will be affected by genotypic differences between the many breeds and mixed breeds. Targeting research to specific breeds on the basis of their propensity for developing the disease or behavior of interest can be advantageous, e.g., the Labrador Retriever for obesity.

There are fewer breeds of cats to contend with in DOHaD research, but overall the knowledge gaps are greater than in dogs. There appear to be fewer longitudinal field data in cats compared with dogs, and there is probably less public awareness of the potential impact of developmental programming on chronic diseases.

## Concluding remarks

There is direct evidence for early-life risk factors associated with obesity and behavioral problems in dogs and cats, and to

a much lesser extent CE in dogs. However, multidisciplinary prospective long-term research is needed to confirm DOHaD in these species. Extensive data from other species provide a scientific foundation to help prioritize early-life events and exposures for investigation. The diversity of dog and cat breeds, breeding management and lifestyles adds complexity to such research. It is believed that breeders, owners and veterinary surgeons each have a critical window of opportunity in one or more of the life stages from pre-conception to the end of the dog or cat's growth phase in which to promote programming beneficial to long-term health. An appreciation by each of these groups of the overall window of development may also help to foster shared responsibility, transparency and information sharing.

Dogs and cats are considered to be family members, and yet veterinary medicine struggles to treat common conditions that adversely impact pets' quality of life, the special owner–pet bond, and the health benefits pets can bring to individuals and society. Preventive medicine and husbandry practices from pre-conception onwards must take a higher priority and be fueled by a better understanding of developmental programming at the population level.

## Author contributions

VG, SC, GE, OF, AG, JS, CV, PC-P, and FP contributed substantially to the conception of the article and to interpretation of data presented. All authors critically reviewed the manuscript for important intellectual content. The authors take full responsibility for the scientific content of the paper and they have all approved the submitted version.

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## Conflict of interest

FP and VG are employees of Royal Canin SAS. SC is a French Government Agent and head of NeoCare ENVT, a Public Research Unit. NeoCare Unit was partially funded by Royal Canin Research Division from 2012 to 2021. As such, SC has presented research results and clinical recommendations at Royal Canin-sponsored conferences. GE provides veterinary clinical advice to Waltham Petcare Science Institute, Waltham-on-the-Wolds, Leicestershire, UK. OF is an employee of Wisdom Panel (Kinship), a Mars Petcare Company. AG is an employee

of the University of Liverpool, but his post is financially supported by Royal Canin, has received financial remuneration for providing educational material, speaking at conferences, and consultancy work from Royal Canin, and is also a member of the Guide Dogs Scientific Advisory Group. JS is an employee of the Gastrointestinal Laboratory at Texas A&M University, which offers diagnostic testing on a fee-for-service basis, and has received speaker honoraria and consulting fees from Royal Canin SAS, Nestlé Purina Petcare, ExeGI Pharma, Nutramax Laboratories, and Hill's Pet Nutrition. CV has done consulting work for a variety of pet food companies (Nestlé Purina PetCare, Royal Canin, Mars Pet Care, and Dechra Specific), has participated as an investigator in clinical trials sponsored by Royal Canin and Affinity Pet Care, develops educational materials for Morris Institute, is part of the Scientific Advisory Board of FEDIAF and a member of the Global Nutrition Committee of the WSAVA, and participates as a speaker or attendee in continuing education events sponsored or organized by Royal Canin, Nestlé Purina PetCare, and Hill's Pet Nutrition.

The remaining author declares that the research was conducted in the absence of any commercial or financial

relationships that could be construed as a potential conflict of interest.

The reviewer AW declared a shared affiliation with the author GE to the handling editor at the time of review.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2022.944821/full#supplementary-material>

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